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CLINICAL STUDY

Neutrophil-to-lymphocyte ratio is involved in the severity of ankylosing spondylitis

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ABSTRACT

OBJECTIVE: Ankylosing spondylitis (AS) is a progressive chronic inflammatory disease mainly characterized by axial skeleton and sacroiliac joint involvement. We aimed to investigate the relation between neutrophil-to-lymphocyte ratio (NLR) and disease severity of AS and to explore its availability in clinical practice.

METHODS: A total of 102 AS patients and 60 individuals who were age- and gender-compatible with the control group were included into the study. Patients were divided into 2 groups according to Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores. Patients with BASDAI scores < 4 were considered to be having mild disease activity, whereas those with scores ≥ 4 were considered to be displaying severe disease activity. Hemogram test during the diagnosis, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and other laboratory values of the control group were recorded.

RESULTS: NLR was observed to be higher in AS patients compared to the controls (2.47 ± 1.33 and 1.72 ± 0.47; respectively; p<0.0001). NLR was observed to be significantly higher in severe AS disease activity compared to the mild AS disease activity (2.72 ± 1.41, 2.20 ± 1.19; respectively; p = 0.001). NLR had statistical significant differences between mild disease activity compared to the controls (2.20 ± 1.19 and 1.72 ± 0.47, respectively; p = 0.263). There was a positive correlation between NLR and BASDAI (r = 0.193, p = 0.041). The performance of NLR evaluating the disease severity by Roc analysis had sensitivity of 69%, specificity of 54% (cut-off value 1.91), and AUC of 0.652 (95% CI, 0.549-0.755) (p = 0.006).

CONCLUSIONS: NLR may be a simple and inexpensive marker to indicate disease activity in patients with AS in daily clinical practice *(Tab. 3, Fig. 3, Ref. 25)*. Text in PDF *www.elis.sk.*

KEY WORDS: ankylosing spondylitis, BASDAI, disease, neutrophil-to-lymphocyte ratio, severity.

Introduction

Ankylosing spondylitis (AS) is a progressive chronic debilitating disease marked by axial skeleton and sacroiliac joint involvement which may also cause peripheral joint involvement. Extra-articular involvement of eyes, skin, vascular structures, and gastrointestinal system may also be seen (1, 2). There are no adjuvant laboratory tests for AS diagnosis, but it is known to be related to HLA-B27 gene. Inflammatory indicators like C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum amyloid A (SAA) and leukocyte are high in the active disease period (3, 4).

Neutrophils and lymphocytes in white blood cells (WBC) and subgroups play a role in the inflammatory process (5). Neutrophil-to-lymphocyte Ratio (NLR) calculated by dividing the neutrophil count to that of lymphocytes has become a marker which has recently started to be used as a systemic indicator of several diseases (5, 6). There are studies performed using NLR for familial Mediterranean fever (FMF), diabetes mellitus (DM) and chronic kidney disease (CKD).

Our purpose in this article was to investigate the relationship between NLR and disease severity in patients with AS and to explore its availability in clinical practice.

Materials and methods

Our study was carried out between April 2009 and April 2011. A total of 112 AS patients diagnosed by means of Modified New York Criteria and 60 people who were age and gender-compatible with the controls. BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) comprises a series of scoring systems designed to measure the disease activity (7). This index scores the reported levels of back pain, fatigue, peripheral joint pain and swelling, localized sensitivity and duration and severity of morning stiffness. Patients were separated into 2 groups according to BASDAI scores. Patients with BASDAI scores less than 4 were considered to be having mild disease activity, whereas patients with scores of 4 or above were considered to be displaying severe disease activity.

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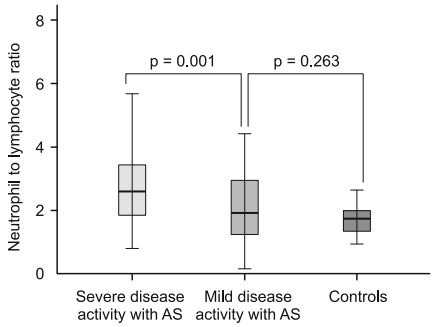

Fig. 1. Comparison of the Neutrophil-to-Lymphocyte Ratio of the groups.

