**ORIGINAL ARTICLE**

# Alarming serum antiprotease levels in axial spondyloarthritis

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*ABSTRACT*

**Objectives:** The objective was to assess the serum levels of secretory leukocyte protease inhibitor (SLPI) and elafin in individuals diagnosed with axial spondyloarthritis (AxSpA) and analyze their diagnostic significance and correlation with disease activity.

**Patients and methods:** The case-controlled, cross-sectional study was conducted between August 2021 and April 2023. Sixty patients diagnosed with AxSpA (n=60) were classified according to imaging results as nonradiographic AxSpA (nr-AxSpA [n=30]; 15 males, 15 females; median age: 30 years; range, 27.6 to 34.1 years) and radiographic AxSpA (r-AxSpA [n=30]; 19 males, 11 females; median age: 33 years; range, 30.6 to 38.1 years), forming two patient groups (the nr-axSpA and r-axSpA groups). A total of 30 age- and sex-matched healthy controls (16 females, 14 males; median age: 33 years; range, 29.2 to 37.1 years) were included. Demographic data, laboratory, and clinical characteristics of the participants were recorded.

**Results:** There was no significant difference between SLPI and elafin serum levels in the disease groups. SLPI and elafin levels in AxSpA and nr-AxSpA groups were significantly higher compared to the control group (p<0.05). Based on receiver operating characteristic analysis, the diagnostic values of both parameters were found to be significant in the Ax-SpA and nr-AxSpA groups (p<0.05). There was no significant correlation between serum levels of SLPI and elafin and disease activity parameters. Significant positive correlations were found between SLPI and elafin in both the nr-AxSpA (p<0.05, r=0.870) and r-AxSpA (p<0.05, r=0.725) groups.

**Conclusion:** The levels of SLPI and elafin were found to be significantly elevated in patients with AxSpA, particularly in those with nr-AxSpA, compared to the control group. Therefore, SLPI and elafin can be used as therapeutic biomarkers for the diagnosis of AxSpA and nr-AxSpA. However, no relationship was found with disease activity.

*Keywords:* Axial spondyloarthritis, elafin, secretory leukocyte protease inhibitor.

Axial spondyloarthritis (AxSpA) is chronic inflammatory arthritis primarily affecting the axial skeleton (sacroiliac joints [SIJ] and spine), which can be categorized into two forms: non-radiographic AxSpA (nr-AxSpA) and radiographic AxSpA (r-AxSpA; ankylosing spondylitis [AS]), with a predominant impact on the axial skeleton.[1,2] Peripheral and extra-articular symptoms, such as dactylitis, enthesitis, uveitis, inflammatory bowel disease (IBD), and psoriasis are closely associated with AxSpA.[1] The disease's long-term course is associated with bone erosion and new bone formation, gradually leading to ankylosis in the affected joints.[2] Despite contributions from the immune system, genetic factors, infections, and some triggering factors to the development of AxSpA, the understanding of the exact pathogenesis of the disease remains incomplete.[3-7] Despite new possibilities for early diagnosis, the time interval between the appearance of initial symptoms and diagnosis remains notable. Therefore, recent research has focused on detecting the disease early and finding a supportive biomarker for assessing disease activity, progression, and treatment response.[8]

Proteases are enzymes primarily produced by inflammatory phagocytes and play a role in various biological processes, including inflammation and tissue damage. An inflammatory process highlights that the immune system can trigger autoimmunity and excessive release of proteases, which are effectors that can lead to

damage in host tissues. In response to protease enzymes, "alarm" or "systemic" inhibitors known as antiproteases are secreted from inflammatory cells, such as neutrophils and macrophages, to neutralize excessive protease burden and protect host tissues.[9] It is also noted that secretory leukocyte protease inhibitor (SLPI) and elafin, important members of the serine antiprotease family, have a variety of biological functions, including antimicrobial, anti-inflammatory, and immunomodulatory functions (in the innate and adaptive immune systems).[10] They are locally synthesized and secreted, produced in response to cytokines, such as interleukin-1 (and tumor necrosis factor (TNF)-alpha, before inflammation, and emphasized to inhibit these cytokines.[11]

