Diagnostic Value of Neutrophil-to-Lymphocyte, Platelet-to-Lymphocyte and Monocyte-to-Lymphocyte Ratio for COVID-19 Screening

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ABSTRACT

COVID-19 is caused by SARS-CoV-2, which can affect all ages. The prevalence of COVID-19 reported by the World Health Organization (WHO) in March 2020 was 3 million cases worldwide. The number of confirmed cases of COVID-19 reported by WHO in June 2020 in Indonesia was 28,233 cases. This research was an observational analytic study with a cross-sectional approach to determine the performance and cut-off of NLR, PLR, and MLR as a screening for COVID-19 infection conducted at the RSDM Clinical Pathology Installation in Surakarta from March 2020 to April 2021. The total subjects of this study were 348 people. The characteristics of the research subjects were presented in descriptive form. The Receiver Operating Characteristic (ROC) curve and the Area Under Curve (AUC) were used to determine the cut-off of NLR, PLR, and MLR. The results were presented in a 2x2 table. A computer program was used for statistical analysis. There was a significant relationship between NLR, PLR, and MLR and the incidence of COVID-19. A cut-off \( \geq 3.010 \), sensitivity 66.5%, specificity 61.9%, PPV 0.773, NPV 0.487, LR (+) 1.744, and LR (-) 0.541 were obtained for NLR as a COVID-19 screening. A cut-off \( \geq 157.035 \), sensitivity 63%, specificity 60.2%, PPV 0.755, NPV 0.455, LR (+) 1.583, and LR (-) 0.614 were obtained for PLR as a COVID-19 screening. A cut-off \( \geq 0.296 \), sensitivity 60%, specificity 58.5%, PPV 0.738, NPV 0.429, LR (+) 1.445, and LR (-) 0.684 were obtained for MLR as a screening for COVID-19. NLR and PLR cannot be used as the main screening biomarkers for COVID-19. Regardless of the clinical manifestations of patients, other biomarkers such as antigen swabs should be considered.

Keywords: COVID-19, NLR, PLR, MLR

INTRODUCTION

Coronavirus Diseases 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The pathogen was originally identified as 2019 novel Coronavirus (2019-nCoV). The risk for transmission of COVID-19 increases at the age of more than 60 years and in people whose comorbidities such as diabetes, cardiovascular disease, chronic kidney disease, and cancer.1 WHO reported a total of 28,233 confirmed COVID-19 cases in June 2020 in Indonesia and the highest number of cases was found in ages > 30-45 years (29.4%), followed by ages > 45-59 years (27.3%), > 17 -30 years (20.5%), ≥ 60 years (14.9%), > 5-17 years (5.5%) and 0-5 years (2.4%), respectively.2,3

The clinical features of COVID-19 range from an asymptomatic state to multi-organ dysfunction. Common clinical features are fever, cough, sore throat, headache, fatigue, and shortness of breath. Some patients by the end of the first week will develop pneumonia, respiratory failure, and death. The development of this disease is associated with an increase in cytokines known as cytokine storms. Complications of COVID-19 can vary from acute lung injury, acute respiratory distress syndrome (ARDS), and shock to acute kidney damage.4,5

METHODS

This study was an observational analytical cross-sectional study to determine performance and the cut-off value of NLR, PLR, and MLR in the screening of COVID-19 infection from patient data treated at RSDM. The research was conducted at the Clinical Pathology Laboratory Installation and Medical Record Installation RSDM Surakarta. The study was conducted from April to May 2021. Retrospective data of research subjects were collected from the medical records of patients treated from April 2020 to May 2021. The sample size was estimated based on the sample size formula for the diagnostic test study design, and a minimum sample size of 348 patients was obtained.6

The target population in this study were all patients at RSDM Surakarta suspected of COVID-19
infection and had COVID-19 PCR test results. The inclusion criteria in this study were patients who were suspected of COVID-19, aged > 18 years who had a PCR COVID-19 swab test results and routine blood count. The exclusion criteria in this study were patients infected with COVID-19 with a history of DM, hypertension, heart disease, kidney disease, liver disease, autoimmunity, and malignancies based on medical records. The study was conducted on patients suspected of COVID-19 who were treated at RSDM Surakarta and who met the inclusion and exclusion criteria selected by consecutive random sampling.

The characteristics of the research subjects were presented in descriptive data. Kolmogorov-Smirnov test was used to determine the normality with a significance of p > 0.05. In addition, data with normal distribution were presented as mean±SD, whereas data with abnormal distribution were presented as median (min-max).