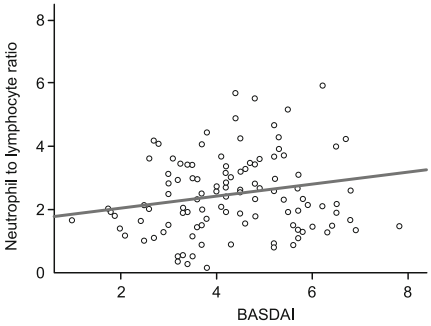

Fig. 2. The correlation between NLR and BASDAI.

The healthy subjects for control group were obtained from hospital records. File and archive records were reviewed and parameters included in the hemogram test during the diagnosis, while inflammatory markers such as ESR and CRP and laboratory values of the controls were recorded. People with immune deficiency, hypertension, diabetes mellitus, acute and/or chronic infection, and coronary artery disease, as well as those with history of malignancy or suspected malignancy, steroid therapy or smoking were excluded from the study. The study protocol was granted by the local ethics committee and was in accordance with the Declaration of Helsinki.

Statistical analysis

The Statistical Package for Social Sciences for Windows 14.0 (SPSS, Inc, Chicago, USA) was used for statistical analyses. Continuous variables were presented as mean ± standard deviation, mean while categorical variables were indicated as number (n) and percent (%). Variables meeting the parametric assumptions were assessed using *t*-test and one-sided ANOVA test in independent groups, Tukey HSD test was used in the inter-group post-hoc evaluation, while categorical variables were reviewed by chi-square test. Pearson correlation analysis was carried out to test the correlation of the data. The comparison of sensitivity and specificity was evaluated by ROC curve graphics. A p value < 0.05 was accepted as significant.

Results

No significant difference was observed for age and gender characteristics of patients and controls (Tab. 1). On diagnosis, mean age of patients was 30.2 ± 10.6 years. Mean duration of disease was 8.2 ± 8.0 years. NLR was observed to be higher in AS patients compared to the controls (2.47 ± 1.33 and 1.72 ± 0.47, respectively; $p < 0.0001$). Demographic and laboratory data of patients and controls are presented in Table 1.

NLR was observed to be significantly higher in severe AS disease activity compared to that in mild AS disease activity (2.72 ± 1.41, 2.20 ± 1.19, respectively; $p = 0.001$). NLR differed statistically significantly between the mild disease activity and controls (2.20 ± 1.19 and 1.72 ± 0.47 respectively; $p = 0.263$). NLR was also observed to be significantly higher in severe disease activity compared to the controls (2.72 ± 1.41 and 1.72 ± 0.47, respectively; $p < 0.0001$) (Fig. 1, Tab. 2). Other laboratory data for mild disease activity, severe disease activity, and controls are introduced in Table 2.

No correlation was observed between NLR and age, age at diagnosis, and duration of the disease. There was a positive cor-

Tab. 1. Demographic and laboratory characteristics of AS patients and controls.

	Patients (n=112)	Controls (n=60)	p value
Age, years	38.4±11.4	35.4±9.5	0.075
Male/Female, n (%)	84 (72.4)/32 (27.6)	35 (58.3)/25 (41.7)	0.063
Hb (g/dL)	13.68±1.77	14.57±1.46	0.001
Plt (x10^9/L)	295.74±83.39	266.00±63.77	0.010
WBC (x10^9/L)	8.60±2.41	6.82±1.17	<0.0001
Neu, x10^9/L	5.22±2.11	3.82±0.94	<0.0001
Lym, x10^9/L	2.49±1.16	2.23±0.52	0.052
ESR, mm/h	26.03±23.39	6.88±4.63	<0.0001
CRP, mg/L	17.78±22.72	2.77±1.60	<0.0001
NLR, %	2.47±1.33	1.72±0.47	<0.0001

Data smean ± SD, Hb: Hemoglobin, Plt: platelets, WBC: white blood cells, Neu: neutrophils, Lym: lymphocytes, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, NLR: neutrophil-to-lymphocyte ratio.

Tab. 2. Comparison of laboratory parameters of the groups.

