

Neutrophil precursors in complete blood count: innovative biomarker for acute pulmonary embolism severity

Karakurt G¹, Özatak C², Güven O³, Naser A⁴, Aynacı E⁵

¹Department of Chest Diseases, Kırklareli Training and Research Hospital, Kırklareli

²Department of Emergency Medicine, Medipol University, Istanbul

³Department of Emergency Medicine, Kırklareli University, Kırklareli

⁴Department of Cardiology, Kırklareli Training and Research Hospital, Kırklareli

⁵Department of Chest Diseases, Beykent University, Istanbul

Türkiye

Abstract

Background: Inflammation plays an important role in the pathogenesis of acute pulmonary embolism (APE), which is a cardiovascular emergency associated with high mortality. The primary determinant of the clinical course in the setting of APE is right ventricular dysfunction (RVD). In this study, we aim to investigate the usefulness of circulating immature granulocytes (IG) as an inflammatory biomarker in predicting RVD in APE.

Methods: We retrospectively analyzed data of 59 patients admitted to the emergency department between January 2019 and June 2022, diagnosed with APE. A complete blood count at admission determined the IG count. According to their echocardiographic evaluation, patients were divided into two groups according to the presence of RVD.

Results: We observed in APE that the mean IG count was significantly higher in patients with RVD than those without RVD ($p=0.001$). The multivariate logistic regression analysis detected a significant ($p=0.006$) and independent effect of the IG count in distinguishing cases with and without RVD.

Conclusions: We found the discriminative effectiveness of the IG 0.05 cut-off value for RVD. IGs, an inflammatory precursor obtained readily and without additional cost as part of a complete blood count, may be a new and valuable biomarker for risk stratification and prognosis assessment by predicting RVD in APE patients. HIPPOKRATIA 2025, 29 (1):20-24.

Keywords: Acute pulmonary embolism, immature granulocytes, neutrophil precursors, right ventricular dysfunction

Corresponding author: Gökhan Karakurt, MD, Department of Chest Diseases, Kırklareli Training and Research Hospital, İstasyon Mahallesi, 28. Edirne Sokak, No 9, Kırklareli, Turkey, tel: +905532904502, e-mail: gokhankarakurt16@gmail.com

Introduction

Acute pulmonary embolism (APE) is a life-threatening cardiovascular disease usually diagnosed in the emergency department¹, and APE patients face a 30-day mortality rate of over 15 %, with sudden death accounting for nearly 11 % of these fatalities^{2,3}. Because of the high mortality rate, prompt identification and appropriate risk stratification of APE patients play an important role in clinical decision-making⁴. Preserved right ventricular function in APE patients carries a good prognosis with anticoagulant therapy alone, while right ventricular dysfunction (RVD) resulting from acute pressure overload is the primary determinant of early clinical course and mortality risk^{5,6}. The risk of an unfavorable outcome is higher in high-risk APE patients presenting with systemic hypotension, cardiogenic shock, or cardiac arrest accompanied by right ventricular dysfunction⁴. Therefore, early identification of patients with poor prognoses may increase the survival rate.

Clinical scoring criteria such as the pulmonary em-

bolism severity index (PESI) scores help to anticipate adverse outcomes in the setting of APE. However, they do not utilize biomarkers and are complex to calculate during an emergency^{7,8}. Biomarkers are valuable for risk stratification and treatment strategies in many cardiopulmonary diseases, but APE-specific markers have not been found^{7,9}. Although troponin levels and cardiac N-terminal pro-B-type natriuretic peptide (NT-proBNP) help predict APE severity and RVD^{4,10}, new, easily accessible, and inexpensive biomarkers will complement these markers.

Emerging evidence underscores inflammation's pivotal role in influencing the severity of APE, leading to extensive research on the association between inflammatory markers and APE severity¹¹⁻¹³. Predicting RVD through inflammatory biomarkers may help improve risk stratification, prioritize cardiac evaluation, and identify APE patients with high mortality risk. For this purpose, straightforward parameters that can be promptly analyzed have been sought in recent years¹⁴.

