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Distribution of human leukocyte antigen B27 (HLA-B27) in Slovak patients

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Abstract
Background and objectives: HLA-B27 is a genetic marker associated with spondyloarthropathies, particularly ankylosing spondylitis and axial spondyloarthritis. While its prevalence varies across populations, no data exist for Slovak patients. This study aimed to determine HLA-B27 prevalence in Slovak patients with suspected spondyloarthropathies and assess differences by sex and age.
Methods: A retrospective cohort of 1,614 patients (888 females and 726 males) was analyzed for HLA-B27 status (positive/negative) using reverse hybridisation (HLA-B27 StripAssay). Statistical analyses included Pearson's Chi-square test and non-parametric Mann–Whitney *U* and Kruskal–Wallis tests for sex- and age-related differences.
Results: HLA-B27 positivity was 20.57%, with a higher proportion in males (23.28%) than females (18.36%, $p = 0.0177$). The less than 20 age group had the highest absolute number of positive cases (126 cases; 17.80%), while the 21–40 group had the highest relative positivity (119 cases; 29.38%). The lowest positivity was in the more than 61 age group (17 cases; 13.08%), though age distribution differences were not statistically significant ($p = 0.7765$). Positivity varies across diagnoses, peaking in musculoskeletal (M) and eye disorders (H), where it exceeds 29%.
Conclusion: HLA-B27 positivity is strongly associated with rheumatologic and ophthalmologic conditions and exhibits age- and sex-related variability. These findings emphasize the diagnostic significance of HLA-B27 testing in Slovak patients, especially for early detection and management of spondyloarthropathies. Further research on HLA-B27 variability and its clinical implications is needed to optimize diagnostic strategies and patient care.

Keywords: Age-related patterns; Gender differences; HLA-B27; Prevalence; Slovak population; Spondyloarthritis

Highlights:
- HLA-B27 prevalence was evaluated in a large cohort of patients from the Slovak Republic over a 12-year retrospective study.
- HLA-B27 positivity was more frequent in males.
- Age-related differences in HLA-B27 prevalence were identified, with higher positivity in the younger age group (<20 years) and lower positivity in older age groups (>61 years).
- The median age for both HLA-B27-positive and negative findings was 27 years.

Introduction

The human leukocyte antigen B27 (HLA-B27) is a class I surface antigen encoded by the B locus on chromosome 6 within the major histocompatibility complex (MHC) (Sharma et al., 2018). Among the HLA class I groups, HLA-B27 exhibits the greatest number of subtype sequences, with its full heavy chain comprising 275 amino acids. Despite having over 200 allelic variants, these molecules are highly conserved, with approximately 90% of positions showing only minor amino acid differences. HLA-B27 primarily functions in presenting antigenic peptides to T cells, playing a critical role in immune responses by forming homodimers that bind to specific receptors involved in immune cell inflammation (Bowness, 2015; Yu et al., 2013).

The presence of HLA-B27 is strongly associated with autoimmune diseases, including ankylosing spondylitis (AS), axial spondyloarthritis (axSpA), reactive arthritis (ReA), acute anterior uveitis (AAU), psoriasis, and psoriatic arthritis (Deodhar et al., 2023; Karakılıç et al., 2024; Zalesak et al., 2024). Individuals with the HLA-B27 allele face a higher risk of these

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conditions due to increased susceptibility to inflammation and tissue damage in joints and other organs (Sharma et al., 2018). HLA-B27 can influence cellular signalling pathways, such as BMP/TGFβ signalling, which may contribute to disease pathogenesis (Grandon et al., 2019). Zhao et al. (2018) suggest that HLA-B27 affects intracellular processing of specific pathogens, such as *Salmonella enteritidis*, in patients with ReA.

HLA-B27 alleles have also been linked to other conditions, such as HIV infection. A closely related allele, HLA-B*5701, has been associated with restricted virus replication in some HIV-infected individuals (Migueles et al., 2000), suggesting that HLA-B27 may influence immune responses beyond AS. Furthermore, Li et al. (2023) reported that while HLA-B27 carrier status does not significantly impact overall survival, patients with HLA-B27-positive AS have increased mortality compared to the general population, indicating a complex interplay of factors affecting clinical outcomes.