Few studies in the literature examine SLPI and elafin serum levels. Studies have examined SLPI and elafin levels in diseases associated with the SpA group, such as psoriasis,[12,13] IBDs, such as ulcerative colitis and Crohn's disease,[14,15] and chronic inflammatory diseases, such as rheumatoid arthritis (RA),[16] systemic sclerosis (SS),[17] and osteoarthritis (OA),[18] and have indicated that they are present in significant amounts in immune system cells and may be associated with disease pathogenesis. In spondyloarthropathies, the significant roles of neutrophils in the early stages of enthesitis formation are well known.[19] In this context, SLPI[20] and elafin,[21] secreted from neutrophils and isolated from synovial fluid, could play a role in AxSpA physiopathogenesis. Furthermore, a study based on gene expression profiles has suggested the potential role of the SLPI gene in AxSpA physiopathogenesis,[22] which supports our view. However, as of our knowledge, no study in the literature examines serum levels of SLPI and elafin in AxSpA, which shares several immunological aspects with etiopathogenetics and other diseases mentioned above. Hence, the objective of this study was to assess the serum levels of SLPI and elafin in patients with AxSpA and analyze their diagnostic significance and correlation with disease activity.

## PATIENTS AND METHODS

This case-controlled, cross-sectional study was conducted at the Department of Physical Medicine and Rehabilitation and the Department of Rheumatology at the Medicine Faculty of Atatürk University between August 2021 and April 2023. Sixty patients diagnosed with AxSpA, assessed using Assessment of SpondyloArthritis International Society Criteria for AxSpA or modified New York criteria for AS, were included in the study.[1,23] The patients diagnosed with AxSpA were classified based on imaging results as the nr-AxSpA group (n=30; 15 males, 15 females; median age: 30 years; range, 27.6 to 34.1 years) and the r-AxSpA group (n=30; 19 males, 11 females; median age: 33 years; range, 30.6 to 38.1 years). Demographic data, including the age and sex of the patients, were recorded. Complete blood count, erythrocyte sedimentation rate (ESR; normal range: 0-20 mm/h), C-reactive protein (CRP; normal range: 0-5 mg/mL) levels, and the presence of HLA (human leukocyte antigen)-B27 were determined from routine blood samples. Current medication use, including nonsteroidal anti-inflammatory drugs (NSAIDs) and biological therapies (TNF-alpha blockers), was evaluated. A healthy control group of 30 age- and sex-matched individuals (16 females, 14 males; median age: 33 years; range, 29.2 to 37.1 years) with no history of rheumatic or autoimmune diseases was included in the study. The following exclusion criteria were applied: age≤18 years, other systemic inflammatory and rheumatological diseases, active infections, neoplasms, metabolic diseases, and pregnancy or lactation.

Disease activities were clinically evaluated using Ankylosing Spondylitis Disease Activity Score (ASDAS), The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and Bath Ankylosing Spondylitis Functional Index (BASFI) scores. For the assessment of spinal mobility, the Bath Ankylosing Spondylitis Metrology Index (BASMI) was utilized.[24,25]

Before blood collection, radiographs of the SIJ, lumbar, and cervical spine were obtained from the patients. Radiographs and SIJ magnetic resonance imaging for the initial disease classification were evaluated by a trained rheumatologist and a central radiologist. SIJs and cases with no radiographic structural damage in the spine (Grade 1 bilaterally or Grade 2 unilaterally) but with acute (active) inflammation on magnetic resonance imaging were classified as nr-AxSpA. Cases with radiographic structural

damage meeting the modified New York criteria were classified as r-AxSpA, creating two distinct forms.[1,2,23]

