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Serum endocan levels in multiple sclerosis relapse and remission

E. AKIL[1], R. ALP[2], M.U. ALUCLU[1], A. ACAR[1], İ. KAPLAN[3]

[1]Department of Neurology, Dicle University Medical School, Diyarbakır, Turkey
[2]Department of Neurology, Diyarbakır Gazi YaÐargil Training and Research Hospital, Diyarbakır, Turkey
[3]Department of Biochemistry, Dicle University Medical School, Diyarbakır, Turkey

Abstract. – OBJECTIVE: Endocan has been defined as an important marker of inflammatory diseases, vascular and endothelial injury, tumour progression, cell adhesion and angiogenesis. In our study, we compared the serum endocan, C-reactive protein (CRP) and neutrophil–lymphocyte ratio (NLR) levels of relapsing-remitting multiple sclerosis (RRMS) patients in remission and in relapse.

PATIENTS AND METHODS: This study included 53 RRMS remission patients, 30 RRMS relapse/post-relapse patients and 44 healthy volunteers. Blood samples were collected once from RRMS patients in remission and from the control group, and twice from RRMS relapse patients: once when relapsing and another 1 month after relapse. The endocan, CRP and NLR levels of the RRMS patients measured while in relapse, 1 month after relapse and while in remission were compared to those of the control group. The studied parameters were compared with the disease duration, relapse frequency, Expanded Disability Status Scale (EDSS) score, applied treatment and lesion burden assessed using magnetic resonance imaging (MRI).

RESULTS: The endocan, CRP and NLR levels were significantly higher in the RRMS group than in the control group ($p < 0.05$). The serum endocan levels were found to be significantly higher in the RRMS relapse group than in the post-relapse and control groups ($p < 0.05$). There were no significant correlations between the disease duration, EDSS score, relapse frequency and lesion burden on MRI and the endocan, CRP and NLR values ($p > 0.05$). According to the correlation analysis, there was a statistically strong positive relationship between the MRI lesion localisation and the EDSS score, disease duration and relapse frequency ($p < 0.001$).

CONCLUSIONS: Endocan increase is a marker of the endothelial injury that develops secondary to the inflammatory process in MS patients. It can thus be considered a moderately good indicator of relapse.

Key Words:
Relapsing-remitting multiple sclerosis, Endocan, C-reactive protein, Neutrophil-lymphocyte ratio.

Introduction

Multiple sclerosis (MS) is an autoimmune, inflammatory, and chronic disease with axonal injury and demyelination that is assumed to occur due to complex interactions between genetic and environmental factors. The mechanisms that may be important in the formation of MS plaques include autoimmunity, genetic predisposition, infections, environmental factors and incidental demyelination[1,2]. Increases in T cells, macrophages, and microglia cells and proinflammatory cytokines and chemokines in active lesions indicate local inflammation.

Two other important causes of MS are endothelial injury and vascular pathology. Endothelial microparticles (EMPs), which emerge as a result of inflammatory endothelial dysfunction and which have recently been determined to be measurable, have drawn clinical attention. EMPs are membranous vesicles released by inflamed endothelial cells in response to the activation of proinflammatory cytokines such as tumour necrosis factor alpha (TNFα) and interferon gamma. Secreted EMPs carry major measurable endothelial molecules such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and platelet endothelial cell adhesion molecule-1. In previous studies, a significant increase in the number of EMP–monocyte complexes, was found in MS relapse patients compared to MS patients in remission, and a correlation was found between this situation and contrast agent uptake on MRI[3,4]. In addition, this complex was found to increase the transendothelial migration rate.

Corresponding Author: Esref Akil, MD; e-mail: esrefakil@gmail.com

First defined as endothelial cell specific molecule-1 (ESM-1), endocan is a proteoglycan structurally composed of a single chain of dermatan sulfate, which is rich in cysteine[5]. Initially detected in endothelial cell cultures, endocan has been found to play a role in angiogenesis, carcinogenesis and lymphogenesis. Endocan is proangiogenic, like vascular endothelial growth factor and fibroblast growth factor, and is induced by proinflammatory molecules such as TNFα and interleukin-1β[6,7].

