

## Association Between Human Leukocyte Antigen-B\*27 and Pathogenesis in Seronegative Spondyloarthropathies in Federation of Bosnia and Herzegovina

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### ABSTRACT

**Objectives:** This study aims to investigate the low-resolution human leukocyte antigen (HLA)-B locus polymorphisms between unrelated healthy individuals and patients with diagnosis of seronegative spondyloarthropathies and determine risky and protective allelic groups and genotypes.

**Patients and methods:** The study included 104 healthy control individuals (52 males, 52 females; median age 43 years; range 2 to 76 years) and 96 patients (43 males, 53 females; median age 28.5 years; range 2 to 67 years) diagnosed with: ankylosing spondylitis (AS) (n=19), reactive arthritis (n=19), psoriatic arthritis (n=28) and undifferentiated spondyloarthropathies (n=30). Genomic deoxyribonucleic acid was extracted from peripheral blood to detect allelic groups of HLA class I and II. Single-specific-primer polymerase chain reaction was used for HLA genotyping and visualization of products after their separation on 1.5% agarose gel for horizontal gel electrophoresis.

**Results:** Significantly increased frequency was found for HLA-A\*02 and HLA-B\*27 allelic variants in all groups of patients. The increased frequency of the HLA-B\*35 allelic group in the control group represents the protective gene variant for the occurrence of AS. The predisposing genotype (HLA-B\*27/B\*44 and B\*27/B\*51) for the onset of disease was only found in AS patients.

**Conclusion:** This study shows the strong association of HLA-B\*27 antigen with spondyloarthropathies, which is considered a risk variant of the gene for the onset of disease. Protective and risky allelic variants and genotypes are rare and their detection as well as increased frequency are possible if larger numbers of patients are involved.

**Keywords:** Genotype; human leukocyte antigen-B\*27; human leukocyte antigen system; protective allelic groups; spondyloarthropathies.

Seronegative spondyloarthropathies or spondyloarthritides (SpA) are a group of inflammatory rheumatic diseases that most commonly occur in genetically predisposed persons, usually in combination with favorable external factors. The occurrence of the disease is associated with the presence of the human leukocyte antigen (HLA)-B\*27 gene, particularly in ankylosing spondylitis (AS).<sup>1</sup> Many epidemiological studies have emphasized the link between several

different forms of arthritis called seronegative spondyloarthropathies and HLA-B\*27 as a genetic marker for these diseases, with exclusion of Whipple's disease and Behçet's syndrome and inclusion of other conditions such as undifferentiated and formes frustes of spondarthritis.<sup>2</sup>

The main role of HLA-B\*27 gene product is to create complexes with  $\beta$ 2-microglobulin which bind short peptide antigens like peptides of

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intracellular microorganisms. After presentation on the cell surface, complexes are specifically recognized by cytotoxic lymphocytes and after lymphocytes activation infected cells are subsequently destroyed. Reactive arthritis (ReA) is characterized with HLA-B\*27 expression and predisposition to the development of infection. Lewis's transgenic rat models for HLA-B\*27:05 and human  $\beta$ 1-microglobulin spontaneously develop a disease similar to SpA in normal housing conditions, but not under pond breeding conditions.<sup>3</sup> However, a similar procedure in mice models did not cause disease development.<sup>4</sup>

Ankylosing spondylitis develops in people with HLA-B\*27, HLA class I gene. Significant association of HLA genes in autoimmune diseases is not found in patients with HLA-B\*27, with increased risk for developing this disease and 35% of genetic risk within the HLA system.<sup>5</sup> Environmental factors that contribute in the development of infectious diseases, particularly in ReA, are most commonly associated with *Salmonella typhimurium*, *Chlamydia trachomatis*, *Shigella flexneri* and others during urogenital or intestinal infections.<sup>6</sup> It is considered that HLA-B\*27 enhances the invasion of *Salmonella* into intestinal epithelial cells.<sup>7</sup> It is almost impossible to find live bacteria or their deoxyribonucleic acid (DNA) in the joints, while fragments of *Chlamydia*, *Yersinia* or *Salmonella* have been found in these patients.<sup>8</sup> In AS, positive correlation is determined for increased antibody titer against *Klebsiella*.<sup>9</sup> There is evidence of association between HLA-B\*27 and SpA, as well as association with infection, which is explained by the theory of molecular mimics. This theory explains the structural similarity between the surface molecules of the microorganisms and HLA-B\*27 structure in SpA.<sup>10</sup> The amino acid homology between HLA-B\*27 and microorganism antigens was observed.<sup>11</sup>

In this study, we aimed to investigate the low-resolution HLA-B locus polymorphisms between unrelated healthy individuals and patients with diagnosis of seronegative SpA and determine risky and protective allelic groups and genotypes.