RESULTS AND DISCUSSIONS

This study involved 348 patients consisting of 230 patients with positive COVID-19 patients and 188 patients with negative COVID-19 and without comorbid disease who were treated at RSDM and carried out PCR swab examinations and routine blood counts at the RSDM Clinical Pathology Laboratory Installation in Surakarta, Central Java from March 2020 to April 2021. Characteristics of research subjects in nominal categorical data (gender) were presented in frequency distribution (%) and analyzed with the Chi-Square test, whereas numerical data (age, hemoglobin (Hb), White Blood Cell (WBC), platelets (PLT) absolute neutrophils, absolute lymphocytes, and absolute monocytes) were expressed as mean±Standard Deviation (SD).

Kolmogorov-Smirnov test was used to determine normality of data and Mann-Whitney test was then used in this study because the data were not normally distributed. The test results of the characteristics of research subjects are shown in Table 1.

There was a significant difference in gender, Hb, WBC, absolute neutrophils, and absolute lymphocytes, whereas there was no significant difference in the variables of age, platelets, and absolute monocytes (Table 1). The ROC curve was then used to determine the cut-off values of NLR, PLR, and MLR (Figure 1).

![ROC Curve](image)

**Figure 1.** The ROC curve of the results of the NLR, PLR, and MLR examinations

The diagnostic test for determining NLR as a screening for COVID-19 can be seen in Table 3. The NLR at cut-off > 3.010 had a sensitivity of 0.665, indicating that NLR > 3.010 would be able to detect 66.5% of positive COVID-19 patients. In addition, the

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean COVID-19</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>121 (52.6%)</td>
<td>43 (36.4%)</td>
</tr>
<tr>
<td>Females</td>
<td>109 (47.4%)</td>
<td>75 (63.6%)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>45.73±13.94</td>
<td>46.5 (17.00-85.00)</td>
</tr>
<tr>
<td><strong>Routine blood</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.96±2.14</td>
<td>12.25±2.47</td>
</tr>
<tr>
<td>Leukocytes (10^9/L)</td>
<td>12.86±43.62</td>
<td>8.63±6.10</td>
</tr>
<tr>
<td>Platelets (10^9/L)</td>
<td>283.00 (26.90-580.00)</td>
<td>273.93±125.68</td>
</tr>
<tr>
<td>Absolute neutrophils</td>
<td>13.09±60.68</td>
<td>5.81±4.32</td>
</tr>
<tr>
<td>Absolute lymphocytes</td>
<td>1.71±2.01</td>
<td>2.04±1.19</td>
</tr>
<tr>
<td>Absolute monocytes</td>
<td>0.60±0.66</td>
<td>0.67±0.95</td>
</tr>
</tbody>
</table>

Note: * p <0.05, there is a statistically significant difference

Table 1. Basic characteristics of research subjects

The AUC value was determined for the cut-off of NLR, PLR, and MLR and the optimal cut-off point was then selected (Table 2).

The diagnostic test for determining NLR as a screening for COVID-19 can be seen in Table 3. The NLR at cut-off > 3.010 had a sensitivity of 0.665, indicating that NLR > 3.010 would be able to detect 66.5% of positive COVID-19 patients. In addition, the
Table 2. Results of the cut-off determination of NLR, PLR, and MLR for COVID-19 patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>1-Specificity</th>
<th>Cut-off</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR</td>
<td>0.695</td>
<td>0.665</td>
<td>0.381</td>
<td>3.010</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PLR</td>
<td>0.662</td>
<td>0.630</td>
<td>0.398</td>
<td>157.035</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MLR</td>
<td>0.617</td>
<td>0.600</td>
<td>0.415</td>
<td>0.296</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: NLR, Neutrophil-to-Lymphocyte Ratio; PLR, Platelet-to-Lymphocyte Ratio; MLR, Monocyte to-Lymphocyte Ratio; COVID, Coronavirus Disease; AUC, Area Under Curve

Table 3. Diagnostic test determination of NLR as a screening for COVID-19

<table>
<thead>
<tr>
<th>Examination</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR (+)</th>
<th>LR (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR (= 3.010)</td>
<td>66.5%</td>
<td>61.9%</td>
<td>0.773</td>
<td>0.487</td>
<td>1.744</td>
<td>0.541</td>
</tr>
</tbody>
</table>

Note: NLR, Neutrophil to-Lymphocyte Ratio; PPV, Positive Predictive Value; NPV, Negative Predictive Value; LR (+), Likelihood Ratio Positive; LR (-), Likelihood Ratio Negative

Table 4. Diagnostic test determination of PLR as a screening for COVID-19

<table>
<thead>
<tr>
<th>Examination</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR (+)</th>
<th>LR (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLR (=157.035)</td>
<td>63.0%</td>
<td>60.2%</td>
<td>0.755</td>
<td>0.455</td>
<td>1.583</td>
<td>0.614</td>
</tr>
</tbody>
</table>

Note: PLR, Platelet-to-Lymphocyte Ratio; PPV, Positive Predictive Value; NPV, Negative Predictive Value; LR (+), Likelihood Ratio Positive; LR (-), Likelihood Ratio Negative