	AS patients with mild disease activity (n=47)	AS patients with severe disease activity (n=65)	Controls (n = 60)	p^1 value	p^2 value
WBC, x10^9/L	8.16±2.01	8.81±2.67	7.01±1.63	0.120	<0.0001
Neu, x10^9/L	4.58±1.83	5.63±2.24	3.94±1.27	0.001	<0.0001
Lym, x10^9/L	2.70±1.52	2.30±0.73	2.45±0.60	0.033	0.946
ESR, mm/h	24.38±23.75	28.04±23.36	7.28±6.40	0.278	<0.0001
CRP, mg/L	15.98±24.47	22.40±22.89	3.77±2.71	0.213	<0.0001
NLR, %	2.20±1.19	2.72±1.41	1.64±0.53	0.001	<0.0001

Data as mean ± SD, WBC: wWhite blood cells, Neu: neutrophils, Lym: lymphocytes, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, NLR: neutrophil-to-lymphocyte ratio. p^{1}value: p value of comparison between mild disease activity with AS and severe disease activity with AS. p^{2}value: p value of comparison between mild disease activity with AS and controls

Tab. 3. Evaluation of NLR, BASDAI and other inflammatory markers in AS patients.

NLR	BASDAI score	WBC	CRP	ESR
rs	0.193	0.269	0.235	0.317
P	0.041	0.004	0.013	0.001

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, WBC: wWhite blood cells, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, NLR:neutrophil-to-lymphocyte ratio.

relation between NLR and BASDAI (Tab. 3, Fig. 2). The correlations between NLR and WBC, CRP and ESR are presented in Table 3. The performance of NLR in evaluating the severity of disease activity with Roc analysis was cut of 1.91, 69 % sensitivity, 54 % specificity, and AUC of 0.652 (95% Cl, 0.549-0.755) (p = 0.006) (Fig. 3).

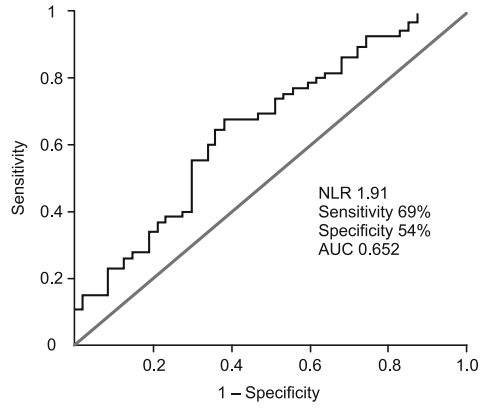

Fig. 3. The Roc analysis of NLR.

Discussion

In this study, the relationship between the severity of disease according to BASDAI scoring system and the relationship between BASDAI and NLR were investigated. NLR was observed to be higher in patients with severe disease activity than in those with mild disease activity in terms of BASDAI scoring system. Also, it was found to be higher in patients with mild disease activity compared to the controls. In our study, NLR was assessed to be a parameter that can indicate the disease severity when the disease activity was evaluated in two categories in BASDAI scoring system.

Although varying between communities, the prevalence of ankylosing spondylitis (AS) in the general population is believed to be 0.5 %. The disease generally onsets in the 2nd or 3rd decade and is observed 2 to 3 times more frequently in males than in females (8, 9). Although its etiology and pathogenesis are not yet fully understood, the genetic predisposition, environmental factors, and bacterial antigens may contribute. HLA B27 and several other genes are believed to contribute to the pathogenesis (2, 8, 10). Due to the effects of HLA-B 27 on the immune system, the opinions center around the damage by host tissues, inflammation development and activation of cytokines (2, 11).

WBC subgroups play an active role in tissue damage, inflammation, cytokine production and in controlling this process. Stress, inflammation, and tissue damage result in an increase in neutrophil counts (6, 12). However, cortisol synthesis has a suppressive effect on lymphocytes. NLR has been included in the daily practice as a frequently used, simple and cheap inflammatory marker (13). Studies on NLR were focused on inflammation and amyloidosis in patients with FMF (14, 15), complications and relevance with disease control in patients with DM (16-19), and disease progression and relationship between cytokines and inflammatory markers like high-sensitivity C-reactive protein (hs-CRP) with CKD (20–22).

