

## Contribution of immature granulocyte level to diagnosis in pleural effusion

*Plevral efüzyonda immatür granülosit düzeyinin tanı üzerine katkısı*

Fatoş Kozanlı<sup>1</sup>, Burcu Akkök<sup>2</sup>

<sup>1</sup>Department of Thoracic Surgery, Kahramanmaraş Sütçü İmam University Faculty of Medicine, Kahramanmaraş, Türkiye

<sup>2</sup>Department of Chest Diseases, Kahramanmaraş Sütçü İmam University Faculty of Medicine, Kahramanmaraş, Türkiye

### ABSTRACT

**Background:** In this study, we aimed to evaluate the diagnostic value of neutrophil and immature granulocyte levels in peripheral blood in cases with pleural effusion.

**Methods:** Between May 2019 and May 2020, a total of 117 patients (43 males, 74 females; mean age: 63.1±18.1 years; range, 18 to 93 years) who had pleural effusion and analysis of pleural fluid were retrospectively analyzed. All patients were evaluated in terms of age, sex, presence of comorbid diseases, approach to the pleural fluid, biochemical values of peripheral blood and pleural fluid, hemogram series of peripheral blood, diagnosis of pleural fluid, and mortality.

**Results:** Of the patients, 66 (54.5%) were diagnosed with benign pleural effusion and 51 (43.5%) were diagnosed with malignant pleural effusion. Number of cases with known primary malignancy was 54 (46.1%). Immature granulocyte count number and percentage of venous blood in the malignant pleural effusion group was significantly higher than the group with benign pleural effusion (p<0.05).

**Conclusion:** As a hemogram parameter, immature granulocyte level is an easily applicable, cheap, and a non-invasive method in the outpatient settings.

**Keywords:** Immature granulocyte, malignant pleural effusion, non-invasive diagnostic method, thoracentesis.

Pleural effusion is caused by increased fluid production and/or decreased fluid absorption.<sup>[1]</sup> Management of pleural effusion is a common clinical condition related to many pulmonary, pleural or extra-pulmonary diseases. A systematic approach

### ÖZ

**Amaç:** Bu çalışmada plevral efüzyonlu olgularda periferik kandaki nötrofil ve immatür granülosit düzeylerinin tanısal değeri incelendi.

**Çalışma planı:** Mayıs 2019 - Mayıs 2020 tarihleri arasında plevral efüzyonu olan ve plevral sıvı tahlili yapılan toplam 117 hasta (43 erkek, 74 kadın; ort. yaş: 63.1±18.1 yıl; dağılım, 18-93 yıl) retrospektif olarak incelendi. Tüm hastalar yaş, cinsiyet, ek hastalık varlığı, plevral sıvıya yaklaşım şekli, periferik kan ve plevral sıvının biyokimyasal değerleri, periferik kan hemogram serisi, plevral sıvının tanısı ve mortalite açısından değerlendirildi.

**Bulgular:** Hastaların 66'sına (%54.5) benign plevral efüzyon ve 51'ine (%43.5) malign plevral efüzyon tanısı kondu. Bilinen primer malignitesi olan olgu sayısı 54 (%46.1) idi. Malign plevral efüzyon grubunda, benign plevral efüzyon grubuna kıyasla, venöz kan immatür granülosit sayısı ve yüzdesi anlamlı düzeyde daha yüksekti (p<0.05).

**Sonuç:** Bir hemogram parametresi olan immatür granülosit düzeyi ayaktan tedavi ortamında kolay uygulanabilen, maliyeti düşük, noninvaziv bir yöntemdir.

**Anahtar sözcükler:** İmmatür granülosit, malign plevral efüzyon, noninvaziv tanı yöntemi, torasentez.

is required for providing a rapid diagnosis and appropriate treatment.<sup>[2,3]</sup> Evaluating the patient with pleural effusion is difficult due to wide range of differential diagnosis which includes both benign and life-threatening conditions.<sup>[4]</sup>

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**Correspondence:** Fatoş Kozanlı, MD, Kahramanmaraş Sütçü İmam Üniversitesi Tıp Fakültesi, Göğüs Cerrahisi Anabilim Dalı, 46040 Onikişubat, Kahramanmaraş, Türkiye.