Table 5. Diagnostic test determination of MLR as a screening for COVID-19

<table>
<thead>
<tr>
<th>Examination</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR (+)</th>
<th>LR (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLR (=0.296)</td>
<td>60%</td>
<td>58.5%</td>
<td>0.738</td>
<td>0.429</td>
<td>1.445</td>
<td>0.684</td>
</tr>
</tbody>
</table>

Note: MLR, Monocyte to-Lymphocyte Ratio; PPV, Positive Predictive Value; NPV, Negative Predictive Value; LR (+), Likelihood Ratio Positive; LR (-), Likelihood Ratio Negative

The expression and distribution of the receptors influence the pathogenesis of viral infections. The ACE2 gene encodes ACE2 as a receptor for SARS-CoV-2. ACE2 receptors are receptors that play a role in SARS-CoV-2 infection. Another in-vitro
study showed a positive correlation between ACE2 expression and SARS-CoV infection. In a study on the expression level and pattern of ACE2 in humans using RNA sequencing showed that Asian males had higher ACE2 expression than females. In addition, there are differences in ACE2 expression among ethnicities. A study conducted on a Chinese population found extreme expression of ACE2 in the human lungs of Asian males.\textsuperscript{9,10}

The mean hemoglobin (Hb) levels for positive COVID-19 patients were than negative COVID-19 patients with $p < 0.007$. These results were in accordance with other studies that found an increase in Hb in patients with positive COVID-19. The increase in Hb in positive COVID-19 is influenced by the presence of comorbidities and habits such as smoking because about 70% of the patients with positive COVID-19 consisted of males.\textsuperscript{11} This study found a correlation with gender, which indicated that there were higher number of male smokers compared to female smokers. This might have led to an increase in the Hb levels in patients with positive COVID-19.

Leukocyte count data showed that patients with positive COVID-19 had a higher mean leukocyte level of 12.86±4.62x10\(^9\)/L compared to that of negative COVID-19 of 8.63±6.10 x10\(^9\)/L. However, this research data obtained a higher value than previous studies, which indicated an increase in leukocytes in patients with positive COVID-19 with a mean of 9.2±3.7x10\(^9\)/L compared to that of negative COVID-19 of 6.0±3.9x10\(^9\)/L.\textsuperscript{12} Other studies have also found a significant increase in leukocyte count and a higher probability of fever and chronic illness in older COVID-19 patients. Increased leukocyte count is associated with a systemic inflammatory response, and increased CRP, IL-6, procalcitonin, and neutrophils.\textsuperscript{13}

IL-6 plays a role in the differentiation of T helper 17 cells from CD4 T-cells. T helper 17 cells can induce an inflammatory response through the production of IL-17A and IL-17F, which act as cytokines for neutrophil migration and activation. The release of granules and the production of cytokines from neutrophils indicate an immune response against the virus. However, an excessive increase in neutrophils can cause a cytokine storm and tissue damage, leading to severe pneumonia and death. Neutrophilia is associated with the development of critical illness, ICU care, and high mortality in COVID-19 patients.\textsuperscript{14} Most of the patients in this study experienced leukocytosis, which might be due to the response to the COVID-19 infection itself or accompanied by another viral or bacterial infection that causes leukocytosis. However, it was difficult to identify any secondary infection/bacterial because culture (blood/sputum/urine) was not performed.

It was found in this study that there was no significant difference in mean platelet count in patients with positive and negative COVID-19. The results of this study were not in line with previous studies that found an increased platelet count in patients with negative COVID-19 of 269.88±66.55 x 10\(^9\)/L compared to that of positive COVID-19 of 233.72±70.13x10\(^9\)/L.\textsuperscript{15} This difference might be because the inclusion criteria for COVID-19 patients were patients admitted to the intensive care unit with symptoms of severe pneumonia, Acute Respiratory Distress Syndrome (ARDS), sepsis, septic shock, arrhythmia, cardiogenic shock, acute renal failure or multiorgan failure. In addition, the treatment room was not considered and patients were not classified into mild or severe symptoms in this study.\textsuperscript{16}

Several mechanisms of the effect of COVID-19 on platelets are as follows: Decreased platelet production: mostly caused by bone marrow aplasia, which causes a decrease in production either due to cytokine effects or the direct effect of coronavirus on bone marrow; Impaired hematopoietic process in bone marrow caused by SARS infection-CoV-2, which leads to low platelet production; Destruction of progenitor hematopoietic cells (including megakaryocytes) by the IL-6 produced during cytokine storms in the bone marrow, which causes low platelets in the peripheral blood.\textsuperscript{17} Because the sample was taken from non-comorbid patients, low platelet count and extensive inflammation were not found supported by a good bone marrow response and cytokines.

