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Cellular Indices and Outcome in Patients with Acute Venous Thromboembolism

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Abstract

Background: Cellular indices provide integrative information about systemic inflammation status which is readily available from routine laboratory parameters. This study aimed to evaluate the prognostic role of three cellular indices in patients with venous thromboembolism (VTE). **Methods:** The RIETE registry database was used to determine the association between the baseline neutrophil-to-lymphocyte-ratio (NLR), platelet-to-lymphocyte-ratio (PLR) and systemic-immune-inflammation-index (SII) for 90-day adverse outcomes in patients with acute VTE. **Results:** From January 2020 to April 2021, 4487 patients with acute VTE were recruited in the RIETE registry. Of these, 2683 presented with symptomatic pulmonary embolism (PE); 283 with incidental PE; 1129 with lower-limb deep vein thrombosis (DVT); 175 with upper-limb DVT; 69 with splanchnic vein thrombosis; 142 with superficial vein thrombosis and 20 with retinal vein thrombosis. Mean values were: NLR 5.9 ± 7.1, PLR 190 ± 158 and SII 1459 ± 2028. During the first 90-days, 38 patients (0.8%) developed recurrent DVT, 45 (1.0%) had recurrent PE, 152 (3.4%) suffered major bleeding, and 484 (11%) died. On multivariable analysis, patients with NLR >4.41 were at an increased risk for major bleeding and patients with NLR >4.96 were at the risk of death, while those with SII >1134.5 were at increased risk for death. **Conclusions:** This study reports the results of a large cohort to date which evaluate the prognostic value of three cellular indices simultaneously in patients with acute VTE. Results support that none of the three baseline cellular indices were sufficient for prediction of VTE recurrences in acute VTE patients. The patients with higher baseline NLR values were at an increased risk of major bleeding or death, those with high SII values were only at an increased risk for mortality.

Keywords

neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), systemic immune inflammation index (SII), venous thromboembolism (VTE), major bleeding, mortality

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Introduction

Recent studies revealed that the immune/inflammatory response may play an important role in the pathogenesis of vascular disease, since it is predisposed to platelet activation and neutrophil recruitment.[1–3] Neutrophil-to-lymphocyte-ratio (NLR), platelet-to-lymphocyte-ratio (PLR) and systemic-immune-inflammation-index (SII) are novel, widely available biomarkers that provide information on immune/inflammatory status. These inexpensive tests have been proposed as useful indicators in a number of diseases in which inflammation plays a critical role, such as cancer, rheumatoid arthritis, chronic lung disease, acute coronary syndrome or COVID-19, among others.[4–9]

The blood cellular indices have been evaluated in various studies in the literature. In these studies, the blood cellular indices provided both clinical diagnostic and prognostic value for acute PE. However, these studies were not uniform, giving different results for different clinical conditions and different outcomes such as PE severity and mortality.[10] Some studies also reported conflicting results on their prognostic role in patients with venous thromboembolism (VTE). It is improtant to note that none of these studies evaluated the predictive value of blood cellular indices on VTE recurrence and bleeding complications.[10] Additionally, clearly defined cut-off points have not yet been established. Most of these recent studies were single-center investigations involving one or two indicators, with varying number of cases and study objectives, thereby making it difficult to reach to consensus conclusions with good clinical guidance.[11–15]

The RIETE (Registro Informatizado Enfermedad TromboEmbólica) registry is an ongoing, multicenter, observational registry of consecutive patients with symptomatic, clinically confirmed acute VTE, with 205 collaborating centers in 27 countries (ClinicalTrials.gov identifier: NCT02832245). The aim of the current study was to evaluate the association between the baseline values of NLR, PLR and SII and 90-day outcomes in patients with acute VTE, in order to clarify its prognostic applicability in VTE, as other laboratory biomarkers (ie, d-dimer, cardiac biomarkers, plasma lactate levels or serum creatinine levels) that have previously shown to predict these adverse outcomes. This study reports the results of a large cohort which evaluate the prognostic value of blood cellular indices simultaneously in patients with acute VTE.

Methods

Data Source

The rationale and methodology of the RIETE registry have been previously reported elsewhere.[16] The protocol for patient enrollment was approved by the ethics committees at the participating sites and all patients (or their healthcare proxies) provided informed consent. Consecutive patients with acute VTE confirmed by objective tests (compression ultrasonography for suspected DVT; helical computed tomography [CT]-scan, ventilation-perfusion lung scintigraphy or contrast angiography for suspected PE) were recruited. Patients were excluded if they were currently participating in a blinded therapeutic clinical trial. All patients (or their relatives) provided informed consent for participation in the registry, in accordance with the local ethics committee requirements.

Variables

The following parameters were recorded in RIETE: demographics; initial VTE presentation; clinical status including chronic heart or lung disease, recent major bleeding and other underlying conditions; risk factors for VTE; blood tests at baseline; the treatment received upon VTE diagnosis and the outcomes appearing during follow-up (at least the first 3 months). Immobilized patients were defined as non-surgical patients who had been immobilized (ie, total bed rest with or without bathroom privileges) for ≥4 days in the 2-month period prior to VTE diagnosis. Surgical patients were defined as those who had undergone an operation in the 2 months prior to VTE. Active cancer was defined as newly diagnosed cancer (less than 3 months before) or when receiving antineoplastic treatment of any type (ie, surgery, chemotherapy, radiotherapy, hormonal, immunotherapy, support therapy or combined therapies). Recent bleeding was defined as a major bleed less than 30 days prior to VTE. Anemia was defined as hemoglobin levels <13 g/dL for men and <12 g/dL for women. Creatinine clearance (CrCl) levels were measured according to the Cockcroft & Gault equation. Haemodynamic instability at diagnosis was defined as a systolic blood pressure (SBP) <90 mm Hg, and/or cardiac arrest. The NLR, PLR and SII were defined as neutrophil/lymphocyte, platelet/lymphocyte, and platelet x neutrophil/lymphocyte counts, respectively.

Study Design

Only patients with objectively confirmed VTE and available information on the cellular indices at baseline were considered for the current study. Neutrophil and lymphocyte counts were included into the RIETE database in January 2020. Thus, this study included patients with acute VTE recruited from January 2020 to April 2021. Major outcomes were symptomatic VTE recurrences, major bleeding and death appearing during the first 90 days of therapy. Recurrent DVT, in patients with clinical suspicion, was defined as a new non-compressible vein segment, or an increase of the vein diameter by at least 4 mm compared with the last available measurement on ultrasonography. Recurrent PE was defined as a new ventilation-perfusion mismatch on a lung scan or a new intraluminal filling defect on spiral CT of the chest. Bleeding events were classified as major if they were overt and required a transfusion of two units of blood or more, or were retroperitoneal, spinal, intracranial, or fatal.