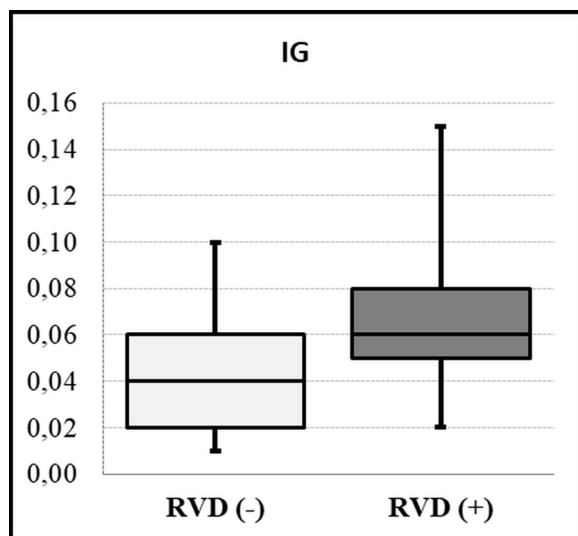


Figure 1: Boxplot showing the mean values of immature granulocyte counts according to the presence of right ventricular dysfunction. The mean immature granulocyte count was significantly higher in patients with right ventricular dysfunction ($p=0.001$).

IG: immature granulocyte, RVD: right ventricular dysfunction.

In recent studies, immature granulocytes (IGs) have been investigated as a marker of inflammation, usually for coronavirus disease 2019 (COVID-19), sepsis, and pancreatitis¹⁵⁻¹⁷. In this study, we aimed to investigate the usefulness of the IG count in whole blood as an inflammatory biomarker in predicting RVD in APE patients.

Methods

Study Design

We conducted a single-center, retrospective cohort study between January 2019 and June 2022, evaluating patients admitted to the emergency department of Medipol University Hospital with APE diagnosis. We assessed the relationship between IG count in whole blood and RVD on echocardiography (ECHO) in patients diagnosed with APE. Data, including case characteristics, laboratory tests, computed tomography (CT) pulmonary angiography (CTPA), and ECHO evaluations, were obtained from the hospital's electronic database.

In the study, we included patients aged over 18 years with APE diagnosed by CTPA, hemogram evaluation on admission, and post-diagnostic ECHO evaluation. We excluded patients with a disease affecting bone marrow neutrophil production. More specifically, the exclusion criteria were hematologic disease, autoimmune disease, immunosuppressive drug use, active cancer, deep vein thrombosis, previous pulmonary embolism, congenital heart disease, history of pulmonary hypertension, infection, or sepsis. The present study was approved by Medipol University ethical decision number 468 (E-10840098-772.02-3115).

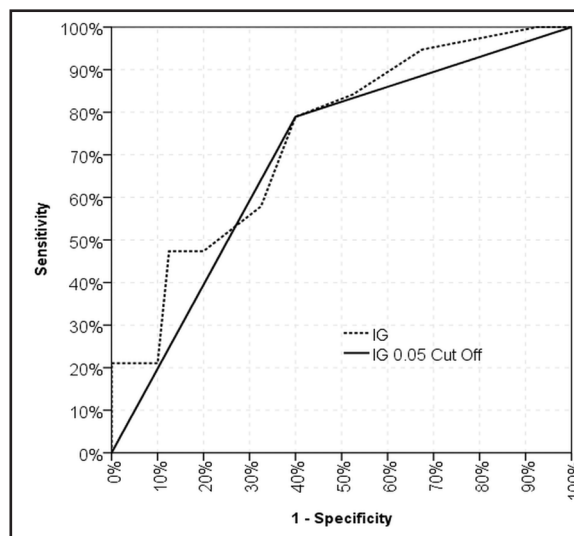


Figure 2: Receiver operating characteristic curve analysis showing the area under the curve for immature granulocyte count (dotted line) and for immature granulocyte count cut-off value of 0.05 to predict right ventricular dysfunction in acute pulmonary embolism patients.

