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# Predictive Role of Blood Cellular Indices and Their Relationship with Endogenous Glycosaminoglycans as Determinants of Inflammatory Biomarkers in Pulmonary Embolism

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## Abstract

**Introduction:** In this study, we profiled the levels of blood cellular indices, endogenous glycosaminoglycans (GAGs) and inflammatory biomarkers in a cohort comprised of pulmonary embolism (PE) patients, to determine their inter-relationships. Identification of this relationship may provide insight to the complex pathophysiology of PE and the predictive role of blood cellular indices in acute PE patients.

**Materials and Methods:** Plasma samples from PE patients and healthy controls were analyzed for thrombo-inflammatory biomarkers (IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-$\gamma$, TNF-$\alpha$, IL-1$\alpha$, IL-1$\beta$, MCP-1, EGF, D-dimer, CRP and MMP-9) using biochip array and ELISA methods. The endogenous GAG levels were quantified using a fluorescence quenching method. The data regarding the blood cellular indices were collected through the review of patient medical records and analyzed to demonstrate their relationship.

**Results:** The levels of inflammatory biomarkers and endogenous GAGs were elevated in acute PE patients compared to controls ($P < .05$). Most of the blood cellular indices have shown significant differences in acute PE patients compared to controls ($P < .05$). The levels of inflammatory biomarkers, endogenous GAGs and the blood cellular indices have shown significant associations in correlation and multivariable analysis. While NLR, PLR and SII were significantly predicting the 30-day mortality, PNR, ELR and EMR were not sufficient to predict 30-day mortality in acute PE.

**Conclusion:** Our results show that the increased thrombo-inflammatory response is associated with the release of GAGs and the changes in blood cellular indices. The predictive role of the blood cellular indices for mortality is dependent on their relationship with the inflammatory response.

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## Introduction

Acute pulmonary embolism (PE) is the third leading cause of cardiovascular mortality. The estimated incidence of acute PE is 600,000 cases annually in the US, accounting for over 100,000 deaths per year.[1] Inflammatory pathways remain a key element in both its development and subsequent complications of acute PE.[2] Many established risk factors (eg, aging, autoimmune diseases, cancer, diabetes, heart and respiratory diseases, infections, obesity, pregnancy, surgery and trauma) have been shown to cause venous thromboembolism (VTE) through the inflammatory pathways.[3] The increased levels of inflammatory biomarkers have been clearly established in laboratory, clinical and epidemiologic studies.[4] However, the complex interplay of inflammation in the pathogenesis of acute PE remains unclear.

Vascular endothelium has important functions in circulatory homeostasis. Endothelial cells are key regulators of the inflammatory response, as they form a physical barrier for blood cells, they regulate the vascular tone and control intravascular coagulation. They also regulate the leukocyte and platelet interactions, smooth muscle cell proliferation and control the vascular inflammation by releasing hormones and other soluble mediators such as cytokines.[5] Endothelial glycocalyx which is expressed on the endothelial cells, is a mixture of glycosaminoglycans (GAGs) attached to proteins. Studies have shown that inflammation can cause glycocalyx dysfunction causing enzymatic degradation (shedding) of its components.[6] Related to this, matrix metalloproteinases (MMPs) form a group of enzymes involved in the degradation of components of the extracellular matrix (ECM). It is possible that inflammatory activation of blood cells and rapid release of granules containing large amounts of MMP-9 during PE may explain how MMPs, especially MMP-9, are involved in pathophysiology of PE.[7] However, studies investigating the relationship between vascular endothelium, blood cells and inflammatory response are scarce in acute PE patients.

Acute PE patients are commonly risk-stratified with an aim to identify those individuals at risk for morbidity and mortality, so that those who are at a low risk to be treated conservatively or in those at the highest risk treated with escalated therapeutic options such as thrombolytic therapy or interventional methods.[8] For this purpose, several institutional guidelines have been published recently.[9–10] However, these risk stratification methods are time consuming in routine clinical settings and costly. Additionally, these risk stratification models have suboptimal accuracy for higher risk patients due to low specificity. Complete blood count with differential testing is a cheap and routinely ordered lab test used throughout the clinical course of patients. Several studies investigated the blood cell counts and ratios for prediction of prognosis of acute PE.[11] These studies were mostly single-center investigations involving one or two parameters. Thus, their predictive role in acute PE remains unclear.

In this study, we profiled the levels of blood cellular markers and endogenous GAGs as determinants of inflammatory biomarkers in a cohort comprised of PE patients. By this way, we sought to determine their relationship in between. Identification of this relationship may provide insight to the complex pathophysiology of PE and the predictive role of blood cellular indices in acute PE patients.

## Materials and Methods

### Patient Selection and Data Collection

Patients 18 years or older were recruited to participate in this study through enrollment conducted in conjunction with an ongoing Institutional Review Board (IRB) approved project by the Pulmonary Embolism Response Team (PERT) registry. Diagnosis of acute PE was confirmed by Computed Tomographic (CT) angiography or ventilation/perfusion imaging. The data regarding the blood cellular counts and ratios, clinical information including the patient demographics are collected through the review of patient electronic medical records (EMR).

### Blood Samples

Whole blood samples were drawn from patients within 24 h of confirmed diagnosis of acute PE and collected under an Institutional Review Board approved protocol. Samples were collected in 3.8% (0.109 mol/L) sodium citrate tubes at the time of PE diagnosis, processed for platelet-poor plasma, and stored at −80 C prior to analysis. Control plasma samples from healthy, non-smoking, adults, aged 19 to 53, were purchased from a commercially available source (George King Biomedical, Overland Park, Kansas).

### Measurement of Endogenous Glycosaminoglycans

Heparin Red method based on fluorescence quenching method was used. The reagents were obtained from Red Probes (Munster, Germany). For determination of endogenous GAG concentrations in plasma samples, the protocol of the provider for a 96-well microplate assay was followed. A mixture of 10 000 µL Heparin Red solution and 90 µL Enhancer solution

was freshly prepared. Twenty microliters of the patient or healthy control sample were pipetted into a microplate well, followed by 80 µL of the Heparin Red–Enhancer mixture. Immediately after mixing, the microplate was introduced in the fluorescence reader (Biotek Cytation-5 microplate reader) and fluorescence recorded within 1 min.

### Measurement of Inflammatory Biomarkers

A Randox Investigator Cytokine and Growth Factors High-Sensitivity Array was utilized to measure IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), IFN-ɣ, tumor necrosis factor (TNF)-α, IL-1α, IL-1β, monocyte chemoattractant protein (MCP)-1 and epidermal growth factor (EGF) (Randox Laboratories, London, United Kingdom) per manufacturer guidelines. Quantification of all factors were tested simultaneously by utilizing a sandwich chemiluminescent immunoassay using a single patient sample. D-dimer, C-reactive protein (CRP) and MMP-9 was measured using an enzyme linked immunosorbent assay (ELISA) based immunoassay (Hyphen Biomedical).

