The predictive value of inflammatory biomarkers in the detection of multiple sclerosis attacks

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Abstract

Multiple sclerosis (MS) is the most prevalent immune-mediated inflammatory demyelinating central nervous system disorder, with a diverse set of clinical signs and symptoms. This study aimed to investigate the diagnostic values of the monocyte/lymphocyte ratio (MLR), red cell distribution width/lymphocyte ratio (RLR), and systemic immune-inflammation index (SII) in detecting multiple sclerosis attacks in patients with Relapsing-remitting MS (RRMS) presenting to the emergency department (ED). This retrospective observational study was conducted among patients with RRMS presenting to the ED of a third-level hospital. The laboratory parameters of 165 patients were compared during the attack and non-attack periods. The paired t-test statistic was used to compare means of inflammatory biomarker measurements between attack and non-attack groups. The neutrophil/lymphocyte ratio (NLR), MLR, RLR, and SII mean of the patients in the MS attack periods were higher than those in the non-attack period. The mean difference of NLR, MLR, RLR, and SII between both groups was 5.40±7.25, 0.37±0.43, 7.77±11.61, 1469.19±1978.88, respectively ($p<0.001$). In ROC analysis, NLR, RLR, MLR, and SII had excellent diagnostic power in detecting MS relapse (AUC: 0.87, 0.81, 0.86, and 0.87, respectively). According to our findings, SII, MLR, NLR, and RLR may be beneficial in confirming the diagnosis of attack in patients with RRMS.

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Introduction

Multiple sclerosis (MS) is the most prevalent immune-mediated inflammatory demyelinating central nervous system disorder, with a diverse set of clinical signs and symptoms.[1,2] MS exhibits significant heterogeneity in radiological and histopathological changes, clinical presentation, disease progression, and treatment response.[3] Regarding its pathogenesis, it has been suggested that the innate and adaptive immune system causes inflammation of a dynamic interaction between glia and neurons, disorders of the blood-brain barrier, demyelination, and neuroaxonal injury to the brain and spinal cord.[4,5]

Since MS patients admitted to the emergency department (ED) have variable clinical symptoms, it is very important to distinguish between those in the attack (exacerbation, relapse, episode) period and those who do not. In this process, inflammatory markers were needed in addition to clinical findings and cranial imaging. The differential count of white blood cells is extensively utilized as a biomarker of systemic inflammation and infection; the latest review article proposed that neutrophils and their phenotype could potentially be linked to the specific disease course of MS.[6–8] Observation of enlargement of CD15+ neutrophils in inactive Relapsing-remitting MS (RRMS) may be helpful for early diagnosis and determination of response to therapy.[6] It was also observed that granulocyte counts decreased in RRMS patients during the remission phase.[6]

The peripheral blood neutrophil-to-lymphocyte ratio (NLR) in autoimmune disease has recently been suggested as a potential,

inexpensive, and effective surrogate biomarker for systemic inflammatory status and, thus, disease activity.[8–12] The value of the monocyte-to-lymphocyte ratio (MLR), red cell distribution width (RDW) to lymphocyte ratio (RLR), and systemic immune inflammation index (SII) as a marker of disease activity in patients with RRMS is unknown. The aim of this study was to investigate the diagnostic values of MLR, RLR, and SII in detecting an MS attack in a cohort of RRMS patients.

Materials and Methods

Participants

In this study, 223 patients with RRMS who were admitted to the emergency department of a third-level hospital between January 2016 and August 2022 were examined. Some patients examined are untreated, and some receive first-line treatment (interferon-beta, sphingosine-1-phosphate inhibitors). However, we do not have any patients who received second-line treatment (antiCd20, natalizumab, cladribine). One hundred sixty-five patients aged 18 years and over who were diagnosed with RRMS according to the 2017 McDonald criteria and were in the MS attack period were involved in the study.[13] An MS attack was defined as a monophasic clinical episode with patient-reported symptoms and objective signs, developing acutely or subacutely in the CNS, lasting at least 24 hours, and reflecting a focal or multifocal inflammatory demyelinating event in the absence of fever or infection.[13] These symptoms and signs were optic neuritis, ophthalmoplegia, focal supratentorial syndrome, focal brainstem or cerebellar syndrome, myelopathy, encephalopathy, headache, altered consciousness, meningismus, or isolated fatigue.[14] Ten patients were excluded for lack of data, 20 due to steroid use within 30 days or recent infection (≤1 month), and 28 for other reasons (stressful co-occurring events in the past six months (*e.g.*, traumatic bone fractures), tumor history, pregnancy, autoimmune comorbidities (rheumatoid arthritis, psoriasis, sjögren's syndrome, etc.; Figure 1).

