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Comparison of the frequency of *HLA B27* gene positivity and negativity and biochemical laboratory findings in patients diagnosed with ankylosing spondylitis

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ABSTRACT

Aim: To compare some routinely studied hematological and biochemical parameters in HLA-B27 positive and negative ankylosing spondylitis (AS) patients and to evaluate their potential as early disease activity biomarkers.

Methods: This retrospective study included 272 AS patients (136 HLA-B27 positive and 136 HLA-B27 negative) admitted to the Medical Genetics Department of Bolu Abant İzzet Baysal University Izzet Baysal Training and Research Hospital between 2018 and 2020. HLA-B27 genotyping was performed using Real-Time PCR. Demographic and laboratory data were collected from records, and statistical analyses compared biomarker levels between groups using appropriate tests.

Results: Significantly higher white blood cell (WBC) count (p=0.033) and lymphocyte (LYM) (p=0.030) were observed in males; females had higher platelet count (PLT) (p=0.013) and procalcitonin (PCT) (p=0.016). The median values of all analyzed biochemical parameters were significantly higher in the HLA-B27 positive group than in the negative group ($p < 0.05$). HLA-B27 positive females had higher PLT and PCT, while negative females showed increased PLT, PCT, neutrophil (NEU), and neutrophil-to-lymphocyte ratio (NLR) ($p < 0.05$).

Conclusion: Our study findings suggest that hematological and inflammatory markers vary by HLA-B27 status and gender. In addition, elevated mean platelet volume (MPV) and NLR may serve as useful biomarkers for early diagnosis, monitoring, and prognosis, highlighting the importance of integrating lab parameters with genetic profiling in AS management.

Keywords: Ankylosing spondylitis, *HLA B27*, complete blood bount parameters, spondyloarthritis.

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1. Introduction

Spondyloarthritis (SpA) is a group of chronic inflammatory diseases characterized by various clinical findings, including sacroiliitis, uveitis, enthesitis, and arthritis. Ankylosing spondylitis (AS), a member of this group, is an example of seronegative spondyloarthritis primarily

affecting the sacroiliac joints and spine. The indications and prevalence of the disease vary according to age, sex, and ethnicity, with global rates ranging from 0.1% to 1.4% [1].

Although the pathogenesis of AS is not fully understood, genetic, environmental, and immunological factors play a significant role. One of the most important genetic factors in the development of the disease is the Human Leukocyte Antigen (HLA)-B27. *HLA-B27*, which is closely associated with AS, is found at varying rates in different populations. For example, the frequency of *HLA-B27* is reported to be 95% in Northern European countries, 83% in Japan, and between 70% and 90% in Turkey [2].

Systemic inflammation has distinct effects on circulating blood cells. Inflammatory markers can be used to assess disease activity. Furthermore, this association reflects the broader role of systemic inflammation in AS pathogenesis. Inflammatory markers such as MPV and NLR have also been studied in other inflammatory and autoimmune conditions, including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, and even malignancies. Their diagnostic and prognostic value in these diseases supports the rationale for evaluating their relevance in AS [3-4]. Additionally, parameters such as white blood cell (WBC) count, mean platelet volume (MPV), red cell distribution width (RDW), and neutrophil-to-lymphocyte ratio (NLR) have also been reported to be associated with AS [5].

In studies, low MPV levels have been identified in active cases of AS and rheumatoid arthritis (RA), with MPV levels returning to normal with appropriate treatment for AS [6]. RDW is a hematological index that reflects variations in red blood cell size and is associated with various conditions, including AS, RA, and osteoarthritis (OA) [7]. One study reported that RDW and NLR are related to autoimmune diseases, including AS [8]. Studies have also shown that platelet count (PLT) values can vary in AS patients [9]. Various laboratory markers, including erythrocyte sedimentation rate (ESR), CRP, complete blood count, platelet count, neutrophils, lymphocytes, platelet-to-lymphocyte ratio (PLR), and NLR, have been found to be significantly elevated in AS patients. Procalcitonin (PCT) has emerged as a useful laboratory marker for assessing the diagnostic importance of rheumatology compared to other biomarkers like CRP and ESR [10].

