GMJ 2024;35:286-290

DOI: http://dx.doi.org/10.12996/gmj.2023.3953

A Retrospective Cohort Study with Blood Parameters for Early Estimation of Multiple Sclerosis: Ratio Suggestion

Multiple Sklerozun Erken Tahmini için Bazı Kan Parametreleriyle Bir Retrospektif Kohort Çalışması: Bir Oran Önerisi

Esra Tekin[1], Sibel Canbaz Kabay[2], Ayşegül Küçük[1]

[1]Department of Physiology, Kütahya Health Sciences University Faculty of Medicine, Kütahya, Türkiye
[2]Department of Neurology, Kütahya Health Sciences University Faculty of Medicine, Kütahya, Türkiye

ABSTRACT

Objective: Multiple sclerosis (MS) is a non-traumatic neurological disease that often affects young adults and causes disability. Because there is no curative treatment that can provide full recovery, it is important to make an early diagnosis and slow the course of disease with current drugs. This study aimed to evaluate and compare some blood parameters and their ratios to each other that can provide an advantage in early diagnosis.

Methods: We compared changes in blood parameters and their ratios to each other between healthy controls (95) and MS patients (95). The MS group was evaluated in three periods: the new diagnosis period, in which patients did not use any medication for MS, the relapse period, and the 6-month attack-free remission period.

Results: The results revealed that the neutrophil-to-lymphocyte ratio (NLR) and erythrocyte-to-lymphocyte ratio increased in MS patients, especially during the attack period. It was remarkable that the erythrocyte-lymphocyte ratio was significantly higher than that in the control group in all three MS periods.

Conclusion: NLR is considered a neuroinflammation marker. It is argued that NLR reflects systemic inflammation better than neutrophil and lymphocyte counts alone. In addition, in our data, the ratio of erythrocytes to lymphocytes was significantly higher in MS patients. Currently, there is no effective laboratory marker for the diagnosis of MS. We have concluded that complete blood count parameters and their ratios to each other can be biomarkers that can provide early diagnosis of MS.

Keywords: Multiple sclerosis, blood biomarkers, neutrophil-to-lymphocyte ratio, erythrocyte-to-lymphocyte ratio

ÖZ

Amaç: Multiple skleroz (MS), sıklıkla genç yetişkinleri etkileyen ve yeti kaybına neden olan travmatik olmayan bir nörolojik hastalıktır. Tam iyileşmeyi sağlayacak küratif bir tedavi olmadığı için erken tanı koymak ve mevcut ilaçlarla hastalığın seyrini yavaşlatmak önemlidir. Erken tanıda avantaj sağlayabilecek bazı kan parametrelerinin ve birbirlerine oranlarının değerlendirilmesi ve karşılaştırılması amaçlandı.

Yöntemler: Sağlıklı kontroller (95) ve MS hastaları (95) arasında kan parametrelerindeki değişiklikleri ve bunların birbirlerine oranlarını karşılaştırdık. MS grubu, MS için herhangi bir ilaç kullanılmayan yeni tanı dönemi, atak dönemi ve 6 aylık ataksız remisyon dönemi olmak üzere 3 dönemde değerlendirildi.

Bulgular: Sonuçlar, MS hastalarında özellikle atak döneminde nötrofil-lenfosit oranının (NLR) ve eritrosit-lenfosit oranının arttığını ortaya koydu. Üç MS döneminin tamamında eritrosit-lenfosit oranının kontrol grubuna göre anlamlı olarak yüksek olması dikkat çekiciydi.

Sonuç: NLR, bir nöroenflamasyon belirteci olarak kabul edilmektedir. NLR'nin sistemik enflamasyonu tek başına nötrofil ve lenfosit sayımlarından daha iyi yansıttığı düşünülmektedir. Ayrıca verilerimizde MS hastalarında eritrosit-lenfosit oranı anlamlı olarak daha yüksekti. MS tanısı için henüz etkili bir laboratuvar belirteci bulunmamaktadır. Bu çalışma sonucunda tam kan sayımı parametrelerinin ve birbirlerine olan oranlarının MS için erken tanı sağlayabilecek biyobelirteç olma potansiyeline sahip olduğu kanısına vardık.