Tel: +90 344 - 300 10 37 e-mail: opdrfatokozanlı@gmail.com

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Pleural effusion affects 3,000 individuals per million population in every year. More than 50 causes for pleural effusion including infection and malignant pleural effusion (MPE) have been known.<sup>[5]</sup> Variety of etiopathogenesis determines treatment protocols. It is a field that requires investigation, as it is common and a clinical condition with many physiopathology.<sup>[1]</sup>

Chest roentgenogram must be initially obtained, if pleural effusion is suspected. Posterior-anterior chest roentgenogram reveals effusions with 200 mL and more volume.<sup>[6]</sup> The first step of differential diagnosis or pathogenesis of pleural effusion is to identify whether the patient has transudative or exudative pleural effusion.<sup>[7]</sup>

Malignant pleural effusion is a condition with morbidity and mortality which affects many individuals worldwide.<sup>[8]</sup> Although it is quite common, there has been no study identifying hospitalization rate of cases with MPE. Clinical experience in cases with MPE is tended toward hospitalization for medical care and symptomatic treatment.<sup>[9]</sup>

Currently, aspiration and cytologic evaluation of pleural fluid is the main diagnostic method. Cytologic evaluation is 60% sensitive for diagnosis. The presence of tumor cells in pleural effusion has diagnostic value in MPE; however, the chance to find tumor cell in fluid is low. Instead, chance of finding tumor markers is higher.<sup>[10]</sup>

In contrast to well-known macrophages related to tumor, importance of granulocytes has recently been recognized. A number of studies has shown that neutrophilic granulocyte count in peripheral blood is increased in different cancer types.<sup>[11]</sup> Large-scale clinical trials have shown that the count of neutrophils and their activities change in cancer.<sup>[12,13]</sup> Similarly, it has been reported that neutrophils have also promoting effects on tumor growth and metastasis.<sup>[14,15]</sup>

In the present study, we aimed to evaluate the diagnostic value of neutrophil and immature granulocyte (IG) levels in peripheral blood in cases with pleural effusion.

## PATIENTS AND METHODS

This single-center, retrospective study was conducted at Kahramanmaraş Sütçü Imam University Faculty of Medicine, Department of Thoracic Surgery between May 2019 and May 2020. Data of patients aged 18 or above who were diagnosed with pleural effusion were retrospectively evaluated. Cases without pleural fluid analysis, those within conclusive diagnosis, and patients age under 18 were excluded from the study.

Finally, a total of 117 patients (43 males, 74 females; mean age:  $63.1 \pm 18.1$  years; range, 18 to 93 years) who had pleural effusion and analysis of pleural fluid were included. All patients were evaluated in terms of age, sex, comorbid disease, approach to pleural fluid, diagnosis of pleural fluid and mortality. Data of cases were obtained through digital data registry module of our hospital.

The patients were evaluated with medical history, physical examination, posteroanterior chest roentgenogram, and routine blood tests. Thoracentesis was initially performed to all cases and, then, additional interventions were decided (tube, surgery, etc.). Pleural fluid samples were sent to pathology laboratory for biochemistry, microbiological and cytologic examination on the same time with blood tests. Differentiation between transudate/exudate was performed according to the Light criteria. After thoracentesis, tube thoracostomy or pleural biopsy were performed in cases with indication. Pleural effusions were classified as malignant, paramalignant, tuberculous pleurisy, parapneumonic effusion, empyema, failure fluids according to their etiology. Hemogram parameters of all patients were recorded during the first admission. The IG levels of our patients were studied with the Sysmax XN\_3000 (Sysmax Corp., Kobe, Japan) hemogram device.

