KASTAMONU MEDICAL JOURNAL

Original Article

DOI: 10.51271/KMJ-0117

Kastamonu Med J. 2023;3(3):155-158

Can immature granulocyte (IG), an inflammatory marker, be used in the differential diagnosis of epilepsy and non-epileptic psychogenic seizure?

Mehmet Akif Bildirici[1], İdris Kocatürk[2]

[1]Department of Biochemistry, Kastamonu Faculty of Medicine, Kastamonu, Turkey
[2]Department of Neurology, Kastamonu Faculty of Medicine, Kastamonu, Turkey

Received: 11/08/2023 ◆ **Accepted**: 25/08/2023 ◆ **Published**: 22/09/2023

ABSTRACT

Aims: Intense neuronal activity during epileptic seizures can cause neuroinflammation. Differential diagnosis of epilepsy and psychogenic non-epileptic seizures (PNES) is a great challenge. Immature granulocyte (IG) is a new inflammatory marker analyzed in hemogram. This study investigated the role of IG in differentiating epilepsy from PNES.

Methods: In this retrospective study, patients who applied to the emergency department for the first time with seizures and were diagnosed with epilepsy/PNES (clinical evaluation and electroencephalography) after included the seizure by the neurology clinical follow-up. Of the 84 patients, 54 had epilepsy, and 30 had PNES. Hemogram analyses were performed within 2 hours of the onset of the seizure.

Results: The IG count was 0.03 x10^9/L (0.02-0.06) and 0.03 x10^9/L (0.02-0.05) in the epilepsy and PNES groups, respectively. The two groups had no statistically significant difference (p=0.291). Serum C-reactive protein (CRP) levels were significantly higher in the epilepsy group (p=0.031). The ROC curve analysis for the CRP test yielded a serum CRP value of 2.35 mg/L, with a sensitivity of 0.57 and a specificity of 0.73, as the optimal cut-off value for distinguishing epilepsy from PNES. The ROC analysis for the AUC yielded an estimate of 0.64 (95% confidence interval: 0.52-0.77).

Conclusion: In conclusion, only CRP was useful in differentiating epilepsy from PNES in the study. However, the IG count did not help to separate the two seizures. Therefore, these findings should be confirmed by further prospective studies with large samples assessing the IG count. This study evaluating the IG count, a new inflammatory marker, will contribute to the literature.

Keywords: Immature granulocyte, epilepsy, psychogenic non-epileptic seizure

INTRODUCTION

Epilepsy, characterized by recurrent seizure attacks, is one of the most common neurological diseases worldwide. Seizures are one of the common reasons for presentations to the emergency department (ED) and constitute 1-3% of the ED visits.[1] Differential diagnosis of epilepsy (generalized tonic-clonic seizures) and psychogenic non-epileptic seizure (PNES) is a great challenge, and misdiagnosis rates can reach 40%.[2,3] Epilepsy is a disease caused by increased nerve cell excitability in the brain and is characterized by seizures.[4] The PNES, also called a dissociative attack, is similar to an epileptic seizure but without epileptiform discharges or electroencephalography (EEG) abnormalities.[5] PNES is a common condition in patients with functional neurological disorders and can cause severe disability in patients.[3] The differential diagnosis of epileptic seizure and PNES is challenging in patients presenting to the ED with first-onset seizures. EEG is the first-choice test to distinguish between these two clinical conditions. The gold standard in the differential diagnosis of these two seizures is video-EEG. However, video EEG monitoring centers are not available everywhere. In addition, due to the high cost of this procedure, it is not possible to apply this process to every seizure patient.[6,7]

A pathological event occurring in the brain tissue can initiate inflammation. Intense neuronal activity is observed during epileptic seizures, which can cause neuroinflammation.[8,9] Immature granulocyte (IG) is a new inflammatory marker routinely analyzed on hemogram, and an increase of IG in peripheral blood indicates bone marrow activation.[10] It has been shown that IG is significantly increased in sepsis and infections when compared to healthy individuals.[11,12] In this

Corresponding Author: Mehmet Akif Bildirici, bildiriciakif@gmail.com

Cite this article as: Bildirici MA, Kocaturk İ. Can immature granulocyte (IG), an inflammatory marker, be used in the differential diagnosis of epilepsy and non-epileptic psychogenic seizure? *Kastamonu Med J.* 2023;3(3):155-158.

study, the role of IG, an inflammatory marker, in differentiating epilepsy from PNES was assessed. In addition, in the differential diagnosis of seizures and PNES, immune-response related indicators: neutrophil-to-lymphocyte ratio (NLR),[13] monocyte-to-lymphocyte ratio (MLR),[14] lymphocyte-to-monocyte ratio (LMR),[15] platelet-to-lymphocyte ratio (PLR),[16] systemic immune-inflammation index (SII),[17] and systemic inflammation response index (SIRI)[18] indices calculated from routine hemogram parameters, and C-reactive protein (CRP) values were also evaluated.

