

Clinical and laboratory features of patients with undifferentiated spondyloarthritis and ankylosing spondylitis

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ABSTRACT

Background: The terms Spondyloarthritis and spondyloarthropathy (Spa) are used to define a group of diseases with related clinical characteristics and genetics. **Aim:** To report the clinical and demographic characteristics of ankylosing spondylitis (AS) and undifferentiated spondyloarthritis (USpA) and to evaluate the frequency of cyclic citrullinated peptide antibody (anti-CCP) positivity. **Material and Methods:** Two hundred patients with USpA or AS, 100 control patients with a diagnosis of rheumatoid arthritis (RA) and 100 healthy volunteers were included. For each patient, their detailed medical histories, physical examination, whole blood counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-CCP, routine biochemical tests, and HLA-B27 test results were evaluated. ASDAS and BASDAI scores and morning stiffness were used to evaluate the disease activity. **Results:** The presenting symptom of 73 (73%) patients in the AS group and 58 (58%) patients in the USpA group was pain in axial joints. A family history of Spa was positive in 32 patients from both groups (32%). A positive HLA-B27 was found in 55% of the AS group and 25% of the USpA group ($p < 0.01$ for the difference between groups). The frequency of positive HLA-B27 was significantly higher in individuals with a family history of SpA ($p = 0.022$). A positive Anti-CCP was found in 56% of the RA group, a significantly higher frequency compared with other groups ($p < 0.001$). The frequency of positive Anti-CCP in patients in AS (9%) and USpA (6%) was significantly higher than in healthy controls ($p < 0.001$). **Conclusions:** The frequency of anti-CCP positivity was higher in SpA patients than in healthy controls. (Rev Med Chile 2021; 149: 1423-1429)

Key words: Anti-Citrullinated Protein Antibodies; Arthritis, Rheumatoid; Spondylarthritis; Spondylitis, Ankylosing.

Características clínicas y de laboratorio de pacientes con espondiloartritis indiferenciada y espondilitis anquilosante

Introducción: Los términos espondiloartritis y espondiloatropatía (Spa) se usan para definir un grupo de enfermedades con características y genética relacionadas. **Objetivo:** Informar las características clínicas y demográficas de la espondilitis anquilosante (EA) y espondiloartritis indiferenciada (USpA) – y

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para evaluar la frecuencia de positividad del anticuerpo péptido citrulinado cíclico (anti-CCP). **Material y Métodos:** En este estudio observacional se incluyeron doscientos pacientes con USpA y EA, 100 pacientes control con diagnóstico de artritis reumatoide (AR) y 100 voluntarios sanos. Se evaluó la historia clínica, examen físico, recuentos sanguíneos completos, velocidad de sedimentación globular (ESR), proteína C reactiva (PCR), anti-CCP, pruebas bioquímicas de rutina y resultados de la prueba HLA-B27. Para evaluar la actividad de la enfermedad se utilizaron las puntuaciones ASDAS y BASDAI y la rigidez matutina. **Resultados:** El síntoma inicial de 73 (73%) pacientes en el grupo de EA y 58 (58%) pacientes en el grupo de USpA fue dolor en las articulaciones axiales. Treinta y dos pacientes de cada grupo (32%) tenían antecedentes familiares de SPA. HLA-B27 fue positivo en el 55% del grupo AS y el 25% del grupo USpA con una diferencia significativa entre los dos grupos ($p < 0.001$). La frecuencia de positividad HLA-B27 fue mayor en individuos con historia familiar de SpA ($p = 0,02$). Se encontraron anti-CCP positivos en el 56% del grupo con AR, una frecuencia significativamente mayor en comparación con otros grupos ($p < 0,01$). La frecuencia de anti-CCP positivo fue mayor los pacientes con AS (9%) y USpA (6%) que en el grupo sano ($p < 0,001$). **Conclusiones:** La frecuencia de positividad anti-CCP fue mayor en los grupos de SpA en comparación con los grupos control sanos.

Palabras clave: Anticuerpos Antiproteína Citrulinada; Artritis Reumatoide; Espondilitis Anquilosante; Espondiloartritis.

The terms spondyloarthritis and spondyloarthropathy (SpA) are used to define a group of diseases with related clinical characteristics and genetics. Disease groups such as undifferentiated spondyloarthritis (USpA), ankylosing spondylitis (AS), reactive arthritis (ReA), spondyloarthritis related with psoriatic arthritis, spondyloarthritis related with Crohn's disease and ulcerative colitis, and juvenile-onset spondyloarthritis are all grouped under the title SpA¹. Although it has been previously believed to be a variant of rheumatoid arthritis (RA), today it is accepted that RA and SpA are different entities. The estimated prevalence of SpA is approximately 0.5–2% in the Caucasian population. There are significant differences in prevalence depending on ethnicity all over the world². While AS and USpA are the most commonly encountered spondyloarthritis types, ReA is encountered less frequently.

