Original Article

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Comparison of Neutrophil-to-Albumin Ratio and Neutrophil-to-Lymphocyte Ratio in Acute Phase Positive and Negative Ankylosing Spondylitis Patients

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Abstract

Aim: Acute phase reactants may not always be positive in ankylosing spondylitis (AS). We herein aimed to investigate whether the neutrophil-to-albumin ratio (NAR) is useful in monitoring AS activity.

Methods: One hundred nineteen patients who were followed up in the internal medicine rheumatology clinic between August 2024 and September 2024 and diagnosed with AS according to the axial spondyloarthropathy classification criteria of the Association for the Assessment of SpondyloArthritis international Society and the 1984 modified New York criteria were included in our study. The C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), NAR and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) status of the patients were recorded at two visits, before and 6 months following treatment.

Results: A significant correlation was found between the initial NLR and the NLR values after 6 months, and between the initial and 6-month BASDAI values in both the CRP positive and CRP negative groups (p=0.000 and p=0.017). It was observed that the changes in individual NAR and NLR values and BASDAI values for each case were parallel (p=0.012).

Conclusion: C-reactive protein values in AS are not always consistent with disease activity, but the NLR may be helpful in such cases. As for the NA ratio, we found that each patient should be evaluated individually.

Keywords: Ankylosing spondylitis, neutrophil-to-albumin ratio, neutrophil-to-lymphocyte ratio

Introduction

Ankylosing spondylitis (AS) is a chronic, systemic inflammatory disease that primarily affects the sacroiliac joint and spine. It represents the archetype of spondyloarthropathies (SpA) (1). Inflammatory low back pain is the most important symptom: Morning stiffness in the hip in the second half of the night is quite specific. Decreased vertebral movements are observed in the advanced stages of the disease. Ankylosing spondylitis can also present as peripheral arthritis, which is often oligoarticular and asymmetric, frequently affecting the lower extremities and limited to the vertebrae. The most common extra-articular involvement is anterior uveitis. Other types of involvement include inflammatory bowel disease, psoriasis, apical pulmonary fibrosis, and aortic valve insufficiency (2). The disease typically begins in the third decade of life, and the male/female ratio for radiographic axial spondyloarthritis is two to one, while for non-radiographic axial spondyloarthritis it is one to one. The most common genetic association is with human leukocyte antigen-B27 (HLA-B27) (3). The incidence estimates of AS range from 0.05% to 1.4%, while the prevalence estimates range from 0.1% to 1.4% per

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10,000 person-years (4). In Türkiye, the overall prevalence of SpA, including AS, is 1.05% (5).

When treatment is ineffective, this disease can lead to permanent disability. Scores are calculated to monitor activity. Among the components of these scores, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are frequently used (6,7). However, ESR and other acute phase reactants are not always associated with disease activity, and changes in ESR are observed in less than 50% of patients. Interleukin 6 and tumor necrosis factor can be considered markers of inflammation in AS, but centers that can routinely test for them are in the minority (1). For this reason, a search for other acute phase reactants has been initiated while easily calculable and cost-effective indicators related to systemic inflammation have been investigated. Based on the knowledge that neutrophil and platelet levels increase with inflammation and lymphocyte levels decrease in autoimmune diseases, neutrophil, monocyte, lymphocyte, and platelet counts and indices derived from these, such as the neutrophil-to-lymphocyte ratio (NLR), monocyte-lymphocyte ratio, and platelet-lymphocyte ratio, have thus gained prominence (1). However, rapid changes in these values, especially during infections, may lead to misleading results independent of AS activity, as they develop in parallel with their half-lives (8). Therefore, we aimed to utilize albumin levels, which have a long half-life and serve as a negative acute phase reactant associated with chronic inflammation (9) and to examine changes in neutrophil-to-albumin ratios (NAR) in AS. In doing so, we used the method of comparison with the NLR emphasized in the previous studies. Our study is the first to evaluate the NAR in AS. In this study, we hypothesized that the NAR may be useful in monitoring the activity of AS.