METHODS

Patients who applied to the ED of Kastamonu Training and Research Hospital with seizures between January 2020 and June 2023 were retrospectively investigated. Patients who presented with seizure for the first time and were diagnosed with epilepsy/PNES (clinical evaluation and EEG) by neurology clinical follow-up after seizure were included in the study. Patients younger than 18, pregnant/breastfeeding women, patients with systemic inflammatory symptoms, patients with a history of systemic disease, and patients without laboratory data were excluded from the study. Patients with hemogram/CRP data were enrolled in the study. The study was approved by the Kastamonu University Clinical Research Ethics Committee (approval number: 2023-KAEK-76, date: 07.05.2023), in accordance with the Declaration of Helsinki.

Hemogram and CRP analyses were performed immediately after patients were admitted to the ED. These analyses were completed in all patients within 2 hours of the onset of the seizure. Hemogram and CRP tests were performed on Sysmex XN-1000 (Sysmex, Kobe, Japan) hematology and Beckman Coulter AU 5800 (Beckman Coulter, Brea, CA, USA) clinical chemistry auto analyzers, respectively. The NLR,[13] MLR,[14] LMR,[15] PLR,[16] SII,[17] and SIRI[18] were calculated as follows:

NLR = Neutrophil count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

MLR = Monocyte count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

LMR = Lymphocyte count ($x10^9$/L) / Monocyte count ($x10^9$/L)

PLR = Platelet count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

SII = Platelet count ($x10^9$/L) x NLR

SIRI = Neutrophil count ($x10^9$/L) x MLR

Statistical analyses were performed using Statistical Package for Social Sciences 18.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were characterized as median and interquartile range (IQR). The chi-square test was used to determine whether there was a significant difference between the two groups regarding gender. The Mann-Whitney U test was used to compare the data between epilepsy and PNES. The receiver operating characteristic (ROC) analysis was performed, and Youden's index was used to determine the area under the curve (AUC), sensitivity, specificity, and optimal cut-off values. Statistically significant p-value <0.05 was considered.

RESULTS

Eighty-four patients who had seizures for the first time and met the inclusion criteria were included in this study. Of these, 54 patients (25 female, 29 male) were diagnosed with epilepsy, and 30 patients (18 female, 12 male) with PNES. Differential diagnosis was arrived in patients who applied to the neurology clinic after seizures. This process was managed with clinical evaluation, EEG, and magnetic resonance imaging (MRI) results.