Treatment and Follow-up

Patients were managed according to the clinical practice of each participating hospital (ie, there was no standardization of treatment).

The type, dose and duration of anticoagulant therapy were recorded. After VTE diagnosis, all patients were followed up on for a minimum of 3 months, although a longer follow-up was advised whenever possible. During each visit, any sign or symptom suggesting VTE recurrences or bleeding complications were noted. Each episode of clinically suspected recurrent DVT or PE was investigated by repeat imaging studies, as appropriate. Most outcomes were classified as reported by the clinical centers. However, if staff at the coordinating center were uncertain how to classify a reported outcome, the event was reviewed by a central adjudicating committee (less than 10% of events).

Statistical Analysis

All of the calculations were performed with SPSS Statistics software (IBM). Continuous variables 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). In univariate analysis, relative risks and corresponding 95% confidence intervals (CI) were calculated for the major outcomes of VTE recurrences, major bleeding, and mortality. A receiver operating characteristic (ROC) curve analysis has been performed for each of the cellular indices and ratios to assess the predictive ability for the major outcomes. The optimal cutoff value of each cellular indices and ratios were assessed by calculating the corresponding area under the curves (AUC) and 95%CIs. Multivariate logistic regression analyses were performed to identify the independent predictors for each of the major outcomes. Hazard ratios (HR) and corresponding 95%CI's were calculated accordingly. The goodness-of-fit of the model was examined by the Hosmer–Leeshawn test. A p value of <0.05 was considered statistically significant.

Identification of Outliers

In both, the neutrophils and lymphocyte counts, the relative outliers are typically in the upper range. In the neutrophils we found 214 outliers, no outliers were found in the lower range, the same trend was noted in lymphocyte counts with 212 outliers in the upper range. Sixty of these patients showed concomitant outlier values in both lymphocyte and neutrophils counts. Interestingly, utilizing this analysis the outliers in the platelets were also mostly in the upper range, but were proportionally higher than the one noted in the neutrophils and lymphocytes, only two patients were found to be in the lower range. The selected outliers were further checked by the RIETE Quality control committee; however, they are not considered as the true outliers.

Results

From January 2020 to April 2021, 4487 patients with acute VTE were recruited. Of these, 2115 (47%) were women, 1956 (43%) were aged >70 years, and 715 (16%) had active cancer. Overall, 2683 patients (60%) initially presented with acute symptomatic PE (with or without concomitant DVT), 283 (6.3%) had incidental PE, 1129 (25%) had lower-limb DVT, 219 (4.9%) had upper-limb DVT, 69 (1.5%) had splanchnic vein thrombosis, 142 (3.2%) had superficial vein thrombosis and 20 (0.4%) had retinal vein thrombosis. Mean values ($\pm$ standard deviation) of the cellular indices were: NLR 5.9 ± 7.1, PLR 190 ± 158 and SII 1459 ± 2028 (Table 1).

Baseline Characteristics

Mean NLR values were higher in men, in patients aged >70 years or weighing <70 kg, and in those with active cancer, recent immobility, chronic lung disease, diabetes, hypertension, anemia or renal insufficiency, but were lower in patients using estrogens, with unprovoked VTE, prior VTE, leg varicosities, or current smokers (Table 1). Mean PLR values were higher in patients weighing <70 kg, and in those with cancer, recent surgery, immobility, or anemia, but were lower in patients using estrogens, with unprovoked VTE, prior VTE, leg varicosities or actively smoking. Mean SII values were higher in patients weighing <70 kg, with cancer, recent surgery, immobility, anemia, or renal insufficiency, but were lower in patients using estrogens, with unprovoked VTE, prior VTE or leg varicosities.

Initial VTE Presentation

Mean NLR values were highest in patients initially presenting with PE (either symptomatic or incidental), particularly in those with tachycardia or hypoxemia, and were lowest in those with superficial vein thrombosis or retinal vein thrombosis (Table 2). Mean PLR values were also highest in patients initially presenting with PE, and lowest in patients with lower-limb DVT, superficial vein thrombosis or retinal vein thrombosis. Mean SII values were highest in patients with symptomatic PE (particularly in those with tachycardia) and lowest in patients with superficial vein thrombosis or retinal vein thrombosis.

90-day Outcomes

During the 90-day study period, 38 patients (0.8%) developed recurrent DVT, 45 (1.0%) had recurrent PE, 152 (3.4%) suffered major bleeding and 484 (11%) died [26 confirmed fatal PE (5.4%), 26 confirmed fatal bleeding (5.4%) rest includes various factors, such as cancer, renal insufficiency, heart failure, infection, cerebral ischemia, multiorgan failure]. Mean NLR, PLR and SII values were higher in patients who bled than in those who did not, and in patients who died rather than in those who did not die during the first 90 days (Table 3). There was no difference in NLR, PLR and SII values between patients developing DVT or PE recurrences and those who did not.

The area under ROC curves for each of the cellular indices and their association with the risk for VTE recurrences, major bleeding or death are depicted in Figures 1–3. The optimal cutoff values of each ratio were: NLR >4.41 for major bleeding

Table 1. Mean Values At Baseline Of The 3 Cellular Indices, According To The Clinical Characteristics Of The Patients.