### Statistical Analysis

The calculations were performed with SPSS Statistics software (IBM). The figures were illustrated with Prism (GraphPad) software. Continuous variables including all the profiled biomarkers and cellular indices were compared using Student's t or Mann–Whitney U tests. Categorical variables were compared using the Chi-square and Fisher's exact tests (two-sided). Multivariable linear regression analysis using backward elimination method was performed to identify the possible predictors of endogenous GAGs, d-dimer, CRP, MMP-9, blood cell counts and cellular indices, in order to determine a causal relationship between the selected variables in acute PE patients. A receiver operating characteristic (ROC) curve analysis has been performed for each of the cellular ratios to assess the predictive ability for the 30-day mortality. Each of the blood cell ratios were assessed by calculating the corresponding area under the curves (AUC) and 95%CIs. A $P$ value of <.05 was considered statistically significant.

**Table 1.** Characteristics of Patients Included in the Analysis. (n: 101)

| Variable | All Patients |
|---|---|
| Age (Median ± standard deviation) | 63 ± 13.74 |
| Gender n (%) | Male: 52 (51.5%)<br>Female 49 (48.5%) |
| Body Mass Index (kg/m2, median ± standard deviation) | 29.96 ± 9.17 |
| Current Smoker n (%) | 12 (11.9%) |
| Hypertension n (%) | 54 (53.5%) |
| Diabetes Mellitus n (%) | 20 (19.8%) |
| Chronic Kidney Disease n (%) | 14 (13.9%) |
| Cancer n (%) | 39 (38.6%) |
| PE Severity n (%) (ACC/AHA) | Low Risk: 20 (19.8%)<br>Sub-massive: 72 (71.3%)<br>Massive: 9 (8.9%) |
| PESI Score n (%) | Low Risk: 26 (25.7%)<br>Intermediate Risk: 19 (18.8%)<br>High Risk: 56 (55.4%) |
| sPESI Score n (%) | Low Risk: 17 (16.8%)<br>High Risk: 84 (83.2%) |
| 30-day Mortality n (%) | No: 88 (87.1%)<br>Yes: 13 (12.9%) |

ACC: American Collage of Cardiology; AHA: American Heart Association; PESI: Pulmonary Embolism Severity Index; sPESI: Simplified Pulmonary Embolism Severity Index.

## Results

### Patient Demographics

Samples from 101 acute PE patients were used in this study. Patient characteristics are presented in Table 1.

The Levels of Inflammatory Biomarkers and Endogenous GAGs in Acute PE Patients and Healthy Controls.

As shown in Figure 1 and Table 2 the levels of inflammatory biomarkers and endogenous GAGs were significantly elevated in acute PE patients compared to normal healthy individuals ($P < .05$).

The Levels of Blood Cell Counts and Ratios in Acute PE Patients and Healthy Controls

As shown in Figures 2 and 3, the blood cell count and ratios were significantly elevated in acute PE patients compared to normal healthy individuals ($P < .05$). This comparison is presented in Table 3.

The Relationship Between the Inflamatory Biomarkers, Endogenous GAGs, and the Blood Cell Counts and Ratios

In the correlation analysis, each of the inflammatory cytokines showed cross correlations within the matrix, and directly or indirectly linked to each other. Table 4 provides the correlation matrix of inflammatory cytokines.

The blood cell counts, and endogenous GAGs have also shown significant correlations with inflammatory cytokines. Spearman coefficients of these correlations are presented in Table 5.

In multivariable analysis of the blood cell counts, IL-6 (β: 0.014; 95% CI: 0.007 - 0.022, $P < .001$) and MCP-1 (β: −0.012: 95%CI: −0.019 – −0.004, $P$: .002) were the significant predictors of white blood cell (WBC) count. IL-6 (β: 0.011; 95% CI: 0.004 - 0.019, $P$: .003), MCP-1 (β: −0.007: 95%CI: −0.014 – −0.0004, $P$: .036) and EGF (β: 0.027, 95% CI: 0.004 - 0.050, $P$: .022) were the significant predictors of neutrophil count. TNF-α (β: 0.136; 95% CI: 0.019 - 0.253, $P$: .023) and IL-10 (β: 0.024; 95% CI: 0.004 - 0.044, $P$: .020) were the significant predictors of lymphocyte count. TNF-α (β: 0.024; 95% CI: 0.002 - 0.047, $P$: .035) was the significant predictor of eosinophil count. IL-6 (β: 0.001; 95% CI: 0.0001 - 0.002, $P$: .019), IL-10 (β: 0.008; 95% CI: 0.002 - 0.014, $P$: .01), MCP-1 (β: - 0.001; 95% CI: - 0.002 – −0.001, $P$: .001) and EGF (β: 0.003; 95% CI: 0.001 - 0.006, $P$: .009) were the significant predictors of monocyte count. IL-10 (β: 0.043; 95% CI: 0.009 - 0.078, $P$: .014) and MCP-1 (β: −0.008; 95% CI: −0.011 – −0.004, $P < .001$) were the significant predictors