Recent investigations[8,9] have shown that endocan can be an important cell marker of inflammatory diseases and of pathologies associated with the endothelium, tumour progression, cell adhesion and angiogenesis. Endocan is secreted from the renal tubule, bronchus and vascular endothelial cells. The detection of high endocan levels in systemic inflammatory diseases and sepsis suggests that endocan can be used as a marker of endothelial cell dysfunction.

In recent years, it has been shown that inflammatory cytokines are triggered by immune mechanisms, which play a key role in the physiopathology of MS, and increase the endothelial injury and endothelial marker levels, which has led to the idea that endocan can be evaluated better in MS patients.

Patients and Methods

The participants in this study had been diagnosed with relapsing-remitting multiple sclerosis (RRMS) at the Neurology Clinic of Dicle University Hospital in Diyarbakır, Turkey, in accordance with the 2010 modified McDonald criteria, and fulfilled the other study inclusion criteria. Fifty-three of them were in remission and 30 were in relapse. Forty-four healthy volunteers who were compatible with this group in terms of age and gender were also enrolled in the study.

Inclusion Criteria

The study inclusion criteria were as follows:

1. A definite diagnosis of MS according to the 2010 modified McDonald criteria;
2. Relapsing-remitting clinical form of MS;
3. For relapse patients, an objective neurological deficit lasting at least 24 h and the presence of new plaques that held contrast in magnetic resonance imaging (MRI);
4. For relapse patients, absence of pseudo-attacks such as infection;
5. For patients in remission, absence of new objective neurological deficits and plaques on MRI in the previous 3 months; and
6. For all patients, non-reception of attack therapy in the previous 3 months.

Exclusion Criteria

The exclusion criteria were as follows:

1. Age under 18 or over 60;
2. Alcohol consumption or substance use;
3. For females, pregnant status or childbirth within the previous 6 months;
4. Presence of any oncological or neurological disease or a known systemic or autoimmune disease (diabetes mellitus, hypertension, chronic obstructive pulmonary disease, congestive heart failure, liver failure, chronic renal failure, hyperlipidemia, vasculitis, etc.);
5. Systemic infection within the previous month;
6. Surgical intervention within the previous month;
7. Refusal to sign the consent form;
8. For the control group, pathological neurological examination findings.

All the individuals who had been informed about the study and had signed the voluntary informed-consent form were included in the study. Approval for this study was obtained from the Clinical Research Ethics Committee of Dicle University Medical Faculty.

The age, gender, and Body Mass Index (BMI) of all the patients and healthy controls included in the study were determined as the main demographic characteristics. The disease-related variables were the MS duration, the number of relapses in the previous year, the Expanded Disability Status Scale (EDSS) score, the lesion burden based on the imaging performed in the previous year and the immunomodulatory or immunosuppressant therapy used. The patients were classified as those who were not treated with medication, those who used first-line immunomodulators or those who received oral therapy. The lesion burden on MRI was classified as 'two-region involvement', 'three-region involvement' or 'four-region involvement' on the basis of how many of the following regions were involved: the periventricular, juxtacortical, infratentorial and medulla spinalis regions. The complete blood count, C-reactive protein (CRP) levels and serum endocan levels of all the participants were studied in the Biochemistry Laboratory of Dicle University Medical

Faculty Hospital. Scans were performed using the Philips Achieva 1.5 Tesla MRI (Amsterdam, The Netherlands).

Commercial enzyme-linked immunosorbent assay kits (SunRed, Shanghai, China) were used to assay the endocan levels of the samples. As per the manufacturer's recommendation, ESM-1 pre-coated with a monoclonal antibody was added to the enzyme well plate and incubated. ESM-1 antibodies that had been biotinylated and combined with streptavidin-horseradish peroxidase enzyme were added to form the immune complex. Incubation and washing were carried out again to remove any uncombined enzymes. Chromogen solutions A and B were added, and the colour of the liquid changed to blue, and in response to the acid, yellow; the darkness of the colour was considered positively correlated with the ESM-1 ratio. The results are presented herein in the form of nanogram (ng)/ml.

The CRP levels of the samples were studied using IMMAGE 800 (Beckman Coulter, Brea, CA, USA) and the nephelometric method. A complete blood count of the samples was performed using the CELL-DYN Ruby device (Abbott, Chicago, IL, USA).

