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COMPARATIVE EVALUATION OF SERUM NEOPTERIN AND BIOPYRRIN IN AXIAL SPONDYLOARTHRITIS: A CROSS-SECTIONAL STUDY

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ABSTRACT

Objective: To compare serum neopterin and biopyrrin levels in axial spondyloarthritis (axSpA) and healthy controls, and to assess their associations with clinical phenotypes and diagnostic performance.

Methodology: This multicenter cross-sectional study included 119 patients with axSpA and 130 healthy controls. Serum neopterin and biopyrrin levels were measured and compared across treatment exposure, C-reactive protein (CRP) status, and radiographic classification. Diagnostic performance was evaluated using receiver operating characteristic (ROC) analysis. Multivariable linear regression was used to assess independent associations after adjustment for age, sex, and body mass index.

Results: Both biomarkers were significantly higher in axSpA compared with controls. Neopterin showed moderate discrimination (AUC 0.74; 95% CI 0.68–0.80; $p<0.001$), while biopyrrin showed weaker performance (AUC 0.60; 95% CI 0.53–0.67; $p=0.018$). Biomarker levels were highest in treatment-naïve patients and remained elevated in CRP-normal disease. Neopterin levels were higher in non-radiographic axSpA. In multivariable analysis, neopterin remained independently associated with axSpA, whereas biopyrrin did not.

Conclusion: Neopterin and biopyrrin are elevated in axial spondyloarthritis, with neopterin demonstrating independent association with disease status and moderate diagnostic performance. Persistent elevation in CRP-normal disease suggests ongoing immuno-oxidative activity beyond conventional inflammatory measures. These findings support neopterin as a promising complementary biomarker for disease characterization, warranting further longitudinal validation.

KEYWORDS: axial spondyloarthritis; neopterin; oxidative stress; biomarker; C-reactive protein; macrophage activation.

1. INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory rheumatic disease with marked clinical and biological heterogeneity[1]. C-reactive protein (CRP) is widely used to assess inflammation and is incorporated into the Ankylosing Spondylitis Disease Activity Score (ASDAS-CRP), a recommended measure of disease activity. However, many patients show active disease despite normal CRP levels, reflecting a discordance between symptoms and measurable systemic inflammation and highlighting the need for complementary approaches[2–4].

Biomarkers may improve diagnosis and disease assessment by identifying inflammatory pathways not captured by routine measures[5,6]. Expanding biomarker evaluation beyond conventional indices may help better understand disease heterogeneity in axSpA.

Macrophage activation plays a key role in inflammatory diseases. Neopterin, released by interferon-γ-stimulated macrophages, reflects cellular immune activation and is linked to both inflammatory and oxidative pathways[7,8]. Its consistent elevation in rheumatic diseases supports its role as a marker of immune-metabolic stress[9]. Oxidative stress also contributes to chronic inflammation and immune dysregulation[10]. Biopyrrins, oxidative metabolites of bilirubin, are stable markers of oxidative stress and may reflect biological activity beyond conventional inflammatory markers[11]. Inflammatory and oxidative pathways are closely interconnected and may contribute to persistent disease activity[12].

However, integrated evaluation of immuno-oxidative pathways across clinical phenotypes and treatment stages in axSpA remains limited. The biological profile of patients with clinically active but CRP-normal disease is poorly understood[9,13]. Differences between radiographic and non-radiographic axSpA may reflect distinct mechanisms, yet comparative biomarker studies are scarce[14].

We hypothesized that immuno-oxidative biomarkers reflect biological activity beyond conventional inflammatory measures. This study evaluated neopterin and biopyrrin levels in axSpA and their associations with treatment exposure, CRP phenotype, and radiographic status.

2. METHODS

2.1 Study design and participants

This cross-sectional study included 119 patients with axSpA and 130 healthy controls. Adult patients (≥18 years) fulfilling the Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axSpA[15] were recruited consecutively from participating rheumatology centers and included both newly diagnosed and established cases. Treatment-naïve patients had no prior therapy, while treated patients were required to be on stable treatment for at least four weeks before sampling[16],[17].

Exclusion criteria included acute infection, malignancy, other systemic autoimmune diseases, chronic kidney or liver disease, pregnancy, and recent vaccination[8]. Healthy controls had no history of inflammatory, autoimmune, metabolic, or chronic disease and had normal CRP levels[18].