HLA-B27 positivity is particularly prevalent among individuals with AAU (Pyare and Majumder, 2022). It is associated with an earlier onset of AS (Rudwaleit et al., 2009) and a higher likelihood of developing AAU (Jaakkola et al., 2006). HLA-B27-positive AAU is often considered a distinct clinical entity frequently linked to systemic diseases such as spondyloarthritis, emphasizing the importance of early detection by ophthalmologists (Werkl et al., 2023). Although the exact role of HLA-B27 in disease initiation and progression remains unclear, it is regarded as the most significant genetic predisposition for AS development (Lim et al., 2019). The prevalence of HLA-B27 varies significantly across populations, reflecting genetic, geographic, and ethnic diversity. Among patients with AS, the highest rates are observed in Caucasian populations, particularly in Northern Europe. For example, approximately 85–95% of Icelanders with AS are HLA-B27-positive (Geirsson et al., 2010). This aligns with broader findings indicating that 90–95% of Caucasian patients with AS are HLA-B27-positive (Nazarinia et al., 2009). In healthy Caucasian populations, prevalence typically ranges from 8% to 14% (Bae and Kim, 2015). In Central Europe, the prevalence of HLA-B27 is around 8%, consistent with the general frequency in populations of European ancestry, which ranges from 6% to 9% (Braun and Sieper, 2023; Mathioudaki et al., 2021). This high prevalence correlates with an estimated AS incidence of 0.1–1.0% among Caucasian adults. Similarly, Mediterranean countries, such as Spain and Italy, exhibit HLA-B27 frequencies comparable to those of Northern European populations. This prevalence has significant implications for the incidence of AS in these regions (Nazarinia et al., 2009).

In contrast to European populations, Brazil demonstrates a considerably lower prevalence of HLA-B27, reported at approximately 4.35% (Longo et al., 2019). This reduction is attributed to Brazil's high levels of racial miscegenation, which dilute genetic predispositions associated with HLA-B27. Comparative studies further underscore this difference, showing lower prevalence rates in Latino groups (71%) compared to European populations (83%) (Jamalyaria et al., 2017).

Asian populations generally exhibit lower prevalence rates of HLA-B27 compared to Caucasians. For instance, in China, HLA-B27 positivity among patients with AAU varies significantly, ranging from 19% to 88% depending on the specific racial group (Qi et al., 2013). Middle Eastern and Arab countries display some of the lowest prevalence rates, ranging from 0.3% to 6.8%, contrasting sharply with rates observed in North America and Europe (Ziade, 2017).

To date, no data on the prevalence of HLA-B27 in Slovak patients with suspected spondyloarthropathies, particularly AS, has been published. Therefore, this study aimed to determine the presence or absence of HLA-B27 alleles (positive/negative) in Slovak patients using the reverse hybridisation technique. The distribution of HLA-B27 alleles (positive/negative) was further analyzed in relation to gender and age.

Materials and methods

Patients' information

This study was conducted in the Slovak Republic from January 2010 to September 2022, involving 1,614 patients referred to a rheumatologist, pediatric rheumatologist, or ophthalmologist, based on clinical symptoms and family history. Informed consent was obtained from all participants before their inclusion in the study. Diagnoses were determined based on medical histories and classified using the International Classification of Diseases, 10th Revision (ICD-10), with the Slovak adaptation (MKCH-10-SK), ensuring consistency and comparability of clinical data. This standard supports robust epidemiological research and effective healthcare decision-making. The study protocol was approved by the Ethics Committee of Constantine the Philosopher University in Nitra, Slovak Republic (UKF/165/2024/191013:003), and the Ethics Committee of the University Hospital Nitra, adhering to the principles of ICH GCP 135/95 and relevant national legislation.