CT

We performed pulmonary angiography using a 256-slice multidetector CT (Brilliance iCT, Philips Healthcare, Best, The Netherlands) utilizing a standard CTPA protocol according to the European Society of Cardiology guidelines⁴. Reports were read double-blinded by two experienced radiologists specializing in chest imaging. Consensus was sought when there was a difference in interpretation.

ECHO

All patients underwent transthoracic ECHO (TTE) examination within 24 hours after APE diagnosis, performed by the same cardiologist with more than three years of experience. We performed TTE operating an IE Elite ultrasound machine (Philips) with an S 5-1 transducer with 1-5 MHz frequency conversion. We define right ventricular dysfunction as decreased tricuspid annular plane systolic excursion measured with M-Mode (<16 mm), enlarged right ventricle, decreased peak systolic (S') velocity of tricuspid annulus (9.5 cm/s), a right ventricle to left ventricle diameter ratio >1, and a flattened intraventricular septum⁴. Cases with any of the mentioned criteria are considered RVD+ group.

Hematology

Blood samples were collected during emergency department presentation within 24 hours preceding APE diagnosis. We utilized an automated hematology analyzer (XN-1000, SYSMEX, Osaka, Japan) to measure the IG count.

Table 1: Basic characteristics and laboratory data of the 59 patients admitted to the emergency department with a diagnosis of acute pulmonary embolism divided into two groups according to the presence of right ventricular dysfunction.

	RVD (-)	RVD (+)	p
Age (years)	52.6 ± 16.2 (49.5)	52.2 ± 14 (47)	0.929 ^t
Sex			
Female	15 (37.5)	6 (31.6)	0.657 ^{x²}
Male	25 (62.5)	13 (68.4)	
No comorbidities	28 (70)	9 (47.4)	0.093 ^{x²}
Comorbidities	12 (30)	10 (52.6)	
IG (10 ³ /uL)	0.04 ± 0.03 (0.04)	0.07 ± 0.04 (0.06)	0.001 ^t

Values are presented as mean ± standard deviation and median in brackets or number with percentage in brackets. t: Independent sample t test, x²: chi-square test, m: Mann-whitney u test, IG: immature granulocyte, RVD: right ventricular dysfunction.

Table 2: Logistic regression analysis results of immature granulocyte count in differentiating the subjects with and without right ventricular dysfunction.

	Univariate Model			Multivariate Model		
	OR	95 % CI	p	OR	95 % CI	p
IG (10 ³ /uL)	5.63	1.55 - 20.44	0.004	7.27	2.07 - 25.54	0.006

Lojistik Regresyon (Forward LR)

CI : confidence interval, IG: immature granulocyte, OR: odds ratio.

Table 3: Immature granulocyte count and receiver operating characteristic curve analysis results.

	95 % CI	AUC	p
IG (10 ³ /uL) 0.05 cut-off	0.553 - 0.837	0.695	0.016
Sensitivity	78.9 %		
Positive predictive value	48.4 %		
Specificity	60.0 %		
Negative predictive value	85.7 %		

AUC: area under the curve, CI: confidence interval, IG: immature granulocyte.

Statistical analysis

We calculated the continuous variables' distribution using the Kolmogorov-Simirnov test. We present the normally distributed continuous variables as mean ± standard deviation, the non-normally distributed continuous variables as median and range (minimal-maximal values), and categorical data as frequency and percentage. We used ANOVA and independent sample t-tests to analyze and compare normally distributed variables between groups. In contrast, we used the Kruskal-Wallis and Mann-Whitney U tests to analyze non-normally distributed independent variables and the chi-square test to analyze qualitative independent variables. The receiver operating characteristic (ROC) curve investigated the effect level and cut-off value. We analyzed the effect level using univariate and multivariate logistic regression, and a p-value less than 0.05 was considered statistically significant for all analyses. We performed statistical analysis in the present study with the IBM SPSS Statistics for Windows, Version 28.0. (IBM Corp., Armonk, NY, USA).