## PATIENTS AND METHODS

This study included a control group of 104 healthy individuals (52 males, 52 females; median age

43 years; range 2 to 76 years) and 96 patients (43 males, 53 females; median age 28.5 years; range 2 to 67 years) diagnosed with: AS (n=19), ReA (n=19), psoriatic arthritis (PsA) (n=28) and undifferentiated SpA (uSpA) (n=30). This study was conducted between January 2012 and June 2014 at the Department for Molecular Immunogenetics, Institute for Transfusion Medicine of Federation of Bosnia and Herzegovina. The study protocol was approved by the Department for Molecular Immunogenetics, Institute for Transfusion Medicine of Federation of Bosnia and Herzegovina Ethics Committee. A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Peripheral venous blood (10 mL) with heparin as anticoagulant was used for serological testing of the HLA class I antigen. Genomic DNA was extracted from 10 mL of peripheral blood (two 5 mL blood tubes with ethylenediaminetetraacetic acid) to detect allelic groups of HLA class I and II. DNA extraction was performed using the Ready-DNA Spin-Kit (Inno-Train, Molecular Biology Grade, Germany). Single-specific-primer polymerase chain reaction (PCR) was used for HLA genotyping and visualization of products was performed using ultraviolet Transilluminator MultiDoc-It (UVP, Cambridge, UK) after their separation on 1.5% agarose gel for horizontal gel electrophoresis. The results were documented by Polaroid camera and sequence-specific oligonucleotide primed PCR (Fluor-analyzer with microspheres, Luminex Corp., Austin, Texas, USA).

### Statistical analysis

Statistical data analysis was performed using PowerMarker software version 3.25 (BRC, North Carolina, USA) and OpenEpi software version 2.3.1 (CreateSpace, CA, USA). Fisher's exact test was used to analyze the frequency of allelic groups.

## RESULTS

Table 1 presents frequencies of allelic groups in HLA class I (A\*, B\*, C\* loci) and HLA class II antigen (DRB1\*, DRB3\*, DRB4\*, DRB5\*, DQB1\* loci) in AS patients.

When we analyzed HLA-A\* gene locus, 11 out of 20 different allelic groups (Table 1)

**Table 1.** Frequencies of allelic groups of human leukocyte antigen class I and II in ankylosing spondylitis patients

HLA-A*	2n=50	Frequency (fa)	HLA-B*	2n=50	Frequency (fa)	HLA-C*	2n=50	Frequency (fa)	HLA-DRB1*	2n=50	Frequency (fa)	HLA-DRB3*, DRB4*, DRB5*	2n=50	Frequency (fa)	HLA-DQB1*	2n=50	Frequency (fa)
A*01	6	0.120	B*08	4	0.080	C*01	6	0.120	DRB1*01	15	0.300	DRB3*	27	0.587	DQB1*02	4	0.080
A*02	18	0.360	B*18	5	0.100	C*02	18	0.360	DRB1*03	4	0.080	DRB4*	9	0.196	DQB1*03	11	0.220
A*03	8	0.160	B*27	22	0.440	C*03	5	0.100	DRB1*04	2	0.040	DRB5*	10	0.217	DQB1*05	24	0.480
A*11	3	0.060	B*35	2	0.040	C*04	3	0.060	DRB1*07	2	0.040				DQB1*06	11	0.220
A*24	6	0.120	B*40	2	0.040	C*05	1	0.020	DRB1*09	1	0.020						
A*25	2	0.040	B*44	6	0.120	C*07	8	0.160	DRB1*10	1	0.020						
A*30	1	0.020	B*49	1	0.020	C*12	4	0.080	DRB1*11	5	0.100						
A*31	1	0.020	B*51	5	0.100	C*14	1	0.020	DRB1*12	1	0.020						
A*32	2	0.040	B*52	1	0.020	C*15	2	0.040	DRB1*13	6	0.120						
A*68	2	0.040	B*55	1	0.020	C*16	2	0.040	DRB1*14	3	0.060						
A*29	1	0.020	B*57	1	0.020				DRB1*15	4	0.080						
									DRB1*16	6	0.120						

HLA: Human leukocyte antigen; n: Presence of allelic groups in sample; fa: Frequency of allelic group.