	N	Neutrophil Count	Lymphocyte Count	Platelet Count	NLR	PLR	SII
Clinical characteristics,							
All patients	**4487**	**7.7 ± 8.2**	**2.0 ± 3.1**	**246 ± 104**	**5.9 ± 7.1**	**190 ± 158**	**1459 ± 2028**
Female sex	2115	7.7 ± 8.7	2.1 ± 3.1	255 ± 104‡	5.5 ± 6.6‡	189 ± 147	1410 ± 1888
Age >70 years	1956	7.5 ± 7.7	1.8 ± 2.8‡	232 ± 98‡	6.3 ± 6.9‡	195 ± 154	1470 ± 1831
Body weight <70 kg	1425	7.7 ± 8.3	1.9 ± 3.1	255 ± 115‡	6.3 ± 7.8†	210 ± 179‡	1626 ± 2308‡
Risk factors for VTE,							
Active cancer	715	7.2 ± 7.7	1.8 ± 3.5*	252 ± 131	6.4 ± 7.4*	230 ± 196‡	1687 ± 2402†
Recent surgery	381	7.5 ± 6.5	1.9 ± 2.5	295 ± 160‡	5.6 ± 5.2	222 ± 164‡	1687 ± 1844*
Immobility for ≥4 days	1384	8.0 ± 8.1	1.8 ± 2.7†	254 ± 106‡	7.2 ± 9.2‡	221 ± 180‡	1819 ± 2564‡
Estrogen intake	210	9.1 ± 11.8	2.9 ± 4.7†	263 ± 85*	4.4 ± 3.8‡	160 ± 123‡	1149 ± 1161‡
Pregnancy/postpartum	37	8.8 ± 3.4	1.9 ± 1.2	287 ± 104*	5.6 ± 3.6	197 ± 137	1738 ± 1682
Unprovoked	2067	7.5 ± 8.0	2.2 ± 3.1†	230 ± 85‡	5.0 ± 5.4‡	160 ± 127‡	1160 ± 1445‡
Prior VTE	524	7.7 ± 9.2	2.4 ± 4.4*	230 ± 89‡	4.9 ± 5.3‡	161 ± 120‡	1134 ± 1415‡
Leg varicosities	695	7.3 ± 7.8	1.9 ± 2.0	232 ± 89‡	5.3 ± 5.9†	168 ± 132‡	1242 ± 1630‡
Concomitant diseases,							
Chronic heart failure	283	7.6 ± 6.3	1.8 ± 2.5	226 ± 110‡	6.4 ± 5.3	193 ± 154	1425 ± 1589
Chronic lung disease	453	8.6 ± 9.9*	1.9 ± 2.5	240 ± 97	6.7 ± 7.9*	194 ± 163	1648 ± 2327
Diabetes	689	8.1 ± 9.4	1.9 ± 2.1	240 ± 106	6.7 ± 8.0†	202 ± 172	1575 ± 1866
Hypertension	2025	7.9 ± 9.1	1.9 ± 2.6*	236 ± 96‡	6.3 ± 7.4†	191 ± 156	1477 ± 1972
Current smoking	473	8.6 ± 9.3†	2.4 ± 3.1†	253 ± 114	5.0 ± 5.0‡	156 ± 128‡	1300 ± 1725
Prior myocardial infarction	234	7.3 ± 7.3	1.7 ± 2.5	235 ± 113	6.4 ± 6.9	205 ± 183	1579 ± 2310
Prior ischemic stroke	278	8.8 ± 11.3	2.1 ± 3.1	244 ± 108	6.1 ± 5.9	201 ± 173	1505 ± 1574
Peripheral artery disease	119	7.1 ± 6.0	1.7 ± 2.1	234 ± 107	6.4 ± 6.3	201 ± 166	1408 ± 1598
Recent major bleeding	119	10.2 ± 14.4	2.1 ± 3.0	257 ± 138	7.0 ± 7.0	207 ± 157	1746 ± 1918
Anemia	1553	7.7 ± 8.6	1.8 ± 2.7‡	270 ± 131‡	6.9 ± 8.8‡	238 ± 186‡	1837 ± 2482‡
CrCl levels <60 mL/min	1210	8.1 ± 8.5*	1.7 ± 2.2‡	233 ± 103‡	6.9 ± 7.6‡	200 ± 164*	1598 ± 1951†

Cellular counts are expressed as values × 1000/mm^3

Comparisons between patients with- versus without the variable: *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

Abbreviations: VTE, venous thromboembolism; CrCl, creatinine clearance; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammation index (neutrophils x platelets/ lymphocytes).

Table 2. Mean Values At Baseline Of The 3 Cellular Indices, According To The Initial Vte Presentation.

	N	Neutrophil Count	Lymphocyte Count	Platelet Count	NLR	PLR	SII
All patients	**4487**	**7.7 ± 8.2**	**2.0 ± 3.1**	**246 ± 104**	**5.9 ± 7.1**	**190 ± 158**	**1459 ± 2028**
Symptomatic PE	2683	8.2 ± 8.3‡	2.0 ± 3.2	247 ± 101	6.6 ± 7.4‡	202 ± 166‡	1639 ± 2188‡
In patients with PE,							
SBP <90 mm Hg	74	11.1 ± 12.2*	2.7 ± 4.2	221 ± 81*	7.3 ± 7.0	171 ± 143	1679 ± 2270
Heart rate >110 bpm	380	9.6 ± 7.6‡	1.8 ± 1.7	257 ± 108*	8.2 ± 10.1‡	210 ± 170	2122 ± 3036‡
Sat O_2 levels <90%	287	8.0 ± 5.7	1.7 ± 1.8	235 ± 105	7.5 ± 8.6†	214 ± 183	1764 ± 2053*
Incidental PE	283	6.8 ± 4.7	1.5 ± 0.8‡	263 ± 153*	6.2 ± 7.2	214 ± 160†	1570 ± 1918
Lower-limb DVT	1129	7.8 ± 9.2	2.2 ± 3.3	234 ± 93	5.2 ± 6.2	165 ± 125	1225 ± 1664
Proximal	905	7.9 ± 9.0	2.2 ± 3.5	231 ± 91†	5.1 ± 5.6	161 ± 120†	1194 ± 1598
Distal	205	7.6 ± 10.2	2.1 ± 2.8	246 ± 101†	5.5 ± 7.7	186 ± 145†	1304 ± 1687
Upper-extremity DVT	175	7.1 ± 8.8	1.9 ± 2.5	252 ± 113	6.0 ± 10	208 ± 191	1473 ± 2371
Splanchnic thrombosis	69	6.9 ± 4.3	1.8 ± 1.7	281 ± 158	5.6 ± 6.7	218 ± 223	1837 ± 3499
Superficial thrombosis	142	5.7 ± 6.9†	2.4 ± 3.4	245 ± 86	3.0 ± 2.5‡	151 ± 143†	760 ± 818‡
Retinal vein thrombosis	20	4.3 ± 1.6	2.2 ± 0.7	221 ± 45*	2.1 ± 0.8‡	109 ± 34‡	466 ± 202‡

Cellular counts are expressed as values × 1000/mm^3

Comparisons between patients with- versus without the variable: *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

Abbreviations: VTE, venous thromboembolism; PE, pulmonary embolism; SBP, systolic blood pressure; bpm, beats per minute; DVT, deep vein thrombosis; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammation index (neutrophils x platelets/ lymphocytes).

and >4.96 for mortality, PLR >166.47 for major bleeding and >167.96 for mortality, and SII >1154.81 for major bleeding and >1134.50 for mortality. No cellular indices were relevant to predict VTE recurrences (Figure 1). The overall accuracy of NLR, PLR and SII for major bleeding events by 90 days were: 0.669 (95%CI: 0.623-0.715), 0.624 (95%CI: 0.577-0.671), and

Table 3. Mean Values At Baseline Of The 3 Cellular Indices, According To The 90-Day Outcomes.