Anahtar Sözcükler: Multiple skleroz, kan biyobelirteçleri, nötrofil-lenfosit oranı, eritrosit-lenfosit oranı

Address for Correspondence/Yazışma Adresi: Esra Tekin, MD, Department of Physiology, Kütahya Health Sciences University Faculty of Medicine, Kütahya, Türkiye
E-mail / E-posta: esratekin105@gmail.com
ORCID ID: orcid.org/0000-0002-9684-3277

Received/Geliş Tarihi: 27.07.2023
Accepted/Kabul Tarihi: 27.09.2023

INTRODUCTION

Multiple sclerosis (MS) is a central nervous system disease that does not have a blood-based biomarker that can provide a diagnosis and causes disability especially by affecting young adults (1). Because there is no curative treatment, it is important to make an early diagnosis and slow the course of the disease with current drugs. Early diagnosis and treatment of MS are important to avoid permanent neurological damage (2).

MS causes visual disturbances (such as optic neuritis), brain stem and spinal cord damage, and cortical damage in patients. Patients usually consult a doctor for weakness in the extremities, loss of sensation, and vision problems (3). The worldwide increase in the incidence of MS, which can cause these serious problems in patients, brings along socioeconomic problems (4). Therefore, it is important to conduct studies for developing curative treatment, early diagnosis, and rapid initiation of treatment.

It is stated that various genes, inflammatory processes, virus infections, and various exposures may be effective among the possible causes of MS, but its exact etiology is still unknown (5). Systemic inflammation is thought to be a factor in MS as in many other diseases. Systemic inflammation, which causes chronic neurodegeneration and activates proinflammatory cytokines and the immune system, also plays an important role in MS (6).

The ratios of complete blood count parameters to each other, especially the neutrophil-to-lymphocyte ratio (NLR), are being investigated for use in the early and easy diagnosis of various diseases (7,8). It has been argued that the NLR, which is considered a neuroinflammation marker, reflects systemic inflammation better than neutrophil and lymphocyte counts alone (9). In addition, different blood parameters such as platelet-to-lymphocyte ratio or monocyte-to-lymphocyte ratio have been tested as MS diagnostic markers (10,11). We suggest that the red blood cell (RBC) to lymphocyte ratio can be used to predict MS, and various other ratios.

For MS, which is difficult to diagnose, blood parameters that may be associated with the disease can be scanned during medical examination, and patients who are deemed necessary can be directed for further investigations. The use of these parameters may be beneficial in the referral of people with clinical symptoms of MS, who are at risk and suspected of the disease, for further examinations without wasting time. This study aimed to evaluate the suitability of blood parameters that can be used for the diagnosis of MS.

MATERIALS AND METHODS

Data Source

The blood test results of patients who were treated at Kütahya Health Sciences University, Evliya Çelebi Training and Research Hospital between 1 December 2017 and 1 December 2022 were retrospectively scanned for this study. Ethical approval for this study was obtained from the Ethics Committee of Non-Interventional Clinical Researches of Kütahya Health Sciences University Faculty of Medicine (approval number: 2022/07-05, date: 22.06.2022). In this study, which was conducted as a retrospective archive investigation, informed consent was not required. The individuals included in the study are described in Figure 1. This study was conducted in accordance with the Declaration of Helsinki.

Serum iron, hemoglobin, hematocrit, red cell distribution width (RDW), white blood cell (WBC), RBC, neutrophil, lymphocyte, NLR, RBC/lymphocyte, and RBC/neutrophil values of patients and healthy controls were investigated. Blood parameters were determined in the hospital laboratory using an AutoAnalyzer.

Study Cohort

Ninety-five healthy controls were included in this study. While selecting the control group, attention was paid to the fact that the individuals were between the ages of 20 and 70 years, had no MS diagnosis, and had no diagnosis of chronic heart, liver, and kidney disease or malignancy. The MS group included 95 patients aged between 20 and 70 years with a diagnosis of MS. The control group was formed to match the MS group in terms of age and gender (Table 1). MS group data were evaluated in three periods: the new diagnosis period, when the patients did not use any medication for MS (naive group), the relapse period, and the remission period without an attack for 6 months. The data of the same 95 MS patients included in the group during the drug-free, attack, and remission periods were compared with each other and with the healthy controls.