After 12 h of fasting, blood samples were collected from all groups to measure elafin, SLPI, ESR, and CRP levels. The collected blood samples were kept at room temperature for 30 min and then centrifuged at 3,500 rpm for 10 min to obtain serum samples. The obtained serum samples were stored at −80°C until the measurement day for elafin and SLPI levels. CRP and ESR were analyzed on the same day the blood samples were collected. Serum CRP levels were analyzed using the immunoturbidimetric method on a Beckman Coulter AU-5800 biochemical autoanalyzer (Beckman Coulter Inc., Brea, CA, USA). ESR was measured according to the Westergren method using tubes containing EDTA (ethylenediaminetetraacetic acid). Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used for measuring serum elafin and SLPI levels (Human Elafin ELISA Kit, catalog no: E4762Hu; Human Secretory Leukocyte Protease Inhibitor ELISA Kit, catalog no: E0880Hu; BT LAB Bioassay Technology Laboratory, Shanghai, China). Serum elafin levels were measured using the ELISA method following the manufacturer's instructions. For both analyses, the intra-assay coefficient of variation was <8%, and the interassay coefficient of variation was <10%.

### Statistical analysis

A power analysis was employed using G*Power version 3.1 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) to determine the minimum sample size required. Based on a significance level of 0.05 and a statistical power of 0.80, the sample size was calculated as 25 members in each group. A total of thirty participants were enrolled in each group to enhance the efficacy of the sample size.

The data were analyzed using IBM SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as the number of cases (n), percentage (%), mean ± standard deviation, and median (Q1-Q3) values. Whether numerical variables were normally distributed was evaluated using the Shapiro-Wilk normality test. Group comparisons were performed using a one-way analysis of variance for variables with normal distribution and a Kruskal-Wallis analysis for variables without normal distribution. Tukey's honest significant difference test for normally distributed variables and the Dunn-Bonferroni test for nonnormally distributed variables were used for multiple comparison tests. Pairwise comparisons between groups were performed using the independent sample t-test for normally distributed variables and the Mann-Whitney U test for nonnormally distributed variables. Pearson correlation analysis was used for normally distributed variables, and Spearman correlation analysis was used for nonnormally distributed variables. ROC (receiver operating characteristic) analysis was used to determine the groups' diagnostic cut-off values of relevant parameters in the groups. A p-value <0.05 was considered statistically significant.

## RESULTS

Sixty-eight percent of AxSpA patients included in the study were HLA-B27 positive. Age and sex exhibited similarity between the AxSpA groups (p>0.05). However, a notable disparity emerged between them in relation to the duration of the disease (p<0.05). Body mass index (BMI) value was similar in the disease groups (p>0.05) and was significantly higher in the r-AxSpA group compared to the control group (p<0.05). Table 1 displays the demographic and clinical characteristics of patients with AxSpA.

No notable disparity was observed among the disease groups in terms of SLPI and elafin values (p>0.05). Among the disease groups, the SLPI and elafin serum levels were ranked from highest to lowest: nr-AxSpA, AxSpA, and r-AxSpA.

In the AxSpA group, the SLPI and elafin values were significantly higher than the healthy control group (p<0.05). ROC analysis was performed to determine the diagnostic cut-off values of SLPI and elafin in the AxSpA group. In the AxSpA group, the diagnostic value of SLPI was 65.4% (area under the curve 0.654), and for elafin, it was 69.5% (area under the curve 0.695). Both parameters had statistically significant diagnostic values (p<0.05). The sensitivity of the

**Table 1.** Comparison of demographic, clinical, and laboratory characteristics of patients with AxSpA and healthy controls