Study design and settings

This study was conducted according to a retrospective observational study design. The study was performed retrospectively after approval by the Ethics Committee of the Istanbul Prof Dr. Cemil Tascioglu City Hospital (protocol code: 240, decision number: 240, issue: E-48670771-020 date: 08 August 2022). The present study was conducted in line with the Declaration of Helsinki.

Study protocol

The laboratory parameters of these patients were compared in the emergency service admissions during the attack and non-attack periods. A neurologist evaluated the MS attack status. After the evaluation, patients with MS attacks were included in the study consecutively (Attack period). The same patients were compared to the emergency service applications in the attack-free period (Non-attack period). Two independent observers reviewed the data, and patients were selected based on eligibility criteria. Laboratory tests of patients with and without attacks were evaluated within 60 minutes after admission to the ED. Hematological and biochemical tests taken from the patients were recorded. NLR, MLR, RLR, and SII ratios were calculated individually. SII is computed by multiplying platelet count by NLR.[15]

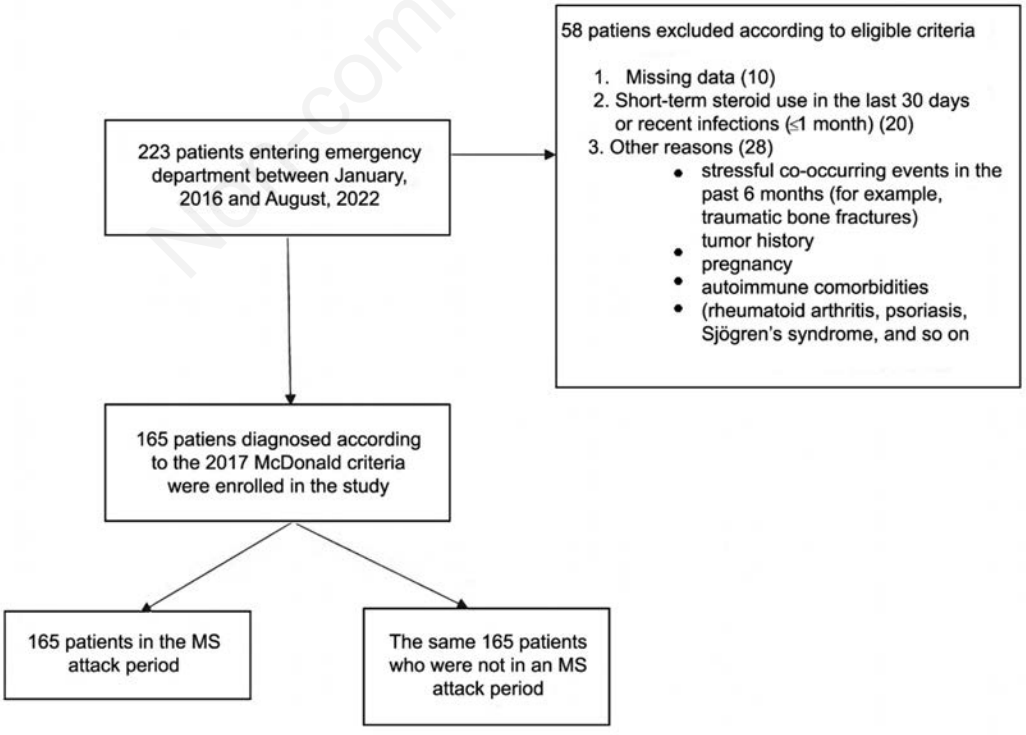

Figure 1. Patients' selection flow chart. MS, multiple sclerosis.

Power analysis

According to the quasi-experimental research design to determine the difference in the mean measurement values of RLR, NLR, MLR, and SII in patients with RRMS who came to the emergency department during the attack and non-attack periods; the number of patients to be included in the study was determined as 165, with an effect size of 0.2 (minimum accepted clinical significance), a maximum type 1 error of 5%, and a minimum power of 80%.