Results

Table 1 presents the basic characteristics and laboratory data of the study population. Nineteen of the 59 patients (32 %) had RVD, and 40 (68 %) did not have RVD. There was no significant difference between patients with and without RVD regarding age and gender distribution or the presence of comorbid diseases (Table 1). The IG count was significantly higher in patients with RVD than those without RVD (p =0.001). Figure 1 illustrates the mean IG counts of patients with and without RVD.

Table 2 displays the results of univariate and multivariate logistic regression analyses. In the univariate model, we observed significant efficiency of the IG count in differentiating the subjects with and without RVD (p =0.004). Other variables were not effective. In the multivariate model, we observed significant and independent efficacy of the IG count in separating the cases with and without RVD (p =0.006).

Figure 2 displays the ROC curve analysis performed to predict RVD in APE. We observed a significant efficiency [area under the curve: 0.737 (0.605-0.869)] of the IG count. Significant efficacy [area under the curve

0.695 (0.553–0.837)] for an IG cut-off value of 0.05 was observed to discriminate between cases with and without RVD ($p = 0.016$). The sensitivity, positive predictive value, specificity, and negative predictive value were 78.9 %, 48.4 %, 60.0 %, and 85.7 %, respectively, for an IG cut-off value of 0.05 (Table 3).

Discussion

Our study demonstrated that an IG ($10^3/\text{uL}$) cut-off value of 0.05 was a significant and independent predictor of RVD in cases admitted to the emergency department with APE diagnosis. Although this finding has not been investigated in the literature, a high IG count may be an unexplored, valuable prognostic marker for assessing pulmonary embolism severity independent of hemodynamic instability.

RVD is the leading cause of poor prognosis in patients with APE. Therefore, prompt and accurate determination of RVD is an important prerequisite³. Inflammation plays an important role in APE and in the development of RVD in APE patients¹⁸. Despite the beneficial effects of delayed neutrophil activation on thrombus resolution, neutrophils are significantly implicated in the pathogenesis of acute right ventricular injury during the early inflammatory phase following APE^{5,18,19}. Watts et al also demonstrated the central role of neutrophils and macrophages in developing right ventricular injury and remodeling after APE²⁰.

In autoimmune diseases, cancer, and severe events such as sepsis, trauma, viral infections, and systemic inflammation can trigger immediate granulopoiesis by increasing the release of immature neutrophils into the blood and neutrophil production²¹. Polymorphonuclear neutrophil granulocytes, induced by granulocyte-colony stimulating factor, develop from progenitor cells and mature into mature segmented neutrophils in several stages in the bone marrow^{22,23}. They pass into the peripheral blood after 7-10 days of maturation. In healthy individuals, these neutrophil precursors, i.e., IGs, are not present in the peripheral blood. Therefore, high levels and frequencies of IGs in peripheral blood reflect bone marrow activation²².

An elevation in circulating IGs may occur as a compensatory response to the sharp decline in activated neutrophil levels²⁴. The half-life of IGs is three hours, which easily reflects the state of inflammation compared with other parameters with longer half-lives^{16,25}. They are also obtained quickly, easily, and inexpensively as part of a complete blood count measurement²⁶.

IGs have been previously investigated as a prognostic parameter in different inflammatory conditions. Daix et al showed that an increased postoperative IG count was associated with postoperative organ failure²⁷. Nahm et al found that IGs may be valuable in evaluating the severity of sepsis and prognosis in patients with suspected sepsis²⁵. Combadière et al associated the increase in the percentage of circulating IGs with the severity of the disease and thromboembolic complications in COVID-19

cases²⁸.

Considering the importance of neutrophils in APE and APE-related RVD injury^{18,20}, the number of circulating IGs may increase after activated neutrophils rapidly decrease. Therefore, it may help predict RVD and disease severity in patients with APE.

However, to our knowledge, only one study has investigated the association of IGs with APE prognosis. Kong et al found that IG elevation was a significant and independent predictor of mortality in APE⁵. However, this study did not address the relationship between the IG level and the right ventricle. In evaluating IGs as biomarkers in APE, the relationship with RVD may be guiding. In our study, we focused on the relationship between IG and RVD. We found a significant and independent relationship between RVD and an elevated IG count in APE.