Statistical Analysis

Statistical analysis was performed using the Statistical Package of the Social Science (SPSS) program with version 20 (IBM SPSS Corp.; Armonk, NY, USA). Quantitative data are given as mean ± standard deviation. The conformity of the data to the normal distribution was determined by the Kolmogorov-Smirnov test. An unpaired t-test was used for normally distributed variables. The Mann-Whitney U test was used for variables that did not show normal distribution. The Wilcoxon test was used for pairwise comparisons of MS groups. P-value <0.05 was considered statistically significant.

RESULTS

Hemoglobin, hematocrit, and RBC values were found to be higher in the MS patient groups ($p<0.05$). Serum iron was significantly higher in the MS-remission group than in the control group ($p<0.05$). The RDW value was found to be higher in the control group than MS groups ($p<0.05$). Lymphocyte counts were low in the MS groups, whereas neutrophil counts were lower in the control group than in the MS groups ($p<0.05$) (Table 2, 3).

There was no difference in the RBC/neutrophil ratio between the groups, whereas the NLR was higher in the MS-relapse and MS-naive groups than in the control group ($p<0.05$). The RBC/lymphocyte ratio was found to be higher in all MS groups than in the control group ($p<0.05$) (Table 4).

In the comparison of MS groups within themselves, neutrophil and WBC counts were lower in the MS-remission group than in the relapse and naive groups. The lymphocyte count was higher in the MS-remission group than in the other groups and close to the control group ($p<0.05$). When the ratios were examined, while the NLR did not show a difference compared with the control in the MS-remission group, the RBC/Lymphocyte ratio had a significant difference ($p<0.05$) (Table 4).

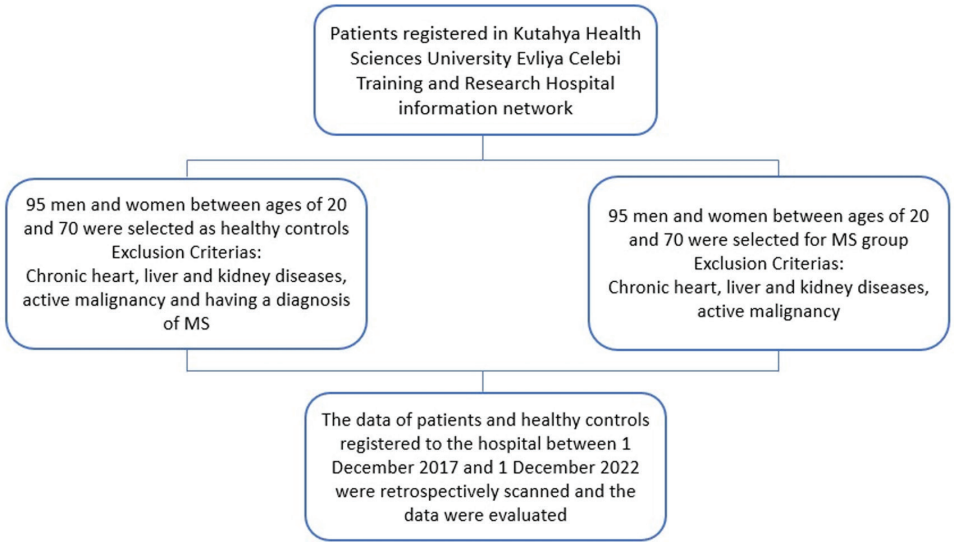

Figure 1. The workflow of this retrospective cohort study and the inclusion and exclusion of individuals.

Table 1. Age and sex characteristics

	MS group	Control group
Age*	37 (22-69) years	35 (21-65) years
Gender (male/female)	33/62	35/60

*Average value and bottom-top values, MS: Multiple sclerosis.

Table 2. Serum Fe, hemoglobin, hematocrit, and RDW levels

	Serum Fe	Hemoglobin	Hematocrit	RDW
Control	73.25±32.7	13.29±1.7	39.32±4.7	14.09±1.9
MS-naive	80.15±36.6	16.4±1.8*	41.61±4.6*	13.74±1.6*
MS-relapse	76.8±32.4*	14.01±1.7*,**	41.57±4.4*	13.68±1.6*
MS-remmission	83.16±36.5*	14.16±1.8*	41.92±4.8*	13.9±1.7

Data are given as mean ± standard deviation. *Compared to control group, p<0.05, **Compared to MS-naive group, p<0.05, MS: Multiple sclerosis, RDW: Red cell distribution width.