	N	Neutrophil Count	Lymphocyte Count	Platelet Count	NLR	PLR	SII
All patients	**4487**	**7.7 ± 8.2**	**2.0 ± 3.1**	**246 ± 104**	**5.9 ± 7.1**	**190 ± 158**	**1459 ± 2028**
DVT recurrences	38	11.7 ± 17.4	3.7 ± 6.2	262 ± 123	5.0 ± 3.8	183 ± 138	1384 ± 1224
PE recurrences	45	10.3 ± 15.1	3.2 ± 5.2	240 ± 112	6.3 ± 6.5	198 ± 219	1631 ± 1865
VTE recurrences	79	11.1 ± 16.4	3.0 ± 4.8	252 ± 120	6.0 ± 5.5	197 ± 188	1581 ± 1613
Major bleeding	152	9.6 ± 10.0†	1.9 ± 5.9	247 ± 111	9.9 ± 9.4‡	271 ± 252‡	2408 ± 2388‡
All-cause death	484	10.3 ± 9.2‡	1.5 ± 2.6‡	245 ± 117	11.5 ± 13‡	265 ± 223‡	2807 ± 3638‡
Any of the above	645	10.1 ± 9.9‡	1.8 ± 3.8*	248 ± 118	10.5 ± 12‡	257 ± 221‡	2582 ± 3337‡
None of the above	3842	7.3 ± 7.8‡	2.1 ± 3.0*	245 ± 101	5.1 ± 5.6‡	179 ± 142‡	1270 ± 1640‡

Cellular counts are expressed as values × 1000/mm^3

Comparisons between patients with- versus without the variable: *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

Abbreviations: DVT, deep vein thrombosis; PE, pulmonary embolism; VTE, venous thromboembolism; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammation index (neutrophils x platelets/ lymphocytes).

0.659 (95%CI: 0.612-0.705), respectively (Figure 2). The overall accuracy of NLR, PLR and SII for mortality events by 90 days were 0.730 (95% CI: 0.706-0.755), 0.632 (95% CI: 0.604-0.660), and 0.696 (95% CI: 0.670-0.772), respectively (Figure 3).

Multivariable Analysis

On multivariable analysis, patients with NLR >4.41 and >4.96 were at increased risk for major bleeding (adjusted odds ratio [aOR]: 1.73; 95%CI: 1.05-2.86) and for death (aOR: 2.50; 95%CI: 1.83-3.42), but not for VTE recurrences (Table 4). Patients with PLR >167.96 and >166.47 were not at increased risk for major bleeding and mortality. Patients with SII >1134.5 were at increased risk for death (aOR: 1.52; 95%CI: 1.08-2.14) but not for VTE recurrences or major bleeding. These results are different from the univariate analysis where all three indices demonstrated an association with major bleeding in contrast to the multivariate analysis, where only NLR exhibited this trend.

Discussion

The blood cellular indices have been evaluated in various studies in the literature. These studies have shown that the blood cellular indices may provide both diagnostic and prognostic information in acute VTE. NLR, PLR and SII are the most studied blood cellular indices in acute VTE. Ates et al have identified NLR as an independent predictor of massive PE in 639 patients comprised of acute PE.[17] In this study, NLR found to have a good diagnostic accuracy with an AUC of 0.893 for this outcome. Kasapoglu et al also reported that NLR levels were significantly higher in patients who died within 30 days in 550 patients comprised of acute PE.[18] Interestingly, NLR was not an independent predictor of death for overall patients in this study. But in subgroup analysis in patients without comorbidities showed that NLR was an independent predictor of mortality. Duman et al have shown that an NLR was predictive for 30-day, 6-month and 1-year mortality in a cohort of 828 PE patients.[19]

Several studies also evaluated the PLR as a diagnostic and prognostic tool for acute VTE. The study of Ates et al identified PLR as an independent predictor of massive acute PE in 639 patients.[17] In this study, PLR found to have a good diagnostic accuracy with an AUC of 0.877 for this outcome. Kasapoğlu et al reported higher levels of PLR in 550 acute PE patients who have deceased within 30-days of acute VTE.[18] However, PLR was not an independent risk factor in multivariable analysis in this study. Duman et al, have reported that there was no significant difference in PLR levels between deceased and surviving subgroups in 828 PE patients.[19] Additionally, PLR was also not an independent risk factor for death in this study. Kundi et al have found that higher levels of PLR in patients with high sPESI scores was independently associated with in-hospital mortality among 646 patients with acute PE.[20] However, Ghaffari et al did not find a difference in PLR between patients with and without major cardiopulmonary adverse events in 492 acute PE patients.[21] But PLR was associated with in-hospital mortality with an AUC of 0.610 in this study.

In the recent literature, PLR were also considered as a predictor of the VTE occurrence in cancer patients and after surgery. Grilz et al identified a significant association between PLR and the occurrence of VTE in 1469 cancer patients.[22] Yao et al identified a higher preoperative PLR than postoperative PLR in 733 patients after total joint replacement.[23] In this study, the postoperative PLR was independently associated with the occurrence of DVT with an AUC of 0.513 and 0.561 for preoperative PLR and postoperative PLR respectively. Furthermore, Kurtipek et al, have 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.[24]

The predictive value of SII was also tested in several previous 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. SII was also higher in patients who had in-hospital mortality.[25] SII was an independent predictor of massive APE with an AUC of 0.957. Peng et al reported an