2.2 Clinical assessment

Demographic data, disease duration, body mass index (BMI), smoking status, and treatment exposure were recorded. HLA-B27 status was obtained from medical records. Patients were classified as radiographic or non-radiographic axSpA according to ASAS criteria.

Clinical assessment and blood sampling were performed on the same day. Disease activity was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[19] and the ASDAS-CRP, calculated according to standard methods[4]. Erythrocyte sedimentation rate (ESR) was measured using routine laboratory methods. CRP-normal axSpA was defined as CRP <5.0 mg/L.

2.3 Treatment exposure groups

Patients were categorized into three groups: treatment-naïve, NSAID-only, and biologic therapy. The biologic group included patients receiving tumor necrosis factor (TNF) inhibitors or interleukin-17 (IL-17) inhibitors, with or without NSAIDs. Treatment classification was based on current medication at the time of sampling.

2.4 Biomarker assessment

Non-fasting venous blood samples were collected, centrifuged, and serum was stored at -80°C until analysis.

Serum neopterin (nmol/L) and biopyrrin (U/L) were measured using commercially available ELISA kits (Sunlong Biotech, China) according to the manufacturer's instructions. Samples were analyzed in duplicate, and mean values were used. Laboratory personnel were blinded to clinical data. Assay performance was within manufacturer-specified ranges.

Biomarker analysis was exploratory and aimed to characterize immuno-oxidative profiles rather than establish diagnostic thresholds.

2.5 Statistical analysis

Statistical analysis was performed using SPSS version 29.0 (IBM Corp., USA). Data distribution was assessed using the Shapiro-Wilk test. Continuous variables are presented as median and interquartile range (IQR).

Group comparisons were performed using the Mann–Whitney U test or Kruskal–Wallis test, with Bonferroni correction for post-hoc analysis. Correlations were assessed using Spearman's rank correlation. Effect sizes were reported as r and η^2.

Multivariable linear regression was used to assess independent associations between axSpA and log-transformed biomarker levels, adjusting for age, sex, and BMI. Model assumptions were checked before analysis.

All analyses were exploratory. A two-sided p-value <0.05 was considered statistically significant. Complete-case analysis was performed without imputation.

2.6 Ethical statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Hawler Medical University (approval number 2/4327, 14/11/2024). Written informed consent was obtained from all participants. The study was not prospectively registered due to its observational design.

3. RESULTS

3.1 Baseline characteristics

A total of 119 patients with axSpA and 130 healthy controls were included. One patient had incomplete imaging data. Among the remaining 118 patients, 58 (49.2%) had radiographic axSpA and 60 (50.8%) had non-radiographic axSpA. HLA-B27 positivity was present in 51 patients (42.9%).

Baseline characteristics are shown in Table 1. Age and body mass index (BMI) were similar between patients and controls. Patients with axSpA showed expected disease activity (median BASDAI 3.80 [IQR 2.82–4.47]; ASDAS-CRP 2.79 [1.96–3.28]).

3.2 Biomarker levels in axSpA versus controls

Both serum neopterin and biopyrrin levels were significantly higher in patients with axSpA compared with healthy controls (Table 2). Elevated levels were also observed in patients with normal CRP.

Neopterin levels were significantly higher in axSpA ($p<0.001$, $r=0.40$). Biopyrrin was also increased ($p=0.009$), although with a smaller effect size ($r=0.17$).

3.3 Diagnostic discrimination analysis

Receiver operating characteristic (ROC) analysis showed that neopterin had moderate ability to distinguish axSpA from controls (AUC 0.74, 95% CI 0.68–0.80, $p<0.001$). Biopyrrin showed weaker discrimination (AUC 0.60, 95% CI 0.53–0.67, $p=0.018$). ROC curves are presented in Figure 2.

3.4 Treatment exposure analysis

Biomarker levels differed across treatment groups (Figure 1). Treatment-naïve patients had the highest levels (neopterin: 13.96 [12.17–18.63] nmol/L; biopyrrin: 72.34 [62.55–87.67] U/L).

Both biomarkers showed significant differences across groups ($p<0.001$ for both). Patients receiving NSAIDs had lower levels, while those on biologic therapy showed reduced variability but persistent elevation.