DISCUSSION

MS is a chronic autoimmune disease that may cause persistent neurological disabilities due to inflammation of the myelin sheath around the nerves (12). When MS is diagnosed, the disease may progress at different severities in patients. The prognosis varies depending on the type of MS and personal factors (13). Although there are drugs that show positive effects in the treatment of MS, there is no curative treatment yet (14). Therefore, it is important to diagnose and start treatment before permanent neurological damage occurs. Easily accessible and inexpensive blood parameters and their ratios can be used for the diagnosis of MS.

While a study found higher RBC levels in MS (15), a group of studies found unchanged RBC levels in MS (16,17). According to these studies, RBC and RBC-related blood parameters may increase in MS, but they do not tend to decrease (15-17). Similarly, in our study, hemoglobin, hematocrit, serum iron, and RBC levels were found to be higher in the MS groups. However, studies have indicated that the functions of erythrocytes may be insufficient and that erythrocyte deformability may decrease in MS patients (18-20).

The elevation in RBC and RBC-related parameters in MS seems to be because erythrocyte count is less affected by neuroinflammation and neuromodulatory drug use than leukocytes. However, despite high RBC, hemoglobin, and hematocrit levels, it is thought that erythrocytes may be dysfunctional in MS. Anemia of chronic disease, for example, causes suppression in the production of erythrocytes and shortening of life span, but leads to moderate anemia (21). As a result, we believe that the presence of inflammation impairs the functions of erythrocytes rather than reducing their number.

In addition, the fact that MS patients consult a doctor and perform tests more frequently compared with age- and gender-matched controls may have prevented possible anemia. Similarly, it is thought that before the diagnosis is made, there are frequent doctor visits in line with the complaints of MS patients.

Compared with leukocyte counts, erythrocyte counts are less affected by neuroinflammation caused by MS (22,23). In addition, neuromodulatory drugs used by MS patients may cause changes in the number of leukocyte types (23,24). Consistent with the higher RBC, hemoglobin, hematocrit, and serum Fe values in the MS groups, the RDW value was also higher in the control group.

In our data, the number of lymphocytes from leukocytes was low in the MS groups, whereas the number of neutrophils was high in the MS-naive and MS-relapse groups. However, the neutrophil count was similar to the control group in the MS-remission group. Therefore, NLR was found to be significantly higher in the MS-naive and MS-relapse groups than in the control group, while there was no

Table 3. WBC, RBC, neutrophils, and lymphocytes

	WBC	RBC	Neutrophil	Lymphocyte
Control	7.46±2.5	4.57±0.6	4.81±2.2	2.1±1
MS-naive	7.44±3.4	4.84±0.4*	4.94±2.9	1.79±0.8*
MS-relapse	7.65±3.4	4.81±0.4*	5.23±2.9	1.72±0.8*
MS-remmission	6.77±2.2*,**,***	4.86±0.3*	4.21±1.6*,**,***	1.94±0.7**

Data are presented as mean ± standard deviation. *Compared with control group, p<0.05, **Compared with MS-naive group, p<0.05. ***Compared with MS-relapse group, p<0.05. MS: Multiple sclerosis, WBC: White blood cell, RBC: Red blood cell.

Table 4. The ratios

	NLR	RBC/lymphocyte	RBC/neutrophil
Control	2.9±1.6	2.49±1	1.13±0.4
MS-naive	3.73±2.2*	3.8±1.6*	1.12±0.4
MS-relapse	4.22±2.1*	3.92±2.2*	1.1±0.3
MS-remmission	2.64±1.6	3.14±2*	1.29±0.4

Data are presented as mean ± standard deviation. *Compared with control group, p<0.05 MS: Multiple sclerosis, NLR: Neutrophil lymphocyte ratio, RBC: Red blood cell.

difference in the MS-remission group from the control. NLR is a marker recommended for use in various diseases to show inflammation (25-27). In our data, NLR was found to be higher in the MS-naive and MS-relapse groups. However, the lack of significant difference in the remission group suggests that it may not be sufficient for the diagnosis of MS patients who have not been diagnosed yet and who are not in the attack phase. In addition to neuroinflammation, the drugs used by MS patients change the number of neutrophils and lymphocyte leukocytes depending on the active substance of the drug. Therefore, it cannot be seen as a reliable biomarker that can make a definitive diagnosis (7,28). As a matter of fact, in various studies in the literature, no significant difference was found between the MS and control groups in terms of NLR (7). These differences may be because the patients are in an attack or remission period or the type of drug they use. However, the high NLR in MS patients seems to be due to a decrease in the lymphocyte count rather than an increase in the neutrophil count. Therefore, we suggest that the ratio of preserved erythrocytes to lymphocytes, which generally decreases in MS patients, although they are not functional, may be a more useful indicator.