## Statistical analysis

Statistical analysis was performed using the IBM SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). Continuous data were presented in mean  $\pm$  standard deviation (SD) or median (min-max), while categorical data were expressed in number and frequency. The chi-square test was used for categorical data in inter-group comparisons. For comparison of quantitative data, the Mann-Whitney U test and for distribution of variables, the Kolmogorov-Smirnov test was used as appropriate. The receiver operating characteristic (ROC) curve was performed for diagnostic evaluation. Sensitivity, specificity, and positive predictive value and negative predictive value were calculated with the formula by calculating the area under the curve (AUC) and identifying cut-off value. A *p* value of  $<0.05$  was considered statistically significant.

## RESULTS

Of all patients, 66 (54.5%) were diagnosed with benign pleural effusion and 51 (43.5%) were diagnosed with MPE. Fifty-four (46.1%) patients had primary known malignancy. The number of right, left, and

**Table 1. Baseline characteristics, side of pleural effusion (right/left/bilateral), and hemogram/biochemistry results**

	Benign				Malign				<i>p</i>
	n	%	Mean±SD	Median	n	%	Mean±SD	Median	
Age (year)			61.3±20.1	65.0			65.3±15.0	70.0	0.263*
Sex									0.148†
Female	38	57.6			36	70.6			
Male	28	42.4			15	29.4			
Side									0.749†
Bilateral	3	4.5			3	5.9			
Right	38	57.6			32	62.7			
Left	25	37.9			16	31.4			
Comorbodite									0.009†
(-)	17	25.8			3	5.9			
(+)	49	74.2			48	94.1			
Mortality									0.036†
(-)	56	84.8			35	68.6			
(+)	10	15.2			16	31.4			
WBC			10.5±4.9	9.3			12.1±6.8	10.0	0.370*
Neutrophil			8.5±8.1	6.6			9.7±6.4	7.5	0.186*
Neutrophil (%)			71.5±16.4	73.7			77.9±14.8	80.4	0.008*
Lymphocyte			1.4±0.7	1.4			1.2±0.8	1.3	0.106*
Lymphocyte (%)			15.3±7.7	15.2			12.1±8.6	10.3	0.017*
Monocyte			2.1±9.1	0.8			0.8±0.5	0.8	0.603*
Monocyte (%)			7.9±2.9	8.1			6.9±3.6	7.1	0.121*
Eosinophil			0.1±0.2	0.1			0.1±0.2	0.1	0.887*
Eosinophil (%)			1.5±1.8	0.7			1.2±1.5	0.8	0.595*
IG			0.1±0.2	0.1			0.3±0.4	0.1	0.000*
IG (%)			0.7±0.7	0.5			2.0±1.9	1.5	0.000*
Hemoglobin			12.1±2.1	12.0			11.3±2.5	10.7	0.034*
Hematocrit			36.7±6.4	37.1			35.2±9.0	33.1	0.104*
MPV			12.5±19.0	10.1			10.1±1.8	10.3	0.717*
RDW			46.3±7.1	45.2			46.0±15.5	46.2	0.502*
PLT			303.2±130.4	290.0			268.9±161.1	243.0	0.099*
PDW			17.4±39.4	11.2			11.9±2.5	11.2	0.721*
Sedimentation			39.8±22.7	38.0			40.9±34.3	35.5	0.624*
CRP			85.7±75.7	62.9			104.7±122.8	63.0	0.685*
Serum albumin			35.0±7.0	36.2			33.1±6.3	32.9	0.153*
Serum protein			66.3±9.1	67.5			61.0±10.4	63.5	0.007*
Serum glucose			129.3±48.6	120.0			147.2±64.0	125.5	0.191*
Serum LDH			295.3±131.5	250.5			417.9±364.7	285.0	0.056*
Pleura albumin			23.7±8.3	24.9			22.2±7.2	22.4	0.279*
Pleura protein			46.6±14.2	48.0			43.1±15.4	43.0	0.093*
Pleura glucose			92.6±45.0	92.5			119.6±67.1	103.5	0.047*
Pleura LDH			579.2±674	343.0			664.8±591.0	437.0	0.0313*

