Platelet-derived immuno-inflammatory indices show best performance in early prediction of COVID-19 progression

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Abstract

Background: Coronavirus disease 2019 (COVID-19) profoundly affects the immune and hematopoietic systems with various degrees of reactive changes in the blood cell counts. Immuno-inflammatory indices are considered a simple and effective tool in the prediction of COVID-19 outcomes. We aimed to evaluate and compare the usefulness of leukocyte and platelet counts-based immuno-inflammatory indices on admission to hospital in predicting COVID-19 progression and mortality.

Methods: A total of 945 patients were enrolled. In addition to blood cell counts, we assessed hemogram-derived immuno-inflammatory indices in relation to COVID-19 progression and death. The indices were tested by analysis of variance, receiver operating characteristic curve analysis, and binomial logistic regressions.

Results: Patients with severe COVID-19 had significantly higher counts of neutrophils, eosinophils, and large immature cells (LIC), while decreased counts of platelets and monocytes. Lymphopenia was found in all of the patients, but without significant association with the outcomes. Patients with a LIC count ≥0.265 x 10⁹/L had 54.7% more odds of having COVID-19 progression. In multivariable analyses, platelets/neutrophil-to-lymphocyte ratio (P/NLR) and platelets-to-neutrophil ratio (P/N) were significant independent predictors of COVID-19 progression and mortality. The odds of a poor outcome were two times higher in cases with P/NLR < 43 x 10⁹/L and P/N < 29 x 10⁹/L.

Conclusion: Indices that include platelet count in combination with neutrophil and/or lymphocyte counts displayed the best discriminatory ability and prognostic value of COVID-19 outcomes. Additionally, LIC showed promising results in the early identification of severe COVID-19.

Keywords
lymphoid and myeloid progenitor cells, lymphopenia, neutrophils, severe acute respiratory syndrome coronavirus 2, thrombocytopenia
INTRODUCTION

Since the outbreak of the coronavirus disease 2019 (COVID-19), tremendous efforts have been made to delineate its pathogenesis and disclose factors that predispose or might point to a severe disease course and fatal outcome. In general, older age (>60 years), male sex, and chronic diseases, such as hypertension and diabetes mellitus, are considered major risks for severe cases of COVID-19.1–3

A healthy immune system is necessary for the proper elimination of viral infections. Otherwise, an inadequate immune response leads to viral dissemination, uncontrolled and excessive systemic inflammation, and, eventually, multiple end-organ damage. Likewise, exaggerated inflammatory response plays an integral part in the pathogenesis of severe COVID-19.2–8

Complete blood count (CBC) parameters and their combined ratios are considered good markers of immune response and useful for risk stratification in bacterial infections, pneumonia, sepsis, and certain cancers.2,4–12 COVID-19 profoundly affects the immune and hematopoietic systems with various degrees of reactive changes in the blood cell counts. Lymphopenia and thrombocytopenia are commonly associated with severe COVID-19 and poor outcomes.2,4–13 Furthermore, increased neutrophil count, levels of CRP, ferritin, lactate dehydrogenase (LDH), procalcitonin, and many cytokines (eg, interleukin [IL]-2, IL-6, TNF-α) correlated with severe disease.2,6,14,15

The most common CBC finding in COVID-19 patients is lymphopenia. There is a significant decrease in lymphocyte counts in critical cases, including CD4+ and CD8+ T cells, B cells (CD19+), and natural killer (NK) cells (CD16/56+).7,16–18 These features appear to be the result of direct viral effects on lymphocytes, inefficient T cell activation and proliferation, leukocyte redistribution with pulmonary entrapment, and processes of functional exhaustion, especially of CD8+ T and NK cells.4,5,14,16,19

Thrombocytopenia in COVID-19 is supposed to be a result of decreased production and increased consumption of platelets in damaged lungs. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may infect hematopoietic progenitors, thereby inducing their growth retardation or apoptosis.2,6,14,16

The blood cell count-derived immuno-inflammatory indices’ ability to predict severity and prognosis of COVID-19 is in the focus of current investigations.2,3,18 The neutrophil-to-lymphocyte ratio (NLR) is considered a simple and reliable tool in evaluating systemic inflammation among critically ill patients and survival in various clinical settings (oncological, septic, cardiovascular).11,12,20 Systemic immune inflammation index (SII) is based on neutrophil, platelet, and lymphocyte counts and reflects their differential roles in viral infections.2,22 It is crucial to assess the severity of a SARS-CoV-2 infection early on in order to provide adequate treatment in a timely fashion.