Materials and Methods

Compliance with Ethical Standards

The ethics committee application was received from Erzincan Binali Yildirim University Non-Interventional Clinical Research Ethics Committee (approval no.: 2024-09/02, date: 11.07.2024). Our work is in accordance with the provisions of the Helsinki Declaration (revised in Brazil in 2013). Informed consent has been obtained in writing from the patients.

Study Design

One hundred nineteen patients who were followed up in our hospital's internal medicine rheumatology clinic between August 2024 and September 2024 and diagnosed with AS according to the axial spondyloarthropathy classification criteria of the Association for the International Assessment of Spondyloarthritis and the 1984 modified New York criteria (7) were included in our study.

The patients selected during these months had applied to our clinic for the first time 6 months ago and were newly diagnosed at that time. The criteria for inclusion in the study also included being at least 18 years old, not being pregnant, and not breastfeeding. The patients' gender, age (years), medications, comorbidities, disease duration (months), and HLA-B27 status were evaluated. C-reactive protein status, NLR, NAR, and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) were recorded at two visits, before and 6 months following treatment. In this study, it was important that the variations in the BASDAI score and acute phase reactants varied in parallel with the BASDAI status, rather than the effectiveness of the treatment or which treatment the patient received.

Total blood count values were measured by flow cytometry, albumin values by spectrophotometric methods, and CRP values by nephelometric methods. NLR and NAR were calculated with mathematical ratios. For CRP, values of 5 mg/L and above were considered positive, while values below this were considered negative. The BASDAI score is obtained by calculating patients' general fatigue, neck, back, and hip pain, peripheral arthritis, tenderness, and morning stiffness on a scale of 0 to 10. The BASDAI score of 4 and above was considered positive, while values below this were evaluated as negative. The BASDAI score positivity, CRP value positivity, and possible increases in the NLR and the NAR were associated with inflammation and disease activity.

Statistical Analysis

Patients were grouped as positive and negative based on their initial CRP values. The initial BASDAI status of these groups was statistically compared with the initial median values of NAR and NLR, as well as the BASDAI status after 6 months with the median values of NAR and NLR from that same period. Additionally, the initial median values of NAR and NLR for each case were compared with the median values 6 months following treatment. Chi-square, paired t-test, Wilcoxon two-related-samples t-test, and Kruskal-Wallis test were used with SPSS version 22. A p-value of 0.05 or below was considered significant. The study was conducted retrospectively.

Results

Demographic Data

The total number of patients was 119. Sixty-four patients were male (54%), and 55 patients were female (46%). The average age of the patients was 41.9±10.3. The distribution of patients according to the medications they used is as follows: ten patients using methotrexate (8.5%), 18 patients using duloxetine (15%), 3 patients using hydroxychloroquine (2.5%), 84 patients using sulfasalazine (70.6%), 10 patients using colchicine (8.5%),