Clinical and laboratory data

Data collection included three components: demographics, clinical diagnosis, and DNA analysis. Demographic data encompassed region, gender, and age (Table 1). Clinical diagnoses were classified using ICD-10 with the Slovak adaptation (MKCH-10-SK), ensuring consistency and comparability of clinical data across studies.

DNA analysis of HLA-B27 allele distribution

Approximately 500µl of peripheral blood was collected from participants and stored in vacuum containers with ethylenediaminetetraacetic acid (EDTA). The presence of HLA-B27 alleles (positive/negative) was determined using the HLA-B27 StripAssay® (ViennaLab Diagnostics GmbH, Vienna, Austria) via reverse hybridisation. This assay consists of three steps: DNA isolation, PCR amplification using biotinylated primers, and hybridisation of amplification products with a test strip containing allele-specific oligonucleotide probes immobilised as parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and coloured substrates. Genomic DNA was extracted from 100µl of peripheral blood, following the kit's procedure. HLA-B27 positivity indicates the presence of one or more HLA-B27 alleles, whereas HLA-B27 negativity indicates their absence. Further technical details are available in ViennaLab Technical Notes (https://www.viennalab.com/home/unterlagen/technical-notes/440-productnote02-hla-b27-2022-06/file).

As demonstrated in Table 2, an analysis of the distribution of HLA-B27 findings is provided according to specific diagnoses. The table presents both the number and percentage of positive and negative findings for each diagnosis, along with their proportion relative to the total patient population.

Table 1. Demographic description of respondents

Region	Gender	Age at disease manifestation	Type of finding HLA-B27		Σ *N*
			HLA-B27 negative	HLA-B27 positive	
Banskobystrický	F	<20	12	–	12
		21–40	6	2	8
		41–60	5	–	5
	M	<20	2	2	4
		21–40	2	–	2
		41–60	1	–	1
Bratislavský	F	<20	5	–	5
		21–40	–	1	1
		41–60	3	–	3
		>61	1	1	2
	M	<20	7	–	7
		21–40	1	1	2
		41–60	2	1	3
Košický	F	>61	1	–	1
	M	<20	1	–	1
		41–60	1	–	1
Nitriansky	F	<20	293	57	350
		21–40	142	51	193
		41–60	152	32	184
		>61	59	7	66
	M	<20	223	60	283
		21–40	119	58	177
		41–60	120	34	154
		>61	40	7	47
Prešovský	F	<20	–	1	1
		21–40	1	–	1
	M	<20	1	–	1
		41–60	1	–	1
		>61	1	–	1
Trenčiansky	F	<20	10	3	13
		21–40	2	1	3
		41–60	5	2	7
		>61	3	–	3
	M	<20	7	–	7
		21–40	5	1	6
		41–60	2	–	2
		>61	2	–	2
Trnavský	F	<20	12	2	14
		21–40	5	1	6
		41–60	3	1	4
		>61	4	–	4
	M	<20	9	1	10
		21–40	2	2	4
		41–60	5	–	5
		>61	2	1	3
Žilinský	F	41–60	1	–	1
		>61	–	1	1
	M	21–40	1	1	2
Σ *N*			1282	332	1614

It is evident that negative findings for HLA-B27 make up the majority of cases, totalling 79.98%. However, the proportion of negative findings varies across individual diagnoses. For instance, diagnoses such as dg. Z (Factors influencing health status) and dg. S (Injuries, poisonings, and external causes) report a 100% negative rate. In contrast, diagnoses such as dg. C (Neoplasms) and dg. M (Diseases of the musculoskeletal system and connective tissue) exhibit a combination of both positive and negative findings.