Importantly, inflammatory indices are easily calculated from a CBC, making them inexpensive and widely accessible tools.2,13,16,21 Therefore, our study aimed at evaluating and comparing the usefulness of leukocyte and platelet counts-based immuno-inflammatory indices on admission to hospital in the prediction of COVID-19 progression and death.

MATERIALS AND METHODS

A total of 945 adult patients hospitalized at the University Clinical Center of Nis (Serbia) were involved in this single-centered, prospective, observational study during April in 2021. All of the patients suffered from an acute respiratory tract infection and were treated per the instructions of the guidelines and current protocols for COVID-19. The alpha variant (B.1.1.7) of SARS-CoV-2 was most prevalent at the time.22

The inclusion criteria for the study were as follows: a confirmed diagnosis of COVID-19 based on a positive result by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis or SARS-CoV-2 antigen test using nasal and pharyngeal swab samples and/or chest radiological (X-rays or CT) findings compatible with SARS-CoV-2 pneumonia. The second criterion was an available CBC analysis performed within 24 h of admission to the hospital. Routine laboratory examinations consisted of CBC and biochemical parameters analyses performed using the CELL-DYN Ruby Hematology Analyzer—Abbott Diagnostics automated hematology analyzer.

The exclusion criteria were severe illness at hospital admission, including cardiac arrest, respiratory failure with hemoglobin O2 saturation (SatO2) levels of less than 50%, a respiratory rate over 30/min, hypotension with systolic blood pressure less than 60mmHg.

In addition to a routine laboratory analysis of the CBC and commonly assessed biochemical markers, we calculated and evaluated CBC-derived immuno-inflammatory indices, specifically: NLR, monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), eosinophil-to-lymphocyte ratio (ELR), SII (platelet counts × neutrophil counts/lymphocyte counts), lymphocyte-to-CRP ratio (LCR), lymphocyte-to-leukocyte ratio (LY/L), platelets-to-neutrophil ratio (P/N), platelet-to-NLR (P/NLR), and platelets-to-CRP ratio (P/CRP). The indices were calculated on admission to hospital and their prognostic value was further evaluated.

The obtained data were compared between patients with and without COVID-19 progression as well as survivors and nonsurvivors. Disease progression was defined as a worsening of COVID-19 that required admission to an intensive care unit (ICU) or resulted in a lethal outcome.

The study was performed according to the Declaration of Helsinki, all of the patients signed an informed consent and the study was approved by the Institution Ethics Review Board of the University Clinical Center of Nis, Serbia (No. 11170 from April 2, 2021).

2.1 Statistical analysis

The normality of distribution was tested using the Kolmogorov-Smirnov test. Continuous variables are presented as a median ± interquartile range (IQR) and categorical variables as numbers (n) and percentages (%). Categorical variables were analyzed using a Chi-square test. One-way analysis of variance (ANOVA) was applied to compare associations between COVID-19 progression.
and death and the values of CBC and immuno-inflammatory indices. The variables, which differed significantly under comparative statistics, were included in an area under the receiver operating characteristic (ROC) curve analysis to assess their discriminative ability and cut-off points. Youden’s index was used to determine cut-off values.

Univariable and multivariable binomial logistic regression was subsequently performed for variables with a statistically significant area under the curve (AUC) in the ROC curve analysis to identify variables associated with COVID-19 progression and in-hospital mortality. Variables were assessed for collinearity and outliers. Statistical significance was set at $p < 0.05$. Statistical calculations were performed using IBM SPSS 25.0 software (SPSS Inc.).

3 | RESULTS

3.1 | Baseline characteristics of the patients

A total of 945 consecutive patients were included in the study, 532 (56.3%) males and 413 (43.7%) females, with the median age of $70 \pm 18$ years old. The median duration of symptoms was 8 days. At admission to the hospital, the median SatO$_2$ was $93.0 \pm 8.0\%$. At least one comorbidity was recorded in 78.8% of the patients, the most prevalent being hypertension in 60.7% and diabetes mellitus type 2 in 21.5%.

The disease progressed in 374 patients (39.6%), of whom 183 (19.4%) required ICU admission, while 351 patients (37.2%) died. Older age ($60+\,$), lower SatO$_2$ level (<95%), as well as higher CRP levels (median $93.2 \pm 93.6\,$) were significantly associated with disease progression and death in all of the patients ($p = 0.000$). Gender and comorbidities did not show significant associations with COVID-19 progression nor in-hospital mortality in our study group (Table 1).

3.2 | Comparison of the parameters in relation to COVID-19 outcomes (one-way ANOVA test)

We evaluated the association between patients’ CBC parameters at hospital admission and the progression of COVID-19 and mortality using a one-way ANOVA test. The results of the ANOVA test are presented in detail in Table 1.