Statistical analysis

According to the central limit theorem, continuous measurements (such as hematological data) should test whether the means are normally distributed, not the data.[16] This study's standard deviations were not higher than the mean for continuous measurements. Therefore, this theory was found suitable, and parametric tests were used. The minimum and maximum values of the variables, as well as the mean and standard deviation, were used in the data analysis to perform the statistics on the continuous data. Frequency and percentage values were used to identify the categorical data. The mean of inflammatory biomarker measurements between attack and non-attack groups were compared using the paired t-test statistic. The receiver operating characteristic (ROC) analysis was used to ascertain the cut-off point in diagnostic value measurements. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) statistics were used to identify statistical significance. AUC values between 0.5 and 0.6 were evaluated as poor, between 0.6 and 0.7 as fair, between 0.7 and 0.8 as acceptable, between 0.8 and 0.9 as excellent, and above 0.9 as outstanding. The level of statistical significance of the data is considered $p<0.05$. Data evaluation and study power analysis were performed using the www.e-picos.com New York software and the MedCalc statistical package program.

Results

A total of 165 patients, 106 female (64.2%), were included in our study. The mean age of the patients was 39.3±11.7 years (Tables 1, 2).

The mean of NLR was 8.22±7.35 in patients with MS attack, and the mean of NLR of patients in the non-attack period was 2.81±1.65. The difference between the mean of NLR of both groups was 5.40±7.25 and was statistically significant ($p<0.001$; Table 3, Figure 2). The mean of MLR was 0.67±0.43 in patients with MS attack, and the mean of MLR of patients in the non-attack period was 0.30±0.17. The difference between the mean of MLR of both groups was 0.37±0.43 and was statistically significant ($p<0.001$; Table 3, Figure 2). The mean of RLR was 15.45±12.06

Table 1. Distribution of descriptive characteristics (n=165).

Characteristics	Groups	Count (n)	Percent (%)
Sex	Female	106	64.2
	Male	59	35.8

Table 2. Evaluation of the difference in biochemistry and hemogram parameters between attack and non-attack periods (n=165).

Characteristics	Min-Max	Median	Mean ±S.D
Age	19-69	38	39.3±11.7

Min, minimum; Max, maximum; S.D, standard deviation.

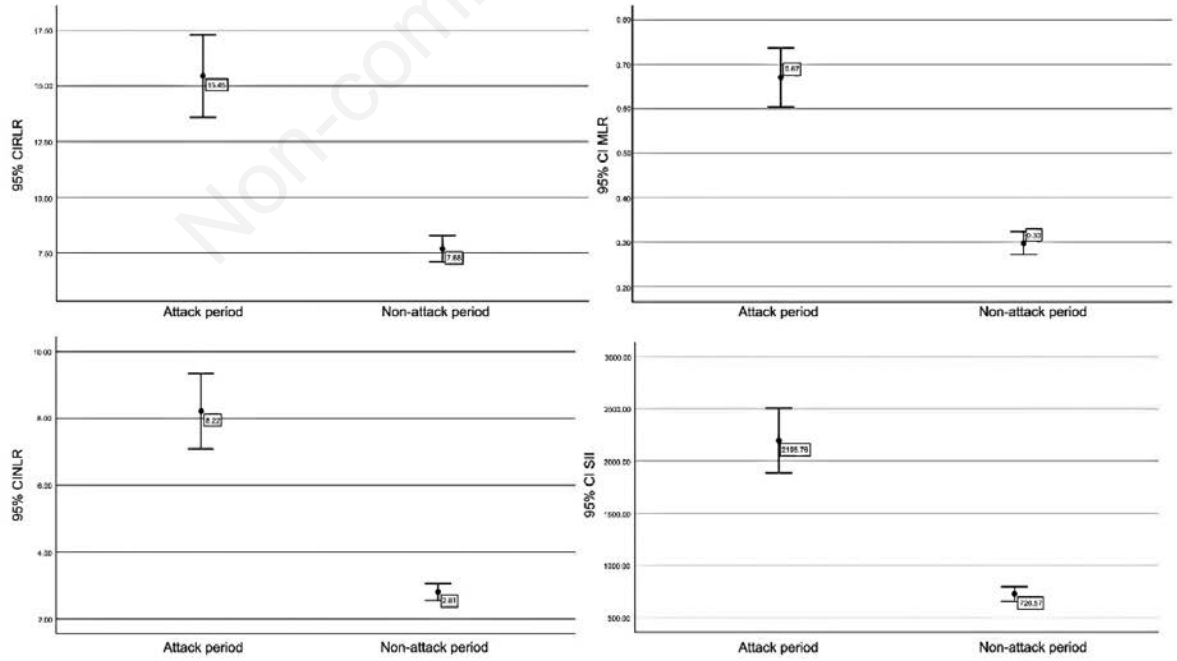

Figure 2. The mean difference of NLR, MLR, RLR and SII between MS attack and non-attack groups. CI, confidence interval; MS, multiple sclerosis; RLR, red blood cell distribution width to lymphocyte ratio; MLR, monocyte to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; SII, systemic immune inflammation index.

in patients with MS attack, and the mean of RLR of patients in the non-attack period was 7.68±3.88. The difference between the mean of RLR of both groups was 7.77±11.61 and was statistically significant (p<0.001; Table 3, Figure 2). The mean of SII was 2195.76±2011.06 in patients with MS attack, and the mean of SII of patients in the non-attack period was 726.57±457.92. The difference between the mean of SII of both groups was 1469.19±1978.88 and was statistically significant (p<0.001; Table 3, Figure 2).