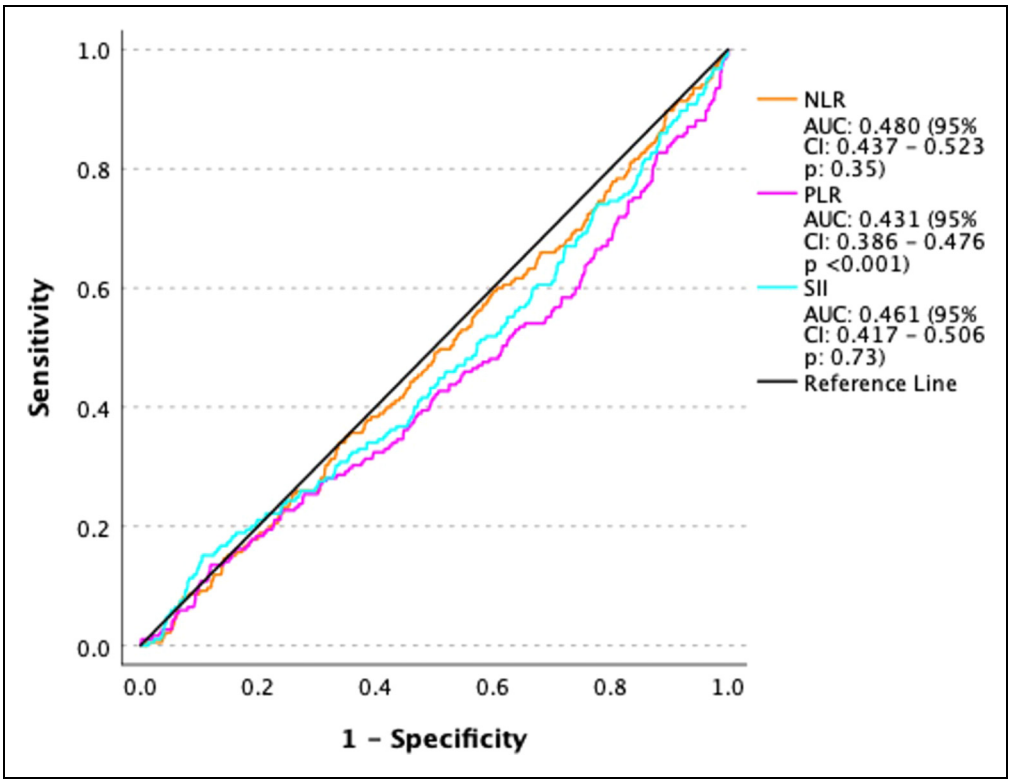

Figure 1. ROC curves of VTE recurrences for cellular indices.

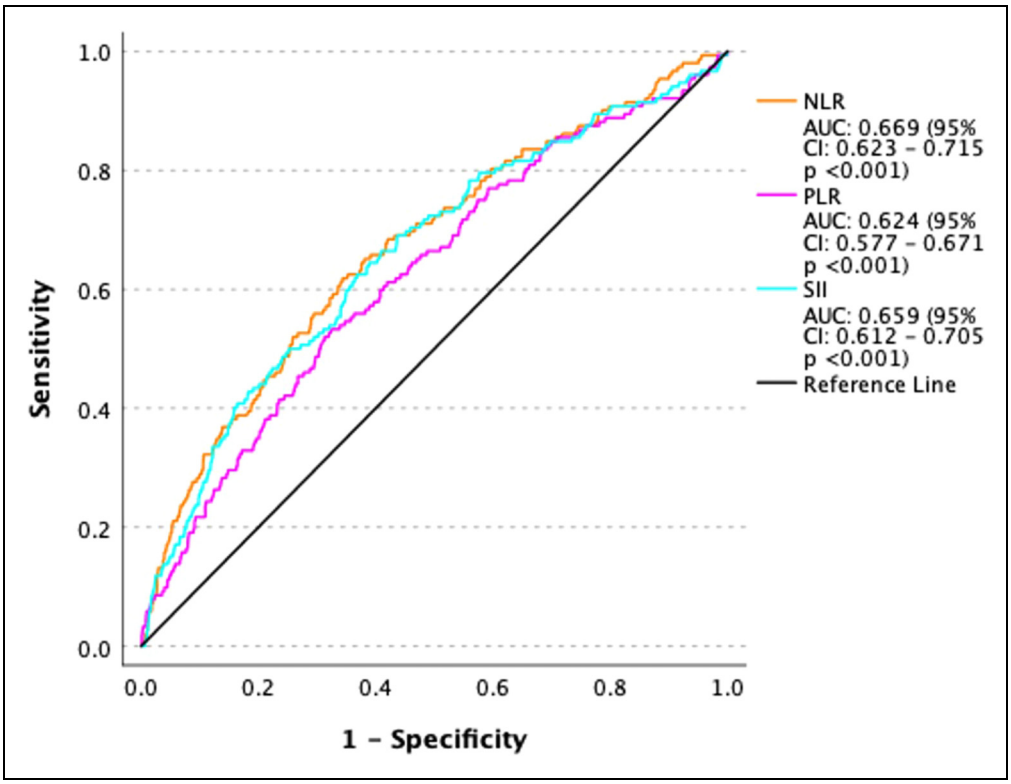

Figure 2. ROC curve of major bleeding for cellular indices.

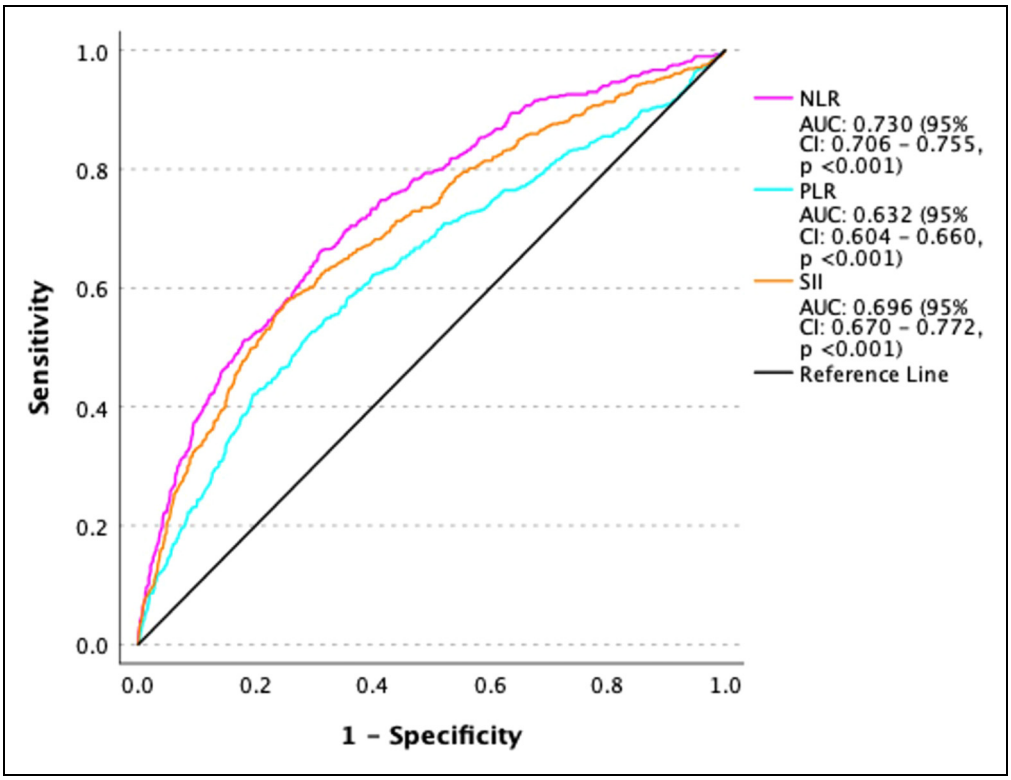

Figure 3. ROC curve of mortality for cellular indices.