Immune-mediated mechanisms, particularly involving interactions among T cells, B cells, osteoblasts, and osteoclasts, have also been shown to play a role in the pathogenesis of AS. Neutrophils play a crucial role in the pathogenesis of AS [11]. One study suggested that neutrophil activation may contribute to oxidative stress, which is implicated in the pathogenesis of AS [12]. The lymphocyte ratio is a measure used to determine disease activity in AS patients and is used alongside other ratios such as NLR, PLR, and mean platelet volume [13]. In this study, we aimed to investigate the changes in biochemical blood count parameters in HLA-B27 positive and negative patients with AS. Given the known associations of these markers with inflammation in a variety of diseases, including autoimmune and chronic inflammatory disorders, our study aims to shed light on whether these parameters can be used as potential predictors in the context of AS.

2. Materials and methods

The sample group of our study consisted of patients diagnosed with AS who were referred to or directly admitted to the Medical Genetics Department of Bolu Abant İzzet Baysal University (BAIBU) Izzet Baysal Training and

Research Hospital. The sample was formed from patients who visited the Medical Genetics Department between 2018 and 2020, selected based on complete genetic results and relevant data. The study included 136 AS patients characterized by *HLA-B27* positive tissue type and 136 AS patients characterized by *HLA-B27* negative tissue type. The demographic characteristics of the patients and biochemical measurement values such as CRP, RDW, MPV, WBC, neutrophils, lymphocytes, NLR, ESR, PLT, and PCT were retrospectively obtained and evaluated from patient files. The *HLA-B27* genetic analyses of the patients were performed using Real-Time PCR.

Written informed consent forms were obtained from all patients. Ethical approval for our study was granted by the Clinical Research Ethics Committee of Bolu Abant İzzet Baysal University on 22/08/2023, with decision number 2023/250.

2.1. Sample Calculation: Considering equal distribution among groups, the sample size was calculated using GPower 3.1.9.7 software with an effect size of 0.5 and a power of 0.95, indicating a necessary minimum total sample size of 210. It was concluded that a minimum of 105 participants was required for each group. Consequently, it was decided to include 136 individuals in each group.

2.2. Statistical Analysis: All statistical computations were carried out with the aid of SPSS (v20.0.0). The distribution of the variables was assessed using the Shapiro-Wilk test. For data conforming to a normal distribution, results were expressed as the arithmetic mean accompanied by standard deviation, whereas non-normally distributed data were summarized using the median and interquartile range. Differences in demographic data and biochemical parameters between groups were assessed using Student's t-test or Mann-Whitney U test, depending on the distribution. The significance level was taken as $p < 0.05$.

3. Results

Among the 272 AS patients included in the study, 53.3% were male and 46.7% were female. In the *HLA-B27* negative group, 62 (45.6%) of patients were male and 74 (54.4%) were female, whereas in the positive group, 83 (61%) were male and 53 (39%) were female.

The average age of patients in the *HLA B27* positive group was found to be 35.51 ± 13.14, while the average age of patients in the negative group was 34 ± 14.97. No statistically significant difference was found in the average ages of the *HLA B27* positive and negative groups (p=0.741).

3.1. Comparison of biochemical parameters in HLA B27 negative and positive patients: Descriptive statistics for the biochemical parameters of *HLA B27* negative and positive patients, as well as the results of the comparative analysis of the groups, including minimum, maximum, median, and IQR values, are presented in Table 1.

Our analysis revealed that the median WBC value in the positive group was significantly higher than in the negative group (U=6,638.0; p<0.001). The median MPV value was also significantly elevated in the positive group compared to the negative group (U=6,828.0; p<0.001). Additionally, the median RDW value was greater in the positive group (U=6,451.0; p<0.001). The positive group showed a higher median PLT value than the negative group, with significant differences (U=7,915.5; p=0.040). The median PCT value was higher in the positive group (U=6,723.5; p<0.001). Similarly, the median NEU value was significantly greater in the positive group (U=4,760.5; p<0.001), as was the median LYM value (U=1,983.0; p<0.001). The median CRP value was also significantly

Table 1. Descriptive statistics and comparison of biochemical parameters in *HLA B27* negative and positive patients.

	***HLA B27* negative**				***HLA B27* positive**				**U***	***p***
	Min	**Max**	**Median**	**IQR**	**Min**	**Max**	**Median**	**IQR**		
WBC	2,350	16,70	7,00	3,43	4,38	15,45	7,99	2,73	6.638,0	***<0,001***
MPV	1,04	10,50	7,75	1,54	5,83	16,600	8,17	1,56	6.828,0	***<0,001***
RDW	10,90	19,00	15,00	2,65	8,00	21,00	15,80	2,67	6.451,0	***<0,001***
PLT	62,20	431,00	250,00	88,75	135	541	266	87,50	7.915,5	***0,040***
PCT	0,054	0,386	0,206	0,074	0,139	0,620	0,236	0,101	6.723,5	***<0,001***
NEU	0,012	0,890	0,1065	0,051	0,046	0,219	0,121	0,054	4.760,5	***<0,001***
LYM	31,00	49,50	40,00	5,30	40,90	55,60	46,55	3,925	1.983,0	***<0,001***
CRP	0,100	75,60	3,50	6,60	0,100	87,32	5,435	6,605	7266,5	***0,002***
ESH	4,00	78,00	17,00	16,75	4,00	84,00	21,50	21,00	6.627,0	***<0,001***
NLR	0,012	0,890	0,10650	0,051	0,046	0,219	0,12100	0,054	7.157,0	***0,001***