Because the RBC/Lymphocyte ratio is both easily accessible and applicable and can be obtained in a minimally invasive way by venous blood collection, it may be beneficial for people with suspected MS. In people with symptoms suggestive of MS, the evaluation of this rate in primary health care institutions and referral for further evaluations if it is found to be high may facilitate the diagnosis. Studies should be carried out to be able to call the found value high and to determine the alarm value.

Conclusion

In this retrospective study, blood-based parameters and their ratios to each other were analyzed and evaluated to facilitate the diagnosis of MS. It was found that the MS groups at different periods differed from the control in various blood parameters. It was concluded that RBC/Lymphocyte, which can be used as a marker, may be more reliable for MS prediction than NLR. However, further studies should be conducted to determine at what value the RBC/Lymphocyte ratio should be referred to a neurologist for MS evaluations. In addition, the potential of this ratio to provide differential diagnosis of MS with other diseases and its specificity and sensitivity as a biomarker should be evaluated. The possible effects on post-diagnosis treatment strategies should also be determined by further studies.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Ethics Committee of Non-Interventional Clinical Researches of Kütahya Health Sciences University Faculty of Medicine (approval number: 2022/07-05, date: 22.06.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: E.T., S.C.K., A.K., Design: E.T., S.C.K., A.K., Supervision: E.T., S.C.K., A.K., Resources: S.C.K., Materials: S.C.K., Data Collection or Processing: E.T., Analysis or Interpretation: E.T., A.K., Literature Search: E.T., A.K., Writing: E.T., A.K., Critical Review: E.T., S.C.K., A.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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

REFERENCES

1. Oh J, Vidal-Jordana A, Montalban X. Multiple sclerosis: clinical aspects. Curr Opin Neurol. 2018; 31: 752-9.
2. Lavorgna L, Borriello G, Esposito S, Abbadessa G, Capuano R, De Giglio L, et al. Impact of early diagnosis on clinical characteristics of an Italian sample of people with multiple sclerosis recruited online. Mult Scler Relat Disord. 2019; 27: 239-46.
3. Crayton HJ, Rossman HS. Managing the symptoms of multiple sclerosis: a multimodal approach. Clin Ther. 2006;28:445-60.
4. Dobson R, Giovannoni G. Multiple sclerosis - a review. Eur J Neurol. 2019; 26: 27-40.