Positive findings constituted 20.02% of all cases, with a clear distinction in their distribution across individual diagnoses. Of particular note is dg. N (Genitourinary system diseases), which exhibits a 50% positive finding rate, but due to the small sample size ($n = 6$), this result should be interpreted with

Table 2. Distribution of HLA-B27 findings according to ICD-10, Slovak version of MKCH-10

MKCH-10	HLA-B27 positive	HLA-B27 negative	Total patients
I. Infectious and parasitic diseases (A00–B99)	7 (25.93%)	20 (74.07%)	27 (1.67%)
II. Neoplasms (C00–D48)	1 (7.14%)	13 (92.86%)	14 (0.87%)
IV. Endocrine, nutritional, and metabolic diseases (E00–E90)	2 (15.38%)	11 (84.62%)	13 (0.81%)
VI. Diseases of the nervous system (G00–G99)	7 (14.58%)	41 (85.42%)	48 (2.97%)
VII. Diseases of the eye and adnexa (H00–H59)	47 (29.19%)	114 (70.81%)	161 (9.98%)
IX. Diseases of the circulatory system (I00–I99)	3 (12.00%)	22 (88.00%)	25 (1.55%)
X. Diseases of the respiratory system (J00–J99)	1 (33.33%)	2 (66.67%)	3 (0.19%)
XI. Diseases of the digestive system (K00–K93)	3 (13.04%)	20 (86.96%)	23 (1.43%)
XII. Diseases of the skin and subcutaneous tissue (L00–L99)	1 (9.09%)	10 (90.91%)	11 (0.68%)
XIII. Diseases of the musculoskeletal system and connective tissue (M00–M99)	209 (21.22%)	776 (78.78%)	985 (61.03%)
XIV. Diseases of the genitourinary system (N00–N99)	3 (50.00%)	3 (50.00%)	6 (0.37%)
XVIII. Symptoms, signs, and abnormal clinical and laboratory findings, not classified elsewhere (R00–R99)	48 (16.27%)	247 (83.73%)	295 (18.28%)
XIX. Injuries, poisoning, and other consequences of external causes (S00–T98)	0 (0.00%)	1 (100.00%)	1 (0.06%)
XXI. Factors influencing health status and contact with health services (Z00–Z99)	0 (0.00%)	2 (100.00%)	2 (0.12%)

caution. In comparison, dg. H (Diseases of the eye and adnexa) exhibited the highest proportion of positive cases (29.19%), followed closely by dg. C (Neoplasms) with 29.17%. Furthermore, no positive findings were recorded in diagnoses such as dg. S (Injuries, poisonings, and external causes) and dg. Z (Factors influencing health status). This distribution underscores the variability in HLA-B27 positivity across different clinical conditions, with a stronger association between positive findings and specific diagnoses, such as neoplasms and diseases of the eye and adnexa.

A more detailed breakdown of musculoskeletal and connective tissue diagnoses (M00-M99) is provided in Table 3. The highest prevalence of HLA-B27 positivity was found in spondylopathies (M45–M49) at 52.63%, reinforcing its strong association with conditions such as ankylosing spondylitis. Inflammatory polyarthropathies (M05–M14) showed a 27.13% positivity rate, while other joint disorders (M20–M25) had a 22.07% positivity rate. Notably, no HLA-B27 positivity was detected in infectious arthropathies (M00–M03), muscle disorders (M60–M63), or osteopathies (M86–M90).

The findings confirm the strong association between HLA-B27 and inflammatory joint diseases, particularly spondyloarthropathies, while underlining the importance of alternative diagnostic markers for other musculoskeletal disorders.

Statistical analysis

Statistical analyses were conducted using R software, version 3.6.3. Differences in HLA-B27 status (positive/negative) between genders and age groups of disease manifestation (<20, 21–40, 41–60, >61) were analyzed using Pearson's chi-square test. The Shapiro–Wilk test assessed the normality of data distribution. Due to the violation of normality, the non-parametric Mann–Whitney *U* test was employed to compare the age distribution between HLA-B27-positive and negative findings. A significance level of $p < 0.05$ was applied to all statistical tests, ensuring robust analysis of the categorical and non-normal data distribution.

Results

Gender differences in HLA-B27 findings

Using Pearson's chi-square test, a statistically significant difference was observed in the distribution of HLA-B27-positive and negative findings between genders ($p = 0.0177$, df = 1, $\chi^2 = 5.626$). HLA-B27-negative findings constituted 79.43% of the total, with the highest proportion observed in women (81.64%). Among men, this proportion was 4.92 percentage points lower, constituting 76.72%. HLA-B27-positive findings accounted for 20.57% of the total, with a nearly equal representation between men (23.28%) and women (18.36%) (Table 4).