SD: Standard deviation; WBC: White blood cell; IG: Immature granulocyte; MPV: Mean platelet volume; RDW: Red blood cell distribution width; PLT: Platelet; PDW: Platelet distribution width; CRP: C-reactive protein; LDH: Lactate dehydrogenase; \* Mann-Whitney U test; † Chi-squared test.

**Table 2. Diagnosis of pleural fluid**

Diagnosis of pleural fluid	Number of cases (n)	Percentage of cases (%)
Malignant pleural effusion	51	43.5
Failure fluids*	21	17.9
Inflammation	16	13.7
Empyema	13	11.1
Parapneumonic pleural effusion	6	5.1
Tuberculous pleurisy	4	3.5
Paramalignant pleural effusion	3	2.6
Secondary to pulmonary thromboembolism	3	2.6
<i>Total</i>	117	100

\* Congestive heart failure, kidney failure, liver failure.

**Table 3. Type of intervention**

Type of intervention	Number of cases (n)	Percentage of cases (%)
Thoracentesis	68	58.1
Thoracic tube	34	29.1
Catheter thoracostomy	9	7.7
*VATS	6	5.1
<i>Total</i>	117	100

VATS: Video-assisted thoracic surgery.

**Table 4. Immature granulocyte in malignant and benign pleural effusions**

	Univariate model			Univariate model		
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>
IG	83.00	4.08-1689	0.004			
IG (%)	3.25	1.79-5.89	<0.005	5.374	2.211-13061	<0.005

OR: Odds ratio; CI: Confidence interval; IG: Immature granulocyte.

bilateral pleural effusion cases was 70 (59.8%), 41 (35.1%), and six (5.1%), respectively. There was no significant difference ( $p>0.05$ ) between the groups with benign and MPE in terms of age, sex, and side of pleural effusion (right/left/bilateral) (Table 1).

**Table 5. Immature granulocyte AUC value in malignant and benign pleural effusions**

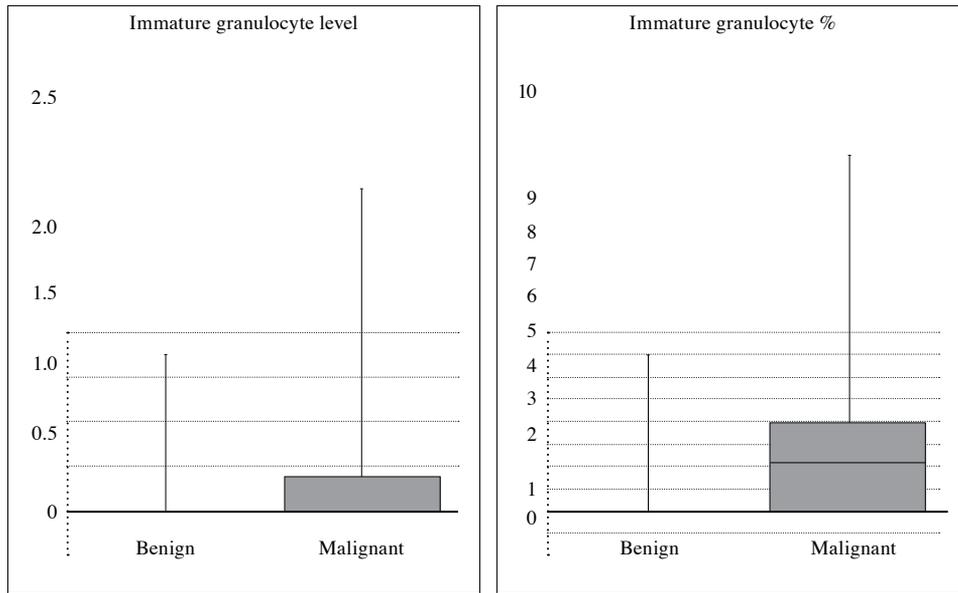
	AUC	95% CI	<i>p</i>
IG	0.740	0.647- 0.832	<0.005
IG (%)	0.775	0.684-0.867	<0.005

AUC: Area under the curve; CI: Confidence interval; IG: Immature granulocyte.