Statistical Analysis

All the obtained data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows version 21 software (SPSS Inc., Armonk, NY, USA). The data were presented as mean ± standard deviation (SD). The one-way analysis of variance, Mann–Whitney U and chi square tests were used to compare the study groups. The compliance of the variables with normal distribution was examined using visual and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk tests). The correlation coefficients and statistical significance were calculated using Spearman's correlation for the relationships between the variables, at least one of which was not normally distributed or was ordinal. For statistical significance, the type 1 error rate was set to 5%. The diagnostic decision-making features of the serum endocan levels for predicting MS relapse were examined via receiver operating characteristics curve analysis. In the presence of significant threshold values, the sensitivity, specificity, and positive and negative predictive values of these thresholds were calculated. In the evaluation of the area under the curve, the diagnostic value of the test was considered statistically significant in cases with a type 1 error rate below 52%.

Results

In total, 83 RRMS patients and 44 healthy volunteers who met the study inclusion criteria were included in the study. As can be seen in Table I, of the 83 patients in the RRMS group, 21 (25.4%) were men and 62 (74.6%) were women, and 53 were in remission (42 female, 79.8%; 11 male, 20.2%) while 30 were in relapse (10 male, 33.3%; 20 female, 66.6%). As for the control group, there were 9 men (20.5%) and 35 women (79.5%). There was no significant difference in gender distribution between the RRMS and control groups ($p = 0.364$ and $p > 0.05$, respectively). In terms of age, there was no significant difference either between the two groups (35.79 ± 10.50 and 35.88 ± 10.47, respectively; $p = 0.963$ and $p > 0.05$). Neither was there a significant difference between the groups in terms of BMI (23.79 ± 2.92 and 23.9 ± 3.53, respectively; $p > 0.05$).

Among the RRMS patients included in the study, 43 (51.8%) had two-region involvement on MRI, 25 (30.1%) had three-region involvement and 15 (18.1%) had four-region involvement. Nine patients (10.85%) did not use medication. Of the

Table I. Sociodemographic data and disease-defining features of the MS and control groups.

	RRMS (n = 83)	Control (n = 44)
Gender (M/F)	21 (25.4%)/62 (74.6%)	9 (20.5%)/35 (79.5%)
Age (years)	35.79 ± 10.50	35.88 ± 10.47
BMI	23.79 ± 2.92	23.9 ± 3.53
Disease duration (years)	5.33 ± 5.07	–
Relapse frequency	1.53 ± 0.73	
EDSS score	3.01 ± 1.70	

Note: Values were entered as mean ± standard deviation. MS: multiple sclerosis; RRMS: relapsing-remitting multiple sclerosis; M: male; F: female; BMI: body mass index; EDSS: Expanded Disability Status Scale.

Table II. Comparison of the endocan, CRP and NLR levels in the RRMS and control groups.

	RRMS (n = 53)	Control (n = 44)	*p*-value
Endocan	295.3 ± 220.7	225.83 ± 132.64	$p < 0.05$
CRP	0.74 ± 1.31	0.19 ± 0.21	$p = 0.007$
NLR	3.15 ± 2.40	2.01 ± 0.54	$p = 0.002$

CRP: C-reactive protein; NLR: neutrophil–lymphocyte ratio; RRMS: relapsing-remitting multiple sclerosis.

patients using medication, 59 (71.08%) were using first line immunomodulators and 15 (18.07%) were receiving oral therapy.

As shown in Table II, the endocan levels were higher in the RRMS group (295.3 ± 220.7) than in the control group (225.83 ± 132.64; $p < 0.005$). The CRP levels were also significantly higher in the RRMS group (0.74 ± 1.31) than in the control group (0.19 ± 0.21; $p = 0.007$). As for the neutrophil–lymphocyte ratio (NLR) levels, they were also found to be significantly higher in the RRMS group (3.15 ± 2.40) than in the control group (2.01 ± 0.54; $p = 0.002$).

As can be seen in Table III, the serum endocan levels were significantly higher in the relapse group (396.5 ± 260) than in the post-relapse and control groups (225.83 ± 132.64; $p < 0.05$). There were significant differences in the serum CRP and NLR levels between the relapse and control groups ($p < 0.05$).