| Characteristics | AxSpA (n=60) | nr-axSpA (n=30) | r-axSpA (n=30) | HC (n=30) | p |
|---|---|---|---|---|---|
| Age (year) [median (IQR)] | 31 (30.2-33.1) | 30 (27.6-34.1) | 33 (30.6-38.1) | 33 (29.2-37.1) | NS |
| Sex | | | | | NS |
| Male | 34 | 15 | 19 | 14 | |
| Female | 26 | 15 | 11 | 16 | |
| BMI (kg/m$^2$) [median (IQR), Mean±SD] | 24.9 (24-26) | 23.8±2.9 | 26.5±4.9 | 21.4 (21-23) | $\gamma^*$ |
| CRP (mg/L) median (IQR) | 3.1 (4.6-11.2) | 2.6 (2.6-6.1) | 4.1 (5.2-17.6) | 0.96 (1.2-2.5) | $\gamma^*$ |
| ESR (mm/saat) median (IQR) | 9 (10.8-18.2) | 8 (6.7-14.7) | 12 (12-24.3) | 5 (4.3-8.1) | $\gamma^*$ |
| Disease duration (year) [Mean±SD] | 6.58±4.8 | 3.69±3.403 | 9.37±4.311 | - | $\alpha^*$ |
| HLA-B27 positivity [n (%)] | 41 (68) | 21 (70) | 20 (66) | - | NS |
| SLPI level [median (IQR)] | 738 (799-1136) | 933 (833-1378) | 579 (629-1041) | 498 (513-842) | $\beta^*$ Q$^*$ |
| Elafin level [median (IQR)] | 86.5 (93.7-147) | 89.5 (91.7-181) | 80.4 (73.7-136) | 63.5 (50-79.5) | $\beta^*$ Q$^*$ |
| BASDAI [median (IQR)] | 3.1 (1.4-4.8) | 3.2 (1.35-4.72) | 2.5 (1.4-5.45) | - | NS |
| ASDAS-CRP [Mean±SD] | 2.36±1.18 | 2.22±1.16 | 2.49±1.2 | - | NS |
| ASDAS-ESR [Mean±SD] | 2.2±1.14 | 2.08±1.12 | 2.31±1.18 | - | NS |
| BASMI [median (IQR)] | 6 (6-6.9) | 5 (5.3-5.8) | 6.5 (6.5-8) | - | NS |
| BASFI [median (IQR)] | 1.3 (1.5-2.5) | 1.2 (1.2-2.7) | 1.6 (1.3-2.8) | - | NS |
| Treatment | | | | | |
| NSAID [n (%)] | 24 (40) | 17 (57) | 7 (23) | - | NS |
| TNF-α blockers [n (%)] | 36 (60) | 13 (43) | 23 (77) | - | NS |

AxSpA: Axial spondyloarthritis; nr-axSpA: Non-radiographic axial spondyloarthritis; r-axSpA: Radiographic axial spondyloarthritis; HC: Healthy controls; IQR: Interquartile range; SD: Standard deviation; BMI: Body mass index; CRP: C-reactive protein level; ESR: Erythrocyte sedimentation rate; HLA-B27: Human leukocyte antigen-B27; SLPI: Secretory leukocyte protease inhibitor; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; ASDAS: Ankylosing Spondylitis Disease Activity Score; BASMI: Bath Ankylosing Spondylitis Metrology Index; BASFI: Bath Ankylosing Spondylitis Functional Index; NSAID: Non-steroidal anti-inflammatory drugs; 95% confidence interval, α=0.05; * p<0.05; α: Comparison of nr-AxSpA and r-axSpA; β: Comparison of nr-AxSpA and HC; γ: Comparison of r-axSpA and HC; Q: Comparison of AxSpA and HC.

SLPI parameter was 76.7%, and the specificity was 61.1%, while the sensitivity of the elafin parameter was 76.1%, and the specificity was 69.3% (Figure 1).

In the nr-AxSpA group, the SLPI and elafin values were significantly higher than the healthy control group (p<0.05). According to the ROC analysis results, the diagnostic value of SLPI in the nr-AxSpA group was 73.2% (area under the curve 0.732), while the diagnostic value of elafin was 69.5% (area under the curve 0.695). Both parameters had statistically significant diagnostic values (p<0.05). The sensitivity of the SLPI parameter was 79.3%, and the specificity was 59.5%, while the sensitivity of the elafin parameter was 82.8%, and the specificity was 64.5% (Figure 2).