increased SII in patients with VTE compared to those without VTE. In this study, SII was an independent predictor of VTE after hip fracture of elderly patients.[26]

Reported data, obtained from a large series of consecutive patients with acute VTE, reveal that the cellular indices may help to predict adverse outcomes at 90 days, thus supporting the role of the immune/inflammatory response in the pathogenesis of VTE. In this cohort, none of the three cellular indices predicted the risk for VTE recurrences within the first 90 days, but patients with NLR >4.41, >4.96 were at increased risk for major bleeding (68.4% sensitivity and 68.0% specificity) or death (66.9% sensitivity and 66.8% specificity), and those with SII >1134.5 were at an increased risk of death (64.9% sensitivity and 64.9% specificity). One in every 30 patients (3.4%) suffered major bleeding within the first 90 days, and one in every four such patients died of bleeding. Thus, the clinical relevance of these indices at baseline should not be underestimated. On univariate analysis, all 3 cellular indices were associated to a higher risk for major bleeding, as were older age, cancer, recent immobility, hypertension, recent major bleeding, anemia, renal insufficiency or initial VTE presentation as PE. Most of these clinical variables have been identified and validated in the literatuire as independent risk factors for bleeding in patients with VTE.[27–29] Interestingly however, only NLR, anemia and renal insufficiency independently predicted the risk for major bleeding. Moreover, both NLR and SII independently predicted the risk of death.

A meta-analysis including 2323 patients from 7 retrospective studies found the NLR and the PLR to independently predict the risk of death (in the short- and in the long term) in patients with acute PE.[15] The prognostic role of the SII (including neutrophils, lymphocytes and platelets) is currently less known, although a retrospective report involving 442 patients with PE suggested its association with worse outcomes, including hemodynamic compromise and in-hospital mortality.[25]

In another study on patients with acute PE, NLR and PLR correlated with the severity of PE evaluated by the PESI or simplified PESI scores.[30] In contrast, high NLR values were not associated with more severe risk profiles in a more recent small cohort of less than 100 patients with PE.[31] This study reports t PE patients with tachycardia or hypoxemia to have high NLR and SII values. Thus, the prognostic role of these cellular indices in patients with acute PE needs to be further investigated.

In this study, the value of cellular indices in predicting outcome in patients with acute VTE has been addressed as novel biomarkers based on the utilization of blood counts. The molecular basis of these relationships requires detailed molecular and cellular investigations. Thrombo-inflammatory biomarkers have been extensively investigated and reported previously from a previous study, from our group.[32] A clear relationship between upregulation of inflammatory cytokines has been demonstrated in this comprehensive study. This study also provides an opportunity to demonstrate that, the variations in thrombo-inflammatory biomarkers may be relevant to the changes in the cellular indices, such as NLR, PLR and SII. The RIETE Registry, cohort did not address thrombo-inflammatory biomarkers except for the d-dimer,

Table 4. Univariate- And Multivariate Analyses For Vte Recurrences, Major Bleeding And Death Within The First 3 Months. Results Are Expressed As Odds Ratio And 95% Confidence Limits.

	VTE Recurrences		Major Bleeding		Mortality	
Variable	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
Cellular indices,						
NLR (>optimal cutoff)	NS	-	2.99 (2.11-4.23)	1.73 (1.05-2.86)	4.06 (3.33-4.97)	2.50 (1.83-3.42)
PLR (>optimal cutoff)	NS	-	2.17 (1.56-3.02)	NS	2.42 (1.99-2.93)	NS
SII (>optimal cutoff)	NS	-	2.86 (2.05-4.00)	NS	3.41 (2.80-4.15)	1.52 (1.08-2.14)
Clinical characteristics,						
Gender	NS	-	NS	-	NS	-
Age >70 years	NS	-	1.58 (1.14-2.19)	NS	2.16 (1.78-2.62)	NS
Weight <70 kg	NS	-	NS	-	1.60 (1.32-1.94)	NS
Risk Factors for VTE,						
Active Cancer	1.74 (1.23-2.46)	2.28 (1.26-4.15)	1.73 (1.19-2.54)	NS	5.09 (4.15-6.23)	4.87 (3.36-7.05)
Recent Immobility	0.47 (0.32-0.68)	NS	1.72 (1.24-2.38)	NS	1.75 (1.44-2.12)	1.95 (1.38-2.75)
Unprovoked VTE	1.43 (1.06-1.92)	NS	0.44 (0.30-0.62)	NS	0.27 (0.22-0.34)	NS
Prior VTE	NS	-	NS	-	NS	-
Leg Varicosities	1.98 (1.41-2.78)	1.73 (1.21-2.47)	NS	-	NS	-
Concomitant Diseases,						
Chronic Heart Failure	NS	-	NS	-	2.34 (1.73-3.17)	NS
Chronic Lung Disease	1.85 (1.24-2.76)	1.67 (1.07-2.59)	NS	-	1.76 (1.34-2.30)	NS
Diabetes	NS	-	NS	-	1.99 (1.59-2.49)	1.47 (1.13-1.93)
Hypertension	NS	-	1.40 (1.01-1.94)	NS	1.57 (1.30-1.90)	NS
Current Smoking	NS	-	NS	-	NS	
Prior Myocardial Infarction	NS	-	NS	-	1.49 (1.03-2.16)	NS
Prior Ischemic Stroke	NS	-	NS	-	1.81 (1.30-2.50)	NS
Recent Major Bleeding	NS	-	3.05 (1.61-5.81)	NS	2.26 (1.44-3.56)	NS
Anemia	NS	-	2.41 (1.74-3.34)	1.56 (1.08-2.26)	2.83 (2.34-3.43)	1.46 (1.16-1.84)
CrCl levels <60 mL/min	NS	-	2.14 (1.54-2.97)	1.68 (1.13-2.51)	2.51 (2.07-3.05)	1.65 (1.28-2.14)
Initial VTE presentation,						
Symptomatic PE	1.52 (1.12-2.06)	NS	2.15 (1.52-3.06)	NS	6.56 (5.04-8.55)	2.46 (1.78-3.40)
Incidental PE	0.40 (0.16-0.99)	0.40 (0.16-0.99)	NS	-	1.48 (1.05-2.09)	NS
Lower Limb DVT	NS	-	0.62 (0.44-0.87)	NS	0.56 (0.46-0.69)	0.62 (0.50-0.78)
Upper Limb DVT	NS	-	NS	-	NS	-
Splanchnic Vein Thrombosis	NS	-	NS	-	NS	-
Superficial Vein Thrombosis	2.67 (1.51-4.73)	2.06 (1.12-3.78)	NS	-	0.06 (0.01-0.41)	0.11 (0.02-0.84)
Retinal Vein Thrombosis	NS	-	NS	-	NS	-