As shown in Table IV, there were no significant differences between the serum endocan, CRP and NLR levels of the post-relapse and control groups ($p > 0.05$). Additionally, no significant differences were found between the serum endocan, CRP and NLR levels of the remission and control groups ($p > 0.05$).

Comparison of the Serum Endocan, CRP and NLR Levels in the RRMS Remission and Relapse Groups

As can be seen in Table V, the serum endocan levels in the relapse group (396.5 ± 260) were

Table III. Comparison of the endocan, CRP and NLR levels in the RRMS relapse and control groups.

	MS relapse (n = 30)	Control (n = 44)	*p*-value
Endocan	396.5 ± 260	225.83 ± 132.64	$p = 0.034$
CRP	0.99 ± 1.96	0.19 ± 0.21	$p = 0.007$
NLR	3.39 ± 2.86	2.01 ± 0.52	$p = 0.007$

CRP: C-reactive protein; NLR: neutrophil–lymphocyte ratio; RRMS: relapsing-remitting multiple sclerosis; MS: multiple sclerosis.

Table IV. Comparison of the endocan, CRP and NLR levels in the RRMS post-relapse and control groups.

	MS relapse (n = 30)	Control (n = 44)	*p*-value
Endocan	225.83 ± 132.64	232.3 ± 257	$p > 0.05$
CRP	0.19 ± 0.21	0.5 ± 0.7	$p > 0.05$
NLR	2.01 ± 0.52	3.1 ± 0.5	$p > 0.05$

CRP: C-reactive protein; NLR: neutrophil–lymphocyte ratio; RRMS: relapsing-remitting multiple sclerosis.

Table V. Comparison of the endocan, CRP and NLR levels in the RRMS relapse and remission groups.

	RRMS relapse (n = 30)	RRMS remission (n = 44)	*p*-value
Endocan	396.5 ± 260	271.3 ± 147.9	$p < 0.05$
CRP	0.99 ± 1.96	0.60 ± 0.7	$p > 0.05$
NLR	3.39 ± 2.86	3.0 ± 2.1	$p > 0.05$

CRP: C-reactive protein; NLR: neutrophil–lymphocyte ratio; RRMS: relapsing-remitting multiple sclerosis.

Table VI. Comparison of the disease parameters in the RRMS group.

	Disease duration	EDSS score	MRI lesion burden	Relapse frequency
Disease duration		$p < 0.01$	$p < 0.01$	$p < 0.05$
	r = 1	r = 694	r = 392	r = 229
EDSS score	$p < 0.01$		$p < 0.01$	$p < 0.01$
	r = 694	r = 1	r = 617	r = 489
Lesion burden on MRI	$p < 0.01$	$p < 0.01$		$p < 0.01$
	r = 392	r = 617	r = 1	r = 318
Relapse frequency	$p < 0.05$	$p < 0.01$	$p < 0.01$	
	r = 229	r = 489	r = 318	r = 1

Note: The values after the decimal point were written for the r results. RRMS: relapsing-remitting multiple sclerosis; EDSS: Expanded Disability Status Scale; MRI: magnetic resonance imaging; r: Spearman's rank correlation coefficient; *$p < 0.05$.

significantly higher than those in the remission group (271.3 ± 147.9; $p < 0.05$). There was no significant difference in the CRP levels between the remission group (0.60 ± 0.7) and the relapse group (0.99 ± 1.96; $p > 0.05$). There was also no significant difference in the NLR levels between the remission group (3.0 ± 2.1) and the relapse group (3.39 ± 2.86; $p > 0.05$).

Comparison of Disease-Defining Parameters with the Serum Endocan, C-Reactive Protein and Neutrophil-Lymphocyte Ratio Levels and Correlations in the Relapsing-Remitting Multiple Sclerosis Group

In terms of therapeutic agents, the patients were classified as being treated with interferon, glatiramer acetate or oral therapy. As can be seen in Table VI, no significant difference was found in terms of the endocan ($p = 0.6$), CRP ($p = 0.9$) and NLR ($p = 0.2$) levels. There was a statistically strong positive correlation between the MRI lesion localization and the EDSS score, disease duration and relapse frequency ($p < 0.01$). There was no significant correlation between the endocan, CRP and NLR levels in any of the groups ($p > 0.05$). In the RRMS group, there was a statistically weak positive correlation between the CRP and NLR levels (r = 407; $p = 0.03$). There was no significant difference in the number of relapses between the remission (1.6 ± 0.2) and relapse groups (1.4 ± 0.2) among the RRMS patients ($p = 0.196$). There was also no significant difference in the lesion burden on MRI between the remission and relapse groups among the RRMS patients ($p = 0.196$).