3.5 CRP phenotype analysis

Patients were stratified into CRP-normal (n=96) and CRP-elevated (n=23) groups. Neopterin levels were similar between CRP-normal (11.23 nmol/L) and CRP-elevated (12.01 nmol/L) patients ($p=0.514$), and both were higher than controls ($p<0.001$).

Biopyrrin levels also did not differ significantly between CRP groups ($p=0.512$).

3.6 HLA-B27 status

No significant differences in neopterin or biopyrrin levels were observed between HLA-B27-positive and HLA-B27-negative patients (all $p>0.05$).

3.7 Radiographic versus non-radiographic axSpA

Patients with non-radiographic axSpA had higher neopterin levels compared with radiographic disease (12.40 [9.22–15.79] vs 10.09 [8.38–12.39] nmol/L, $p=0.003$).

Biopyrrin levels were not significantly different between groups (64.36 [47.49–79.84] vs 54.99 [35.13–68.56] U/L, $p=0.09$) (Table 2).

3.8 Correlation with clinical disease activity

Both biomarkers showed weak and non-significant correlations with disease activity measures. Neopterin had minimal correlation with BASDAI ($\rho=0.10$, $p=0.291$) and ASDAS-CRP ($\rho=0.09$, $p=0.333$).

Biopyrrin showed similarly weak correlations with BASDAI ($\rho=0.13$, $p=0.176$) and ASDAS-CRP ($\rho=0.12$, $p=0.215$). Correlations with CRP and ESR were also weak (all $\rho<0.20$).

3.9 Multivariable exploratory analysis

Multivariable regression analysis (Table 3) showed that axSpA was independently associated with higher log-neopterin levels ($\beta=0.327$, $p<0.001$), but not with biopyrrin ($\beta=0.017$, $p=0.759$).

Male sex was associated with lower levels of both neopterin ($\beta=-0.190$, $p<0.001$) and biopyrrin ($\beta=-0.120$, $p=0.03$), while age and BMI were not significantly associated with either biomarker.

4. DISCUSSION

This study shows that biomarkers of macrophage activation and oxidative stress are elevated in axSpA. Neopterin demonstrated a consistent and independent association with disease status, whereas biopyrrin showed weaker associations. Importantly, both biomarkers remained elevated in patients with normal CRP, suggesting persistent biological activity beyond conventional inflammatory markers.

CRP is widely used in clinical practice and is incorporated into the ASDAS-CRP score, a validated measure of disease activity in axSpA[19,4]. However, our findings support previous evidence that CRP-based assessment may not fully capture disease activity in all patients[2,3]. The persistence of elevated biomarkers in CRP-normal disease indicates additional biological pathways beyond acute-phase responses.

These results are consistent with studies showing increased neopterin levels in spondyloarthritis[20] and the role of oxidative stress in ankylosing spondylitis[21,22], and broader evidence of altered oxidative stress and immune activation in rheumatic diseases[10,22,9]. Biomarker levels were independent of HLA-B27 status[2,15] and showed weak correlations

with disease activity measures, including ASDAS-CRP, suggesting that immuno-oxidative activity reflects complementary biological processes.

The findings support the biological heterogeneity of axSpA. Neopterin reflects macrophage-driven immune activation and links inflammation with oxidative stress[7]. Higher levels in treatment-naïve patients likely reflect untreated inflammation, while increased neopterin in non-radiographic axSpA may indicate more active immune processes in earlier disease stages.

Clinically, persistent immuno-oxidative activation despite normal CRP and ASDAS-CRP may explain the discordance between symptoms and objective markers in axSpA. These biomarkers may therefore provide complementary information for disease characterization, although they are not yet suitable for routine clinical use and require further validation[5,23].

This study has several limitations. Its cross-sectional design does not allow assessment of causality or disease progression. The study was conducted in a single region, which may limit generalizability. Oxidative stress biomarkers may also be influenced by unassessed metabolic or lifestyle factors, and measurements at a single time point do not capture longitudinal disease activity.

5. CONCLUSION

ASDAS-CRP may not fully capture biological activity in axial spondyloarthritis. Elevated immuno-oxidative biomarkers, particularly neopterin, indicate persistent immune activation even in patients with low inflammatory activity. These findings support the role of immuno-oxidative markers as complementary tools for disease characterization. Further longitudinal and comparative studies are needed to define their value in disease monitoring and biomarker-based stratification.

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