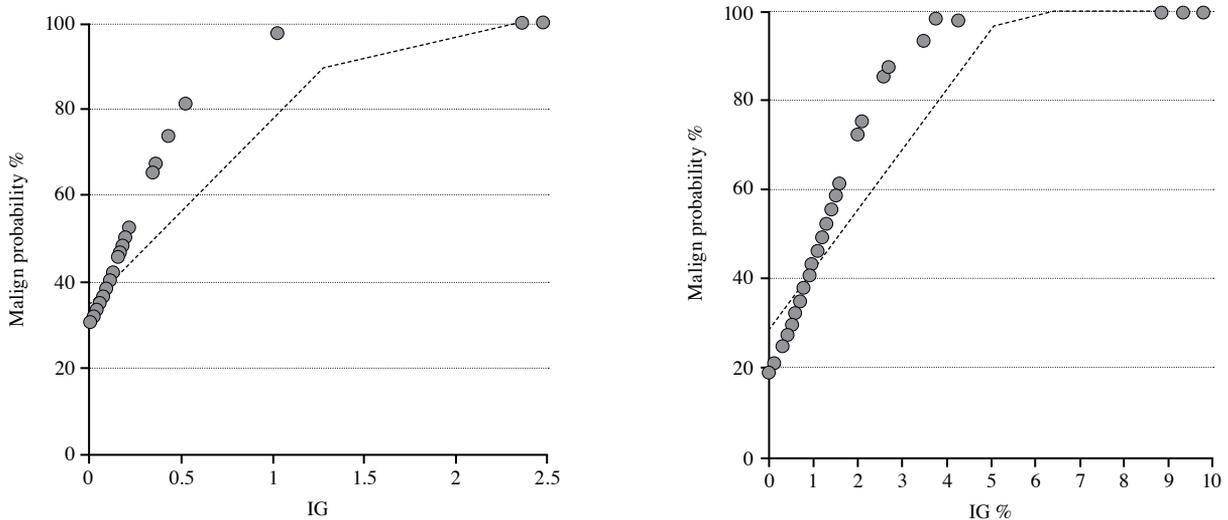
Our cases with pleural effusion listed below (Table 2) had malignant effusion, failure fluid, inflammation, empyema, parapneumonic effusion, tuberculous pleurisy, and pleural effusion secondary to pulmonary thromboembolism, respectively.

Thoracentesis was performed in 68 (58.1%) cases, thoracic tube was inserted in 34 (29.1%) cases, catheter thoracostomy was performed in nine (7.7%) cases, and video-assisted thoracoscopic surgery (VATS) was performed in six (5.1%) cases (Table 3).

The number and percentage of venous blood IG was significantly higher ( $p<0.05$ ) in MPE group than the group with benign pleural effusion. In the univariate analysis, the number and percentage of



**Figure 1.** Immature granulocyte levels and percentages.



**Figure 2.** Immature granulocyte levels and percentages in cases with malignant pleural effusion.

IG: Immature granulocyte.

venous blood IG were efficient in predicting patients with malignant and benign pleural effusion ( $p < 0.05$ ) (Table 4). In the multivariate analysis, IG% was independently efficient in significantly predicting patients with malignant and benign pleural effusion ( $p < 0.05$ ) (Figure 1). Significant efficiency ( $p < 0.05$ ) of IG value was shown in predicting patients with MPE (AUC 0.740 [0.647-0.832]) (Table 5).