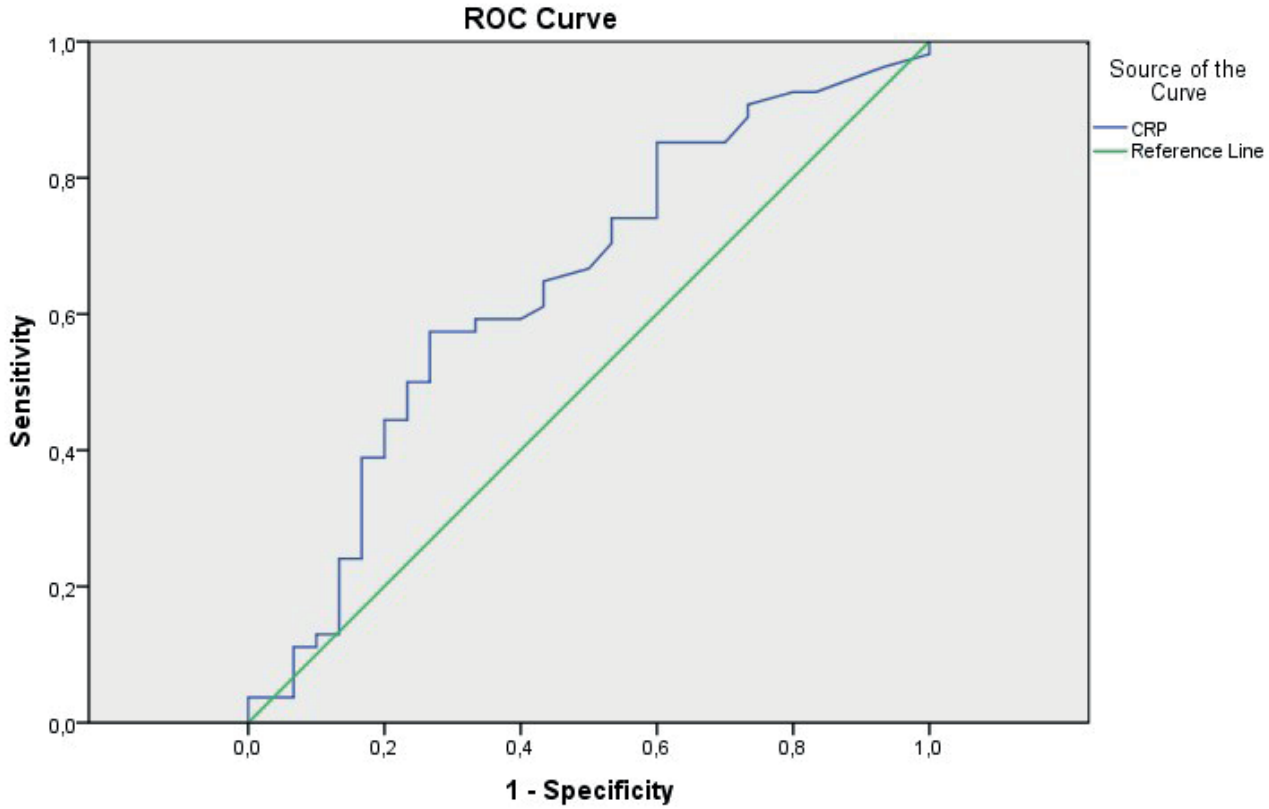

Figure 1. The ROC analysis of the CRP in the differential diagnosis of epilepsy and PNES.

Table 1. Age and gender characteristics, IG count, immune response-related markers (NLR, MLR, LMR, PLR, SII, and SIRI), and serum CRP levels in epilepsy and PNES groups.

	Generalized tonic-clonic epileptic seizure	PNES	P-value
Age (years)	39 (24.75-57.50)	34 (22.75-47.50)	0.163
Female/Male (gender)	25/29	18/12	0.231
Leukocyte count (x10^9/L)	9.14 (6.78-11.87)	8.39 (7.34-10.41)	0.621
Neutrophil count (x10^9/L)	5.70 (3.79-8.21)	5.25 (3.93-7.47)	0.478
Lymphocyte count (x10^9/L)	2.06 (1.29-2.98)	2.30 (1.66-3.21)	0.265
Monocyte count (x10^9/L)	0.61 (0.47-0.75)	0.58 (0.52-0.73)	0.823
Platelet count (x10^9/L)	227 (189-295)	250 (224-300)	0.155
IG count (x10^9/L)	0.03 (0.02-0.06)	0.03 (0.02-0.05)	0.291
NLR	2.33 (1.55-6.89)	2.46 (1.50-3.40)	0.375
MLR	0.26 (0.21-0.42)	0.24 (0.19-0.33)	0.241
LMR	3.82 (2.40-4.85)	4.10 (3.07-5.26)	0.227
PLR	112 (76-174)	116 (84-152)	0.955
SII	592 (309-1263)	617 (354-924)	0.608
SIRI	1.52 (0.88-3.69)	1.20 (0.84-2.48)	0.320
CRP (mg/L)	2.85 (0.90-7.83)	1.55 (0.40-3.03)	0.031

PNES: psychogenic non-epileptic seizure, IG: immature granulocyte, NLR: neutrophil-to-lymphocyte ratio, MLR: monocyte-to-lymphocyte ratio, LMR: lymphocyte-to-monocyte ratio, PLR: platelet-to-lymphocyte ratio, SII: systemic immune-inflammation index, SIRI: systemic inflammation response index, CRP: C-reactive protein.