Age differences in HLA-B27 findings

Pearson's chi-square test also revealed a statistically significant difference in HLA-B27 findings across age groups ($p = 2.2e\text{-}16$, df = 3, $\chi^2 = 456.06$). The highest absolute number of HLA-B27-positive cases was observed in the <20 age group (126 cases, 17.80%), while the lowest number was in the >61 age group (17 cases, 13.08%). However, in terms of proportion within each age group, the highest prevalence of HLA-B27 positivity was found in the 21–40 age group (29.38%). HLA-B27-negative cases were most frequent in the <20 age group (582 cases, 82.20%), while the highest relative proportion of HLA-B27-negative cases was recorded in the >61 age group (86.92%) (Table 5).

Age and HLA-B27 findings

The Shapiro–Wilk test indicated a violation of the normality assumption ($p = 0.0022$). Therefore, the non-parametric Mann–Whitney *U* test was applied, revealing no significant difference ($p = 0.7765$, W = 214961) in the presence of HLA-B27 (positive/negative) alleles across patient age groups (Fig. 1). The median age for both (positive and negative) findings was 27 years (Fig. 1).

Table 3. Prevalence of HLA-B27 findings in Musculoskeletal and connective tissue disorders (M00–M99) according to ICD-10 classification

Diseases of the musculoskeletal system and connective tissue (M00–M99)		HLA-B27 negative	HLA-B27 positive	Percentage of HLA-B27 positive	Total patients
M00–M25 Arthropathies	M00–M03 Infectious arthropathies	5 (100.00%)	0 (0.00%)	0.00%	5 (0.51%)
	M05–M14 Inflammatory polyarthropathies	94 (72.87%)	35 (27.13%)	16.75%	129 (13.10%)
	M15–M19 Arthroses	6 (85.71%)	1 (14.29%)	0.48%	7 (0.71%)
	M20–M25 Other joint disorders	346 (77.93%)	98 (22.07%)	46.89%	444 (45.08%)
M30–M36 Systemic connective tissue disorders		18 (78.26%)	5 (21.74%)	2.39%	23 (2.34%)
M40–M54 Dorsopathies	M40–M43 Deforming dorsopathies	1 (100.00%)	0 (0.00%)	0.00%	1 (0.10%)
	M45–M49 Spondylopathies	27 (47.37%)	30 (52.63%)	14.35%	57 (5.79%)
	M50–M54 Other dorsopathies	249 (86.76%)	38 (13.24%)	18.18%	287 (29.14%)
M60–M79 Soft tissue disorders	M60–M63 Muscle disorders	9 (100.00%)	0 (0.00%)	0.00%	9 (0.91%)
	M65–M68 Disorders of synovial membrane and tendons	10 (90.91%)	1 (9.09%)	0.48%	11 (1.12%)
	M70–M79 Other soft tissue disorders	6 (100.00%)	0 (0.00%)	0.00%	6 (0.61%)
M80–M94 Osteopathies and chondropathies	M80–M85 Disorders of bone density and structure	2 (66.67%)	1 (33.33%)	0.48%	3 (0.30%)
	M86–M90 Other osteopathies	2 (100.00%)	0 (0.00%)	0.00%	2 (0.20%)
	M91–M94 Chondropathies	1 (100.00%)	0 (0.00%)	0.00%	1 (0.10%)
M95–M99 Other disorders of the musculoskeletal system and connective tissue		0 (0.00%)	0 (0.00%)	0.00%	0 (0.00%)
Total patients		776 (78.78%)	209 (21.22%)	100.00%	985 (100.00%)

Table 4. Distribution of probands by gender

Gender	HLA-B27 negative	HLA-B27 positive	Total
Female	725 (81.64%)	163 (18.36%)	888 (55.02%)
Male	557 (76.72%)	169 (23.28%)	726 (44.98%)
Σ	1282 (79.43%)	332 (20.50%)	1614 (100.00%)