Patients with COVID-19 progression had significantly higher absolute counts of leukocytes, neutrophils, eosinophils, large immature cells (LIC), and platelet distribution width (PDW), but decreased counts of platelets, monocytes, and MCHC values. Similarly, patients who died had significantly higher absolute counts of leukocytes, neutrophils, eosinophils, LIC, and values of MCV, red cell distribution width (RDW), and PDW, while decreased counts of platelets, monocytes, and MCHC levels (Table 1).

Although the patients’ CBC showed that there was no true thrombocytopenia (platelets <150x10$^9$/L), the absolute count of platelets was significantly lower in patients who developed COVID-19 progression later on ($196.00 \pm 136.00$ vs $216.20 \pm 120.00$) or eventually died ($194.00 \pm 132.50$ vs $215.60 \pm 122.25$). All of the patients who had been admitted to the hospital had lymphopenia ($0.980 \pm 0.690$ x10$^3$/L), more prominently in the subgroups with progression ($0.910 \pm 0.670$) and patients who passed away ($0.870 \pm 0.660$). However, there was no significant difference compared to the patients who recovered (Table 1).

We determined significantly higher values of NLR, ELR, SII ($p = 0.000$), and MLR ($p < 0.001$) in the patients with COVID-19 progression and the deceased. PLR was higher only among patients with disease progression ($p = 0.011$). The values of LY/L, P/N, P/NLR ($p = 0.000$), and P/CRP ($p < 0.039$) were significantly lower among patients who had experienced progression or died, while the LY/CRP ratio was not significantly associated with the outcomes (Table 2).

3.3 | The ROC curve analysis of CBC and immuno-inflammatory indices

Variables that were found significant in the comparison tests were further analyzed by the ROC curve analysis in order to determine their diagnostic ability and optimal cut-off points for predicting an outcome. The best accuracy identified was for SatO$_2$ level, with an AUC value of 0.734 ($p = 0.000$; 95%CI:0.688–0.769) for progression and 0.724 ($p = 0.000$; 95%CI:0.688–0.761) for prediction of death.

Among immuno-inflammatory indices, NLR, LY/L, P/N, and P/NLR provide us with the best ability to distinguish patients with a higher risk for disease progression and in-hospital mortality. Moreover, among the CBC parameters, LIC and RDW values showed significant results in identifying COVID-19 severity (Figure 1).

We observed significant results in the diagnostic ability of disease progression and AUC above 0.600, for the following variables: P/NLR (AUC = 0.661), NLR (AUC = 0.649), LY/L (AUC = 0.648), P/N (AUC = 0.645), and age (AUC = 0.615) (Figure 1A).

The significant results in the diagnostic ability of COVID-19 mortality and AUC above 0.600, were found for P/NLR (AUC = 0.658), age (AUC = 0.649), P/N (AUC = 0.644), NLR (AUC = 0.640), LY/L (AUC = 0.639), and P/CRP (AUC = 0.600) (Figure 1B).

RDW provided a significant result of AUC = 0.596 for disease progression and AUC = 0.616 for mortality ($p = 0.000$), while LIC showed AUC = 0.605, but AUC = 0.590 for mortality ($p = 0.000$). The systemic immune-inflammation index showed significant ($p = 0.000$) but low accuracy of AUC = 0.578 and AUC = 0.564, while other variables displayed poor performance.

We determined two types of cut-off points, one for which we arbitrarily choose the sensitivity of around 80% to reduce the false-negative rates and the second one based on Youden’s correction, which emphasizes both sensitivity and specificity and thus should indicate better performance.
| Parameter          | All patients | Progression | Death | p value | All patients | Progression | Death | p value |
|--------------------|--------------|-------------|-------|---------|--------------|-------------|-------|---------|
|                    |              | Yes (39.6%) | No (60.4%) |         | Yes (37.2%) | No (62.8%) |         |
| Age (years)        | 70 ± 18      | 73 ± 17     | 68 ± 18 | 0.000*  | 74 ± 15      | 67 ± 18     | 0.000* |
| Males, n (%)       | 531 (56.3%)  | 225 (42.4%) | 306 (57.6%) | 0.058   | 207 (39.0%)  | 324 (61.0%) | 0.195  |
| Comorbidity (%)    | 744 (78.8%)  | 293 (39.4%) | 451 (60.6%) | 0.774   | 277 (37.2%)  | 467 (62.8%) | 0.952  |
| Saturation (%)     | 93.0 ± 8.0   | 88.0 ± 14.0 | 94.0 ± 6.0 | 0.000*  | 88.0 ± 14.5 | 94.0 ± 5.0 | 0.000* |
| CRP (mg/L)         | 93.2 ± 93.6  | 105.2 ± 104.6 | 87.1 ± 84.9 | 0.018*  | 1094 ± 108.8 | 88.7 ± 82.3 | 0.009* |