Age and gender characteristics, IG count, immune response-related markers (NLR, MLR, LMR, PLR, SII, and SIRI), and serum CRP levels in epilepsy and PNES groups are presented in **Table 1**. The IG count was 0.03 x109/L (IQR: 0.02-0.06) in the epilepsy group and 0.03 x109/L (IQR: 0.02-0.05) in the PNES group. The two groups had no statistically significant difference (p=0.291). No significant difference existed in any immune-response related biomarkers (**Table 1**). Only serum CRP levels significantly differed between the two groups (p=0.031). The serum CRP level was 2.85 mg/L (IQR: 0.90-7.83) in the epilepsy group, and the serum CRP level was 1.55 mg/L (IQR: 0.40-3.03) in the PNES group. The ROC curve analysis was performed for the CRP test. It yielded a serum CRP value of 2.35 mg/L with a sensitivity of 0.57 and a specificity of 0.73 as the optimal cut-off value to distinguish epilepsy from the PNES. The ROC analysis for the AUC yielded an estimate of 0.64 (95% confidence interval: 0.52-0.77) (**Figure 1**).

DISCUSSION

In this study, the data of patients who had seizures for the first time were analyzed retrospectively. In the differential diagnosis of epileptic seizures and PNES, IG count, an inflammatory marker, immune response-related biomarkers (NLR, MLR, LMR, PLR, SII, and SIRI), and CRP levels were evaluated. When the literature was examined, we could not find any study that evaluated the IG count in the differential diagnosis of epilepsy and PNES. Therefore, we hope this study will significantly contribute to the literature.

Epilepsy is a common neurological disease characterized by seizures.[4] The view that inflammatory mediators are released by both brain cells and peripheral immune cells in the epileptogenic process has been supported by studies. These inflammatory mediators are secreted in the brain by glia, neurons, and endothelial cells of the blood-brain barrier.[9] Inflammatory processes developing in the brain contribute to both the onset of epilepsy and the recurrence of seizures by causing neuronal hyperexcitability by mechanisms that are not fully understood.[19] The role of inflammation in epilepsy has been shown in many studies.[9,20,21] However, we could not found any epilepsy study on the IG count, a new inflammatory marker. Various studies have investigated whether the IG count can be a marker for the diagnosis and prognosis of some neurological diseases. In their study, Korkut et al. showed that the IG count effectively shows 30-day mortality in ischemic stroke patients.[22] Bedel et al. found that the IG count was significantly associated with poor prognosis in spontaneous intracerebral hemorrhage.[23] In addition, in a recent study, it was shown that the IG count is effective in predicting hospital mortality in spontaneous intracerebral hemorrhage.[24] Hence, we compared our results with studies on immune response-related markers (NLR, MLR, LMR, PLR, SII, and SIRI), especially the NLR. There are many studies, especially on the relationship between NLR and epilepsy. NLR in epilepsy was found to be significantly higher in several studies compared to healthy controls.[25-27] On the other hand, studies with no significant difference were also published.[7,28,29] In our study, especially IG count and the NLR, and other immune response-related markers were not to be significant in differentiating epilepsy and PNES. In a study evaluating the potential use of leukocytosis in the differentiation of PNES and epileptic seizures, it was observed that leukocyte elevation started 2 hours after the event, and there was a significant difference in epilepsy to the PNES.[30] The fact that hemogram analyses were performed in the early phase of the seizure (within 2 hours of the seizure onset) in our study may have caused these results.

The CRP is one of the major acute phase reactants in humans,[31] and a significant difference was found in the epilepsy group only for the CRP test from the parameters in this study (p = 0.031). However, the results had low predictive properties (AUC: 0.64, sensitivity: 0.57, specificity: 0.73). Early sampling time (within 2 hours after the event) may have led to these findings, as expressed in the study mentioned above,[30] designed by dynamic blood collection after the seizure event.

To distinguish a generalized epileptic seizure from PNES, studies on parameters such as serum lactate level, prolactin level, postictal ammonia, and anion gap are available in the literature.[30,32-34] These tests also have some limitations. Lactate and ammonia levels should be analyzed at an early period of seizure and they are more expensive tests than the hemogram.[32,34] It has been shown that prolactin level can also increase after PNES.[35] In addition, prolactin analysis is not very useful in emergency conditions. However, the hemogram is rapid and inexpensive. Therefore, IG count, other hemogram parameters, and immune response-related markers (NLR, MLR, LMR, PLR, SII, and SIRI) that can be easily calculated from these parameters may be more beneficial for differential diagnosis. Consequently, further prospective studies with large sample sizes and well-designed according to the onset of the seizure time should be performed.