**Table 2.** Frequencies of allelic groups of human leukocyte antigen class I and II in psoriatic arthritis patients

HLA-A*	2n=56	Frequency (fa)	HLA-B*	2n=56	Frequency (fa)	HLA-C*	2n=56	Frequency (fa)	HLA-DRB1*	2n=56	Frequency (fa)	HLA-DRB3*, DRB4*, DRB5*	2n=56	Frequency (fa)	HLA-DQB1*	2n=56	Frequency (fa)
A*01	11	0.196	B*07	3	0.053	C*01	2	0.035	DRB1*01	6	0.107	DRB3*	14	0.250	DQB1*02	16	0.285
A*02	12	0.214	B*08	4	0.071	C*02	6	0.107	DRB1*03	12	0.214	DRB4*	29	0.517	DQB1*03	13	0.232
A*03	5	0.089	B*13	5	0.089	C*03	6	0.107	DRB1*04	5	0.089	DRB5*	9	0.160	DQB1*04	2	0.035
A*11	4	0.071	B*14	1	0.017	C*04	5	0.089	DRB1*07	6	0.107				DQB1*05	12	0.214
A*23	1	0.017	B*18	3	0.053	C*05	3	0.053	DRB1*08	2	0.035				DQB1*06	13	0.232
A*24	7	0.125	B*27	6	0.107	C*06	9	0.160	DRB1*11	1	0.017						
A*25	1	0.017	B*35	5	0.089	C*07	6	0.107	DRB1*12	3	0.053						
A*26	2	0.035	B*37	1	0.017	C*08	5	0.089	DRB1*13	9	0.160						
A*30	3	0.053	B*38	3	0.053	C*12	2	0.035	DRB1*14	2	0.035						
A*31	1	0.017	B*39	5	0.089	C*14	4	0.071	DRB1*15	5	0.089						
A*32	2	0.035	B*40	3	0.053	C*15	5	0.089	DRB1*16	5	0.089						
A*33	1	0.017	B*44	3	0.053	C*16	3	0.053									
A*69	1	0.017	B*47	1	0.017												
			B*48	1	B*48												
			B*49	1	B*49												
			B*51	5	B*51												
			B*55	2	B*55												
			B*57	3	B*57												
			B*58	1	B*58												

HLA: Human leukocyte antigen; n: Presence of allelic groups in sample; fa: Frequency of allelic group.

**Table 3.** Frequencies of allelic groups of human leukocyte antigen; class I and II in reactive arthritis patients

HLA-A*	2n=56	Frequency (fa)	HLA-B*	2n=56	Frequency (fa)	HLA-C*	2n=56	Frequency (fa)	HLA-DRB1*	2n=56	Frequency (fa)	HLA-DRB3*, DRB4*, DRB5*	2n=56	Frequency (fa)	HLA-DQB1*	2n=56	Frequency (fa)
A*01	5	0.131	B*07	3	0.078	C*01	3	0.026	DRB1*01	8	0.210	DRB3*	20	0.526	DQB1*02	7	0.184
A*02	11	0.289	B*08	3	0.078	C*02	8	0.250	DRB1*03	5	0.131	DRB4*	4	0.125	DQB1*03	12	0.315
A*03	8	0.250	B*15	1	0.026	C*03	4	0.125	DRB1*04	2	0.052	DRB5*	6	0.157	DQB1*04	1	0.026
A*11	4	0.125	B*18	4	0.125	C*04	5	0.131	DRB1*07	2	0.052				DQB1*05	14	0.367
A*24	4	0.125	B*27	7	0.184	C*05	1	0.026	DRB1*08	1	0.026				DQB1*06	4	0.125
A*25	1	0.026	B*35	3	0.078	C*06	3	0.078	DRB1*09	1	0.026						
A*26	2	0.052	B*39	1	0.026	C*07	9	0.236	DRB1*11	6	0.157						
A*31	1	0.026	B*40	4	0.125	C*12	2	0.052	DRB1*12	2	0.052						
A*32	1	0.026	B*41	1	0.026	C*15	1	0.026	DRB1*13	6	0.157						
A*68	1	0.026	B*44	3	0.078	C*16	1	0.026	DRB1*14	1	0.026						
			B*51	5	0.131	C*17	1	0.026	DRB1*15	1	0.026						
			B*57	3	0.078				DRB1*16	3	0.078						