The values of the complete blood count parameters

| Parameter          | All patients | Progression | Death | p value | All patients | Progression | Death | p value |
|--------------------|--------------|-------------|-------|---------|--------------|-------------|-------|---------|
| RBC (x10^{12}/L)  | 4.520 ± 0.859 | 4.500 ± 0.925 | 4.510 ± 0.773 | 0.277 | 4.500 ± 0.970 | 4.510 ± 0.765 | 0.250 |
| HGB (g/L)         | 136.0 ± 26.0 | 135.0 ± 29.0 | 137.0 ± 22.5 | 0.425 | 134.0 ± 30.0 | 137.0 ± 23.0 | 0.216 |
| HCT (%)           | 40.9 ± 7.5   | 40.8 ± 8.5  | 41.2 ± 6.8  | 0.667 | 40.7 ± 8.7  | 41.3 ± 6.8  | 0.460  |
| MCV (fl)          | 91.0 ± 6.0   | 92.0 ± 7.0  | 91.0 ± 6.0  | 0.064 | 92.0 ± 7.0  | 91.0 ± 6.0  | 0.049* |
| MCH (pg)          | 30.3 ± 2.4   | 30.6 ± 2.6  | 30.4 ± 2.3  | 0.977 | 30.3 ± 2.6  | 30.4 ± 2.3  | 0.985  |
| MCHC (g/L)        | 332.0 ± 9.0  | 331.0 ± 9.0 | 332.0 ± 9.0 | 0.009* | 331.0 ± 9.0 | 332.0 ± 9.0 | 0.001* |
| RDW (%)           | 11.90 ± 1.40 | 11.90 ± 1.40 | 11.60 ± 1.10 | 0.081 | 12.00 ± 1.35 | 11.60 ± 1.20 | 0.029* |
| WBC (x10^{9}/L)   | 8.300 ± 5.630 | 9.100 ± 7.400 | 7.800 ± 4.550 | 0.033* | 8.900 ± 7.500 | 8.000 ± 4.855 | 0.048* |
| NE (x10^{9}/L)    | 6.720 ± 4.990 | 7.400 ± 6.695 | 6.120 ± 4.075 | 0.000* | 7.381 ± 6.780 | 6.250 ± 4.470 | 0.000* |
| MO (x10^{10}/L)   | 0.370 ± 0.300 | 0.370 ± 0.290 | 0.410 ± 0.280 | 0.039* | 0.360 ± 0.290 | 0.400 ± 0.280 | 0.017* |
| EO (x10^{9}/L)    | 0.050 ± 0.058 | 0.070 ± 0.060 | 0.070 ± 0.060 | 0.022* | 0.070 ± 0.070 | 0.060 ± 0.060 | 0.020* |
| BA (x10^{9}/L)    | 0.060 ± 0.060 | 0.060 ± 0.090 | 0.050 ± 0.040 | 0.179 | 0.060 ± 0.090 | 0.050 ± 0.050 | 0.164  |
| LY (x10^{9}/L)    | 0.980 ± 0.690 | 0.910 ± 0.670 | 1.070 ± 0.715 | 0.516 | 0.870 ± 0.660 | 1.050 ± 0.730 | 0.626  |
| LIC (x10^{9}/L)   | 0.200 ± 0.188 | 0.250 ± 0.280 | 0.180 ± 0.160 | 0.000* | 0.210 ± 0.230 | 0.190 ± 0.160 | 0.000* |
| ALY (x10^{9}/L)   | 0.040 ± 0.050 | 0.040 ± 0.040 | 0.040 ± 0.045 | 0.999 | 0.040 ± 0.040 | 0.040 ± 0.040 | 0.947  |
| PLT (x10^{9}/L)   | 208.00 ± 121.75 | 196.00 ± 136.00 | 216.20 ± 120.00 | 0.004* | 194.00 ± 132.50 | 215.60 ± 122.25 | 0.001* |
| PDW (%)           | 17.30 ± 4.0   | 18.00 ± 5.0  | 17.00 ± 4.30 | 0.004* | 17.80 ± 4.15 | 17.20 ± 4.00 | 0.003* |
| PCT (%)           | 17.90 ± 11.60 | 18.30 ± 11.90 | 18.10 ± 10.80 | 0.518 | 18.00 ± 12.30 | 17.80 ± 11.30 | 0.341  |
| MPV (fl)          | 9.00 ± 1.40   | 9.10 ± 1.70  | 9.10 ± 1.45  | 0.987 | 9.10 ± 1.70  | 9.00 ± 1.40  | 0.758  |

Note: The results show median ± IQR, except for HCT that had normal distribution. * significant result.