This study has some limitations. First, the diagnosis of epilepsy/PNES in all samples was made by clinical evaluation and EEG in the neurology clinic after generalized tonic-clonic seizures. In other words, the diagnosis of PNES was not confirmed by the gold standard video-EEG procedure. The other, there were no clear records in the hospital information management system regarding the seizure onset times of the patients. Blood tests analyzed immediately after the ED admission were performed only once. In addition, the fact that our sample size is small (moderate) is another limitation

of this study. In conclusion, further studies need to be conducted in which dynamic blood collection is performed by following the patients for at least 24 hours after the event.

CONCLUSION

In summary, this study aimed to differentiate epilepsy and PNES from a practical and rapid blood test, as video-EEG is not helpful in the ED conditions. Of the parameters we evaluated, only the CRP test was significant in the differential diagnosis. This significance supports the existence of neuroinflammation in epilepsy. However, other inflammatory biomarkers, especially the IG count, did not help in differentiating. Thus, further prospective studies with large samples evaluating the IG count and immune response-related markers (NLR, MLR, LMR, PLR, SII, and SIRI) are required. By determining an appropriate cut-off limit, clinical decision support can be provided to clinicians for the differential diagnosis of generalized seizures, especially in the ED.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Kastamonu University Clinical Researches Ethics Committee (Date: 07.05.2023, Decision No: 2023-KAEK-76)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Pallin DJ, Goldstein JN, Moussally JS, Pelletier AJ, Green AR, Camargo CA Jr. Seizure visits in US emergency departments: epidemiology and potential disparities in care. *Int J Emerg Med*. 2008;1(02):97-105.
2. Griffith NM, Szaflarski JP. Epidemiology and classification of psychogenic nonepileptic seizures. In: Gates JR, Rowan AJ, editors. Nonepileptic seizures. Cambridge: Cambridge University Press; 2010;3:16
3. Alsaadi TM, Marquez AV. Psychogenic Nonepileptic Seizures. *Am Fam Physician*. 2005;72(1):849-856.
4. Saxena S, Li S. Defeating epilepsy: a global public health commitment. *Epilepsia Open*. 2017;2(2):153-155.
5. Brown RJ, Trimble MR. Dissociative psychopathology, non-epileptic seizures, and neurology. *J Neurol Neurosurg Psychiatry*. 2000;69(3):285-289.
6. Karakas C, Modiano Y, Ness PCV, et al. Home video prediction of epileptic vs. nonepileptic seizures in US veterans. *Epilepsy Behav*. 2021;117:107811.
7. Morkavuk G, Koc G, Leventoglu A. Is the differential diagnosis of epilepsy and psychogenic nonepileptic seizures possible by assessing the neutrophil/lymphocyte ratio? *Epilepsy Behav*. 2021;116:107736. doi:10.1016/j.yebeh.2020.107736
8. Dey A, Kang Xu, Qiu J, Du Y, Jiang J. Anti-inflammatory small molecules to treat seizures and epilepsy: from bench to bedside. *Trends Pharmacol Sci*. 2016;37(6):463-484.
9. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. 2011;7(1):31-40.
10. Senthilnayagam B, Kumar T, Sukumaran J, M J, Rao K R. Automated measurement of immature granulocytes: performance characteristics and utility in routine clinical practice. *Patholog Res Int*. 2012;2012:483670. doi:10.1155/2012/483670
11. Ansari-Lari MA, Kickler TS, Borowitz MJ. Immature granulocyte measurement using the Sysmex XE-2100. Relationship to infection and sepsis. *Am J Clin Pathol*. 2003;120(5):795-799. doi:10.1309/LT30-BV9U-JJV9-CFHQ
12. Nigro KG, O'Riordan M, Molloy EJ, Walsh MC, Sandhaus LM. Performance of an automated immature granulocyte count as a predictor of neonatal sepsis. *Am J Clin Pathol*. 2005;123(4):618-624. doi:10.1309/73H7-K7UB-W816-PBJ
13. Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. *J Surg Oncol*. 2005;91(3):181-184.