## DISCUSSION

Differentiation between malignant and benign fluid is important and difficult due to differences between

treatment and prognosis of cases with pleural effusion. Only one-third of cases with definitive diagnosis of cancer have pleural effusion as the initial sign.<sup>[16,17]</sup> About 12 to 24% of all pleural effusion cases are malignant effusion.<sup>[10]</sup> In our study, 51% of our cases had malignant effusion. We believe that this difference was due to our regional endemic features. Patients without pleural fluid sampling were excluded from our study. This may explain the lower incidence of our cases with pleural effusion secondary to congestive heart failure. We consider that congestive heart failure resolves with appropriate medical treatment and pleural fluid also

resolves without aspiration; therefore, its incidence was lower in our study.

We have started to research for new procedures which may contribute to the diagnosis due to costly and time-consuming diagnostic methods and more frequently observed MPE in our region. Thoracentesis is a simple and safe procedure. However, interest to non-invasive diagnostic methods has been increasing in recent years. Using tumor markers rather than tumor cell itself has become more frequent.<sup>[10]</sup> Therefore, we started to search for a non-invasive, easily-applicable, safe and new procedure. There were studies indicating neutrophils and, particularly, IG promote angiogenesis, tumor migration and metastasis in the literature were searched.<sup>[14,15,18]</sup> However, we did not find a similar study related to the prediction of malignancy in particularly pleural effusions.

In the last year, we conducted a statistical study on hemogram, biochemical, microbiology, and cytology results of pleural fluid and on venous blood hemogram and biochemical results. We observed that IG level in hemogram worked on venous blood was significantly higher in cases with MPE ( $p < 0.05$ ). Using the ROC curve, it was calculated as 0.740 for AUC IG level and 0.775 for IG percentage, and the accuracy rate for diagnosis was high. It was found to be between 0.647 and 0.832 for the IG level at the 95% confidence interval and between 0.684 to 0.867 for the IG percentage, which was significant in terms of predicting MPE. Unfortunately, a single cut-off value could not be found in the statistical study we conducted in terms of predicting MPE in IG levels and percentages. ROC curve was drawn showing multiple cut-off values. There is no other study related to calculating IG levels for predicting MPE in the literature. Further studies with larger series are needed for this area. Management of pleural effusion includes difficult and costly protocols. However, most of the cases may not be diagnosed.<sup>[19]</sup> Even cytologic tests can only diagnose 60% of cases.<sup>[10]</sup>

Hemogram test worked on venous blood is an easily-applicable, non-invasive, and rapidly performed method. We believe that IG measured in venous blood may contribute to plan the diagnostic methods in cases who could not be diagnosed even with all necessary evaluations. Therefore, we present the efficiency of level and percentage of IG for predicting MPEs in our study along with the literature data.

The limitations of our study were the low number of cases, since immature granulocyte levels have been studied for the last year.

In conclusion, most commonly seen pleural effusion type was malignant pleural effusion in our series. Blood immature granulocyte level was statistically significantly higher in predicting malignant pleural effusion. Non-invasive interventions have gained attention as diagnostic methods in recent years. As a hemogram parameter, immature granulocyte level is an easily applicable, cost-effective, and non-invasive method in the outpatient settings. We believe that immature granulocyte level may contribute to diagnosis in malignant pleural effusion cases. Further studies with larger samples are needed in this field due to the pleural effusion cases without a diagnosis even after all diagnostic methods.

**Ethics Committee Approval:** The study protocol was approved by the Kahramanmaraş Sütçü Imam University Faculty of Medicine Clinical Research Ethics Committee (Date: 06/10/2020, No: 24). The study was conducted in accordance with the principles of the Declaration of Helsinki.

**Patient Consent for Publication:** A written informed consent was obtained from each patient.

**Data Sharing Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Author Contributions:** F.K., study concept, design, data analysis and data interpretation, writing, rationale; B.A., data collection, illustrations and figures, critical review.

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