Abbreviations: ALT, atypical lymphocytes; BA, basophils; CRP, C reactive protein; EO, eosinophils; HCT, hematocrit; HGB, hemoglobin; LIC, large immature cells; LY, lymphocytes; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin; MCV, mean cell volume; MO, monocytes; MPV, mean platelet volume; NE, neutrophils; PCT, plateletcrit; PDW, platelet distribution width; PLT, platelets; RBC, erythrocytes; RDW, red cell distribution width; WBC, leukocytes.
### TABLE 2  Comparison of immuno-inflammatory indices between patients according to COVID-19 outcome (one-way ANOVA)

| Index   | All patients | Progression | Death | p value | p value |
|---------|--------------|-------------|-------|---------|---------|
|         | Yes          | No          |       |         |         |
| NLR     | 6.770 ± 6.765| 8.306 ± 8.476| 5.699 ± 4.890| 0.000* | 7.906 ± 7.249| 5.778 ± 4.842| 0.000* |
| MLR     | 0.383 ± 0.295| 0.432 ± 0.341| 0.385 ± 0.245| 0.000* | 0.417 ± 0.312| 0.389 ± 0.241| 0.001* |
| PLR     | 217.901 ± 167.244| 218.841 ± 173.711| 210.294 ± 153.994| 0.011* | 210.884 ± 160.404| 213.861 ± 153.357| 0.057 |
| ELR     | 0.064 ± 0.065| 0.077 ± 0.085| 0.059 ± 0.052| 0.000* | 0.073 ± 0.074| 0.060 ± 0.051| 0.000* |
| SII     | 1456.71 ± 1786.75| 1685.53 ± 1884.94| 1394.42 ± 1414.83| 0.000* | 1685.53 ± 1838.87| 1384.00 ± 1412.18| 0.000* |
| LY/L    | 0.122 ± 0.094| 0.104 ± 0.081| 0.135 ± 0.089| 0.000* | 0.104 ± 0.078| 0.136 ± 0.092| 0.000* |
| LY/CRP  | 0.011 ± 0.017| 0.009 ± 0.012| 0.012 ± 0.016| 0.421  | 0.009 ± 0.012| 0.012 ± 0.016| 0.295  |
| P/N     | 32.47 ± 23.68| 28.52 ± 22.39| 35.69 ± 35.45| 0.000* | 27.97 ± 22.51| 35.67 ± 23.92| 0.000* |
| P/NLR   | 30.98 ± 33.14| 27.23 ± 24.54| 34.52 ± 35.45| 0.000* | 27.24 ± 25.90| 35.46 ± 35.78| 0.000* |
| P/CRP   | 2.26 ± 2.85  | 1.89 ± 2.28  | 2.54 ± 3.00  | 0.039* | 1.840 ± 2.216| 2.568 ± 3.030| 0.016* |

Note: The results show median ± IQR. * significant result.

Abbreviations: ELR, eosinophil-to-lymphocyte ratio; LY/CRP, lymphocyte-to-CRP ratio; LY/L, lymphocyte-to-leukocyte (WBC) ratio; MLR, monocyte-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio; P/CRP, platelets-to-CRP ratio; P/NLR, platelets-to-NLR ratio; P/N, platelets-to-neutrophil ratio; PLR, platelets-to-lymphocytes ratio; SII, systemic immune inflammation index.

![FIGURE 1](image-url)  Title: The ROC curve analyses of variables in discrimination of patients with high risk of COVID-19 progression and death. Legend: A. The ROC curve analyses in relation to COVID-19 progression. B. The ROC curve analyses in relation to COVID-19 mortality. AUC, area under the curve; LIC, large immature cells; RDW, red cell distribution width; NLR, neutrophil to lymphocyte ratio; SII, systemic immune inflammation index; LY/L, lymphocyte to leukocyte ratio; P/N, platelets to neutrophil ratio; P/NLR, platelets to NLR ratio)
3.4 Logistic regression analyses of CBC and immuno-inflammatory indices

We analyzed variables that provided significant discriminatory ability in the ROC curve analysis with an AUC of more than 0.600. The variables were tested as continuous and at the cut-off points. All regression analyses were adjusted for age and SatO2 level.