14. Warimwe GM, Murungi LM, Kamuyu G, et al. The ratio of monocytes to lymphocytes in peripheral blood correlates with increased susceptibility to clinical malaria in Kenyan children. *PLoS One*. 2013;8(2):e57320. doi:10.1371/journal.pone.0057320
15. Merekoulias G, Alexopoulos EC, Belezos T, Panagiotopoulou E, Jelastopulu DM. Lymphocyte to monocyte ratio as a screening tool for influenza. *PLoS Curr*. 2010;2:RRN1154.
16. Smith RA, Bosonnet L, Ghaneh P, et al. The platelet-lymphocyte ratio improves the predictive value of serum CA19-9 levels in determining patient selection for staging laparoscopy in suspected periampullary cancer. *Surgery*. 2008;143(5):658-666.
17. Hu B, Yang XR, Xu Y, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. *Clin Cancer Res*. 2014;20(23):6212-6222.
18. Jia CP, Chen H, Sun B. *Zhonghua Wai Ke Za Zhi*. 2019;57(11):862-865. doi:10.3760/cma.j.issn.0529-5815.2019.11.013
19. Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and brain inflammation. *Exp Neurol*. 2013;244:11-21.
20. Vieira ÉLM, de Oliveira GNM, Lessa JMK, et al. Peripheral leukocyte profile in people with temporal lobe epilepsy reflects the associated proinflammatory state. *Brain Behav Immun*. 2016;53:123-130. doi:10.1016/j.bbi.2015.11.016
21. Terrone G, Balosso S, Pauletti A, Ravizza T, Vezzani A. Inflammation and reactive oxygen species as disease modifiers in epilepsy. *Neuropharmacology*. 2020;167:107742. doi:10.1016/j.neuropharm.2019.107742
22. Korkut M, Bedel C, Zortuk Ö, Selvi F. Can immature granulocytes indicate mortality in patients with acute ischemic stroke? *Neurology Asia*. 2021;26(4):665-670.
23. Bedel C, Korkut M, Selvi F, Zortuk Ö. The immature granulocyte count is a new predictor of the 30-day mortality in intracerebral haemorrhage patients: preliminary study. *Indian Journal of Neurosurgery*. 2021;10(02):114-120.
24. Kocatürk İ, Gülten S. Immature granulocyte and other markers in prediction of mortality in spontaneous intracerebral hemorrhage. *Journal of Health Sciences and Medicine*. 2023;6(2):368-373.
25. Aslan A, Cevik E. "Critical Analysis of Blood Parameters in Epilepsy Patients" 2020;11:193-196.
26. Güneş M, Büyükgöl H. Relationship between generalized epileptic seizure and neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and neutrophil mediated inflammation. *Int J Neurosci*. 2020;130(11):1095-1100.
27. Faruk Ozdemir A, Kemerdere R, Orhan B, et al. Serum endocan and preoperative systemic inflammatory markers in patients with epilepsy. *Neurochirurgie*. 2020;66(1):29-35.
28. Özdemir HH, Akil E, Acar A, et al. Changes in serum albumin levels and neutrophil-lymphocyte ratio in patients with convulsive status epilepticus. *Int J Neurosci*. 2017;127(5):417-420.
29. Eroglu T, Turkoglu SA, Bolac ES, Yildiz S, Yildiz N. Hemogram parameters in epilepsy may be indicators of chronic inflammation and hypoxemia. *J Neurol Clin Neurosci*. 2017;1(1):17-20.
30. Li Y, Matzka L, Flahive J, Weber D. Potential use of leukocytosis and anion gap elevation in differentiating psychogenic nonepileptic seizures from epileptic seizures. *Epilepsia Open*. 2019;4(1):210-215.
31. Kushner I. The phenomenon of the acute phase response. *Ann NY Acad Sci*. 1982;389:39–48.
32. Doğan EA, Ünal A, Ünal A, Erdoğan Ç. Clinical utility of serum lactate levels for differential diagnosis of generalized tonic-clonic seizures from psychogenic nonepileptic seizures and syncope. *Epilepsy Behav*. 2017;75:13-17.
33. Shah AK, Shein N, Fuerst D, Yangala R, Shah J, Watson C. Peripheral WBC count and serum prolactin level in various seizure types and nonepileptic events. *Epilepsia*. 2001;42(11):1472-1475.
34. Albadareen R, Gronseth G, Landazuri P, He J, Hammond N, Uysal U. Postictal ammonia as a biomarker for electrographic convulsive seizures: a prospective study. *Epilepsia*. 2016;57(8):1221-1227.
35. Alving J. Serum prolactin levels are elevated also after pseudo-epileptic seizures. *Seizure*. 1998;7(2):85-89.