** Mann-Whitney U*

higher in the positive group (U=7,266.5; p=0.002). Moreover, the median ESH value was higher in the positive group (U=6,627.0; p<0.001). Finally, the median NLR value was significantly greater in the positive group (U=7,157.0; p=0.001).

In conclusion, significant differences in biochemical parameters were observed in individuals with a positive HLA B27 result (p<0.05), aligning with existing literature.

3.2. Comparison of biochemical parameters in male and female patients: The statistics for the biochemical parameters of female and male patients included in the study, along with the results of the comparison between genders, are presented in Table 2.

The results of our analysis indicated that the median WBC value in males was significantly higher than in females (U=7,828.5; p=0.033). In contrast, the difference in median MPV values between the groups was not statistically significant (U=9,337.0; p=0.841), nor was the difference in median RDW values (U=10,003.5; p=0.219). However, the median PLT value was higher in females, and this difference was statistically significant (U=10,818.5; p=0.013). Similarly, the median PCT value was significantly higher in females (U=10,760.5; p=0.016). The median NEU values showed no significant difference between groups (U=9,388.5; p=0.780). Conversely, the median LYM value was significantly higher in males (U=7,807.0; p=0.030). The difference in median CRP values was not statistically significant (U=8,359.0; p=0.190), and the ESH values were also nonsignificant between the groups (U=9,422.5; p=0.740). Lastly, the analysis of NLR did not yield a statistically significant result (U=9,822.5; p=0.342). In conclusion, while MPV, RDW, NEU, CRP, ESH, and NLR values were similar between genders, males had significantly higher WBC and LYM values, whereas females had significantly higher PLT and PCT values.

Table 2. Descriptive statistics and comparison of biochemical parameters in males and females.

	Male				**Female**				**U***	***p***
	Min	**Max.**	**Median**	**IQR**	**Min**	**Max.**	**Median**	**IQR**		
WBC	2,35	16,10	7,77	3,01	2,83	16,70	7,18	2,99	7.828,5	***0,033***
MPV	1,04	16,60	7,90	15,56	5,37	13,60	8,030	1,63	9.337,0	*0,841*
RDW	10,90	19,40	15,40	3,10	8,00	21,00	15,50	2,70	10.003,5	*0,219*
PLT	135,00	479,00	251,00	80,50	62,200	541,00	268,00	93,00	10.818,5	***0,013***
PCT	0,119	0,600	0,210	0,083	0,054	0,620	0,226	0,087	10.760,5	***0,016***
NEU	0,012	0,890	0,110	0,054	0,012	0,418	0,118	0,055	9.388,5	*0,780*
LYM	31,00	55,60	44,00	24,60	32,90	53,200	43,50	6,500	7.807,0	***0,030***
CRP	0,100	87,32	5,37	6,97	0,100	58,400	4,00	6,300	8.359,0	*0,190*
ESH	5,00	78,00	18,00	17,00	4,00	84,00	19,00	16,00	9.422,5	*0,740*
NLR	0,012	0,890	0,110	0,054	0,012	0,418	0,118	0,055	9.822,5	*0,342*

** Mann-Whitney U*

The results obtained from the analysis of biochemical parameters by gender in the *HLA B27* positive patient group are presented in Table 3.

The difference in median WBC values between the groups was found to be statistically non-significant (U=2,006.0; p=0.388). When examining the difference in median MPV values,

Tablo 3. Descriptive statistics and comparison of biochemical parameters of males and females in HLA B27 positive patients.