RDW and P/CRP were not significant in univariable analyses and were excluded from further testing. Values of LIC were significant (p = 0.035) in predicting progression of COVID-19 (OR = 1.934, 95%CI:0.862–0.909). The LIC count cut-off at ≥0.265 (AUC sensitivity 46.2%, specificity 73.8%) was significant and could predict disease progression (p = 0.019) (OR = 1.547, 95%CI: 1.075–2.226). Patients with a LIC count ≥0.265x10⁹/L had a 54.7% greater likelihood of experiencing disease progression.

Univariable analyses showed a significant association of NLR, LY/L, P/N, and P/NLR indices with the outcomes. In a multivariable analysis using continuous values, we determined P/NLR to be a significant predictor of COVID-19 progression with p = 0.000 (B = −0.021, odds = 0.979, 95%CI: 0.969–0.990). Every increase in the P/NLR value reduced the odds of COVID-19 progression by 2.1%.

In the analysis of data by cut-off points we determined significant results for P/NLR and P/N variables. The P/NLR cut-off point <43 (AUC sensitivity 80.2%) was significant (p = 0.001) with OR = 2.084 (95%CI: 1.325–3.276), meaning that persons with P/NLR levels <43x10⁹ cells/L have 2.08 times more likelihood of having COVID-19 progression. The NLR value ≥4.96x10⁹/L was on the border of statistical significance (p = 0.052).

The P/N cut-off point of <29 (AUC sensitivity 56.7%) showed a significant predictive value of disease progression (p = 0.008) with the OR = 1.630 (95%CI: 1.135–2.341). The result points to a 63% higher probability of having COVID-19 progression if the P/N values are <29x10⁹ cells/L. The LY/L value of <0.11x10⁹ cells/L was on the border of the statistical significance (p = 0.053) (Table 3).

Similarly, in the analysis of correlations with a lethal outcome, the continuous values of the P/NLR index were significant (p = 0.000), increasing the odds by 2.1% (B = −0.021, odds = 0.979, 95%CI: 0.968–0.990). The model’s sensitivity was 46.2%, its specificity 90.1%, with a positive predictive value of 74.8%.

Once more, P/NLR and P/N variables demonstrated a significant association with mortality in the analysis of cut-off points. The P/NLR cut-off of <43 (AUC sensitivity 80.6%) was found to be significant (p = 0.001) with OR = 2.135 (95%CI: 1.345–3.389). The P/N cut-off point of <29 (AUC sensitivity 56.2%) showed a significant predictive value of mortality (p = 0.017) with OR = 1.581 (95%CI: 1.086–2.302). The result showed a 58.1% higher risk of death from COVID-19 when P/N values were <29x10⁹/L (Table 3).

4 | DISCUSSION

Destructive changes in hematological and immunological systems caused by SARS-CoV-2 appear to play a crucial role in the pathogenesis of hyperinflammation and multiorgan injury in severe COVID-19.3-5 Interactions between macrophages, monocytes, neutrophils, platelets, and lymphocytes are driven by inflammatory cytokines and produce characteristic changes in the CBC of COVID-19 patients.13,16,20 We confirmed a significantly elevated inflammatory status among patients with COVID-19 progression and those who died.

Overwhelming data identify lymphopenia and thrombocytopenia as hallmarks of severe COVID-19.4,13,16 Besides variable total leukocyte counts, sustained lymphopenia and thrombocytopenia were observed in critically ill patients. Mechanisms leading to profound and persistent lymphopenia in sepsis include redistribution of