HLA: Human leukocyte antigen; n: Presence of allelic groups in sample; fa: Frequency of allelic group.

were determined and the highest frequency was estimated for HLA-A\*02 allelic group (0.360). HLA-B\* gene locus showed 10/37 different allelic groups and highest frequency was detected for HLA-B\*27 (0.440), while for the HLA-C\* gene locus 10/14 allelic groups were found and HLA-C\*02 allele group had the highest frequency (0.360). The HLA-DRB1\* gene locus was determined by the presence of 12 different allelic groups and by the absence of HLA-DRB1\*08; the highest frequency was estimated for HLA-DRB1\* 01 allele group (0.300). Among HLA-DRB3\*, DRB4\*, DRB5\* class II gene loci, the highest frequency was detected for DRB3\* (0.587). DQB1\*04 allele group was absent in the HLA-DQB1\* gene locus and HLA-DQB1\*05 was the most frequent allele group (0.480).

Results regarding frequencies of allelic groups in HLA class I (A\*, B\*, C\* loci) and HLA class II antigen (DRB1\*, DRB3\*, DRB4\*, DRB5\*, DQB1\* loci) in PsA patients are presented in Table 2. In the group of patients with PsA (Table 2), 14 different allelic groups for the HLA-A\* gene locus were found and the HLA-A\*02 allele group had the highest frequency (0.214). When we analyzed HLA-B\* gene locus, 19 different allelic groups were determined and the HLA-B\*27 was the allelic group with the highest frequency (0.107). Twelve different allelic groups were found in the HLA-C\* gene locus and the HLA-C\*06 group had the highest frequency (0.160). Only HLA-C\* 17 allele group was not found. In the HLA-DRB1\* gene locus, 11 allelic groups with the highest frequency for HLA-DRB1\*03 (0.214) were detected, while HLA-DRB1\*09 and DRB1\*10 allelic groups were absent. Gene loci HLA-DRB3\*, 4\*, 5\* were represented with the following allelic frequencies of DRB3\* (0.250), DRB4\* (0.517) and DRB5\* (0.160). The HLA-DQB1\* gene locus was represented with five different allelic groups and the highest frequency was estimated for the DQB1\*02 (0.285).

Table 3 presents values of allelic group frequencies in HLA class I (A\*, B\*, C\* loci) and HLA class II antigen (DRB1\*, DRB3\*, DRB4\*, DRB5\*, DQB1\* loci) in ReA patients. HLA-A\* gene locus was characterized by 10 different allelic groups and the highest frequency was found for HLA-A\*02 (0.289). HLA-B\* gene locus contained 12 different allelic groups and the highest frequency was detected for HLA-B\*27

(0.184). Two allelic groups, HLA-C\*08 and C\*14, were not determined in ReA patients, while among the 11 estimated allelic groups, HLA-C\*07 had the highest frequency (0.236). Out of the 11 allelic groups that were detected in the HLA-DRB1\* gene locus, DRB1\*01 allele group had the highest frequency (0.210). The absence of HLA-DRB1\*10 allelic group was confirmed. HLA-DRB3\*, DRB4\*, DRB5\* gene loci were represented by the following allelic frequencies: HLA-DRB3\* (0.526), DRB4\* (0.125) and HLA-DRB5\* (0.157). HLA-DQB1\* gene locus had five different allelic groups and the highest frequency was obtained for HLA-DQB1\* 05 allelic group (0.367).