***HLA B27* Positive**	**Male**				**Female**				**U***	***p***
	Min	**Max.**	**Median**	**IQR**	**Min**	**Max.**	**Median**	**IQR**		
WBC	4,70	13,100	7,980	2,650	4,380	15,450	8,100	2,955	2.006,0	*0,388*
MPV	5,83	16,60	8,190	1,710	6,210	13,600	8,160	1,475	2.242,5	*0,848*
RDW	11,60	19,40	15,600	2,600	8,000	21,00	16,100	2,90	2.528,5	*0,142*
PLT	135,00	479,00	256,00	77,000	174,00	541,00	288,00	109,50	2.673,0	***0,035***
PCT	0,144	0,60	0,227	0,085	0,139	0,620	0,250	0,118	2,711,0	***0,022***
NEU	0,046	0,219	0,126	0,055	0,046	0,198	0,119	0,058	2.070,0	*0,563*
LYM	40,900	55,600	47,00	4,500	41,00	53,20	45,90	3,50	2.150,5	*0,827*
CRP	0,100	87,320	5,700	6,210	0,110	58,40	5,00	6,50	2.153,5	*0,837*
ESH	6,00	77,00	22,00	19,00	4,00	84,00	21,000	21,00	2.343,5	*0,520*
NLR	0,046	0,219	0,126	0,055	0,046	0,198	0,119	0,058	2.083,0	*0,603*

** Mann-Whitney U*

no statistically significant result was obtained (U=2,242.5; p=0.848). The analysis of median RDW values between the groups also revealed no significant difference (U=2,528.5; p=0.142). The median PLT value in females was significantly higher than that in males, and this difference was statistically significant (U=2,673.0; p=0.035). The median PCT value in females was higher than in males, and this difference was also significant (U=2,711.0; p=0.022). No statistically significant difference was observed in the comparison of median NEU values between the groups (U=2,070.0; p=0.563). Similarly, the analysis of median LYM values did not yield a statistically significant result (U=2,150.5; p=0.827). The difference in median CRP values between the groups was also found to be statistically non-significant (U=2,153.5; p=0.837). The median ESH values did not show a statistically significant difference between the groups (U=2,343.5; p=0.520). The analysis of median NLR values between the groups also did not reveal a significant difference (U=2,083.0; p=0.603). In conclusion, the values of WBC, MPV, RDW, NEU, LYM, CRP, ESH, and NLR were similar between male and female patients with *HLA B27* positivity; however, the PLT and PCT values in females were found to be significantly higher than those in males.

In our study, the analysis results of biochemical parameters according to gender in *HLA B27* negative patients are presented in Table 4. In our research, the biochemical parameters analyzed by gender in *HLA B27* negative patients are summarized in Table 4. There was no notable variation in median WBC values among the groups (U = 2,016.5; p = 0.225). Similarly, the difference in median MPV values was not significant (U=2,514.5; p=0.335), nor were the median RDW values (U=2,647.5; p=0.096). A statistically significant difference was observed in median PLT values, with higher levels detected in females than in males (U=2,752.0; p=0.045). Additionally, the median PCT value in females was also significantly greater than in males (U=2,759.5; p=0.042). The median NEU

Tablo 4. Descriptive statistics and comparison of biochemical parameters for male and female patients with HLA B27 negative.

***HLA B27* negative**	**Male**				**Female**				**U***	***p***
	Min	**Max.**	**Median**	**IQR**	**Min**	**Max.**	**Median**	**IQR**		
WBC	2,350	16,100	7,230	3,707	2,83	16,70	6,79	2,85	2.016,5	*0,225*
MPV	1,040	10,33	7,45	1,525	5,37	10,50	7,835	1,508	2.514,5	*0,335*
RDW	10,90	18,20	14,55	3,150	11,10	19,00	15,100	2,40	2.647,5	*0,096*
PLT	147,00	413,00	242,50	66,50	62,200	431,00	262,00	86,25	2.752,0	***0,045***
PCT	0,119	0,386	0,195	0,067	0,054	0,360	0,216	0,079	2.759,5	***0,042***
NEU	0,012	0,890	0,10	0,041	0,012	0,418	0,117	0,049	2.946,5	***0,004***
LYM	31,00	49,50	40,05	4,875	32,90	49,20	40,000	6,10	2.123,0	*0,455*
CRP	0,100	75,600	4,350	7,280	0,100	37,30	3,00	6,49	2.071,0	*0,330*
ESH	5,00	78,00	15,000	15,00	4,00	45,00	18,00	19,25	2.464,5	*0,456*
NLR	0,012	0,890	0,100	0,041	0,012	0,418	0,1175	0,049	2.892,5	***0,009***