Based on this new finding, an elevated IG count in APE may predict RVD and reflect mortality. In addition, our findings suggest that the high sensitivity and negative predictive values also guide differentiating patients without RVD in APE and patients with priority for evaluation. Considering the literature and our findings, IG is a new biological parameter that should be further investigated in the evaluation of disease severity independent of hemodynamic instability in patients with APE.

Our study has several limitations: the single-center and retrospective study design, the limited number of patients, and the lack of long-term follow-up limit our results. Long-term clinical outcomes and mortality need to be assessed in future research. Another limitation is the lack of comparison with other parameters reflecting RVD, such as NT-proBNP and troponin, and the lack of PESI data. Finally, although the effects of systemic inflammation have been previously investigated, we could not compare immature granulocytes with other inflammatory markers (such as proinflammatory cytokines). Large, multicenter, prospective studies are needed to confirm the clinical usefulness of IGs as a prognostic marker in patients with APE.

Conclusion

In conclusion, in our study, a high IG count was associated with RVD in APE and was a significant and independent biomarker for predicting RVD in APE. Given the easy availability and cost of IGs and our findings, IGs may serve as a novel and valuable marker for risk stratification and prognosis prediction in patients with APE.

Conflict of interest

The authors declare no competing interests.

References

1. Láinez-Ramos-Bossini AJ, Moreno-Suárez S, Pérez-García MC, Gálvez-López R, Garrido Sanz F, Rivera-Izquierdo M. Acute pulmonary embolism: Appropriateness of emergency department management according to clinical guidelines. *Radiologia (Engl Ed)*. 2022; 64: 291-299.
2. Ince O, Altintas N, Findik S, Sariaydin M. Risk stratification