Table 1. Demographic characteristics of patients	
Age (year), mean ± *SD, (min-max)	41.9±10.3 (19-69)
Female, (n%)	55 (46)
Male, (n%)	64 (54)
Methotrexate using, (n%)	10 (8.5)
Duloxetine using, (n%)	18 (15)
Hydroxychloroquine using, (n%)	3 (2.5)
Sulfasalazine using, (n%)	84 (70.6)
Colchicine using, (n%)	10 (8.5)
**NSAID using, (n%)	85 (71.4)
Adalimumab using, (n%)	33 (28)
İnfliximab using, (n%)	15 (12.5)
Certolizumab using, (n%)	7 (6)
Golimumab using, (n%)	13 (11)
Etanercept using, (n%)	11 (9)
Secukinumab using, (n%)	8 (6.5)
‡FMF, (n%)	7 (6)
Behçet's disease, (n%)	4 (3.5)
Uveitis, (n%)	13 (11)
Psoriasis, (n%)	1 (0.8)
¶UC, (n%)	4 (3.2)
Crohn's disease, (n%)	2 (1.6)
†Type 2 DM, (n%)	2 (1.6)
‡‡HT, (n%)	2 (1.6)
Fibromyalgia, (n%)	39 (33)
Peripheral arthritis, (n%)	81 (68)
§§NLR (beginning), median (min-max)	1.970 (0.55-12.44)
§§NLR (after 6 months), median (min-max)	1.762 (0.62-6.86)
¶¶NAR (beginning), median (min-max)	0.111 (0.06-2.41)
¶¶NAR (after 6 months), median (min-max)	0.101 (0.01-1.70)
Duration of disease (month), mean ± *SD (min-max)	56.2±55.8 (0-228)
††BASDAI positive cases, (n%)	107 (90)
††BASDAI positive cases (after 6 months), (n%)	46 (31)
***CRP positive cases, (n%)	59 (49)
***CRP positive cases (after 6 months), (n%)	29 (25)
‡‡‡HLA-B27 positive cases, (n%)	60 (51)

Explore and frequency test were used
*SD: Standard deviation, **NSAIDs: Non-steroidal anti-inflammatory drugs, ‡FMF: Familial mediterranean fever, ¶UC: Ulcerative colitis, †Type 2 DM: Type 2 diabetes mellitus, ‡‡HT: Hypertension, §§NLR: Neutrophil-to-lymphocyte ratio, ¶¶NAR: Neutrophil-to-albumin ratio, ††BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, ***CRP: C-reactive protein, ‡‡‡HLA-B27: Human leukocyte antigen-B27

85 patients using non-steroidal anti-inflammatory drugs (71.4%), 33 patients using adalimumab (28%), 15 patients using infliximab (12.5%), 7 patients using certolizumab (6%), 13 patients using golimumab (11%), 11 patients using etanercept (9%), and 8 patients using secukinumab (6.5%). Seven patients had familial Mediterranean fever (6%); 4 patients had Behçet's disease (3.5%); 13 patients had uveitis (11%); 1 patient had psoriasis (0.8%); 4 patients had ulcerative colitis (3.2%); 2 patients had Crohn's disease (1.6%); 2 patients had type 2 diabetes mellitus (1.6%); 2 patients had hypertension (1.6%); 39 patients had fibromyalgia (33%); and 81 patients had peripheral arthritis (68%) as comorbidities. The initial NLR median (min-max) was 1.970 (0.55-12.44); the NLR median after 6 months was 1.762 (0.62-6.86); the initial NAR median (min-max) was 0.111 (0.06-2.41); and the NAR median after 6 months was 0.101 (0.01-1.70). The disease duration was 56.2±55.8 months. At the beginning, 107 patients had positive BASDAI scores (90%), while 6 months later, 46 patients had positive BASDAI scores (31%). There were 59 patients with positive CRP values at the beginning (49%), and 6 months later, there were 29 patients with positive CRP values (25%). There were 60 patients (51%) who were HLA-B27 positive (Table 1).

Relation Between Acute Phase Reactants and BASDAI Scores

Initial CRP positivity was correlated with a high initial BASDAI score, and CRP positivity after 6 months correlated with changes in BASDAI score at that time (Table 2).