As shown in Table VII, when the cut-off value of the CRP level was determined as 0.28, it was found that it predicted RRMS relapse with 66.7% sensitivity and 61% specificity (AUC, 0.676 [0.572–0.781]; $p = 0.004$). When the cut-off value of the serum endocan level was determined as 259.9, it was found that it predicted RRMS relapse with 66.7% sensitivity and 66.9% specificity (AUC, 0.695 [0.594–0.797]; $p = 0.001$) (Figure 1).

Discussion

Our study was the first in the literature to investigate the endocan, CRP and NLR levels in patients with RRMS. In our study, the endocan, CRP and NLR levels of RRMS patients measured during the relapse period, 1 month after relapse and during the remission period were compared with those of the healthy control group. The endocan, CRP and NLR levels were found to be

Table VII. ROC curve analysis of the CRP and endocan levels in the prediction of RRMS relapse.

	Cut-off value	Sensitivity	Specificity	AUC	*p*-value
Endocan	259.9	66.7	66.9	0.695 (0.594–0.797)	0.001
CRP	0.28	66.7	61	0.676 (0.572–0.781)	0.004

ROC: receiver operating characteristics; CRP: C-reactive protein; RRMS: relapsing-remitting multiple sclerosis; AUC: area under the ROC curve.

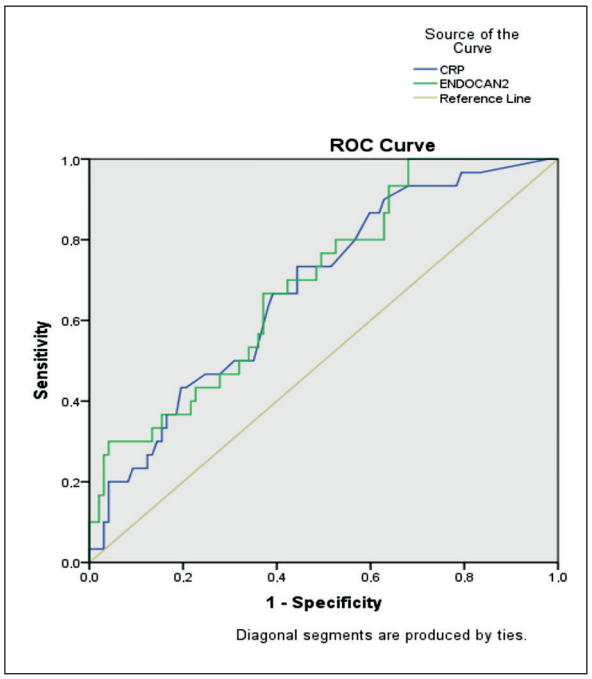

Figure 1. Receiver operating characteristics curve of the C-reactive protein and endocan levels in the prediction of relapsing-remitting multiple sclerosis relapse.

significantly higher in the relapse group than in the remission and post-relapse groups ($p < 0.05$). When the cut-off value of the CRP level was 0.28, it predicted RRMS relapse with 66.7% sensitivity and 61% specificity (AUC, 0.676 [0.572–0.781]); $p = 0.004$). When the cut-off value of the serum endocan level was 259.9, it predicted RRMS relapse with 67% sensitivity and 66.9% specificity.

A study conducted by Balta et al[10] showed a positive correlation between the serum endocan and CRP levels in Behçet's disease patients. The endocan levels were found to be associated with the disease activity and erythrocyte sedimentation rate; consequently, it was concluded that endocan can be used as a marker of disease activity in Behçet's disease patients. In another study, disease activity and cardiovascular risk in psoriasis vulgaris patients were found to be directly proportional to high endocan levels and could be related[11]. In studies investigating malignancy, it was shown that high endocan levels are associated with disease prevalence, metastasis, and angiogenesis. In small-cell lung cancer and renal cell carcinoma patients, there was a positive correlation between the serum endocan levels and the disease prognosis[12,13]. The endocan levels in non-alcoholic fatty liver disease were found to be independently correlated with both the fatty liver index and the BARD score[14]. In their study, Scherpereel et al[15] suggested that in sepsis patients, the serum endocan levels are related to the disease severity and may indicate serious endothelial cell dysfunction. In another report, it was determined that the endocan level could be a predictor of the sepsis and acute respiratory distress syndrome prognoses[16]. In our study, the serum endocan levels were higher in the RRMS relapse group than in the RRMS remission, post-relapse and control groups ($p < 0.05$). This finding is consistent with those for other autoimmune diseases (Behçet's disease, psoriasis) in the literature and suggests that the MS disease activity may be associated with the endocan levels.