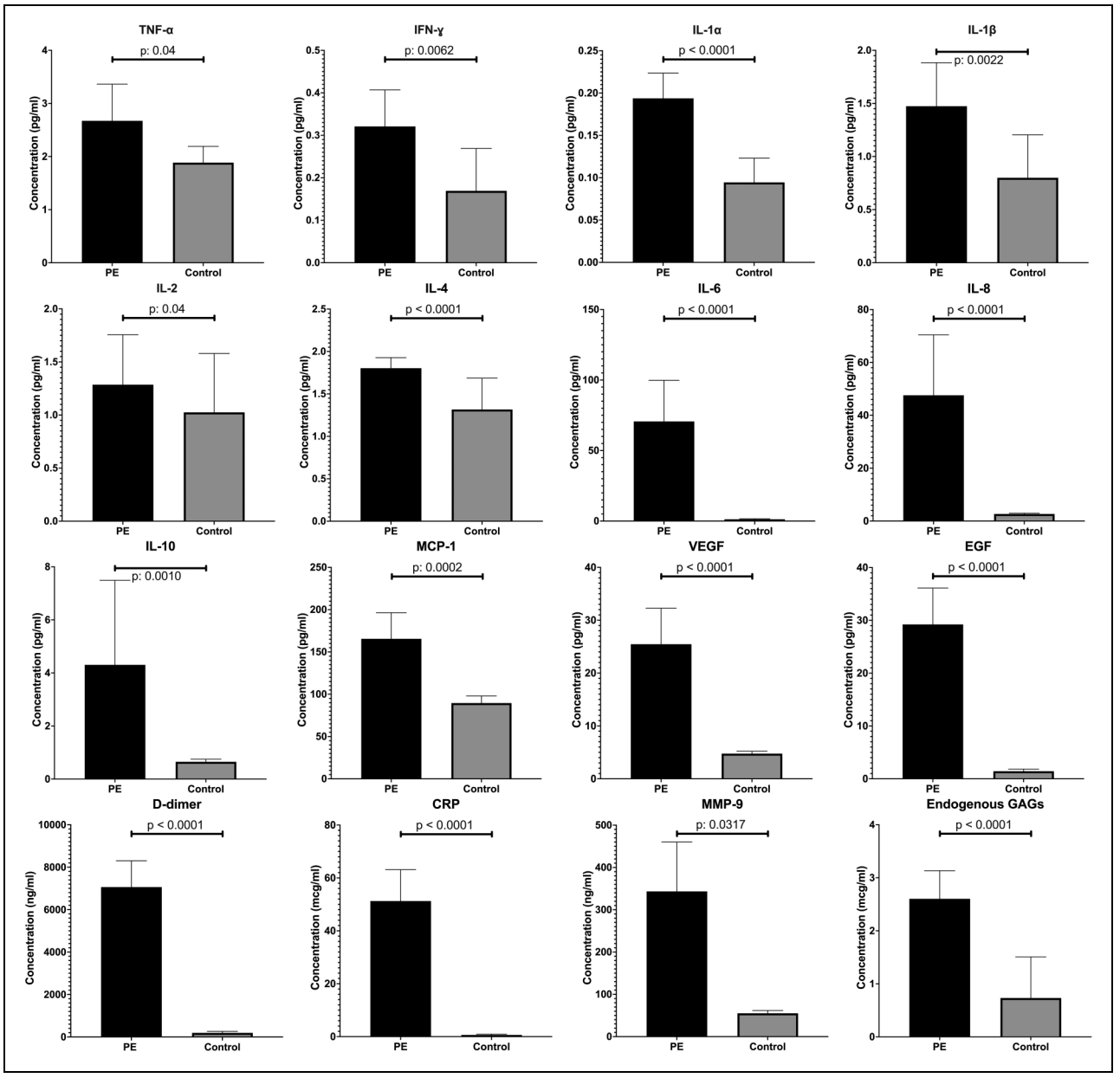


**Figure 1.** The Levels of Inflammatory Biomarkers and Endogenous GAGs in Acute PE Patients (n: 101) and Healthy Controls (n: 40). Data is represented as mean with 95% CI. PE indicates pulmonary embolism; CI: confidence interval.

of hemoglobin count. And IL-6 (β: 0.177; 95% CI: 0.0002 - 0.353, *P*: .05), IFN-ɣ (β: - 49.79; 95%CI: −95.32 – −4.26, *P*: 0.032) and MCP-1 (β: −0.35: 95%CI: −0.52 – −0.18, *P* < .001) were the significant predictors of platelet count.

Additionally, IL-4 (β: −0.801; 95%CI: −1.386 – −0.217, *P*: .008), IL-8 (β: 0.005; 95%CI: 0.002 - 0.008, *P*: .003), IL-10 (β: 0.207; 95%CI: 0.168 - 0.246, *P* < .001) and EGF (β: 0.012; 95%CI: 0.003 - 0.022, *P*: .010) were the significant predictors of endogenous GAGs. TNF-α (β: −113.82; 95%CI: −165.31 – −62.34, *P* < .001), IL-1α (β: −1057.51; 95%CI: −1831.78 – −283.24, *P*: .008) and IL-1β (β: 317.88; 95%CI: 219.41-426.34, *P* < .001) were the significant predictors of MMP-9. IL-10 (β: 180.28; 95%CI: 108.92 - 251.64, *P*: .008) was a significant predictor of D-dimer. And IL-1α (β: 93.29; 95%CI: 19.34 - 167.24, *P*: .014), IL-2 (β: 7.98; 95%CI: 2.43 - 13.52, *P*: .047), MCP-1 (β: −0.103; 95%CI: −0.205 – −0.002, *P*: .047) and EGF (β: 0.511; 95%CI: 0.195 - 0.827, *P*: .002) were the significant predictors of CRP.

Furthermore, the correlations between blood cellular indices and inflammatory cytokines were also significant. Spearman coefficients of these correlations are presented in Table 6.

In multivariable regression of the blood cellular indices, TNF-α (β: −0.76, 95% CI: −1.49 – −0.02, *P*: .043), IL-2 (β: 2.87, 95% CI: 1.60 — 4.15, *P* < .001), IL-10 (β: −0.259, 95% CI: −0.441 — −0.077, *P*: .006) and MCP-1 (β: 0.051, 95% CI: 0.027 — 0.075, *P* < .001) were the significant predictors of

**Table 2.** The Levels of Inflammatory Biomarkers and Endogenous GAGs in Acute PE Patients and Healthy Controls.

| Biomarker | PE Patients (Mean ± SEM) (n: 101) | Healthy Controls (Mean ± SEM) (n:40) | Fold Increase | *P* value |
|---|---|---|---|---|
| TNF-α | 2.69 ± 0.33 | 1.88 ± 0.15 | 1.43 | **.04** |
| IFN-ɣ | 0.3208 ± 0.04 | 0.1693 ± 0.04 | 1.89 | **.0062** |
| IL-1α | 0.19 ± 0.01 | 0.09 ± 0.01 | 2.11 | **< .0001** |
| IL-1β | 1.47 ± 0.19 | 0.79 ± 0.19 | 1.86 | **.0022** |
| IL-2 | 1.28 ± 0.23 | 1.02 ± 0.27 | 1.25 | **.04** |
| IL-4 | 1.80 ± 0.06 | 1.31 ± 0.18 | 1.37 | **<.0001** |
| IL-6 | 70.64 ± 14.25 | 1.26 ± 0.19 | 56.06 | **< .0001** |
| IL-8 | 47.57 ± 11.17 | 2.67 ± 0.14 | 17.81 | **< .0001** |
| IL-10 | 4.30 ± 1.55 | 0.64 ± 0.04 | 6.71 | **.0010** |
| MCP-1 | 165.60 ± 15.04 | 89.56 ± 4.13 | 1.84 | **.0002** |
| VEGF | 25.46 ± 3.33 | 4.75 ± 0.2231 | 5.36 | **< .0001** |
| EGF | 29.22 ± 3.37 | 1.42 ± 0.19 | 20.57 | **< .0001** |
| D-dimer | 7063.00 ± 624.50 | 190.90 ± 34.83 | 36.99 | **<.0001** |
| CRP | 51.25 ± 5.983 | 0.6278 ± 0.1651 | 81.63 | **<.0001** |
| MMP-9 | 343.3 ± 54.13 | 55.23 ± 2.04 | 6.21 | **.0317** |
| Endogenous GAGs | 2.603 ± 0.23 | 0.7328 ± 0.37 | 3.55 | **<.0001** |

Bold values are statistically significant ($P < .05$). SEM: Standard Error of the Mean

NLR, IL-2 (β: 39.11, 95% CI: 13.96-64.25, p: .003) was the significant predictor of PLR, IL-1β (β: −886.70; 95% CI: −1665.86 — −107.55, *P*: .026) and IL-2 (β: 504.15; 95% CI: 72.03 - 936.27, *P*: .023) were the significant predictors of SII. In multivariable analysis, there were no significant relationship between the inflammatory biomarkers with PNR, ELR and eosinophil-to-neutrophil ratio (ENR).