- in submassive pulmonary embolism via alveolar-arterial oxygen gradient. *Hippokratia*. 2014; 18: 333-339.
3. Jia D, Liu F, Zhang Q, Zeng GQ, Li XL, Hou G. Rapid on-site evaluation of routine biochemical parameters to predict right ventricular dysfunction in and the prognosis of patients with acute pulmonary embolism upon admission to the emergency room. *J Clin Lab Anal*. 2018; 32: e22362.
 4. Konstantinides SV, Meyer G, Becattini C, Bueno H, Geersing GJ, Harjola VP, et al; 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). *Eur Heart J*. 2020; 41: 543-603.
 5. Kong T, Park YS, Lee HS, Kim S, Lee JW, Yu G, et al. Value of the Delta Neutrophil Index for Predicting 28-Day Mortality in Patients With Acute Pulmonary Embolism in the Emergency Department. *Shock*. 2018; 49: 649-657.
 6. Yetgin GO, Aydin SA, Koksali O, Ozdemir F, Mert DK, Torun G. Clinical probability and risk analysis of patients with suspected pulmonary embolism. *World J Emerg Med*. 2014; 5: 264-269.
 7. Bontekoe E, Brailovsky Y, Hoppensteadt D, Bontekoe J, Siddiqui F, Newman J, et al. Upregulation of Inflammatory Cytokines in Pulmonary Embolism Using Biochip-Array Profiling. *Clin Appl Thromb Hemost*. 2021; 27: 10760296211013107.
 8. Zhang Y, Zhang Z, Wei R, Miao X, Sun S, Liang G, et al. IL (Interleukin)-6 Contributes to Deep Vein Thrombosis and Is Negatively Regulated by miR-338-5p. *Arterioscler Thromb Vasc Biol*. 2020; 40: 323-334.
 9. Hijazi Z, Oldgren J, Siegbahn A, Wallentin L. Application of Biomarkers for Risk Stratification in Patients with Atrial Fibrillation. *Clin Chem*. 2017; 63: 152-164.
 10. Meyer T, Binder L, Hruska N, Luthe H, Buchwald AB. Cardiac troponin I elevation in acute pulmonary embolism is associated with right ventricular dysfunction. *J Am Coll Cardiol*. 2000; 36: 1632-1636.
 11. Kayrak M, Erdoğan HI, Solak Y, Akilli H, Gül EE, Yildirim O, et al. Prognostic value of neutrophil to lymphocyte ratio in patients with acute pulmonary embolism: a retrospective study. *Heart Lung Circ*. 2014; 23: 56-62.
 12. 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: e22072.
 13. Venetz C, Labarère J, Jiménez D, Aujesky D. White blood cell count and mortality in patients with acute pulmonary embolism. *Am J Hematol*. 2013; 88: 677-681.
 14. Ateş H, Ateş İ, Bozkurt B, Çelik HT, Özol D, Yldrm Z. What is the most reliable marker in the differential diagnosis of pulmonary embolism and community-acquired pneumonia? *Blood Coagul Fibrinolysis*. 2016; 27: 252-258.
 15. Ayres LS, Sgnaolin V, Munhoz TP. Immature granulocytes index as early marker of sepsis. *Int J Lab Hematol*. 2019; 41: 392-396.
 16. Georgakopoulou VE, Makrodimetri S, Triantafyllou M, Samara S, Voutsinas PM, Anastasopoulou A, et al. Immature granulocytes: Innovative biomarker for SARS-CoV-2 infection. *Mol Med Rep*. 2022; 26: 217.
 17. Huang Y, Xiao J, Cai T, Yang L, Shi F, Wang Y, et al. Immature granulocytes: A novel biomarker of acute respiratory distress syndrome in patients with acute pancreatitis. *J Crit Care*. 2019; 50: 303-308.
 18. Watts JA, Gellar MA, Obratsova M, Kline JA, Zagorski J. Role of inflammation in right ventricular damage and repair following experimental pulmonary embolism in rats. *Int J Exp Pathol*. 2008; 89: 389-399.
 19. Saghadzadeh A, Hafizi S, Rezaei N. Inflammation in venous thromboembolism: Cause or consequence? *Int Immunopharmacol*. 2015; 28: 655-665.
 20. Watts JA, Zagorski J, Gellar MA, Stevinson BG, Kline JA. Cardiac inflammation contributes to right ventricular dysfunction following experimental pulmonary embolism in rats. *J Mol Cell Cardiol*. 2006; 41: 296-307.
 21. Scapini P, Marini O, Tecchio C, Cassatella MA. Human neutrophils in the saga of cellular heterogeneity: insights and open questions. *Immunol Rev*. 2016; 273: 48-60.
 22. Lipiński M, Rydzewska G. Immature granulocytes predict severe acute pancreatitis independently of systemic inflammatory response syndrome. *Prz Gastroenterol*. 2017; 12: 140-144.
 23. Nierhaus A, Klatt S, Linssen J, Eismann NM, Wichmann D, Hedke J, et al. Revisiting the white blood cell count: immature granulocytes count as a diagnostic marker to discriminate between SIRS and sepsis—a prospective, observational study. *BMC Immunol*. 2013; 14: 8.
 24. Bermejo-Martin JF, Andaluz-Ojeda D, Almansa R, Gandía F, Gómez-Herreras JI, Gomez-Sanchez E, et al. Defining immunological dysfunction in sepsis: A requisite tool for precision medicine. *J Infect*. 2016; 72: 525-536.
 25. Nahm CH, Choi JW, Lee J. Delta neutrophil index in automated immature granulocyte counts for assessing disease severity of patients with sepsis. *Ann Clin Lab Sci*. 2008; 38: 241-246.
 26. Myari A, Papapetrou E, Tsaousi C. Diagnostic value of white blood cell parameters for COVID-19: Is there a role for HFLC and IG? *Int J Lab Hematol*. 2022; 44: 104-111.
 27. Daix T, Guérin E, Tavernier E, Marsaud JP, Hacan A, Gauthier F, et al. Immature Granulocytes: A Risk Factor of Infection after Cardiac Surgery. *Cytometry B Clin Cytom*. 2018; 94: 887-894.
 28. Combadière B, Adam L, Guillou N, Quentric P, Rosenbaum P, Dorgham K, et al. LOX-1-Expressing Immature Neutrophils Identify Critically-Ill COVID-19 Patients at Risk of Thrombotic Complications. *Front Immunol*. 2021; 12: 752612.