Table 5. Distribution of probands by age at disease manifestation

Age at disease manifestation	HLA-B27 negative	HLA-B27 positive	Total
<20	582 (82.20%)	126 (17.80%)	708 (43.87%)
21–40	286 (70.62%)	119 (29.38%)	405 (25.09%)
41–60	301 (81.13%)	70 (18.87%)	371 (22.99%)
>61	113 (86.92%)	17 (13.08%)	130 (8.05%)
Σ	1282 (79.43%)	332 (20.57%)	1614 (100.00%)

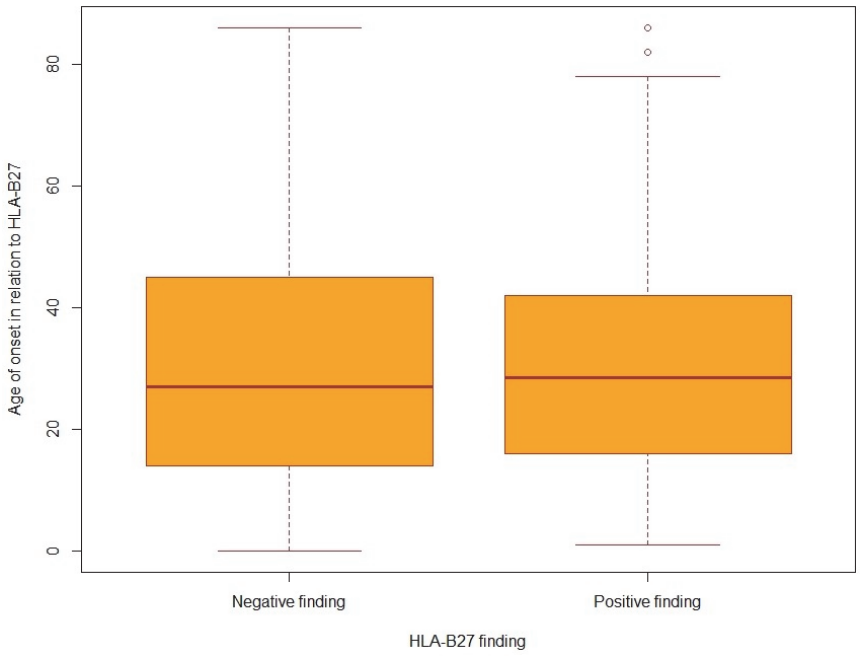

Fig. 1. Distribution of patients based on the state of findings in relation to age

Discussion

Gender differences in HLA-B27 prevalence

This study identified a statistically significant difference in the prevalence of HLA-B27 negativity between genders, with women showing a higher proportion (81.64%) compared to men (76.72%). Although HLA-B27 positivity was present in both genders, it was slightly more frequent in men (23.28%) than in women (18.36%). The higher rate of HLA-B27 negativity in women underscores the need for diagnostic strategies that account for gender differences. These findings align with Tournadre et al. (2013), who observed that women with axSpA often present atypical clinical features and are more likely to be HLA-B27 negative, potentially leading to diagnostic delays.

The observed gender differences in HLA-B27 prevalence align with previous studies, which suggest that men with spondyloarthritis are more frequently HLA-B27-positive, whereas women exhibit a higher prevalence of HLA-B27-negative disease variants (Tournadre et al., 2013). This may reflect sex-based variations in genetic susceptibility, as well as differences in clinical presentation and disease progression.

Notably, HLA-B27-positive men are more likely to develop axial disease, whereas women, regardless of HLA-B27 status, exhibit higher rates of enthesitis and peripheral arthritis (Rusman et al., 2018). These variations could contribute to the delayed diagnosis often observed in female patients, as their symptoms may not align with the classic axial phenotype traditionally associated with AS (Feldtkeller et al., 2003).

Additionally, treatment response differs by gender, with HLA-B27-positive men demonstrating superior responses to TNF inhibitors (TNFi) compared to women, who tend to exhibit higher disease activity and more persistent symptoms (Baraliakos et al., 2015; Deodhar and Yu, 2017). This underscores the need for sex-specific therapeutic strategies that account for these variations.