In the correlation analysis between endogenous GAGs, MMP-9, D-dimer, CRP and blood cell counts neutrophil count (Spearman r: 0.401), NLR (Spearman r: 0.222) and PNR (Spearman r: −0.276) was significantly corelated with MMP-9 and neutrophil count (Spearman r: 0.260), monocyte count (Spearman r: 0.271) NLR (Spearman r: 0.250), PLR (Spearman r: 0.212) and SII (Spearman r: 0.276) were significantly correlated with CRP. Spearman coefficients of these correlations are presented in Table 7.

In multivariable analysis, none of the blood cell counts and indices were significant predictors of endogenous GAGs and CRP levels. PLR (β: 11.76; 95% CI: 1.66 - 21.86, *P*: .02) and SII (−1.148; 95% CI: −2.194 – −0.102, *P*: .03) were significant predictors of d-dimer. Neutrophil count (β: 22.91; 95% CI: 8.27 - 37.55, *P*: .003), eosinophil count (β: 722.92; 95% CI: 354.85 - 1094.99, *P*: < .001), PNR (β: −1.763; 95% CI: −3.182 – −0.344, *P*: .01) and ELR (β: 873.86; 95% CI: 473.95 - 1273.78, *P*: < .001) were significant predictors of MMP-9.

### *Predictive Role of Blood Cell Counts and Ratios in 30-day Mortality of Acute PE*

The receiver operating characteristic (ROC) curve analysis of blood cell counts was performed to evaluate their relevance in prediction of 30-day mortality. None of the blood cell counts were significant in predicting 30-day mortality of acute PE except hemoglobin count. The area under the curve (AUC) for hemoglobin count was 0.747 (95% CI: 0.631-0.864, *P*: .004).

The receiver operating characteristic (ROC) curve analysis of NLR, PLR, SII, PNR, ELR and EMR in predicting 30-day mortality were also performed. The areas under the ROC curve for NLR, PLR, and SII were 0.765 (95% CI: 0.593-0.936, *P*: .007), 0.729 (95% CI: 0.526-0.933, *P*: .020) and 0.758 (95% CI: 0.605-0.912, *P*: .009), respectively. In ROC curve analysis, PNR, ELR and EMR were not significant in predicting 30-day mortality of acute PE.

The receiver operating characteristic (ROC) curve analysis for each of the cellular ratios to assess the predictive ability for the 30-day mortality is represented in Figure 4.

## Discussion

The primary findings of this study can be summarized as: (1) The levels of inflammatory biomarkers and endogenous GAGs were significantly elevated in acute PE patients in comparison to normal healthy individuals. (2) Most of the blood cell count and ratios have shown significant differences in acute PE patients compared to healthy controls. (3) The levels of all inflammatory biomarkers, endogenous GAGs and the blood cellular indices have shown significant correlations with each other, showing a direct or indirect inter-relationship. (4) The multivariable analysis has shown significant causal associations between inflammatory biomarkers, endogenous GAGs and the blood cellular indices. (5) In our patient cohort, while NLR, PLR and SII were significantly predicting the 30-day mortality, PNR, ELR and EMR were not significant in predicting 30-day mortality of acute PE.

The role of inflammatory biomarkers in venous thromboembolism has been investigated in several previous studies.[2–5] However, in none of these studies were inflammatory biomarkers, endogenous GAGs and the blood cellular indices were considered together as determinants of pathogenesis of this serious disease. In our current study we were able to evaluate all the tested biomarkers completely in our patient cohort and healthy controls. The levels of all the tested inflammatory biomarkers and endogenous GAGs were higher in comparison to healthy controls. Among them CRP (81.63), IL-6 (56.06-fold), d-dimer (36.99-fold), EGF (20.57-fold), IL-8 (17.81-fold), IL-10 (6.71-fold), MMP-9 (6.21-fold) and VEGF (5.36-fold) have shown the most prominent increases in acute PE patients. These findings clearly represent the increased thrombo-inflammatory response in acute PE patients.

We also investigated the levels of the blood cellular indices in our patients and healthy controls. WBC count (1.35-fold) and the neutrophil count (1.66-fold) were higher in PE patients compared to healthy controls. Lymphocyte count (0.76-fold), eosinophil count (0.68-fold) and the hemoglobin levels 0.86-fold) were lower in PE patients. We did not observe any significant difference in monocyte and platelet counts in PE patients compared to healthy controls. Additionally, NLR (8.22-fold), PLR

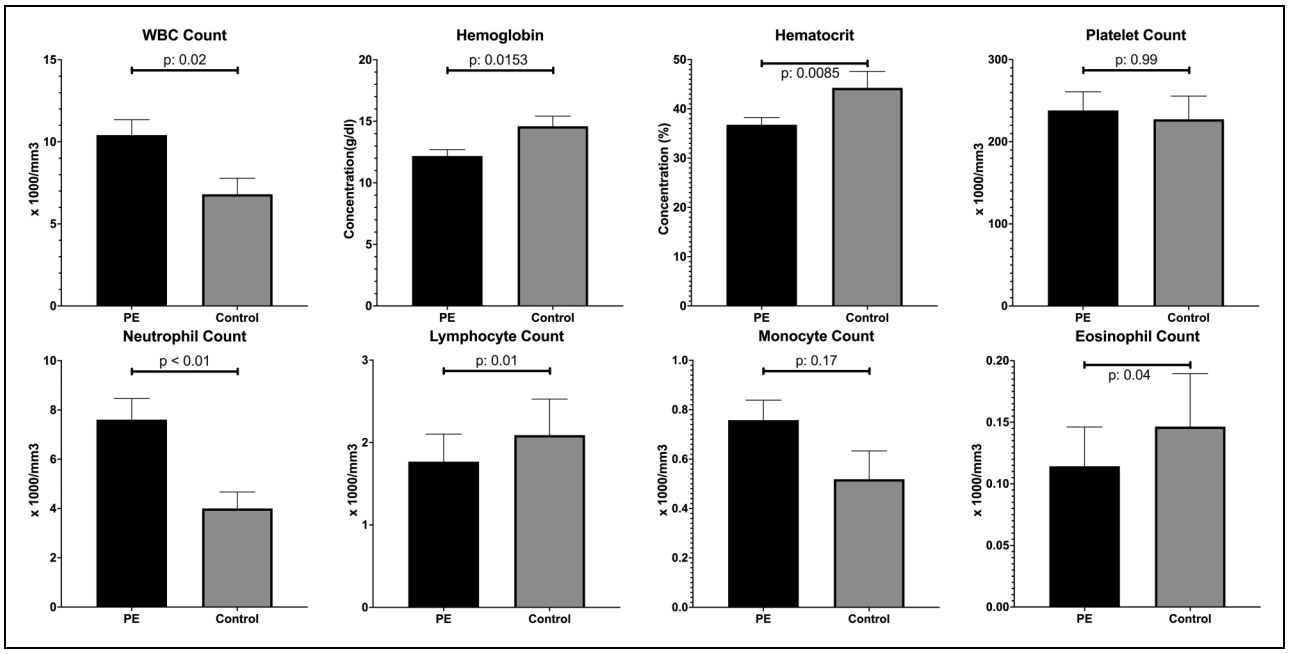


**Figure 2.** The Levels of Blood Cell Counts in PE Patients (n:101) and Healthy Controls (n:15). Data is represented as mean with 95% CI. PE indicates pulmonary embolism; CI: confidence interval.