| TABLE 3 | Binomial logistic regressions of independent variables association with COVID-19 progression and mortality |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Progression** | **Cut-off, sensitivity 80%** | **Odds** | **95%CI** | **Wald** | **p** | **Death** | **Odds** | **95%CI** | **Wald** | **p** |
| NLR (≥ 4.96)    | 3.594           | 0.988           | 13.072         | 3.769          | 0.052          | NLR (≥ 4.96) | 2.747     | 0.786           | 9.599         | 2.506          | 0.113 |
| LY/L (<0.16)    | 0.378           | 0.101           | 1.411          | 2.096          | 0.148          | LY/L (<0.16) | 0.472     | 0.131           | 1.694         | 1.327          | 0.249 |
| P/N (<23)       | 0.969           | 0.648           | 1.450          | 0.023          | 0.879          | P/N (<23)   | 0.965     | 0.640           | 1.456         | 0.028          | 0.866 |
| P/NLR (<43)     | 2.084           | 1.325           | 3.276          | 10.113         | 0.001*         | P/NLR (<43) | 2.135     | 1.345           | 3.389         | 10.354         | 0.001* |
| **Cut-off, Youden’s index** | **Odds** | **95%CI** | **Wald** | **p** | **Death** | **Odds** | **95%CI** | **Wald** | **p** |
| NLR (≥ 7.14)    | 0.629           | 0.266           | 1.491          | 1.108          | 0.293          | NLR (≥ 6.13) | 1.317     | 0.875           | 1.983         | 1.739          | 0.187 |
| LY/L (<0.11)    | 2.354           | 0.989           | 5.598          | 3.748          | 0.053          | LY/L (<0.09) | 1.073     | 0.678           | 1.698         | 0.091          | 0.763 |
| P/N (<29)       | 1.630           | 1.135           | 2.341          | 7.010          | 0.008*         | P/N (<29)   | 1.581     | 1.086           | 2.302         | 5.707          | 0.017* |
| P/NLR (<31)     | 1.231           | 0.812           | 1.864          | 0.960          | 0.327          | P/NLR (<25) | 1.423     | 0.907           | 2.231         | 2.360          | 0.124 |

Note: All analyses were adjusted for age and hemoglobin O2 saturation. * significant result.

Abbreviations: B, logistic regression coefficient β; CI, confidence interval; LY/L, lymphocyte to leukocyte ratio; NLR, neutrophil to lymphocyte ratio; OR, odds ratio; P/NLR, platelets to NLR ratio; P/N, platelets to neutrophil ratio.
lymphocytes and accelerated apoptosis, with selective susceptibility of Th1 T cells. In addition, neutrophilia also represented a prognostic factor of acute respiratory distress syndrome (ARDS) in COVID-19 patients. Within the lungs, the SARS-CoV-2 infection initiates pulmonary infiltration by neutrophils early in the course of the disease. Despite their actions against the virus, neutrophils might cause lung injury through the degranulation and generation of neutrophil extracellular traps.

An absolute lymphocyte count was capable of predicting COVID-19 disease severity and it was found to be significantly lower in patients who died than among survivors. There was a nearly threefold increased risk of severe COVID-19 with a lymphocyte count <1.5 x 10^9/L. However, certain studies failed to determine any significant correlations between absolute lymphocyte count and severe disease, such as the study by Kaal et al. We found lower lymphocyte counts in patients who experienced disease progression and death; however, the results were without statistical significance. The result is evidently a consequence of commonly present lymphopenia in most of our patients at admission (median 0.980 x 10^9/L).

Higher leukocyte, neutrophil, and eosinophil counts showed a significant correlation with COVID-19 progression and death. Although a WBC count below 4 x 10^9/L may define systemic inflammatory response syndrome the same way as its high values above 12 x 10^9/L, we observed only increased values to be associated with a poor prognosis in patients with COVID-19.

Thrombocytopenia is another common sign among COVID-19 patients and is correlated with in-hospital death. The mechanism for thrombocytopenia in COVID-19 is multifactorial. Megakaryocytopenia might be directly affected by SARS-CoV2 or by inflammatory cytokines, while the lungs, as an important site of platelet production, suffer infection-induced damage. Platelet hyperreactivity in COVID-19 patients is reflected in the presence of larger and younger platelets in the circulation. Platelet activation and aggregation within damaged endothelium cause their massive consumption and thus decrease their numbers in the blood.

Even an early decrease in platelet count was associated with a poor prognosis for COVID-19 patients. Patients’ relative risk of death increased as platelet counts dropped. In support of the previous reports, we have demonstrated that a decrease in one’s platelet count is significantly correlated with a worse outcome among COVID-19 patients.

Given the strong influence of pro-inflammatory cytokines on the bone marrow (TNF-α, IL-1, IL-6), there are certain specificities in the CBC as a reflection of their effect on hematopoietic cell proliferation and maturation. The cytokines decrease the production of erythrocytes, while stimulating generation and accelerating the release of immature myeloid populations. Predominating immature myeloid cell populations have been observed in critically ill COVID-19 patients. In this regard, higher RDW and PDW levels at admission were observed among severe COVID-19 patients and those who passed away compared to survivors.

A large immature cell population consists of various immature forms of myeloid and lymphoid cells. In our study, an increase in LIC count was highly associated with worse outcomes and represented an independent predictor of COVID-19 progression and mortality. In addition, we detected derangements of RDW, PDW, and MCHC parameters, however, with poor discriminatory abilities in the ROC curve analysis.