Although SLPI and elafin levels were higher in the r-AxSpA group than in the healthy control group, they were not statistically significant (Table 1).

However, there was no significant correlation between SLPI and elafin values and disease duration, BASDAI, ASDAS-ESR, ASDAS-CRP, BASMI, and BASFI index values in the disease groups (p>0.05). However, both nr-AxSpA (p<0.05, r=0.870) and r-AxSpA (p<0.05, r=0.725) groups showed significant positive correlations between SLPI and elafin levels. There was no significant correlation between SLPI and elafin levels in the healthy control group. Based on the medication use, there were no notable variations observed in the levels of SLPI and elafin among the patient groups (Table 1).
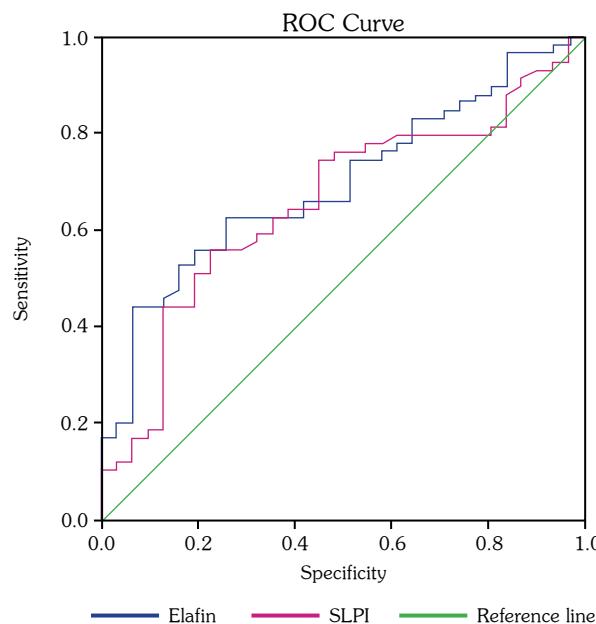
**Figure 1.** The results of SLPI and elafin ROC analysis in AxSpA group.

SLPI: Secretory leukocyte protease inhibitor; ROC: Receiver operating characteristic; AxSpA: Axial spondyloarthritis.
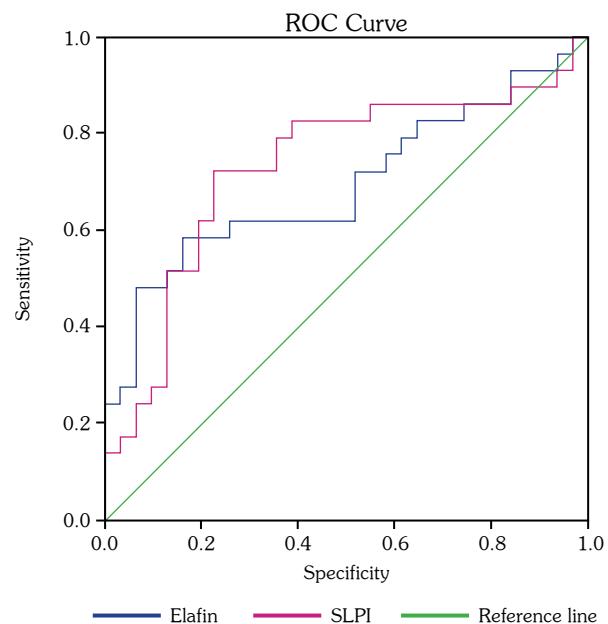


**Figure 2.** The results of SLPI and elafin ROC analysis in nr-axSpA group.

SLPI: Secretory leukocyte protease inhibitor; ROC: Receiver operating characteristic; nr-axSpA: Non-radiographic axial spondyloarthritis.

## DISCUSSION

This study found that SLPI and elafin serum levels were significantly higher in the AxSpA and nr-AxSpA groups compared to the control group (p<0.05). In accordance with the ROC analysis, the diagnostic properties of SLPI and elafin levels in the AxSpA and nr-AxSpA groups exhibit notable statistical significance (p<0.05). There was no significant correlation between SLPI and elafin values and disease activity parameters in the disease groups. A significant correlation was also observed between SLPI and elafin levels in both the nr-AxSpA and r-AxSpA groups.