Results regarding frequencies of allelic groups in HLA class I (A\*, B\*, C\* loci) and HLA class II (DRB1\*, DRB3\*, DRB4\*, DRB5\*, DQB1\* loci) in SpA patients are presented in Table 4. Ten different allelic groups with the highest frequency for the HLA-A\*02 (0.233) were found in the HLA-A\* gene locus (Table 4). The HLA-B\* gene locus was characterized by 18 different allelic groups and the highest frequency was detected for the HLA-B\*27 allelic group (0.150). The HLA-C\* gene locus had 12 allelic groups while HLA-C\*07 allelic group had the highest frequency (0.300). The absence of C\*16 allele group was established for this gene locus. The HLA-DRB1\* gene locus analysis revealed the existence of 12 different allelic groups and the highest frequency was found for HLA-DRB1\*15 (0.233). The absence of HLA-DRB1\*09 allelic group was also found in SpA patients. HLA-DRB3\*, DRB4\*, DRB5\* gene loci were represented by the following allelic frequencies: HLA-DRB3\* (0.533), DRB4\* (0.150) and DRB5\* (0.283). Five different allelic groups were found for the HLA-DQB1\* gene locus and the highest frequencies (0.233) were obtained for DQB1\*05 and DQB1\*06 allelic groups.

Table 5 demonstrates the risky and protective HLA allelic groups found in patients with AS, ReA, PsA and SpA. Significantly increased frequency of the HLA-B\*27 allelic group in AS patients ( $p=0.001$ ) represents a potent and strong predisposing factor for the disease onset and it is considered as a risky allelic variant. However, the significantly increased frequency of the HLA-B\*35 allelic group ( $p=0.018$ ) in the healthy individuals (control group) represents the protective variant of gene in terms of the disease occurrence.

**Table 4.** Frequencies of allelic groups of human leukocyte antigen; class I and II in undifferentiated spondyloarthropathies patients

HLA-A*	2n=56	Frequency (fa)	HLA-B*	2n=56	Frequency (fa)	HLA-C*	2n=56	Frequency (fa)	HLA-DRB1*	2n=56	Frequency (fa)	HLA-DRB3*, DRB4*, DRB5*	2n=56	Frequency (fa)	HLA-DQB1*	2n=56	Frequency (fa)
A*01	12	0.200	B*07	5	0.083	C*01	3	0.050	DRB1*01	3	0.500	DRB3*	32	0.533	DQB1*02	11	0.183
A*02	14	0.233	B*08	7	0.233	C*02	9	0.150	DRB1*03	8	0.133	DRB4*	9	0.150	DQB1*03	19	0.316
A*03	7	0.116	B*13	3	0.050	C*03	5	0.083	DRB1*04	6	0.100	DRB5*	17	0.283	DQB1*04	2	0.033
A*11	5	0.083	B*15	1	0.016	C*04	4	0.066	DRB1*07	4	0.066				DQB1*05	14	0.233
A*23	2	0.033	B*18	4	0.066	C*05	4	0.066	DRB1*08	2	0.033				DQB1*06	14	0.233
A*24	9	0.150	B*27	9	0.150	C*06	8	0.133	DRB1*10	1	0.016						
A*26	3	0.050	B*35	3	0.050	C*07	18	0.300	DRB1*11	6	0.100						
A*30	2	0.033	B*37	3	0.050	C*08	1	0.016	DRB1*12	1	0.016						
A*32	3	0.050	B*38	1	0.016	C*12	2	0.033	DRB1*13	8	0.133						
A*68	3	0.033	B*40	2	0.033	C*14	1	0.016	DRB1*14	5	0.083						
			B*41	1	0.016	C*15	4	0.066	DRB1*15	14	0.233						
			B*44	8	0.133	C*17	1	0.016	DRB1*16	2	0.033						
			B*47	1	0.016												
			B*49	1	0.016												
			B*51	8	0.133												
			B*52	1	0.016												
			B*55	1	0.016												
			B*57	1	0.016												

HLA: Human leukocyte antigen; n: Presence of allelic groups in sample; fa: Frequency of allelic group.