The relation between the initial NLR values and initial BASDAI scores and the NLR values and BASDAI scores six months later was also compared between cases with initial CRP positive and negative statuses. Both the CRP positive and CRP negative groups showed that the initial NLR values changed in correlation with the initial BASDAI scores, and the NLR values after 6 months changed in correlation with the BASDAI scores after 6 months. However, when we analyzed the NAR values, this situation was not valid for either group (Table 3). In Table 4, the change in acute phase reactants was examined based on the first and 6-month BASDAI values of each case in groups with initial CRP values that were positive or negative. Accordingly, the NLR and NAR values changed in parallel with the case-specific BASDAI scores. However, there was no significant increase in NAR values in cases where the initial CRP

Table 2. Relationship between BASDAI scores at baseline and 6-month follow-up and CRP				
Acute phase reactant	**Relationship with ¶BASDAI (p-value)**	**¶BASDAI is high (n%)**	**¶BASDAI (after 6 months) (p-value)**	**n (%)**
****CRP positivity**	0.048*	56 (47)	-	-
****CRP positivity (after 6 months)**	-	-	0.001*	19 (16)

Chi-square test was used
*: p-value is significant (≤0.05), **CRP: C-reactive protein, ¶BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

Table 3. Relationship between BASDAI scores at baseline and 6 month follow-up and acute phase reactants

Acute phase reactant	In ‡‡CRP positive group relationship with ¶BASDAI (p-value)	Relationship with ¶BASDAI in ‡‡CRP positive group (after 6 months) (p-value)	Median values of beginning (min-max)	Median values after 6 months (min-max)	In ‡‡CRP negative group relationship with ¶BASDAI (p-value)	Relationship with ¶BASDAI in ‡‡CRP negative group (after 6 months) (p-value)	Median values of beginning (min-max)	Median values after 6 months (min-max)
****NLR (beginning)**	0.032*	-	1.97 (0.55-6.65)		0.002*	-	1.95 (0.9-12.44)	-
****NLR (after 6 months)**	-	0.024*	-	1.69 (0.65-4.74)	-	0.05*		1.79 (0.62-6.86)
‡NAR (beginning)	0.083	-	0.122 (0.06-2.41)		0.159	-	0.103 (0.06-1.63)	-
‡NAR (after 6 months)	-	0.3	-	0.107 (0.05-1.61)	-	0.13	-	0.099 (0.01-1.7)

Paired t-test, Wilcoxon two related samples test, and Kruskal-Wallis test were used
*: p-value is significant (≤ 0.05), **NLR: Neutrophil-to-lymphocyte ratio, ‡NAR: Neutrophil-to-albumin ratio, ‡‡CRP: C-reactive protein, ¶BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

Table 4. Changes in NLR and NAR values according to BASDAI changes in patients with positive and negative initial CRP values

	‡‡CRP positive cases			‡‡CRP negative cases		
Acute phase reactant	**Cases with high initial ¶BASDAI**	**Cases with low ¶BASDAI after 6 months**	***p-value**	**Cases with high initial ¶BASDAI**	**Cases with low ¶BASDAI after 6 months**	***p-value**
****NLR (median, min-max)**	2.17 (0.86-6.65)	1.60 (0.65-4.74)	0.000*	2.19 (1.05-12.44)	1.75 (0.62-3.74)	0.014*
‡NAR (median, min-max)	0.12 (0.06-2.41)	0.092 (0.05-1.61)	0.000*	0.118 (0.06-1.55)	0.086 (0.05-1.24)	0.000*
	Cases with low initial ¶BASDAI	**Cases with high ¶BASDAI after 6 months**	***p-value**	**Cases with low initial ¶BASDAI**	**Cases with high ¶BASDAI after 6 months**	***p-value**
****NLR (median, min-max)**	1.13 (0.86-1.56)	1.61 (1.59-2.10)	0.017*	1.31 (1.05-2.41)	1.99 (1.38-6.86)	0.149
‡NAR (median, min-max)	0.089 (0.07-0.11)	0.12 (0.09-1.18)	0.109	0.094 (0.07-1.63)	0.12 (0.07-1.7)	0.012*

Paired t-test, Wilcoxon two related samples test, and Kruskal-Wallis test were used
*: p-value is significant (≤0.05), **NLR: Neutrophil-to-lymphocyte ratio, ‡NAR: Neutrophil-to-albumin ratio, ‡‡CRP: C-reactive protein, ¶BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

value was positive and the first BASDAI score was low, even if the BASDAI score was high after 6 months. Similarly, in cases where the initial CRP value was negative and the first BASDAI score was low, despite an increase in the BASDAI score after 6 months, no significant increase in the NLR value was observed.