This is the first study to measure serum levels of elafin and SLPI using AxSpA, analyze their diagnostic significance, and investigate their correlation with disease activity. While HLA-B27 is considered the most valuable diagnostic marker in AxSpA patients, CRP is used to assess disease activity and predict disease progression or treatment response.[8] Furthermore, high BMI values have been reported to be associated with disease activity in AxSpA patients.[26] In our study, the HLA-B27 rate in the AxSpA groups was around 66 to 70%, consistent with the literature.[27]

CRP values were within the normal range for all disease groups. However, a statistically significant difference was found between the r-AxSpA and control groups. In this context, average CRP values might indicate no significant inflammation due to the patients receiving treatment and the disease activity is low or in remission. Given that the CRP values fell within the normal range, the slight increase in CRP observed in the r-AxSpA group can be deemed inconsequential from a clinical standpoint. Additionally, in our study, BMI values were within the normal range for the patient groups (normal range: 18.5-24.9 kg/m$^2$). However, despite being within the normal range, the r-AxSpA group had significantly higher BMI values than the control group. The occurrence of a high BMI value may be attributed to previous distressing conditions and restrictions in joint functionality caused by ankylosis and a sedentary lifestyle.

There are a few studies examining the serum levels of SLPI and elafin. In the literature, SLPI and elafin serum levels have been evaluated in diseases associated with the SpA group, such as psoriasis, IBD, and other rheumatological diseases. A study on psoriasis reported that

SLPI and elafin levels were higher in patients compared to healthy controls, indicating a protective effect of SLPI against the disease.[13] Additionally, they mentioned that elafin levels were associated with disease severity.[12] Another study related to IBD reported that SLPI has antiprotease activity against neutrophil elastase enzyme-induced functional impairment in the intestinal epithelium, thus protective against colitis.[18] Increased elafin expression in the colonic mucosa of IBD patients, particularly those with ulcerative colitis, has been demonstrated.[28,29] However, there are also studies in the literature reporting a decrease in elafin expression in the mucosa of IBD patients, which could lead to increased elastolytic activity in the colon tissue,[30] and stating that elafin levels decrease in IBD and correlate negatively with disease activity.[14] These studies suggest that the low elafin levels could be within systemic or local intestinal protease/antiprotease imbalance, potentially leading to increased elastase proteolytic activity and intestinal inflammation. Additionally, they suggest that the decrease in elafin could be a consequence of IBD progression rather than a cause and that chronic inflammation could lead to elafin consumption, ultimately implying a protective role of elafin in IBD.[14,30]

In an *in vitro* study modeling RA in mice, it was reported that SLPI, when secreted from synovial fibroblasts over time, reduces immunoglobulin (Ig) G/IgM production and the severity of arthritis and cartilage damage in mice treated with SLPI. This finding highlighted a new endogenous anti-inflammatory pathway with therapeutic potential in RA.[16] Moreover, while SLPI levels increased in OA joint chondrocytes, it did not seem to modulate OA development in mice by itself; however, it could potentially be a biomarker for OA in humans and animal models.[18] Another study on SS disease reported that SLPI and elafin levels were higher in SS and RA patients than in healthy controls. However, no statistical relationship existed among inflammation markers, such as ESR and CRP. They suggested that they could play a role in the pathogenesis of SS and could be considered candidates for serum biomarkers in SS with lung involvement.[17] Furthermore, SLPI and elafin, important in inflammation and having roles in the early stages of enthesis development, could indicate critical roles in AxSpA pathogenesis.[19,31]

Additionally, SLPI and elafin inhibit TNF-alpha synthesis; it has been suggested that they may play an essential role in the pathogenesis of AxSpA disease, which often responds significantly to TNF-alpha inhibition.[22]