Based on the MS disease parameters, we found a statistically strong positive correlation between the MRI lesion localization and the EDSS score, disease duration and relapse frequency. In the RRMS group, there was a statistically weak positive correlation between the CRP and NLR levels. In the group comparisons, no significant correlations were found between the endocan, CRP and NLR levels.

In our study, the endocan, CRP and NLR levels were found to be significantly higher in the RRMS group than in the healthy control group. Finding high endocan levels in the activation of RRMS suggested that endocan can be used as a marker to detect relapse. However, when the number of patient relapses, the disease duration, the EDSS score, the lesion burden on MRI and the applied treatment were evaluated, no significant results were found regarding the studied parameters ($p > 0.05$). In terms of therapeutic agents taken, the patients were classified as those who took interferon, glatiramer acetate or oral therapy. No statistical differences were found between the groups in terms of the endocan, CRP and NLR levels ($p > 0.05$).

In this study, the basal CRP levels were found to be high in the RRMS patients. Moreover, Polachini et al[17] found that the CRP levels of the MS patients, along with other inflammatory markers, were significantly higher than those of the healthy controls. In the study by Soilu-Hanninen et al[18], the high-sensitivity CRP levels in the RRMS and healthy control groups were found to be similar. However, the high-sensitivity CRP levels were found to be higher in the relapse patients than in the remission patients and lower in the group receiving high-dose interferon-1a than in the placebo group. In our study, the serum CRP levels

were found to be significantly higher in the RRMS group (0.74 ± 1.3) than in the healthy control group (0.19 ± 0.21), and a significant difference was found between the relapse and control groups ($p < 0.05$). No significant differences were found between the relapse, remission and post-relapse groups ($p > 0.05$). The fact that the CRP levels were higher in the MS group than in the healthy control group could have been due to the inclusion of the relapse and post-relapse groups in the study. The lack of significant differences between the groups was considered due to the inclusion of patients using methylprednisolone in the study.

Defined in recent years, NLR is a potential disease predictor that has been found to be significantly higher in many systemic inflammatory diseases. It is suggested that NLR is an indicator of systemic inflammation in serious diseases. In studies conducted on neurodegenerative diseases such as Alzheimer's and Parkinson's disease, the NLR levels were found to be significantly high[19]. In the study by Demirci et al[20], the NLR levels in the MS patients and their correlation with the disease severity were investigated. The NLR levels of the MS patients and the healthy control group were compared, and those of the MS patients were found to be significantly higher ($p < 0.001$). Furthermore, the NLR levels were higher in the relapse patients than in the remission patients, and a positive correlation was found when they were compared using the EDSS scores. Consequently, it was proposed that a high NLR level can be considered an independent marker of progressive disability. NLR can be a simple, inexpensive, fast and repeatable marker of MS prognosis[19]. In our research, the NLR levels were found to be significantly higher in the patient group (3.15 ± 2.4) than in the healthy control group (2.01 ± 0.54; $p = 0.002$). There was also a significant difference between the serum NLR levels of the relapse and control groups ($p < 0.05$).

Our study supports the hypothesis that endothelial injury accompanies the inflammatory process in the MS relapse period. The endocan, CRP and NLR levels of the RRMS patients in our study were found to be higher than those of the healthy controls.

Conclusions

We conclude that endocan may have value in predicting MS relapse, and endothelial injury developing secondary to the inflammatory process may be a guide to new therapeutic objectives. Endocan can therefore be considered a simple, inexpensive, accessible, and moderately good diagnostic marker for predicting MS relapse. As the studies on this subject are insufficient, more investigations with a broad range of patients are necessary.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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