**Table 5.** Risky and protective human leukocyte antigen allelic group

Diagnosis	HLA allelic group	Control		Patients		Statistics			Risk/protective
		2n	fa	2n	fa	OR	95%CI	p	
Ankylosing spondylitis	HLA-B*27	5	0.024	22	0.440	31.9	11.18-91	0.001*	Risk protective
	HLA-B*35	32	0.153	2	0.167	0.224	0.051-0.968	0.018*	
Psoriatic arthritis	HLA-B*27	5	0.024	6	0.107	4.872	1.42-16.61	0.027*	Risk
	HLA-C*08	1	0.004	5	0.089	20.29	2.32-177.5	0.003*	Risk
Reactive arthritis	HLA-B*27	5	0.024	7	0.184	23.68	6.54-85.76	0.00*	Risk
Spondyloarthropathies	HLA-B*27	5	0.024	9	0.150	17.7	5.33-56.73	0.00*	Risk

HLA: Human leukocyte antigen; n: Presence of allelic group in sample; fa: Frequency of allelic group; OR: Odds ratio; CI: Confidence interval; \* Statistically significant at 0.05 level.

**Table 6.** Comparison of values of frequencies for most common human leukocyte antigen-B\*27 gene loci in ankylosing spondylitis patients

Diagnosis	Genotypes	Control		Patients		Statistics			Risk/protective
		n	fa	n	fa	OR	95% CI	p	
Ankylosing spondylitis	B*27/B*44	-	-	5	0.200	-	-	0.0004*	Risk
	B*27/B*51	-	-	4	0.160	-	-	0.0025*	Risk

n: Presence of allelic group in sample; fa: Frequency of allelic group; OR: Odds ratio; CI: Confidence interval; \* Statistically significant at 0.05 level.

In the patients, HLA-B\*27 allelic group is also risky and based on the estimated ratio (odds ratio [OR]=4.872), it presents a five-fold higher risk for disease development. The HLA-C\*08 allelic group in these patients is also a risky variant. ReA and SpA patients also have a high risk HLA-B\*27 allele group which is also highly significant ( $p=0.00$ ).

The frequency analysis of the different genotypes within the HLA-B\* gene locus showed statistically significantly higher frequencies of HLA-B\*27/B\*44 ( $p=0.0004$ ) and B\*27/B\*51 ( $p=0.0025$ ) genotypes in AS patients (Table 6). These genotypes are considered to be predisposing risk factors for AS development. However, in case of the three other diseases, none of the risky genotypes have been confirmed. Protective genotypes were not detected for any of the four analyzed diseases.

## DISCUSSION

The recurrent maximum frequency of allelic variants may be observed in the analyzed patients.

For all four groups, the highest frequencies are present for the allele groups HLA-A\*02 and HLA-B\*27. The highest frequency of the allele group HLA-C\*07 is present in ReA and SpA, the highest frequency of allelic variant HLA-DRB1\*01 is present in AS and ReA, and the HLA-DRB3\* and HLA-DQB1\*05 allelic variant with the highest frequency is present in AS, ReA and SpA patients. Interestingly, in rheumatoid arthritis,<sup>12</sup> HLA-B\*27 is not present with the highest frequency and this class is HLA-B\*35. Antigen HLA-B\*27 has been the subject of research for more than 30 years due to its association with AS. At the beginning of the seventies of the last century, an increased incidence of HLA-B27 antigens was found among AS patients.<sup>13</sup> Later research has confirmed that HLA-B\*27 antigen is present in 90-95% patients with AS in nearly all populations, making this linkage one of the strongest known linkages between HLA and disease genes. HLA-B\*27 has been associated with other SpA. The relationship between the gene HLA-B\*27 and the different SpA is not uniform, as is the difference between the different ethnic groups.<sup>14</sup> Our values are similar to another in which the incidence of HLA-B\*27 antigen is approximately 4-8%. Unlike



ReA, “trigger” for the beginning of the disease was not found in AS. Some studies have shown that the beginning of ReA is related to bacterial infections of the urogenital system, intestine and respiratory system and that 40 to 80% of patients with ReA are positive for HLA-B\*27 antigen.<sup>15</sup> The strength of the allelic group HLA-B\*27 varies in different racial and ethnic populations.<sup>16</sup>