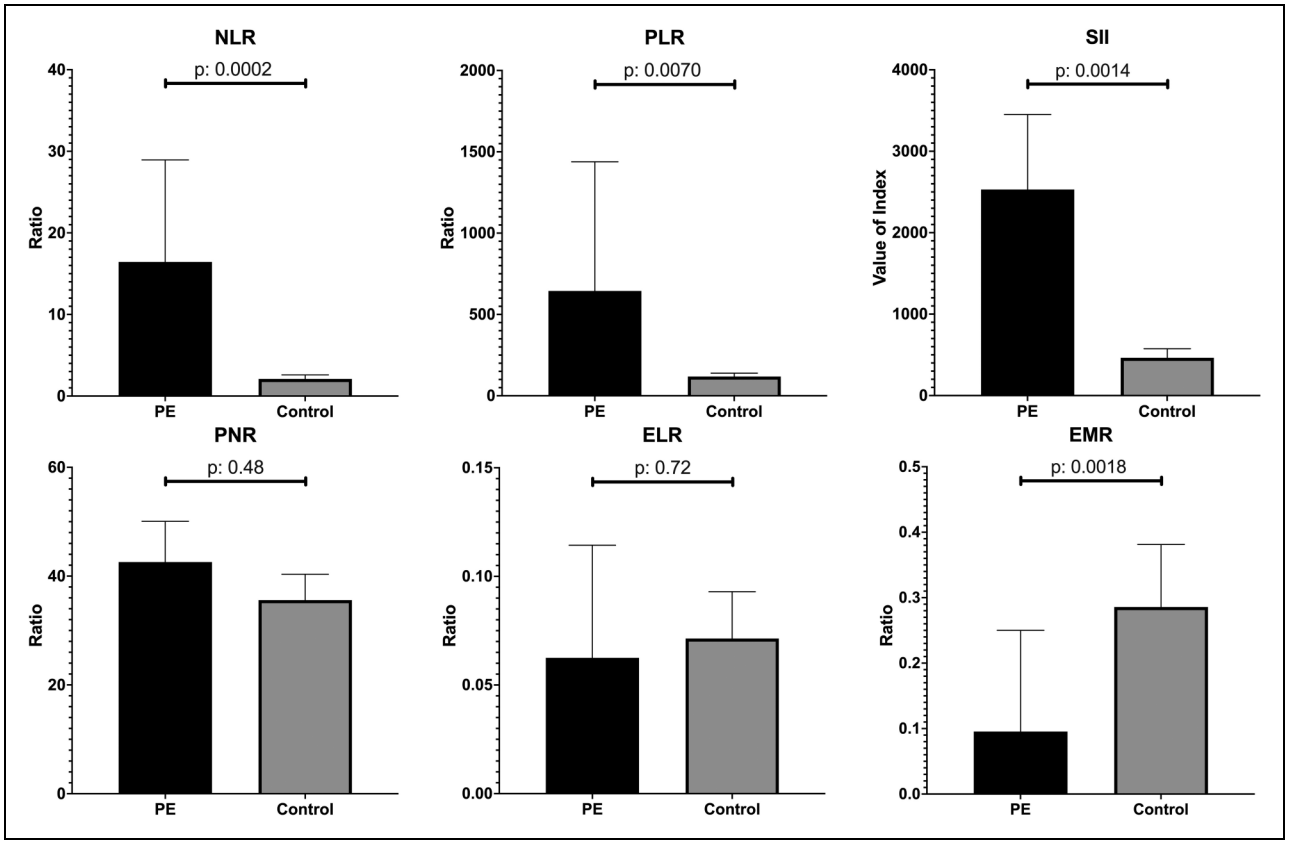


**Figure 3.** The Levels of Blood Cellular Ratios in PE Patients (n:101) and Healthy Controls (n:15). Data is represented as mean with 95% CI. PE indicates pulmonary embolism; CI: confidence interval; NLR: Neutrophil-to-lymphocyte Ratio; PLR: Platelet-to-lymphocyte Ratio; SII: Systemic-immune-inflammation-index; PNR: Platelet-to-neutrophil Ratio; ELR: Eosinophil-to-lymphocyte Ratio; EMR: Eosinophil-to-monocyte Ratio.

**Table 3.** The Levels of Blood Cell Counts and Cellular Indices in PE Patients and Healthy Controls.

| Cellular Index | PE Patients (Mean ± SEM) (N: 101) | Healthy Controls (Mean ± SEM) (n: 15) | Fold Increase | *P* value |
|---|---|---|---|---|
| WBCs | 10.09 ± 0.44 | 7.43 ± 0.48 | 1.35 | **.02** |
| Neutrophil | 7.19 ± 0.37 | 4.32 ± 0.33 | 1.66 | **<.01** |
| Lymphocyte | 1.76 ± 0.16 | 2.31 ± 0.24 | 0.76 | **.01** |
| Monocyte | 0.73 ± 0.03 | 0.57 ± 0.06 | 1.28 | .17 |
| Eosinophil | 0.11 ± 0.01 | 0.16 ± 0.02 | 0.68 | **.04** |
| NLR | 16.69 ± 6.26 | 2.03 ± 0.23 | 8.22 | **.0002** |
| PLR | 706.30 ± 400.1 | 108.60 ± 10.88 | 6.50 | **.0070** |
| SII | 2523 ± 448.30 | 456.8 ± 52.78 | 5.52 | **.0014** |
| PNR | 43.48 ± 3.42 | 33.44 ± 2.65 | 1.30 | **.48** |
| ELR | 0.09 ± 0.015 | 0.07 ± 0.011 | 1.28 | .72 |
| EMR | 0.166 ± 0.025 | 0.288 ± 0.033 | 0.57 | **.0018** |
| Hemoglobin | 12.21 ± 0.26 | 14.11 ± 0.45 | 0.86 | **.0153** |
| Hematocrit | 36.87 ± 0.7420 | 43.40 ± 2.091 | 0.84 | **.0085** |
| Platelet | 238.60 ± 11.27 | 233.60 ± 18.16 | 1.02 | 0.99 |

Bold values are statistically significant ($P < .05$). NLR: Neutrophil-to-lymphocyte Ratio; PLR: Platelet-to-lymphocyte Ratio; SII: Systemic-immune-inflammation-index; PNR: Platelet-to-neutrophil Ratio; ELR: Eosinophil-to-lymphocyte Ratio; EMR: Eosinophil-to-monocyte Ratio; SEM: Standard Error of the Mean.