There was a statistically significant difference in the mean of white blood cells, RDW, platelets, neutrophils, lymphocytes, monocytes, and eosinophils between the attack and non-attack groups (Table 3).

While there was a statistically significant difference between the means of glucose, alanine aminotransferase, aspartate aminotransferase, and C-reactive protein in both groups, there was no statistically significant difference between the means of urea, creatinine, hemoglobin, and hematocrit (Table 3).

In ROC analysis, NLR, RLR, MLR, and SII had excellent diagnostic power in detecting MS relapse (AUC: 0.87, 0.81, 0.86, and 0.87, respectively; Table 4).

Discussion

It is very crucial to determine that patients with RRMS who applied to the emergency department are in the attack period. Although many biomarkers have been used for differential diagnosis, the search for the perfect biomarker continues. Although laboratory parameters obtained from peripheral blood are inexpensive and easily accessible, biomarkers obtained from cerebrospinal fluid (CSF) are more valuable in diagnostic terms. However, CSF analysis requires additional technical knowledge and is more costly. In addition, there is a risk of developing complications during the procedure[17] Therefore, low cost and easy to calculate hematological inflammatory biomarkers have gained prominence.

NLR, which represents the balance between neutrophil and lymphocyte levels, has been recently proposed as an informative

Table 3. Evaluation of the difference in biochemistry and hemogram parameters between attack and non-attack periods.

	Attack period (Mean ±S.D)	Non-attack period (Mean ±S.D)	Mean difference (Mean ±S.D)	95% Confidence interval of the difference	p
Glucose	108.55±27.99	100.37±23,51	8.17±28.74	3.76-12.59	<0.001
Urea	29.47±10.16	27.83±7.64	1.64±10.15	0.75-3.19	0.4
Creatininen	0.96±3.30	0.70±0.23	0.25±3.33	-0.26-0.76	0.33
ALT	28.87±30.63	19.37±14.02	9.46±30.83	4.76-14.24	<0.001
AST	28.38±22.29	20.95±10.17	7.42±20.74	4.24-10.61	<0.001
CRP	6.84±8.46	4.88±7.78	1.97±10.38	0.37-3.56	0.02
WBC	9.76±3.32	7.79±2.03	1.97±3.31	1.46-2.47	<0.001
HGB	12.98±1.73	13.08±1.71	-0.11±1.72	-0.37-1.62	0.45
HCT	39.09±4.78	39.34±4.65	-0.25±4.76	-0.98-0.48	0.5
PLT	270.25±57.66	258.88±56.08	11.37±57.29	2.56-20.18	0.01
RDW	14.06±1.51	13.47±1.01	0.58±1.26	0.39-0.78	<0.001
NEU	7.52±3.11	4.99±1.76	2.52±3.31	2.01-3.03	<0.001
LYM	1.26±0.61	2.11±0.85	-0.85±0.76	-0.96- (-0.72)	<0.001
MON	0.67±0.26	0.54±0.16	0.13±.025	0.09-0.17	<0.001
EOS	0.18±0.14	0.15±0.11	0.03±0.14	0.01-0.05	0.009
RLR	15.45±12.06	7.68±3.88	7.77±11.61	5.98-9.55	<0.001
MLR	0.67±0.43	0.30±0.17	0.37±0.43	0.31-0.44	<0.001
NLR	8.22±7.35	2.81±1.65	5.40±7.25	4.29-6.52	<0.001
SII	2195.76±2011.06	726.57±457.92	1469.19±1978.88	1165.01-1773.38	<0.001

Paired t test (p<0.05 significance); S.D, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; WBC, white blood cells; HGB, hemoglobin; HCT, hematocrit; PLT, platelets; RDW, red cell distribution width; NEU, neutrophil; LYM, lymphocyte; MON, monocyte; EOS, eosinophil; RLR, RDW to lymphocyte ratio; MLR, monocyte to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; SII, systemic immune inflammation index.

Table 4. Diagnostic accuracy of inflammatory parameters for differentiation of multiple sclerosis (MS) attack.