The present study found that SLPI and elafin serum levels were significantly higher in the AxSpA and nr-AxSpA groups compared to the control group. However, this elevation was not significant in the r-AxSpA group. The highest to lowest serum levels in patient groups were nr-AxSpA, AxSpA, and r-AxSpA, respectively. The potential cause for the increased levels of SLPI and elafin could be attributed to the ongoing inflammatory process experienced by patients or the emergence of an anti-inflammatory response as a result of said process. Moreover, the presence of enzyme elevations in etiopathogenetically similar diseases (e.g., psoriasis and IBD) suggests that this situation may not be specific to AxSpA and might be related to tissue damage and activation of local cells and inflammation in the region.

Furthermore, it is possible that the elevated serum level observed in nr-axSpA patients is a consequence of their reduced exposure to disease and chronic inflammation. The duration of the disease was notably reduced in the nr-AxSpA group. In patients with r-AxSpA, the potential cause for the lowest value could be attributed to increased consumption resulting from heightened exposure to disease and chronic inflammation. The duration of the disease was markedly elevated in the r-AxSpA group. However, the absence of a correlation between disease duration and serum alarm antiprotease levels was observed within the disease groups.

In simpler terms, SLPI and elafin enzymes can act as a serum biomarker that forms during inflammation or as a protective anti-inflammatory biomarker that increases in response to inflammation.

Therefore, it is revealed that SLPI and elafin possess the potential to unveil the contributions of the antiprotease enzyme system towards the development of diseases and its pivotal roles in the process.

In the study, there was no relationship between disease activity parameters and SLPI and elafin in the disease groups. This may

be due to the fact that patients are under treatment and have low disease activity or remission. Furthermore, the participants in the investigation were categorized into two groups: those undergoing NSAID or TNF-alpha blocker therapy. The observed groups did not exhibit any notable disparity in SLPI and elafin levels. The potential implication of this observation suggests that both medication interventions play an important role in relation to SLPI and elafin.

The positive correlation between SLPI and elafin in the groups might indicate harmony between these proteins in their functions and joint functional activities. For instance, both are potent serine protease and significant neutrophil elastase inhibitors. Additionally, it is known that SLPI inhibits cathepsin G but not proteinase 3, while elafin is a proteinase 3 inhibitor but does not inhibit cathepsin G.[32] Therefore, while performing their functions, it is conceivable that when one protein cannot perform a task, the other takes on that task and works together in a unified manner.

In the literature, the most potent biomarker currently used for diagnosing AxSpA is HLA-B27. The prevalence of HLA-B27 varies among different continents and ethnic/racial populations. For example, in the USA, HLA-B27 shows moderate to high sensitivity and low specificity in AS patients, whereas in Lebanon, it can exhibit low sensitivity (41.1%) and high specificity (96.2%).[33,34] In Türkiye, the prevalence of HLA-B27 in AS patients has been reported to be approximately 70%, similar to the data from our study.[35] Therefore, the sensitivity and specificity of HLA-B27 can vary in different geographical and ethnic/racial populations among AxSpA patients. In our study, the sensitivity and specificity values of SLPI and elafin are close to HLA-B27, the most potent biomarker currently used for diagnosis. Thus, if the serum levels of SLPI and elafin are above the determined cut-off value, they could serve as supportive biomarkers in disease diagnosis or monitoring treatment response. They could play an alarming role, particularly in the early stages of the disease, as indicated in the article's title.

The strengths of our study include being the first to evaluate the possibility of SLPI and elafin serum levels as supportive biomarkers in disease diagnosis and their potential relationship with disease activity. The limitations of our study include a measurement of SLPI and elafin levels while patients were under treatment, a lack of long-term follow-up, and an absence of bone change assessment.

In conclusion, the levels of SLPI and elafin were found to be significantly elevated in patients with AxSpA, particularly in those with nr-AxSpA, compared to the control group. Therefore, SLPI and elafin can be used as therapeutic biomarkers for the diagnosis of AxSpA and nr-AxSpA. However, no relationship was found with disease activity.

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