FMF is an auto-inflammatory, autosomal recessive disease accompanied by self-limiting attacks. The clinical course of the disease and complications indicate that not only is the disease not limited with episodes, it also involves chronic systemic inflammation. One of the most significant complications of the disease is amyloidosis secondary to systemic inflammation. Amyloidosis is more common in patients with homozygous M694V mutation (14, 15). NLR was shown to be higher in FMF patients with amyloidosis (14) and with homozygous M694V mutation (15) compared to other patients.

Diabetes mellitus (DM) is a disease involving systemic inflammation observed with a decrease in insulin secretion and/or impairment in plasma glucose after the resistance occurs in periphery. A study by Shiny et al (16) on patients with DM, subjects with impaired glucose tolerance (IGT) and normal glucose tolerance (NGT) reported that NLR was higher in DM patients compared to subjects with IGT, and higher in subjects with IGT compared to subjects with NGT. The control of plasma glucose had a significant place in DM patients. HbA1c was included in parameters used in the treatment during the follow-up. In their study with DM patients, Sefil et al (17) divided the patients with DM into two groups, namely those with HbA1c $\leq$ 7 (regulated DM) and >7 (non-regulated DM). NLR was reported to be higher in HbA1c > 7 patients compared to HbA1c $\leq$ 7 patients. A relationship between HbA1c and NLR was shown in the same study. The occurrence of free oxygen radicals, cytokines, vascular damage, and endothelium dysfunction play a role in the development of complications. Microvascular and macrovascular complications may develop in patients with DM following chronic systemic inflammation. Öztürk et al (18) indicated that NLR was higher in DM patients with microvascular complications compared to those with no complications. Renal involvement which may be observed during the clinical course of the disease manifests with albumin excretion in urine. This was a significant marker of renal and extra-renal prognosis. Afsar's study (19) showed that NLR was associated with albumin secretion in urine in patients with DM.

Chronic kidney disease (CKD) is a proof of systemic inflammation when complications in the clinical course are taken into account. In their study consisting of patients in the stage of predialysis, peritoneal dialysis, hemodialysis, and a healthy control group, Okyay et al (20) reported that NLR was higher in all three groups compared to the healthy control group. Again in the same study, they have demonstrated a correlation between interleukin-6 and hs-CRP. In their recent study on patients with renal impairment, Turkmen et al (21) reported a correlation between NLR and tumor necrosis factor alpha. In a study carried out on patients with stage 4

CKD, Kocyigit et al (22) reported that the disease progression was faster in patients with NLR = 3 compared to patients with NLR < 3.

As neutrophils, lymphocytes, and macrophages are important elements of the defense system, they have significant roles in the control of immune mechanisms of the pathogenesis of AS patients. Studies performed and the results of sacroiliac joint sampling suggest that cytokines are also included in these mechanisms in AS patients (7, 8, 11, 23). Korkosz et al (7) reported that ESR and CRP were higher in patients with high disease activity (BASDAI ≥ 4) compared to patients with mild disease activity (BASDAI < 4) and to control group. Also, IL-6 and TNF alpha levels were shown to be not different among two groups, but statistically significantly higher compared to the control group. SAA is the acute-phase protein synthesized in the liver in response to pro-inflammatory cytokines. Jung et al (4) have shown that SAA levels of AS patients were higher compared to the control group. A correlation between BASDAI scoring and SAA levels was also shown in the same study (4).

Nowadays studies investigating NLR in AS are described in literature. Boyraz et al found that there was no NLR difference when compared with the control group (24). In another study, Gökmen et al.suggested that NLR in AS group was higher than in controls and correlated with CRP (25). Accordingly, we found that NLR was higher in AS compared to control group, and correlated with BASDAI, WBC, CRP and ESR. In our study, we divided the patients into two groups according to their NLR disease activities and observed significant differences between the two groups and the control group. We believe that NLR could be a promising biomarker presenting the disease activity of AS patients due to elevated levels existing in active patients and correlated with BASDAI. However, our study has limitations, namely that it is a retrospective cross-sectional study and does not investigate the relationship between NLR and cytokines, as well as possible effects of drugs on NLR.

In conclusion, this study suggests that NLR could be a simple and cheap inflammatory marker with a potential to demonstrate the disease activity of AS. Prospective studies need to be performed to enlighten the significance of NLR in patients with AS as well as its clinical availability.

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