MS attack :169 MS non-attack :169	AUC	Cut-off	Sensitivity %	Specificity %	AUC 95% CI	p	PPV %	NPV%
NLR	0.87	>3.33	84.6	76.9	0.83-0.90	<0.001	78.6	83.3
RLR	0.81	>8.56	75.1	72.8	0.76-0.85	<0.001	73.4	74.5
MLR	0.86	>0.36	82.25	77.51	0.82-0.89	<0.001	78.5	81.84
SII	0.87	>807.92	87.6	70.4	0.83-0.90	<0.001	74.7	85.1

AUC, Area under curve; SE, Standard error; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; NLR, neutrophil to lymphocyte ratio; RDW, red cell distribution width; RLR, RDW to lymphocyte ratio; MLR, monocyte to lymphocyte ratio; SII, systemic immune inflammation index.

and non-invasive peripheral biomarker to determine systemic inflammatory status in various chronic inflammatory diseases.[18,19] In our study, we found higher NLR rates in patients with MS attacks. This could be due to an inflammatory process in MS, which accelerates during MS attacks. Bisgaarrd *et al*. discovered higher NLR in patients with optic neuritis and MS compared to the healthy control group. In addition, similar to our study, higher NLR was reported in patients in relapse than in patients in remission.[20] Demirci *et al.* revealed that NLR predicted disease activity with 0.68 AUC, 67% sensitivity and 97% specificity in MS.[11] In a retrospective study of RRMS patients at an MS center in Italy, a high NLR was found to be related to disease activity.[10] In this study, NLR was able to predict the active period in MS supporting the literature.

In MS pathogenesis, peripherally activated T-cells recruit a diverse array of myeloid cells, including monocytes/macrophages, to promote and drive an inflammatory reaction, often causing axonal transection and irreversible focal central nervous system (CNS) damage.[2] LMR has been demonstrated to be a marker of the systemic inflammatory response and a potential prognostic factor in a number of cancers.[21] In this study, the MLR was higher in the attack periods compared to the attack-free period may be the reflection of the inflammatory state in the central nervous system in MS to the peripheral immune status. In the study of Hemond *et al.* in MS patients, high MLR was significantly associated with physical disability status score (EDSS) and brain atrophy. That is, MLR was high in MS when clinical and neuroimaging were poor.[22] Similarly, in this study, MLR was high during the MS attack period when the clinic was exacerbated.

SII is a new inflammatory index that exhaustively demonstrates the host immune and inflammatory state balance.[23] A high SII score has been linked to poor outcomes in cancer patients, heart failure, and coronary artery disease.[15] However, the relationship between SII and MS attacks is unclear. This study found SII to be closely associated with disease activity. An all-encompassing inflammatory biomarker like SII elevated during an MS attack could be related to the peak of inflammatory activity during this time. In a recent study, inflammatory markers such as interleukin 4, parathormone, homocysteine, and interleukin 17 were associated with disability and disease activity in MS.[24]

RLR is one of the novel defined inflammatory indices. Wu *et al.* discovered that RLR has high sensitivity and specificity in predicting hepatic impairment in patients with the hepatitis E virus.[25] Meng *et al.* demonstrated that RLR could predict the severity of primary biliary cirrhosis due to its high diagnostic specificity.[26] The relationship between RLR and MS attack has not been addressed in any previous study. According to this study's results, RLR was observed to be higher in the MS attack period.

Limitations

The most critical limitation of our study is the small number of patients. The reason for this is the low frequency of MS disease in the community and, therefore, the low number of emergency department admissions. Another limitation of our study is that it was conducted retrospectively. However, we think that prospective studies planned in the long term will support the findings of our research. The study only included patients with RRMS from various MS types. Therefore, the results we found are valid only for RRMS. Moreover, since the EDSS score was not calculated in the study, the relationship between it and inflammatory markers could not be determined. In addition, the duration of the onset of MS attack at the time of being admitted to the emergency department is uncertain. Thus, it is unclear in which part of the attack period the hematological markers were obtained. The relapse status of the patients was evaluated clinically and history, and MRI lesions were not included in the study. The attack period status was not evaluated according to the type of treatment received by the patients. This issue may be investigated in future studies.

Conclusions

It is crucial in the emergency department to identify the patients with RRMS who are in the attack phase. Inflammatory markers contribute to this process. According to our findings, SII, MLR, NLR, and RLR may be beneficial in confirming the attack diagnosis in patients with RRMS who present to the emergency department. However, large-scale studies are required for more conclusive results.

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