** Mann-Whitney U*

value was higher in females as well, with a statistically significant difference (U=2,946.5; *p*=0.004). The median LYM values did not show a significant difference (U=2,123.0; *p*=0.455), and the median CRP values were also not statistically significant (U=2,071.0; *p*=0.330). Similarly, the differences in median ESH values between the groups were not significant (U=2,464.5; *p*=0.456). Notably, the median NLR value was significantly higher in females compared to males (U=2,892.5; *p*=0.009). WBC, MPV, RDW, LYM, CRP, and ESR values were similar in *HLA-B27* negative males and females, while PLT, PCT, NEU, and NLR were significantly higher in females

4. Discussion

Ankylosing spondylitis is a long-term inflammatory disorder that predominantly involves the axial skeleton, particularly the spine and sacroiliac joints. The prevalence of *HLA B27* varies globally, ranging from 0.32% to 1.4%, with a reported prevalence of 0.49% in Turkey. This genetic marker is found in 90% of AS patients compared to less than 8% in the general population, underscoring the link between *HLA B27* positivity and AS, particularly due to specific *HLA-B* gene variants [14].

AS is characterized by chronic inflammation that can lead to significant pain in the back, lumbar region, hips, and legs. This systemic inflammatory response may alter hematological parameters, potentially serving as indicators of disease activity and associated risks. Differences in blood parameters between *HLA B27* positive and negative patients have been noted, yet insufficient data exist on this comparison, limiting the understanding of *HLA B27*'s impact on these parameters [15]. Consequently, recent studies have aimed to investigate the effects of *HLA B27* positivity and negativity on biochemical blood parameters in AS patients. Our study seeks to explore the relationship between *HLA B27* genetic analysis results and biochemical blood count parameters in AS patients.

Our research first assessed patient age and gender. No significant difference was found in average ages between the *HLA B27* positive and negative groups, although a statistically significant difference was noted in age distribution by gender. Öztürk et al. reported similar findings regarding age distribution in *HLA B27* positive individuals [16], and Taşkın et al. found no statistical difference in average ages between positive and negative patients [17].

Regarding biochemical parameters, the WBC level reflects disease activity and inflammation severity in rheumatic diseases like AS. Therefore, WBC levels are expected to be an important parameter in the management of AS concerning disease progression and treatment response [18]. Our study revealed statistically significant higher WBC levels in *HLA B27* positive patients, suggesting an acute reaction. Supporting literature indicates significantly lower WBC levels in *HLA B27* negative groups [19]. Our analysis showed significant differences in WBC levels by gender; however, no significant results emerged when comparing WBC levels among *HLA B27* positive males and females, nor between *HLA B27* negative males and females. Previous studies also reported no significant gender differences in WBC levels among AS patients, [20] which aligns with our findings. Although WBC levels generally remain stable across genders, they can effectively assess disease activity or treatment response [21].

Another parameter highlighted in our study is the mean platelet volume (MPV), which reflects platelet activation and function. Factors such as inflammation levels and treatment status can influence MPV, suggesting it may serve as a monitor for disease status and treatment response

[22]. Our analysis showed significantly higher MPV levels in the *HLA B27* positive group, consistent with findings from other studies. Notably, literature indicates a significant decrease in MPV levels during the active phase of AS [23]. However, no statistically significant differences in MPV were found when comparing males and females within *HLA B27* positive or negative groups.

Significant results were also found in red cell distribution width levels, with the *HLA B27* positive group exhibiting higher RDW. Gorial et al. reported increased RDW values in AS patients compared to healthy controls, [24] while another study indicated RDW could differentiate between *HLA B27* positive and negative cases [25]. No significant gender differences in RDW were observed in AS patients, supporting our findings.

Our study also revealed significant differences in platelet levels between groups. While previous research indicated that PLT levels could rise during disease flares, the significance of *HLA B27* positivity on this increase remains unclear [26]. In a study, higher platelet counts were found in AS patients, suggesting potential implications for coagulation function in *HLA B27* positive individuals.[18] Significant differences were noted in PLT levels when analyzing groups by gender, though another study reported no significant gender difference [27].

PCT, indicative of platelet function in coagulation, showed marked differences depending on the presence or absence of HLA B27. Luo et al. [28] suggested that PCT, alongside PLT and MPV, may serve as a diagnostic marker for AS, while other studies have reported conflicting results regarding PCT levels by gender, highlighting a lack of consensus [29]. Significant results were obtained in the analysis of PCT according to gender within the groups.