(6.50-fold) and SII (5.52-fold) were elevated in PE patients compared to healthy controls. EMR (0.57-fold) was lower in PE patients. We did not observe any difference in the levels of PNR and ELR in PE patients compared to healthy controls. These results clearly show that the levels of the blood cellular indices in patients with acute PE are significantly different compared to the control group.

The levels of inflammatory biomarkers, endogenous GAGs and the blood cellular indices have shown multiple correlations with each other in the correlation analysis. Furthermore, the multivariable analysis has shown significant associations between inflammatory biomarkers, endogenous GAGs and the blood cellular indices. In the ROC curve analysis, except for hemoglobin count, while none of the blood cell counts were significant in predicting 30-day mortality of acute PE, NLR, PLR and SII were the significant blood cell ratios that can predict this outcome. It is important to highlight that, among the other blood cellular indices, NLR, PLR and SII have shown the most prominent increases compared to healthy individuals in our patient cohort. Additionally, they also showed stronger associations with inflammatory biomarkers in correlation and multivariable analysis. This finding reminded us that the prediction accuracy of the blood cell counts and ratios, is more likely to be associated with how they reflect the inflammatory status to the clinical outcomes.

Another important finding that we observed in our study was related to the levels of endogenous GAGs. The levels of endogenous GAGs (3.55-fold) have shown a less prominent increase in comparison to healthy controls. The levels of endogenous GAGs have shown significant relationship with inflammatory biomarkers in correlation and multivariate analysis. However, their levels did not show such a relationship with the blood cell counts and ratios. This finding may lead us to think that the functions and not the quantity of these blood cells are more important determinants on the release of endogenous GAGs to the circulation. These findings would be worthwhile to investigate further.

The blood cell counts, and ratios have been evaluated in various studies in the literature. In these studies, the blood cell counts, and ratios provided both clinical diagnostic and prognostic value for acute PE. However, these studies were not uniform, giving different results for different outcomes.[11] NLR is maybe the most studied cell index in acute PE. These studies have shown that the NLR can be used as a diagnostic and prognostic tool. For instance, in the study that Ates et al have published, NLR were identified as an independent predictor of massive PE in 639 patients comprised of acute PE.[12] In this study, NLR had good clinical diagnostic value for this outcome (AUC = 0.893). In the study of Kasapoglu et al, NLR levels were significantly higher in patients who died within 30 days in 550 patients comprised of acute PE.[13] However, NLR was not an independent predictor of death for overall patients in this study. But a subgroup analysis in patients without comorbidities showed that NLR was an independent predictor of mortality. Duman et al found that an NLR was predictive for 30-day, 6-month and 1-year mortality in a cohort of 828 PE patients.[14]

Several studies have also evaluated the PLR as a diagnostic and prognostic tool for acute PE. Regarding this, Ates et al identified PLR as an independent predictor of massive acute PE in 639 patients.[12] In this study, PLR had also good clinical diagnostic value for this outcome (AUC: 0.877). Kasapoğlu et al reported higher levels of PLR in 550 acute PE patients who have deceased within 30-days.[13] However, PLR was not an independent risk factor in multivariable analysis. Interestingly, Duman et al have reported that there was no significant difference in PLR levels between deceased and surviving subgroups in 828 PE patients.[14] Additionally, PLR was not an independent risk factor for death. Kundi et al found higher levels of PLR in patients with high sPESI scores and PLR was independently associated with in-hospital mortality with an AUC of 0.860, among 646 patients with acute PE.[15] Ghaffari, et al did not find a difference in PLR between patients with and without major cardiopulmonary adverse events in 492 patients comprised of acute PE,[16] but PLR was associated with in-hospital mortality with an AUC of 0.610.

PLR may also be a predictor of the occurrence of VTE in cancer patients and after surgery. Grilz, et al identified a significant association between PLR and the occurrence of VTE in 1469 cancer patients.[17] Yao et al identified a higher preoperative PLR than postoperative PLR in 733 patients after total joint replacement.[18] In this study, the postoperative PLR was independently associated with the occurrence of deep venous thrombosis (DVT) with an AUC of 0.513 and 0.561 for preoperative PLR and postoperative PLR respectively. Furthermore, Kurtipek et al reported higher PLR values in 71 patients with acute PE compared to healthy controls, suggesting that PLR may be associated with pulmonary artery endothelial cell dysfunction.[19] Importantly, Wang et al conducted a meta-analysis of seven

**Table 4.** Spearman Correlation Coefficients for Inflammatory Biomarkers in Acute PE Patients.

| | IFN-γ | TNF-α | IL-1α | IL-1β | IL-2 | IL-4 | IL-6 | IL-8 | IL-10 | MCP-1 | VEGF | EGF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IFN-γ | 1.000 | **0.287** | 0.079 | 0.186 | **0.310** | 0.103 | -0.114 | 0.124 | 0.043 | 0.040 | 0.075 | **0.205** |
| TNF-α | **0.287** | 1.000 | 0.083 | **0.375** | **0.225** | 0.126 | **0.358** | **0.555** | **0.428** | **0.446** | 0.099 | 0.010 |
| IL-1α | 0.079 | 0.083 | 1.000 | **0.490** | **0.205** | **0.300** | 0.043 | 0.060 | 0.129 | -0.090 | -0.043 | 0.017 |
| IL-1β | 0.186 | **0.375** | **0.490** | 1.000 | **0.378** | **0.491** | **0.279** | **0.458** | **0.358** | **0.230** | 0.158 | 0.007 |
| IL-2 | **0.310** | **0.225** | **0.205** | **0.378** | 1.000 | **0.239** | 0.151 | 0.157 | **0.230** | 0.046 | 0.122 | **-0.343** |
| IL-4 | 0.103 | 0.126 | **0.300** | **0.491** | **0.239** | 1.000 | 0.118 | **0.281** | **0.230** | 0.197 | 0.120 | -0.124 |
| IL-6 | -0.114 | **0.358** | 0.043 | **0.279** | 0.151 | 0.118 | 1.000 | **0.552** | **0.434** | **0.359** | **0.251** | 0.018 |
| IL-8 | 0.124 | **0.555** | 0.060 | **0.458** | 0.157 | **0.281** | **0.552** | 1.000 | **0.523** | **0.513** | **0.242** | 0.106 |
| IL-10 | 0.043 | **0.428** | 0.129 | **0.358** | **0.230** | **0.230** | **0.434** | **0.523** | 1.000 | **0.401** | 0.174 | -0.073 |
| MCP-1 | 0.040 | **0.446** | -0.090 | **0.230** | 0.046 | 0.197 | **0.359** | **0.513** | **0.401** | 1.000 | 0.168 | -0.019 |
| VEGF | 0.075 | 0.099 | -0.043 | 0.158 | 0.122 | 0.120 | **0.251** | **0.242** | 0.174 | 0.168 | 1.000 | **0.242** |
| EGF | **0.205** | 0.010 | 0.017 | 0.007 | **-0.343** | -0.124 | 0.018 | 0.106 | -0.07 | -0.019 | **0.242** | 1.000 |

Bold values are statistically significant ($P < .05$).