Finally, when we investigated acute phase reactants that could help predict disease activity, we concluded that the initial CRP and initial NLR values are predictors of the BASDAI score (Table 5).

Discussion

In this study, in which we investigated the ability of NLR and NAR values to detect inflammation in acute phase positive and negative AS cases, we only recorded the patients' CRP values. The reason for this is that CRP is more specific to inflammation, compared to ESR, and fewer factors affect its levels. While CRP stands out in the follow-up of inflammatory arthritis, ESR is more valuable in connective tissue diseases (10).

When we grouped a total of 119 patients according to acute phase reactants, 59 cases (49%) had a positive initial CRP value, while 60 cases (51%) were negative. In the literature, ESR and CRP have been found to be positive in about 40% of AS patients (11). In this regard, our study is consistent with the literature.

In our study, among the patient group with a positive initial CRP value, CRP and NLR stood out as acute phase reactants that showed a change associated with the BASDAI scores at baseline and 6 months later. In this group, the initial CRP and NLR values of patients having a high baseline BASDAI score

Table 5. Predictors of BASDAI score			
Acute phase reactant	**p-value**	**Exp (B)**	**95% confidence interval**
Baseline **CRP	0.044*	8.8	1.06-73.09
Baseline ‡NLR	0.006*	79.1	3.47-1802.13
Baseline §NAR	0.545	51.5	0.056-3.65

Paired t-test, Wilcoxon two related samples test, and Kruskal-Wallis test were used
*: p-value is significant (≤0.05), BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, **CRP: C-reactive protein, ‡NLR: Neutrophil-to-lymphocyte ratio, §NAR: Neutrophil-to-albumin ratio

were significantly higher than those of patients having a low baseline BASDAI score, and this was also valid for the visits at 6-month follow-up. The study conducted by Karoli et al. (12) on 200 patients diagnosed with AS in India found that cases with a high BASDAI score (>4) also had elevated CRP and NLR values. In this regard, our study is consistent with the literature. In line with this information, a positive CRP value at the time of diagnosis in an AS patient may predict the presence of inflammation with a high BASDAI score and indicate that CRP can be used as an activity marker. Additionally, studies indicating that NLR values parallel CRP, indicates that the NLR can also be used for this purpose just like CRP.

However, in the group with a negative initial CRP value, we identified the NLR as the only acute phase reactant that showed a significant difference between patients with high and low initial BASDAI scores, and it followed a parallel trend with the BASDAI score. According to the meta-analyses by Xu et al. (13), which encompass a total of 10 studies involving 765 AS patients and 701 healthy individuals, it has been noted that the NLR values in patients are related to BASDAI and CRP levels, and that NLR values may indicate disease activity. In addition, a study conducted by Kucuk et al. (14) with 102 AS patients in Türkiye found that the NLR values in cases with severe disease activity were significantly higher than those in cases with mild disease activity, and there was a positive correlation with the BASDAI score. In this regard, it can be concluded that the NL ratio may serve as an alternative acute phase reactant in the follow-up of AS cases where CRP values are negative and CRP is insufficient in determining disease activity. The group with a negative initial CRP value and a high initial BASDAI score showed a decrease in BASDAI score and a decrease in the NL ratio after 6 months of treatment compared to initial values. However, in the group with a negative initial CRP value and a low initial BASDAI score, no increase was observed in the NL rate compared to the initial values, despite a high BASDAI score after 6 months of treatment. It was a surprising finding in our research. When we investigated the reason for this, we discovered that non-inflammatory pathologies could lead to an increase in the BASDAI score 6 months after treatment in selected cases. Fibromyalgia and mechanical joint diseases are the main causes that can lead to a misleading increase in the BASDAI score. According to the study conducted by Gao (15) with 121 patients diagnosed with AS in China, the BASDAI score in patients accompanied by fibromyalgia was higher compared to those without. The lack of an increase in the NL ratio has allowed us to determine that the condition is not inflammatory, thus making it a reliable parameter. However, there are also studies in the literature indicating that the NL ratio does not show a linear relationship with the BASDAI score and disease activity (16,17). Therefore, more studies are needed to determine whether the NLR can be used in the context of negative acute phase reactant cases in AS.