Another finding of the study is the level of NEU. In one study, significant differences in neutrophil levels were identified between *HLA B27* positive and negative groups of patients with AS [9]. A large-scale study also reported an increase in neutrophil counts [30]. These results are consistent with our findings. According to our study, there was no significant difference in NEU levels between genders. Comparisons between genders within the positive and negative groups also did not yield significant results. In a similar study examining the gender factor, higher levels were observed in the female group, resulting in a significant difference [31]. Further studies are needed to determine whether neutrophil levels vary by gender in patients with AS.

A study on *HLA B27* positivity and negativity in AS reported that HLA B27 peptide complexes activate T lymphocytes, potentially affecting lymphocyte levels and leading to autoimmunity [32]. Lymphocyte levels were significantly higher in *HLA B27* positive patients, supporting findings by Boyraz et al. that reported increased lymphocyte counts.[33] However, some studies have indicated a decrease in lymphocyte counts in this population. Our analysis showed no significant differences in LYM levels by gender.

Another parameter examined was CRP, and significant results were observed when analyzing the CRP levels of *HLA B27* negative and positive patients. Omar et al. studied the CRP levels of *HLA B27* positive and negative groups, noting that the levels in the positive group were higher, although this difference was not significant [34]. In our study, no significant difference was found in CRP levels between genders. Another study reported that CRP levels varied in AS patients based on both gender and smoking status [35].

According to the research findings, the differences appear to be unclear.

Another parameter analyzed is the ESR value. In our study, significant results were found in the analysis of ESR levels between groups. Öztürk et al. reported that ESR levels in AS patients showed significant variability based on *HLA B27* positivity and negativity [36]. Supporting our findings. However, no significant results were obtained for ESR analysis by gender. Kutlucan et al. noted that ESR did not vary significantly with gender in AS patients [37].

The final parameter evaluated in our study is the NLR. Statistical analyses revealed significant differences in values between the positive and negative groups. However, there is no specific information regarding a direct relationship between *HLA-B27* positivity and negativity and NLR levels. Some studies suggest that NLR may be higher in *HLA-B27* positive AS patients [38]. In a study, NLR levels in AS were investigated, and significant results were obtained. Our study did not find significant differences in NLR when analyzed by gender. In a study, NLR levels in AS patients were examined regarding seasonal variations, and no significant gender differences were reported [39]. Similarly, Sun et al. found that gender did not significantly alter NLR levels [40]. Regarding gender, while estrogen levels may enhance lymphocyte activation, testosterone levels can increase neutrophil activity. Thus, it is possible for NLR levels in AS patients to vary by gender, but further research is needed in this area. In summary, our study provides valuable insights into the relationship between *HLA B27* status and various biochemical parameters in AS patients. We believe that further research would be beneficial to clarify the impact of *HLA B27* positivity and negativity on these parameters.

4.1. Study limitations and Conclusions: Our results are largely consistent with the literature. Expanding the sampling to cover a wider geographic area and including a larger number of samples in the research will enhance the significance of the results. We believe that evaluating complete blood count parameters that may be useful in the diagnosis of Ankylosing Spondylitis will provide ease in terms of cost and feasibility, and contribute to the diagnostic process of AS.

The retrospective and cross-sectional design of our study inherently imposes certain limitations in assessing changes in biomarker levels over time, their relationship with disease flares, or treatment responses. We acknowledge the current limitations of our research and trust that these constraints do not diminish the contribution of our study to the literature but rather illuminate new avenues for further investigation.

In this study, only HLA-B27 positive and negative AS patients were compared; healthy controls and other rheumatologic disease groups were not included. This limitation restricts the ability to discern whether the observed biomarker changes are specific to AS or related to a general inflammatory response or concomitant clinical conditions. However, since the primary objective of our study was to evaluate the impact of HLA-B27 genetic variation on biochemical parameters in individuals diagnosed with AS, the focus was intentionally placed on these two patient groups. Accordingly, our study aims to elucidate the hematological and inflammatory marker differences between HLA-B27 positive and negative AS patients and to assess the potential association of this genetic determinant with disease activity. Additionally, as the study was retrospective and cross-sectional, disease activity indices such as BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) and ASDAS (Ankylosing Spondylitis Disease Activity Score) were not routinely recorded in patient files, thus

could not be included in the analysis. This limits assessing a direct relationship between biomarker levels and disease severity or clinical activity. Nonetheless, as the main objective was to investigate the impact of HLA-B27 status on hematological and inflammatory parameters, this limitation should not be viewed as undermining the study's overall purpose. Future prospective longitudinal studies with systematic collection and analysis of disease activity scores would enhance the clinical relevance and diagnostic value of these biomarker findings.

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