5. Ward M, Goldman MD. Epidemiology and Pathophysiology of Multiple Sclerosis. Continuum (Minneap Minn). 2022; 28: 988-1005.
6. Voet S, Prinz M, van Loo G. Microglia in Central Nervous System Inflammation and Multiple Sclerosis Pathology. Trends in Molecular Medicine. 2019; 25; 112-23.
7. Gelibter S, Pisa M, Croese T, Dalla Costa G, Orrico M, Preziosa P, et al. Neutrophil-to-lymphocyte ratio: a marker of neuro-inflammation in multiple sclerosis? J Neurol. 2021; 268: 717-23.
8. Atılgan CÜ, Şendül SY, Kösekahya P, Çağlayan M, Alkan A, Güven D, et al. Evaluation of Neutrophil-to-Lymphocyte Ratio and Mean Platelet Volume in Patients with Active and Inactive Thyroid Orbitopathy. Sisli Etfal Hastan Tip Bul. 2018; 52: 26-30.
9. Hasselbalch IC, Søndergaard HB, Koch-Henriksen N, Olsson A, Ullum H, Sellebjerg F, et al. The neutrophil-to-lymphocyte ratio is associated with multiple sclerosis. Mult Scler J Exp Transl Clin. 2018; 4: 2055217318813183.
10. Carnero Contentti E, López PA, Criniti J, Pettinicchi JP, Cristiano E, Patrucco L, et al. Platelet-to-lymphocyte ratio differs between MS and NMOSD at disease onset and predict disability. Mult Scler Relat Disord. 2022; 58: 103507.
11. Huang WC, Lin HC, Yang YH, Hsu CW, Chen NC, Tsai WC, et al. Neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio are associated with a 2-year relapse in patients with multiple sclerosis. Mult Scler Relat Disord. 2022; 58: 103514.
12. Marcus R. What Is Multiple Sclerosis? JAMA. 2022; 328: 2078.
13. Cortese R, Giorgio A, Severa G, De Stefano N. MRI Prognostic Factors in Multiple Sclerosis, Neuromyelitis Optica Spectrum Disorder, and Myelin Oligodendrocyte Antibody Disease. Front Neurol. 2021; 12: 679881.
14. Doshi A, Chataway J. Multiple sclerosis, a treatable disease. Clinical Medicine (London, England). 2016;16: s53-9.
15. Akaishi T, Misu T, Fujihara K, Nakaya N, Nakamura T, Kogure M, et al. White blood cell count profiles in multiple sclerosis during attacks before the initiation of acute and chronic treatments. Sci Rep. 2021; 11: 22357.
16. Kasprzycka W, Nieśpiałowska M, Jakubowska-Solarska. Blood count parameters in the course of multiple sclerosis. Journal of Transfusion Medicine. 2019; 12: 117-23.
17. Hon GM, Hassan MS, Rensburg RS, Erasmus RT, Matsha T. The hematological profile of patients with multiple sclerosis. Open Journal of Modern Neurosurgery. 2012; 2: 36-44.
18. Geiger M, Hayter E, Martin RS, Spence D. Red blood cells in type 1 diabetes and multiple sclerosis and technologies to measure their emerging roles. J Transl Autoimmun. 2022; 5: 100161.
19. Groen K, Maltby VE, Sanders KA, Scott RJ, Tajouri L, Lechner-Scott J. Erythrocytes in multiple sclerosis - forgotten contributors to the pathophysiology? Mult Scler J Exp Transl Clin. 2016; 2: 2055217316649981.
20. Simpson LO, Shand BI, Olds RJ, Larking PW, Arnott MJ. Red cell and hemorheological changes in multiple sclerosis. Pathology. 1987; 19: 51-5.
21. Ganz T. Molecular pathogenesis of anemia of chronic disease. Pediatr Blood Cancer. 2006; 46: 554-7.
22. Chmielewski PP, Strzelec B. Elevated leukocyte count as a harbinger of systemic inflammation, disease progression, and poor prognosis: a review. Folia Morphol (Warsz). 2018; 77: 171-8.
23. Schweitzer F, Laurent S, Fink GR, Barnett MH, Hartung HP, Warnke C. Effects of disease-modifying therapy on peripheral leukocytes in patients with multiple sclerosis. J Neurol. 2021; 268: 2379-89.
24. Manni A, Iaffaldano A, Lucisano G, D'Onghia M, Mezzapesa DM, Felica V, et al. Lymphocyte Count and Body Mass Index as Biomarkers of Early Treatment Response in a Multiple Sclerosis Dimethyl Fumarate-Treated Cohort. Front Immunol. 2019; 10: 1343.
25. Ustuntas G, Basat SU, Calik AN, Sivritepe R, Basat O. Relationship between Epicardial Fat Tissue Thickness and CRP and Neutrophil-Lymphocyte Ratio in Metabolic Syndrome Patients Over 65 Years. Sisli Etfal Hastan Tip Bul. 2021; 55: 405-11.
26. Qin B, Ma N, Tang Q, Wei T, Yang M, Fu H, et al. Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) were useful markers in assessment of inflammatory response and disease activity in SLE patients. Mod Rheumatol. 2016; 26: 372-6.
27. Bisgaard AK, Pihl-Jensen G, Frederiksen JL. The neutrophil-to-lymphocyte ratio as disease actvity marker in multiple sclerosis and optic neuritis. Mult Scler Relat Disord. 2017; 18: 213-7.
28. Dinoto A, Sartori A, Cheli M, Pasquin F, Baldini S, Bratina A, et al. Lymphopenia during treatment with dimethyl fumarate in patients with multiple sclerosis: Prevalence, predicting factors and clinical outcomes. Mult Scler Relat Disord. 2022; 57: 103357.