**Table 5.** Spearman Correlation Coefficients for Blood Cell Counts and Inflammatory Biomarkers in Acute PE Patients.

| Markers | WBC Count | Neutrophil Count | Lymphocyte Count | Eosinophil Count | Monocyte Count | Hb Count | Platelet Count | Endogenous GAGs | MMP-9 | D-dimer | CRP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IFN-γ | **-0.208** | -0.205 | -0.085 | 0.053 | -0.120 | 0.023 | -0.127 | 0.007 | 0.019 | -0.159 | -0.054 |
| TNF-α | 0.044 | -0.025 | 0.018 | 0.053 | -0.032 | **-0.359** | -0.166 | **0.381** | 0.132 | 0.068 | 0.056 |
| IL-1α | 0.110 | 0.059 | 0.216 | -0.025 | 0.121 | 0.051 | 0.087 | 0.234 | -0.020 | **0.218** | 0.168 |
| IL-1β | 0.070 | 0.046 | 0.070 | -0.144 | 0.123 | -0.101 | -0.122 | 0.267 | 0.178 | 0.140 | 0.119 |
| IL-2 | 0.002 | 0.002 | -0.082 | -0.004 | 0.053 | **-0.229** | -0.089 | -0.076 | -0.083 | 0.033 | 0.176 |
| IL-4 | 0.083 | 0.038 | **0.222** | 0.019 | -0.121 | 0.043 | -0.060 | 0.088 | 0.061 | 0.094 | -0.065 |
| IL-6 | **0.309** | **0.322** | -0.170 | -0.090 | **0.249** | **-0.283** | 0.007 | 0.144 | **0.207** | **0.311** | **0.385** |
| IL-8 | 0.022 | 0.041 | -0.119 | -0.107 | 0.041 | **-0.369** | -0.162 | **0.278** | **0.247** | 0.126 | 0.052 |
| IL-10 | 0.226 | **0.234** | -0.204 | **-0.256** | -0.050 | **-0.369** | **-0.258** | **0.290** | **0.310** | **0.258** | 0.026 |
| MCP-1 | -0.064 | -0.056 | -0.052 | -29 | -0.118 | **-0.239** | **-0.314** | **0.204** | **0.238** | 0.096 | **-0.226** |
| VEGF | **0.222** | **0.257** | -0.112 | -0.049 | 0.143 | 0.024 | **0.239** | **0.256** | **0.361** | -0.086 | 0.162 |
| EGF | -0.002 | 0.041 | 0.105 | 0.156 | 0.085 | 0.078 | 0.195 | 0.196 | 0.058 | -0.115 | 0.170 |

Bold values are statistically significant ($P < .05$).

**Table 6.** Spearman Correlation Coefficients for Blood Cellular Indices and Inflammatory Biomarkers in Acute PE Patients.

| Markers | NLR | PLR | SII | PNR | ELR | EMR |
|---|---|---|---|---|---|---|
| TNF-α | −0.051 | −0.061 | −0.058 | −0.079 | 0.121 | −0.043 |
| IFN-γ | −0.049 | 0.073 | −0.098 | 0.058 | 0.049 | 0.075 |
| IL-1α | -0.131 | -0.122 | -0.105 | -0.082 | -0.130 | -0.069 |
| IL-1β | -0.023 | -0.099 | -0.082 | **-0.217** | -0.156 | -0.194 |
| IL-2 | 0.110 | 0.137 | 0.112 | -0.064 | 0.022 | -0.056 |
| IL-4 | -0.110 | -0.182 | -0.142 | -0.142 | -0.059 | 0.002 |
| IL-6 | **0.336** | **0.207** | **0.359** | **-0.267** | 0.071 | -0.173 |
| IL-8 | 0.158 | 0.074 | 0.123 | **-0.219** | -0.020 | **-0.204** |
| IL-10 | **0.270** | 0.110 | 0.189 | **-0.377** | -0.139 | **-0.320** |
| MCP-1 | 0.061 | -0.012 | 0.005 | -0.178 | -0.006 | -0.088 |
| VEGF | **0.212** | **0.256** | **0.414** | -0.029 | 0.059 | -0.123 |
| EGF | -0.069 | 0.012 | 0.015 | 0.130 | 0.142 | 0.111 |

Bold values are statistically significant ($P < .05$). NLR: Neutrophil-to-lymphocyte Ratio; PLR: Platelet-to-lymphocyte Ratio; SII: Systemic-immune-inflammation-index; PNR: Platelet-to-neutrophil Ratio; ELR: Eosinophil-to-lymphocyte Ratio; EMR: Eosinophil-to-monocyte Ratio.

**Table 7.** Spearman Correlation Coefficients for Endogenous GAGs, MMP-9, D-dimer, CRP and Blood Cellular Indices.

| Markers | Endogenous GAGs | MMP-9 | D-dimer | CRP |
|---|---|---|---|---|
| WBC | 0.045 | **0.368** | -0.023 | 0.146 |
| Neutrophil Count | -0.016 | **0.401** | -0.005 | **0.260** |
| Eosinophil Count | 0.093 | 0.107 | -0.072 | -0.127 |
| Lymphocyte Count | 0.037 | -0.041 | -0.017 | -0.127 |
| Monocyte Count | -0.005 | 0.109 | -0.010 | **0.271** |
| Hemoglobin Count | -0.070 | 0.006 | -0.083 | -0.002 |
| Platelet Count | -0.047 | 0.138 | -0.184 | 0.178 |
| NLR | -0.064 | **0.222** | 0.047 | **0.250** |
| PLR | -0.078 | 0.075 | -0.065 | **0.212** |
| SII | -0.117 | 0.194 | -0.016 | **0.276** |
| PNR | -0.081 | **-0.276** | -0.179 | -0.122 |
| ELR | 0.087 | 0.141 | 0.016 | -0.060 |
| EMR | 0.037 | 0.143 | -0.057 | -0.166 |

Bold values are statistically significant ($P < .05$). NLR: Neutrophil-to-lymphocyte Ratio; PLR: Platelet-to-lymphocyte Ratio; SII: Systemic-immune-inflammation-index; PNR: Platelet-to-neutrophil Ratio; ELR: Eosinophil-to-lymphocyte Ratio; EMR: Eosinophil-to-monocyte Ratio.