Of AS patients, 90.3% in Austria and Germany,<sup>17</sup> 90% in Croatia,<sup>18</sup> 93% in Finland<sup>19</sup> and 94% in the UK<sup>20</sup> were HLA-B\*27 positive. Our results show a positive association between the presence of allelic HLA-B\*27 and AS. The increased frequency of HLA-B\*27 allelic groups (OR=31.9%; confidence interval [CI]=11.18-91;  $p < 0.001$ ) is considered a risk allele group. We consider the statistically significant occurrence of genotypes B\*27/B\*44 ( $p = 0.0004$ ), B\*27/B\*51 ( $p = 0.0025$ ) in AS patients to be risky genotypes for the development of AS. The increased frequencies of these genotypes may be due to the presence of allelic HLA-B\*27 group in these genotypes, which is present with high frequency in AS patients. The distribution of allelic sex frequency in patients from Federation of Bosnia and Herzegovina with AS does not follow a common pattern of ratio, where males affected 8-9 times more often. The most common allele groups of both sexes are HLA-A\*02, B\*27, C\*02, DRB1\*01, DQB1\*05 in 1:1 ratio. Metzger reported the first data about the relationship between HLA-B\*27 and PsA.<sup>21</sup> However, other studies have shown the association of PsA with different antigens of some HLA locus, as well as the association with various HLA loci, which ultimately suggests that the disease is caused by the combination of multiple HLA. HLA-B\*27 antigen in combination with HLA-DR7 and B39 antigens causes disease progression, while other authors have found that HLA-DQ3 antigen that is absent in a patient also causes progression of disease.<sup>22</sup> In Croatia, the frequency of HLA-B\*13, -B27 and -B17 antigens was established in patients with PsA.<sup>23</sup> The most powerful correlation in our research is present for all B\*27 and C\*06 allele groups. The ratio indicates that the presence of the allele group HLA-B\*27 causes about five times risk for developing PsA. B\*27 was the most common allele of HLA-B locus (12.1%) among patients with PsA in Croatia but did not show the highest relative risk (RR=2.4) for the disease. Two

allele B\*39 (RR=9.2) and B\*57 (RR=8.4) showed the highest RR for PsA in Croatia.<sup>24</sup>

The HLA-B\*27 allele group (OR=23.68, 95% CI=6.54-85.76,  $p = 0.00001$ ) is considered a risk variant of the gene for the development of ReA. Incidence of ReA in Minnesota (1950-1980) was 3.5/100.000 per year (0.035%) for males aged under 50 years, but was not observed for females. However, 10 to 20 times the incidence of HLA-B\*27 (30-40%) was found in homosexual males and domestic Americans.<sup>25</sup> American homeland (native) Navaho Indians, Greenland Inuit Eskimos, Alaska Inupiat Eskimos and Alaska Yupik Eskimos have a higher prevalence of ReA (1%) according to the high frequency of HLA-B27, intestinal and sexually transmitted diseases.<sup>26</sup> Many studies have demonstrated more frequent ReA in male sex, and in our study, three times more affected females.

Ankylosing spondylitis and uSpA are the most common subtypes of SpA. USpA is more common in males and approximately 70% HLA-B27 patients are positive. The blood donor epidemiological estimates in Berlin showed that the prevalence for AS was 0.86% and 0.67% for uSpA. In clinical practice, these data show that the frequency of uSpA is similar to AS.<sup>15</sup> High HLA-B27 positivity was found in 56% uSpA Eskimo patients from Alaska; while in the population of Lebanon, the positivity was only 6%.<sup>26,27</sup> The HLA-B\*27 allele group (OR=17.4; 95% CI=5.33-56.73;  $p = 0.00006$ ) is considered a risky variant of the uSpA gene.

Our research is limited by a small number of patients. Also, the role of HLA antigen in the etiology of rheumatic diseases is associated with other genes such as HLA (MICA class I MHC chain-related gene and TNF) and microsatellite locus within HLA. Additional research in this region helps to diagnose patient disease faster. Such studies are very important pilot projects and will contribute much to the future.

In conclusion, autoimmune SpA are associated with B\*27 allelic group in the HLA-B locus in the Federation of Bosnia and Herzegovina. Studies with larger number of patients are required as well as typing HLA-B\*27 antigen at high resolution for a clearer picture of the complete molecular basis of the disease and detecting the allele frequencies and their statistical significance.

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