As improvements in the understanding of rheumatologic diseases have evolved, investigation of antibodies against citrullinated antigens has been developed as a more specific and sensitive test in RA diagnosis. Citrulline is formed by deamination of the amino acid arginine. Antibodies

against citrullinated structures have been recognized in RA for a long time. Anti-cyclic citrullinated peptide antibodies (anti-CCPs), thanks to previous studies, have also been discovered. Many studies measuring the diagnostic performance of anti-CCPs have been performed. These antibodies are very specific and they can be detected in the very early phase of disease or even a couple of years before disease onset³. Although anti-CCP is a specific marker for RA, it may be determined as positive even in some healthy individuals, and there is still inadequate information about its clinical significance. The aim of the present study was to determine the frequency and clinical significance of positive tests for cyclic citrullinated peptide antibodies (anti-CCP) among USpA and AS patients, as well as to investigate the correlations between anti-CCP and both disease activity indices in this group of patients.

Materials and Methods

Two hundred patients with USpA or AS, older than 18 years of age who applied to the Rheuma-

tology Outpatient Clinic between January 2010 and January 2014 and were diagnosed according to modified New York criteria, 100 control patients with a diagnosis of RA and 100 healthy volunteers were included in this study. The 2010 ACR/EULAR diagnostic criteria were used for RA. Exclusion criteria were overlap syndrome, pregnancy, malignancy, and acute infectious conditions. Age, gender, complaint at application, age of onset of disease symptoms, age at diagnosis, and time from symptoms onset in the case of delayed diagnosis, presence of familial SpA history, axial joint involvement, peripheral joint involvement, enthesopathy, and eye involvement were evaluated in all patients. Ankylosing Spondylitis Disease Activity Score (ASDAS), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and morning stiffness were used in the evaluation of disease activity. Whole blood counts (CBC), erythrocyte sedimentation rates (ESR), C-reactive protein (CRP), and biochemical tests were performed. Additionally, RF, anti-CCP and HLA B-27 results were assessed. The control group consisted of RA patients without any known additional rheumatologic disease, and whose anti-CCP values had been measured. Anti-CCP analysis was performed with the same technique and laboratory method for all the cases. The healthy control group was formed by healthy volunteers without any known disease. Healthy volunteers were obtained by screening persons who applied to the Rheumatology Outpatient Clinic and who did not find inflammatory rheumatic disease as a result of the evaluations and tests.

Statistical Analysis

Normal distribution of variables such as age, age at diagnosis, disease duration, BASDAI, ASDAS and anti-CCP was evaluated by using the Shapiro-Wilk test. Median values were used in descriptive statistics of not normally and categorical variables. Number (n), and percentage values were given for categorical variables such as gender, anti-CCP group, and presence of infection. Differences in variables such as age, anti-CCP, ESR, CRP, and RF were tested between AS, USpA, RA control, and healthy control groups using the Kruskal-Wallis test. Bonferroni correction paired comparisons were performed to determine the group which caused significant differences among variables at the end of the Kruskal-Wallis test.

Similarly, differences in age at diagnosis between the three groups were examined by using Kruskal-Wallis test. A chi-square test was performed to determine whether there was any difference in presenting symptoms, infection presence, HLA B-27, eye involvement, and anti-CCP between the groups. The Mann-Whitney U test was used for differences in BASDAI and ASDAS values between anti-CCP positive patients.

IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) and MS-Excel 2007 programs were used. The level of statistical significance was determined as $p < 0.05$.

Results

Among the 400 patients included, 64.2% were female ($n = 257$) and 35.8% ($n = 143$) were male. There were 100 individuals in each of the AS, USpA, RA and healthy groups. The median age was 45 (19-79) years in the AS group; 49.5 (20-68) years in the USpA group; 55 (20-80) years in the RA control group; and 42 (18-64) years in the healthy control group. The median ages at diagnosis were 41 (18-74) years and 47 (16-65) years in the AS and USpA groups, respectively. Time delay in diagnosis was 21.5 (0-240) months in the AS group, and 28.5 (0-228) months in the USpA group. There was no significant difference in age at diagnosis or time delay in diagnosis between the two groups ($p > 0.05$). There was no significant difference in disease duration between the AS and USpA groups ($p = 0.193$). Diagnosis durations were 36.5 (0-240) months and 33.5 (1-96) months in the AS and USpA groups, respectively. Diagnosis duration was significantly longer in the AS group ($p = 0.002$). While BASDAI values were significantly different between two groups ($p = 0.017$), there was no significant difference in ASDAS values between the groups ($p = 0.708$). The median of BASDAI was higher in the USpA group compared with the AS group (Table 1).