Abbreviations: NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammation index (neutrophils x platelets/lymphocytes); VTE, venous thromboembolism; CrCl, creatinine clearance; PE, pulmonary embolism; DVT, deep vein thrombosis; NS, non-significant.

however the cellular indices were easily retrievable. Such a relationship between the cellular indices and the thrombo-inflammatory biomarkers will provide interesting insight in the understanding of the pathogenesis of VTE. In another study, Darwish et al, have also reported on the dysregulation of hemostatic and inflammatory biomarkers with reference to adhesion molecules TNF-a, and PAI-1.[33] Ongoing studies on the cellular indices in this group and their correlation with the biomarkers investigated is being addressed. In a lymphoma cohort, some of the cellular indices and thrombo-inflammatory biomarkers have been simultaneously measured. Such measurements have provided useful data in stratifying risk and outcomes in cancer patients.[34] Future studies, to complement cellular indices data, with such biomarkers as inflammatory cytokines, tissue factor, and microparticles will provide additional information on the pathogenesis of VTE.

The role of microparticles in the understanding of the pathogenesis of VTE is well known.[35] Microparticles are drive mainly from platelets, white cells, and endothelial lining. These microparticles are pro-coagulant in nature, and profiling of these microparticles has been addressed in several studies. In the current study, the main focus is on demonstrating value of cellular indices in conjunction with a large registry. This registry, thus also offer an opportunity to quantify microparticles, however the current study is mainly focused on cellular indices and correlations with cellular microparticles can be subject of future studies.

The role on inflammation in inducing hemorrhage in thrombocytopenia has been reported previously. Platelets play an important role in inflammatory responses. Inflammatory processes may result in hemorrhagic response to multiple pathways, where the cytokines play a role including the integrins.[36] In this study, recent bleeding was defined as a major bleed less than 30 days prior to VTE. In-terms of the association of cellular indices with bleeding risk, the NLR

showed an association with bleeding risk in the multivariate analysis, where as the PLR and SII did not reveal any relevance. Additional data, on a larger cohort will provide furthur insights. Therefore the RIETE Registry provides a unique opportunity to validate the diagnostic and prognostic significance of these indices.

This study has several limitations to consider. First of all, reported findings should be treated with caution considering the limitations of observational studies to infer causality. This study did not include a control group. This study did not address the prognostic relevance of repeated measurements of cellular indices, which may have a higher accuracy than a single one. Reported study only represents the outcomes appearing during the first 90 days. Additionally, this study did not completely consider the clinical characteristics of the patients while including in this study. Increased levels of blood cellular indices is detected in several other illnesses (particularly in cancer patients) other than VTE. Moreover, reported study did not compare other inflammatory biomarkers (such as CRP, d-dimer) with the selected variables. Finally, although clinicians might not perceive that this is readily available, and easily calculated indicators with blood cell counts.

The current analysis also has some strengths. To the best of our knowledge, this is the largest cohort study evaluating the prognostic value of three cellular indices simultaneously in patients with acute VTE. Prior publications concerning the prognostic role of these immune/inflammatory indicators were single-center studies. Our data provides a multicenter perspective, which increases the generalizability of the results. In the current literature, there is no specific cut-off values of NLR, PLR or SII for mortality. This study have obtained different optimal cut-off points of the cellular indices for major bleeding outcomes. Thus, reported study offers a new perspective of the problem, and notable data.

In conclusion, the cellular indices represent a consistent and powerful predictor of major bleeding or death within the first 90 days in patients with acute VTE. This should lead to increased awareness about these indicators, particularly NLR. Nonetheless, the prognostic role of these tests in patients with different risk profiles (ie, active cancer, normotensive acute PE, etc) is still underexplored and warrants further investigations.

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References

1. Thomas MR, Storey RF. The role of platelets in inflammation. *Thromb Haemost*. 2015;114(3):449-458.
2. Welsh P, Grassia G, Botha S, Sattar N, Maffia P. Targeting inflammation to reduce cardiovascular disease risk: a realistic clinical prospect? *Br J Pharmacol*. 2017;174(22):3898-3913.
3. Condado JF, Junpaparp P, Binongo JN, et al. Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) can risk stratify patients in transcatheter aortic-valve replacement (TAVR). *Int J Cardiol*. 2016;223:444-449.
4. Diem S, Schmid S, Krapf M, et al. Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. *Lung Cancer*. 2017;111:176-181.
5. Dong CH, Wang ZM, Chen SY. Neutrophil to lymphocyte ratio predict mortality and major adverse cardiac events in acute coronary syndrome: a systematic review and meta-analysis. *Clin Biochem*. 2018;52:131-136.
6. Erre GL, Paliogiannis P, Castagna F, et al. Meta-analysis of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio in rheumatoid arthritis. *Eur J Clin Invest*. 2019;49(1):e13037.
7. Şahin F, Koşar AF, Aslan AF, Yiğitbaş B, Uslu B, Serum biomarkers in patients with stable and acute exacerbation of chronic obstructive pulmonary disease: a comparative study. *J Med Biochem*. 2019;38(4):503-511.