On the other hand, this situation, observed for the NLR and CRP, was not valid for the NAR, which is another variable we investigated. There was no significant difference in the NA ratios between patients with high and low BASDAI scores in both the acute phase positive and negative groups at the first visit. This was also the case after 6 months. However, a significant difference appeared between the NA ratios measured at the beginning of the study and after 6 months, in parallel with the variation in the BASDAI score calculated at the beginning and after 6 months for each case. If a patient's high initial BASDAI score decreased after 6 months, it was also observed that this patient's NAR was also lower after 6 months. The reason for this situation may be the effects of individual factors such as liver functions, gender differences, and nutritional status on albumin, along with the relatively long half-life of albumin, which is about 3 weeks (18). Therefore, the result may be necessary to compare the result with the case itself. In our study, we did not have any patients with liver failure. Since the albumin value was not evaluated independently, differences between genders were not examined, and nutritional status was not assessed. Another finding in our study was that in cases where the BASDAI score was low in the initial CRP positive group and the BASDAI score was high after 6 months, there was no increase in the NAR compared to the initial score. When we investigated the reason for this, we found that the number of cases was insufficient and that statistical analysis could not be conducted.

When we researched the literature on this topic, we could not find any studies evaluating the NA ratio in AS. The lack of studies on the variation of NAR between AS patients and healthy individuals makes it impossible to establish a cut-off value for it or to comment on the existence of a significant difference. However, in the study conducted by Feng et al. (19) on lung cancer cases, it was concluded that the NAR was closely related to inflammation and exhibited an increase. In addition, a study conducted by Karasu et al. (20) in Türkiye has shown that NAR was high in cases of acute myocardial infarction. In line with these studies, it suggests that the level of the NAR increases in inflammatory conditions; thus, it may be used to monitor inflammatory processes. This also supports the idea that NAR can be used in the follow-up of rheumatological diseases, and it indicates that more research is needed.

Finally in our study, acute phase reactants that could predict disease activity were identified as CRP and NLR. That is, in cases where the initial CRP value is positive, the CRP indicates that the BASDAI score will be higher and the disease will progress more aggressively. In cases with negative CRP, NLR served this purpose. In the study conducted by Chen et al. (21) with 156 patients, elevated CRP levels were associated with the disease's poor prognosis, and our research was consistent with the literature in this regard.

Study Limitations

Our limitations include the small number of cases and the lack of cut-off values for CRP, NAR, and NLR. Despite these limitations, this study stands out as the first to comparatively evaluate the NAR alongside established inflammatory markers such as NLR and CRP in relation to BASDAI scores among AS patients. Moreover, the inclusion of both acute-phase–positive and –negative subgroups provides a comprehensive insight into the potential clinical utility of NAR as an alternative activity marker in CRP-negative cases.

Conclusion

We have shown that CRP levels in AS do not always correlate with disease activity, and that the NLR may be helpful in such cases. As for the NAR, further studies are needed to explore whether each patient should be evaluated individually, suggesting that there may be an alternative acute phase reactant in AS cases.

Ethics

Ethics Committee Approval: The ethics committee application was received from the Erzincan Binali Yildirim University Non-interventional Clinical Research Ethics Committee (approval no.: 2024-09/02, date: 11.07.2024).

Informed Consent: Informed consent has been obtained in writing from the patients.

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Footnotes

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