Gender-related differences in disease presentation are supported by studies highlighting a higher prevalence of radiographic axial spondyloarthritis (r-axSpA) in men, with prevalence ratios ranging from 1.2:1 to 2:1 in favor of men (Baumberger and Khan, 2017; Sieper and van der Heijde, 2013). Conversely, non-radiographic axial spondyloarthritis (Nr-axSp) has a more balanced gender distribution (Sieper and van der Heijde, 2013). These findings suggests that the greater prevalence of HLA-B27 negativity in women may reflect differences in disease phenotype or diagnostic pathways. The Assessment of SpondyloArthritis International Society (ASAS) criteria, which emphasize clinical features such as inflammatory back pain, sacroiliitis on imaging, and extra-articular manifestations, remain crucial for diagnosing HLA-B27 negative patients, particularly women (Tournadre et al., 2013).

Age-related patterns

This study revealed significant age-related differences in HLA-B27 prevalence. The highest absolute number of HLA-B27-positive cases was observed in individuals under 20 years of age (126 cases, 17.80%), while the lowest was in those over 61 years (17 cases, 13.08%). These findings are consistent with the literature, suggesting that HLA-B27 positivity is associated with earlier disease onset (Jaakkola et al., 2006; Lin and Gong, 2017).

HLA-B27 status has been strongly associated with age at disease onset, with HLA-B27-positive patients developing AS at a younger age compared to HLA-B27-negative patients (Feldtkeller et al., 2003; Rudwaleit et al., 2009). Among patients with non-radiographic axial SpA (nr-axSpA), HLA-B27-positive men and women tend to be younger than their HLA-B27-negative counterparts. This supports the concept of axSpA as a continuum of disease, with HLA-B27 playing a key role in earlier disease onset (Rudwaleit et al., 2005).

Furthermore, late-onset AS (manifesting after the age of 40) occurs more frequently in HLA-B27-negative patients,

suggesting a different pathogenic mechanism for these cases (Arévalo et al., 2018). In contrast, juvenile-onset AS (before age 16) does not appear to be influenced by HLA-B27 status (Feldtkeller et al., 2003).

Another major difference is diagnostic delay, which is significantly longer in HLA-B27-negative patients (median: 11.4 years) compared to HLA-B27-positive patients (median: 8.5 years) (Feldtkeller et al., 2003). This disparity may be due to the lack of a clear diagnostic marker in HLA-B27-negative individuals, leading to misclassification or delayed recognition of SpA (Chaudhary et al., 2023).

Younger cohorts may show higher positivity rates due to immune system maturation and the earlier clinical manifestation of conditions such as ankylosing spondylitis (AS) (Jayaprakash et al., 2023).

Conversely, the significantly lower prevalence of HLA-B27 positivity in the older population may result from selection bias. Individuals who are HLA-B27-positive are more likely to develop symptoms earlier in life, leading to a reduced representation of these individuals in older age groups due to disease-related mortality or complications (Roberts et al., 2013).

Additionally, the progression of autoimmune conditions and cumulative environmental exposures over a lifetime may further influence this pattern. The absence of significant differences in median age between HLA-B27-positive and negative findings (27 years) suggests that symptom onset may not be strictly tied to HLA-B27 status. This finding aligns with Davodi et al. (2023), who noted that while HLA-B27 positivity is associated with specific clinical features, overall disease presentation can vary significantly regardless of HLA-B27 status. These observations highlight the complexity of spondyloarthritis and the need for additional biomarkers to better understand disease progression.

Limitations

Prevalence and regional/ethnic variability

The prevalence of HLA-B27 positivity in this study was 20.57%, which is consistent with reports from other European populations. However, regional differences within the Slovak Republic were not analyzed in this study. Research by Resende et al. (2023) highlights the importance of considering regional and ethnic variability in HLA-B27 prevalence, as genetic and environmental factors can significantly influence disease patterns. Future studies should include detailed regional analyses to better understand the impact of geographic and demographic diversity.