8. Ye W, Chen G, Li X, et al. Dynamic changes of D-dimer and neutrophil-lymphocyte count ratio as prognostic biomarkers in COVID-19. *Respir Res*. 2020;21(1):169.
9. Fu J, Kong J, Wang W, et al. The clinical implication of dynamic neutrophil to lymphocyte ratio and D-dimer in COVID-19: a retrospective study in suzhou China. *Thromb Res*. 2020;192:3-8.
10. Xue J, Ma D, Jiang J, Liu Y, et al. Diagnostic and prognostic value of immune/inflammation biomarkers for venous thromboembolism: is it reliable for clinical practice? *J Inflamm Res*. 2021;14:5059-5077.
11. Ertem AG, Yayla C, Acar B, et al. Relation between lymphocyte to monocyte ratio and short-term mortality in patients with acute pulmonary embolism. *Clin Respir J*. 2018;12(2):580-586.
12. Telo S, Kuluöztürk M, Deveci F, Kirkil G. The relationship between platelet-to-lymphocyte ratio and pulmonary embolism severity in acute pulmonary embolism. *Int Angiol*. 2019;38(1):4-9.
13. Karataş MB, İpek G, Onuk T, et al. Assessment of prognostic value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in patients with pulmonary embolism. *Acta Cardiol Sin*. 2016;32(3):313-320.
14. Kayrak M, Erdoğan HI, Solak Y, et al. Prognostic value of neutrophil to lymphocyte ratio in patients with acute pulmonary embolism: a restrospective study. *Heart Lung Circ*. 2014;23(1):56-62.
15. Wang Q, Ma J, Jiang Z, Ming L. et al. Prognostic value of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in acute pulmonary embolism: a systematic review and meta-analysis. *Int Angiol*. 2018;37(1):4-11.
16. Bikdeli B, Jimenez D, Hawkins M, et al. *Rationale, Design and Methodology of the Computerized Registry of Patients with Venous Thromboembolism (RIETE)*. *Thromb Haemost*. 2018;118(1):214-224.
17. Ates H, Ates I, Kundi H, Yilmaz FM. Diagnostic validity of hematologic parameters in evaluation of massive pulmonary embolism. *J Clin Lab Anal*. 2017;31(5).
18. Kasapoglu US, Olgun Yıldızeli Ş, Arıkan H, et al. Comparison of neutrophil to lymphocyte ratio with other prognostic markers affecting 30 day mortality in acute pulmonary embolism. *Tuberk Toraks*. 2019;67(3):179-189.
19. Duman D, Sonkaya E, Yıldırım E, et al. Association of inflammatory markers with mortality in patients hospitalized with non-massive pulmonary embolism. *Turk Thorac J*. 2021;22(1):24-30.
20. Kundi H, Balun A, Cicekcioglu H, et al. The relation between platelet-to-lymphocyte ratio and pulmonary embolism severity Index in acute pulmonary embolism. *Heart Lung*. 2015;44(4):340-343.
21. Ghaffari S, Parvizian N, Pourafkari L, et al. Prognostic value of platelet indices in patients with acute pulmonary thromboembolism. *J Cardiovasc Thorac Res*. 2020;12(1):56-62.
22. Grilz E, Posch F, Königsbrügge O, et al. Association of platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio with the risk of thromboembolism and mortality in patients with cancer. *Thromb Haemost*. 2018;118(11):1875-1884.
23. Yao C, Zhang Z, Yao Y, Xu X, Jiang Q, Shi D. Predictive value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for acute deep vein thrombosis after total joint arthroplasty: a retrospective study. *J Orthop Surg Res*. 2018;13(1):40.
24. Kurtipek E, Büyükterzi Z, Büyükterzi M, Alpaydın MS, Erdem SS. Endothelial dysfunction in patients with pulmonary thromboembolism: neutrophil to lymphocyte ratio and platelet to lymphocyte ratio. *Clin Respir J*. 2017;11(1):78-82.
25. Gok M, Kurtul A. A novel marker for predicting severity of acute pulmonary embolism: systemic immune-inflammation index. *Scand Cardiovasc J*. 2021;55(2):91-96.
26. Peng J, Wang H, Zhang L, Lin Z, Construction and efficiency analysis of prediction model for venous thromboembolism risk in the elderly after hip fracture. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*. 2021;46(2):142-148.
27. Ruíz-Giménez N, Suárez C, González R, et al. Predictive variables for major bleeding events in patients presenting with documented acute venous thromboembolism. Findings from the RIETE registry. *Thromb Haemost*. 2008;100(1):26-31.
28. Lecumberri R, Jiménez L, Ruiz-Artacho P, et al. Prediction of Major bleeding in anticoagulated patients for venous thromboembolism: comparison of the RIETE and the VTE-BLEED scores. *TH Open*. 2021;5(3):e319-e328.
29. van Es N, Wells PS, Carrier M. Bleeding risk in patients with unprovoked venous thromboembolism: a critical appraisal of clinical prediction scores. *Thromb Res*. 2017;152:52-60.
30. Phan T, Brailovsky Y, Fareed J, Hoppensteadt D, Iqbal O, Darki A. Neutrophil-to-Lymphocyte and platelet-to-lymphocyte ratios predict all-cause mortality in acute pulmonary embolism. *Clin Appl Thromb Hemost*. 2020;26:1076029619900549.
31. Bi W, Liang S, He Z, et al. *The Prognostic Value of the Serum Levels of Brain Natriuretic Peptide, Troponin I, and D-Dimer, in Addition to the Neutrophil-to-Lymphocyte Ratio, for the Disease Evaluation of Patients with Acute Pulmonary Embolism*. *Int J Gen Med*. 2021;14:303-308.
32. Bontekoe E, Brailovsky Y, Hoppensteadt D, et al. Upregulation of inflammatory cytokines in pulmonary embolism using biochip-array profiling. *Clin Appl Thromb Hemost*. 2021;27:10760296211013107.
33. Darwish I, Fareed J, Brailovsky Y, et al. Dysregulation of biomarkers of hemostatic activation and inflammatory processes are associated with adverse outcomes in pulmonary embolism. *Clin Appl Thromb Hemost*. 2022;28:10760296211064898.
34. Otasevic V, Mihaljevic B, Milic N, et al. Immune activation and inflammatory biomarkers as predictors of venous thromboembolism in lymphoma patients. *Thromb J*. 2022;20(1):20.
35. Zhou L, Qi XL, Xu MX, Mao Y, Liu ML, Song HM. Microparticles: new light shed on the understanding of venous thromboembolism. *Acta Pharmacol Sin*. 2014;35(9):1103-1110.
36. Goerge T, Ho-Tin-Noe B, Carbo C, et al. Inflammation induces hemorrhage in thrombocytopenia. *Blood*. 2008;111(10):4958-4964.

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