Given the high heterogeneity in baseline cell counts, it seems better to assess combinations of the most affected CBC values in terms of predicting COVID-19 outcomes. Recent studies demonstrated significant discriminative abilities of NLR and SII in predicting COVID-19 mortality. Increased NLR was reported as a better predictor of bacteremia at ICU admission than routinely used parameters like CRP level, leukocyte, and neutrophil counts. Likewise, an increased NLR has been observed in severe COVID-19 cases and often provided the best method of identifying patients with an unfavorable outcome. Similarly, SII has been introduced as a novel biomarker with excellent prognostic characteristics in assessing in-hospital mortality and the development of ARDS in COVID-19. Its high values correlated with other inflammatory markers, such as CRP, ferritin, procalcitonin, and cytokines.

We confirmed that there was a significant correlation between NLR and SII and outcomes using the ANOVA test and ROC curve analyses, albeit SII showed low accuracy. NLR performed better than SII in distinguishing COVID-19 outcomes. However, neither of the aforementioned displayed any significant predictive power in the multivariable regression analyses.

In the present study, indices including platelet count, P/N, and P/NLR demonstrated the best diagnostic ability and prognostic value. Interestingly, although P/NLR (Pit x Ly/Neu) and SII (Pit x Neu/Ly) are calculated using the same cell counts, their predictive power differed significantly, emphasizing the importance of their ratios’ type for a final result.

Patients with P/NLR levels below 43 x 10^9/L had two times higher odds of experiencing COVID-19 progression and a lethal outcome. A P/N level below 29 x 10^9/L was coupled with a 1.6 greater probability of experiencing COVID-19 progression and increased risk of dying.

Similar to our research, the study of Chaudhary et al. identifies P/N (mean 43.4 ± 33.8) and PLR (mean 219.6 ± 176.2) as the most reliable platelet parameters in providing a prognosis and managing COVID-19 patients. Severe cases had significantly lower P/N counts compared to moderately severe COVID-19 patients. Eissa et al. identified neutrophil to lymphocyte to platelet ratio (NLPR) >0.011 (OR = 38.751) and CRP/LR > 7.6 (OR = 7.604) as being independent diagnostic factors for COVID-19 among several different inflammatory indices. Another research revealed that neutrophil-to-platelet ratio (NPR) had the highest predictive value at ICU admission among COVID-19 patients in multivariable logistic regression models with NLR, PLR, and SII. Similarly, a study by López-Escobar et al. (n = 2808) showed NLR and NPR to be significantly and independently associated with in-hospital mortality of COVID-19 patients in complex adjusted multivariable regression models.

These results emphasize the importance of comparing platelets to neutrophils, in addition to lymphocyte counts, for the purpose of properly evaluating severe inflammation. Therefore, the combination
of the aforementioned hemogram-derived ratios demonstrates significantly more success comparable to other established inflammatory markers including LDH and CRP.

Finally, it is vital to note that we did not include all of the possible basic CBC parameter combinations. We propose several additional ratios, risk scores, and equations with biochemical parameters (eg, CRP, D-dimer, LDH) that might be important for this issue.\textsuperscript{2,16,23,27} However, they should be simple in order to be usable in everyday clinical practice. Furthermore, therapy for COVID-19 was applied following official protocol, but sometimes adjusted to the patients’ specific needs, which might have influenced CBC parameters.

5 | CONCLUSION

Immuno-inflammatory indices represent a simple, easy to use and inexpensive method to evaluate systemic inflammation. They appear to be a valuable additional tool in the early prediction of COVID-19 progression and could be utilized in the monitoring of COVID-19 patients. Our results confirm that indices combining platelet, neutrophil, and lymphocyte counts performed the best in the process of assessing COVID-19 progression and mortality. In this study, P/NLR (\(<43 \times 10^9/L\)) and P/N (\(<29 \times 10^9/L\)) ratios were reliable markers and independently associated with unfavorable outcomes. In addition, the basic CBC parameter LIC showed promising results in the early identification of severe COVID-19 cases. Overall, these results emphasize the importance of comparing platelets and neutrophils as a marker of severe inflammation.

AUTHOR CONTRIBUTIONS

JM: Conceptualization; Methodology, Formal analysis, Investigation, Writing—Original Draft, Visualization. BD: Conceptualization; Methodology, Validation, Supervision. VS: Investigation, Resources, Formal analysis. BD: Formal analysis, Writing—Original Draft, Visualization. DS: Formal analysis, Writing—Review and Editing, Visualization. SP and IM: Investigation, Resources.

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CONFLICT OF INTEREST

The authors state that there are no conflicts of interest regarding the publication of this article.

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