Based on the ROC curve, the NLR cut-off value in patients with positive COVID-19 was 61.9% with a sensitivity of 66.5% and a specificity of 61.9%. Correlation between NLR and fever was then analyzed. Cut-off NLR $\geq 2.4$ indicates a greater risk of COVID-19 than NLR $<2.4$. Patients with positive COVID-19 accompanied by fever due to bacterial infection had higher NLR than fever due to viral infection. An increase in NLR can occur in cases other than COVID-19 such as pneumonia, acute respiratory infections, multi-organ failure, severe cough and fever. Chronic disease can affect a high NLR and leukocyte count and can be characterized by mild and severe symptoms.\textsuperscript{18} Because this study used a large sample of non-comorbid COVID-19 patients, most patients were still in a healthy state or had good bone marrow function. This might result in a reduced risk of exhausted T lymphocytes as in comorbid cases. This was in line with the theory that
found relatively large numbers of lymphocytosis cases in India (mean lymphocyte count of 40.6%).

The cut-off value for PLR in this study was 157.035, with a sensitivity of 63.0% and a specificity of 60.2%. Previous studies reported cut-off of 138.30 for PLR with a sensitivity of 77.42% and a specificity of 51.57% in patients with COVID-19 accompanied with ARDS. These results suggested that a decreased PLR indicates a decrease in platelet count due to greater tissue damage in COVID-19 patients with ARDS. Therefore, these results supported this study to obtain a higher PLR cut-off because ARDS was not used as additional inclusion criteria. Increased PLR values indicate an active inflammatory response and a worse prognosis. Increased PLR correlates with severe COVID-19 infection. The PLR cut-off value for PLR in this study was higher than the mean PLR of other studies. This is related to the relatively good response of thrombopoietin and proinflammatory cytokines to the thrombocytosis process due to inflammation in the tissues or endothelium. However, patients with comorbid in this study mostly experienced thrombocytopenia due to extensive inflammation and inhibition of stem cell progenitors from megakaryocytes by IL-6, which binds to receptors of SOCS3 (suppressor of cytokine signaling).

Another theory found significant thrombocytopenia in COVID-19 patients compared to the control group. However, PLR was not associated with disease severity. Previous studies found significant differences in PLR between critically ill patients and patients with milder symptoms. Another theory suggests that thrombocytopenia is a common occurrence in critically ill patients and indicates serious organ damage and the development of intravascular coagulopathy. The mechanism for thrombocytopenia is caused by endothelial damage that triggers platelet activation, aggregation and thrombosis. Thrombocytopenia can also be caused by increased consumption of platelets. Coronavirus can also directly infect the bone marrow, causing hematopoiesis abnormalities or triggering an auto-immune response to blood cells.

The cut-off value for MLR in this study was 0.296 with a sensitivity of 60.0% and a specificity of 58.5%. These results were in line with previous studies, which reported that cut-off value for MLR was 0.23 with a sensitivity of 75.79% and a specificity of 90% and there was a positive correlation between MLR and body temperature, an increase in monocytes and a decrease in the number of lymphocytes in patients with respiratory disorders. Some cytokines such as IL-6 and interferon alpha (IFN-α) were correlated with an increase in peripheral monocytes, which was able to increase MLR. This theory supported this study that patients with positive COVID-19 had decreased absolute lymphocytes and increased MLR. Previous studies have correlated proinflammatory cytokines; therefore, the effect of cytokine involvement on the cut-off of MLR was unable to be determined. This study obtained lower sensitivity and specificity than other studies. This was because most of the study samples still had a fairly high absolute lymphocyte value and other studies found normal or slightly decreased monocyte count, which led to insignificant difference in the distribution of MLR in COVID-19 and non-COVID-19 patients.

No classification of COVID-19 based on symptoms and no differentiation of COVID-19 accompanied by fever, administration of therapy, length of treatment and multiorgan failure remained the limitations in this study.

CONCLUSIONS AND SUGGESTIONS

A cut-off ≥ 3.010, sensitivity 66.5%, specificity 61.9, PPV 0.773, NPV 0.487, LR (+) 1.744, LR (-) 0.541 of NLR were obtained as a COVID-19 screening; A cut-off ≥ 157.035, sensitivity 63%, specificity 60.2%, PPV 0.755, NPV 0.455, LR (+) 1.583, LR (-) 0.614 of PLR were obtained as a COVID-19 screening; A cut-off ≥ 0.296, sensitivity 60%, specificity 58.5%, PPV 0.738, NPV 0.429, LR (+) 1.445, LR (-) 0.684 of MLR were obtained as a COVID-19 screening. It can be concluded that NLR and PLR cannot be used as the main screening biomarkers in screening for COVID-19. Regardless the clinical manifestations of patientsother biomarkers such as antigen swabs should be considered.

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