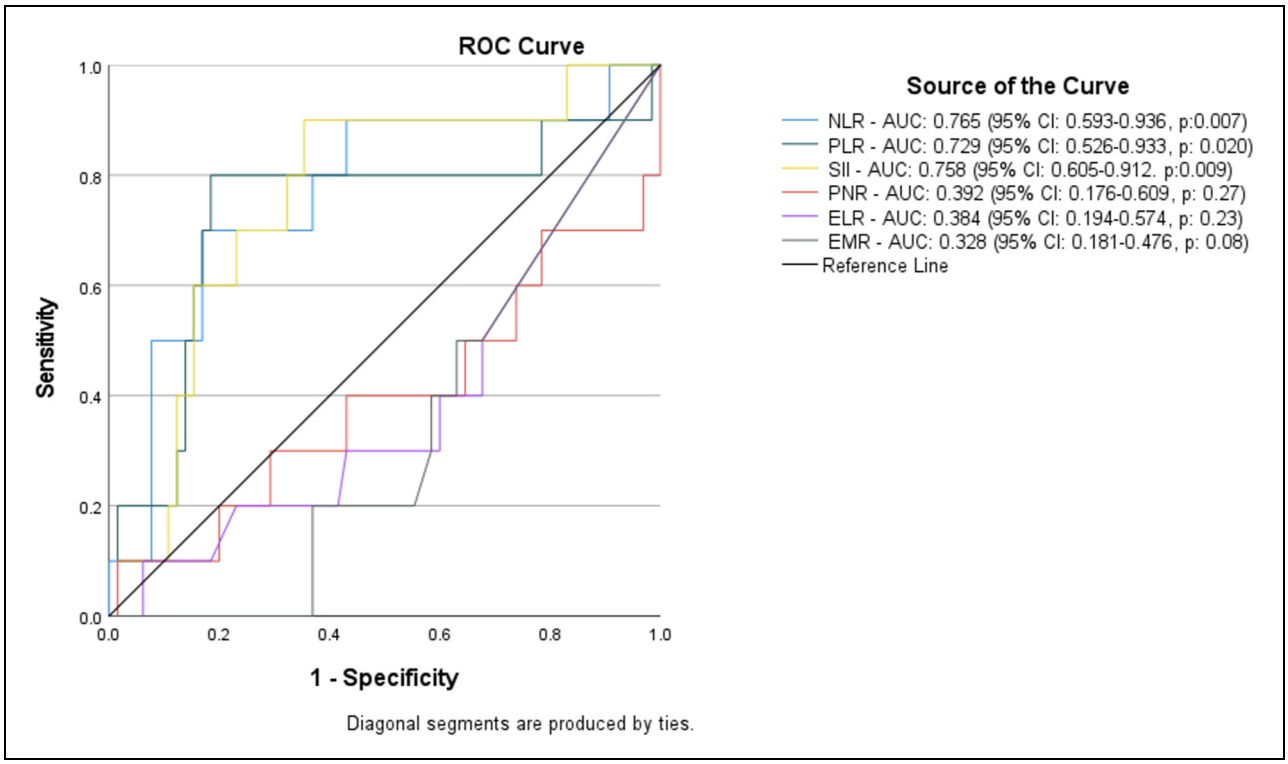


**Figure 4.** The comparison of ROC Curves of blood cell ratios in predicting 30-day mortality of acute PE. NLR: Neutrophil-to-lymphocyte Ratio; PLR: Platelet-to-lymphocyte Ratio; SII: Systemic-immune-inflammation-index; PNR: Platelet-to-neutrophil Ratio; ELR: Eosinophil-to-lymphocyte Ratio; EMR: Eosinophil-to-monocyte Ratio.

studies and found that NLR and PLR were both significantly associated with mortality in patients with PE.[20]

The clinical value of SII was also tested in several studies. These studies have shown that it can also be a useful tool for both the diagnosis and prognosis of mortality in VTE. Gok et al reported elevated SII levels in 442 patients with acute PE.[21] SII was also higher in patients who had in-hospital mortality. SII was an independent predictor of massive acute pulmonary embolism (APE) with an AUC of 0.957. Peng et al reported an increased SII in patients with VTE compared to those without VTE.[22] And SII was an independent predictor of VTE after hip fracture in elderly patients.

It is important to note that platelets and leukocytes are vital in development of thrombosis.[23] The main physiological function of platelets is hemostasis after vascular injury.[24] Platelets can rapidly adhere to the site of vascular injury, forming platelet plug, and promote coagulation. Activated platelets release inflammatory molecules, and express adhesion molecules to interact with leukocytes and endothelial cells leading to vascular inflammation and VTE.[25] Neutrophils and monocytes adhere to intact endothelium within hours of flow reduction.[26] Neutrophils regulate inflammatory and immune responses in VTE by releasing neutrophil-extracellular-traps (NETs) and releasing cytokines, damage associated-molecular-patterns (DAMPs), and extracellular vesicles (EVs) that contribute to neutrophil interactions with other cells.[27–28] Extracellular trap production has also been described in monocytes, eosinophils, and basophils, but neutrophils are believed to be the dominant trap producers in VTE.[29] Monocytes, while classically defined by their phagocytic role in innate immunity, contribute to VTE in multiple ways. The most well-defined roles for monocytes in coagulation are to initiate coagulation through presentation of tissue factor (TF) and to potentiate thrombo-inflammation through inflammasome activation.[30] Eosinophils can act through multiple pathways in development of VTE. They can release major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) which can damage the vascular endothelial cells, they can directly activate tissue factors and platelet-activating factors and direct infiltration of eosinophils can cause vascular endothelial cell damage.[31]

In this study, we found that NLR, PLR, and SII, by better reflecting the inflammatory response, can significantly detect 30-day mortality. On the other hand, although they showed certain relationship with inflammatory response none of the blood cell counts except hemoglobin, PNR, ELR and EMR were sufficient to predict 30-day mortality in acute PE. From this point of view, the predictive role of the blood cellular indices for mortality appears to be dependent on their degree of relationship with the inflammatory response.

## Study Limitations

In this study, the blood sample collection times varied up to 48 h. These variations were partly due to the difference in blood collection times. Additionally, this study is based on a

single sample analysis. Due to logistic reasons and institutional regulations follow-up samples were not collected and analyzed. Future studies should consider collecting sequential samples in follow-up analysis. Additionally, we were not able to completely consider the clinical characteristics of the patients while including in this study. Increased levels of inflammatory biomarkers and blood cellular indices and endothelial dysfunction is detected in several other illnesses (particularly in cancer patients) other than acute PE. We were not able to consider the previous anticoagulant history and the type of anticoagulant used immediately after diagnosis. For this study, it is important to know the history of heparinization in terms of type of heparin and mode of administration. Despite these limitations the study clearly points to the increased thrombo-inflammatory response associated with the release of endogenous GAGs and the changes in blood cellular indices in PE patients.

## Conclusion

In this study, we investigated the levels of blood cellular indices, endogenous GAGs as determinants of inflammatory biomarkers in a group of acute PE patients. Our results, including the relationship between the tested parameters and their relevance in prediction of mortality for acute PE, are of major importance. More studies are needed to understand the predictive role of blood cellular indices and the complex pathophysiology of acute PE.

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### Author Contributions

The main manuscript was prepared by BK. Statistical support and proof-reading, implementation and design of this study were equally shared by all authors.

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