Ethnic differences in HLA-B27 prevalence and its association with AS have been extensively studied. For example, studies in Chinese populations report a stronger association of HLA-B27 with male sex, earlier disease onset, and higher familial aggregation. In contrast, HLA-B27-negative patients exhibit longer diagnostic delays and a higher prevalence of psoriasis (Zhang et al., 2022).

These findings underscore the need for tailored diagnostic and management strategies that account for population-specific patterns.

Methodological considerations

This study utilized reverse hybridization techniques for HLA-B27 detection. While effective, these methods may differ in sensitivity and specificity compared to advanced molecular techniques.

The cross-sectional design of this study limits the ability to assess temporal relationships and disease progression, which are crucial for understanding the long-term impact of HLA-B27 positivity and negativity. Another limitation is the lack of regional stratification, as HLA-B27 prevalence and disease expression may vary within different geographic and ethnic subgroups in Slovakia.

Moreover, selection bias may have influenced the observed age distribution. Older patients with HLA-B27 positivity may be underrepresented due to disease-related mortality, higher symptom burden, or earlier clinical interventions. Longitudinal follow-up studies are necessary to determine whether HLA-B27-positive patients experience faster disease progression and increased mortality risk, as suggested in previous literature (Roberts et al., 2013).

The use of non-parametric statistical tests was necessary due to the non-normal distribution of data, ensuring robust analysis of categorical variables. However, these methods may not fully capture the nuances of allele distribution. Future research should consider longitudinal designs and incorporate more advanced molecular diagnostics to enhance the understanding of HLA-B27's role in disease development.

Clinical implications and future directions

The results of this study reinforce the importance of HLA-B27 testing in the diagnosis and management of spondyloarthropathies. HLA-B27 positivity remains a critical marker for conditions such as AS, as noted by Braun and Sieper (2023). Positive individuals face a higher risk of complications, including AAU and cardiac conduction abnormalities (Milić et al., 2020). Ensuring timely testing and diagnosis, particularly in younger patients and women, can significantly improve clinical outcomes by enabling earlier intervention and targeted management strategies.

Although HLA-B27 testing remains a valuable diagnostic tool, its absence does not exclude axSpA, particularly in women and older patients, where HLA-B27 negativity is more common. Women with axSpA are more likely to present atypical clinical features, including higher rates of enthesitis and peripheral arthritis, which may contribute to diagnostic delays (Rusman et al., 2018; Tournadre et al., 2013). These differences highlight the need for alternative biomarkers and multimodal diagnostic approaches that integrate clinical, imaging, and laboratory findings.

For HLA-B27-negative patients, alternative diagnostic markers and clinical criteria are essential. The ASAS classification criteria, which include imaging and extra-articular manifestations, play a pivotal role in identifying patients with axSpA who do not carry the HLA-B27 allele. This is particularly relevant for women and older patients, where HLA-B27 negativity is more common.

Although this study provides valuable insights into HLA-B27 prevalence, its cross-sectional design offers only a snapshot of the current distribution, without capturing temporal trends or disease progression. Additionally, aggregating data from across the Slovak Republic may obscure potential regional variations. Future research should focus on regional analyses and larger, more diverse cohorts to improve the generalizability of findings and provide a deeper understanding of demographic and genetic variability.

Conclusion

Our findings highlight significant gender- and age-related patterns in HLA-B27 prevalence, with important implications for the diagnosis and management of spondyloarthropathies. The study underscores the critical role of HLA-B27 testing,

while also emphasizing the need for alternative diagnostic approaches for HLA-B27-negative patients. Future research should focus on longitudinal studies, regional stratification, and advanced molecular techniques to further elucidate the complex interplay of genetic, environmental, and demographic factors in spondyloarthritis.

Ethical review

The research was conducted with the approval of the Ethics Committee of Constantine the Philosopher University in Nitra, Slovak Republic (UKF/165/2024/191013:003).

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Conflict of interest

The authors have no conflict of interest to declare.

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