Considering the presenting symptoms in the AS patient group, pain in axial joints was diagnosed in 73% ($n = 73$) of the group; peripheral joint pain in 21% ($n = 21$); heel pain in 3% ($n = 3$); and eye involvement in 3% ($n = 3$). In the USpA group, the presenting symptoms were pain in axial joints in 58% ($n = 58$), pain in peripheral joints in 41% ($n = 41$), and eye involvement in 1% ($n = 1$).

Table 1. Comparison of clinical characteristics and physical examination signs between the AS and USpA groups

		Group		p
		AS median (min-max)	USpA median (min-max)	
Disease duration	(month)	72.0 (12-360)	60.0 (6-360)	0.193
Diagnosis duration	(month)	36.5 (0-240)	33.5 (1-96)	0.002
Diagnosis delay	(month)	21.5 (0-240)	28.5 (0-228)	0.091
Morning stiffness	(minute)	30.0 (0-180)	27.5 (0-200)	0.608
Upper Schober	(cm)	5.0 (2-6)	5.0 (2-8)	0.583
Lower Schober	(cm)	4.0 (1-6)	5.0 (1-7)	0.019
BASDAI		4.8 (1.2-9.3)	5.4 (1-9.3)	0.017

Infection was present in 12% (n = 12) of the AS group, and 21% (n = 21) of the USpA group before SpA diagnosis. There was no statistically significant difference in the presence of infection between the two groups (p = 0.086).

There was no statistically significant difference in family history of SpA between the AS and USpA groups (both 32%, n = 32). While HLA B-27 positivity was 55% (n = 55) in the AS group, it was 25% (n = 25) in USpA group. HLA-B27 positivity was determined as being significantly high (p < 0.001) (Table 2). HLA B-27 positivity rates were 51.6% (n = 33) and 34.6% (n = 47) among patients with and without a family history of SpA, respectively (p = 0.022). HLA B-27 positivity was determined as being significantly higher in patients with a family history of SpA.

While the frequency of positive anti-CCP values were 9% (n = 9), 6% (n = 6), and 56% (n = 56) in the AS, USpA, and RA control groups, respectively there was no anti-CCP positivity in the healthy control group.

The frequency of anti-CCP positivity in the RA control group was statistically significantly higher compared with all other groups. The distribution of anti-CCP was statistically similar in the AS and USpA groups (p = 0.421), while the results of other paired comparisons showed statistically significant differences. When anti-CCP positivity rates of the AS and USpA groups were compared with the healthy control group, they were determined to be significantly higher (p < 0.001, p = 0.004).

When anti-CCP results in the SpA group were compared with disease activity scores, the medians of BASDAI and ASDAS were 4.8 (2-7) and 3 (1.6-4.4) among patients with positive anti-CCP, and 5.1 (1-9.3) and 2.8 (1.2-8) among patients with negative anti-CCP. There was no significant difference between anti-CCP results and each of BASDAI and ASDAS values (p = 0.418 and p = 0.915, respectively) as shown in Table 3.

Table 2. Comparison of AS and USpA groups in terms of HLA B-27

	Group		p
	AS n (%)	USpA n (%)	
Family History			
No	68 (68.0)	68 (68.0)	1.000
Yes	32 (32.0)	32 (32.0)	
HLAB27			
Negative	45 (45.0)	75 (75.0)	< 0.001
Positive	55 (55.0)	25 (25.0)	

Table 3. Comparison of anti-CCP results with both BASDAI and ASDAS values

	SpA		p
	CCP Negative	CCP Positive	
BASDAI	5,1 (1-9,3)	4,8 (2-7)	0,418
ASDAS	2,8 (1,2-8)	3,0 (1,6-4,4)	0,915

Discussion

The present study shows that the frequency of anti-CCP positivity was higher in SpA groups compared to healthy controls. The median ages of the AS and USpA groups were 45 (19-79) years and 49.5 (20-68) years, respectively. In the TRASD study which was performed, the mean age of AS patients was 39 ± 10.7 years⁴. Although median age in USpA was not significantly different, it was slightly higher. This might be caused by milder disease progression in females, and later applications to hospitals. Time delay in diagnosis was 21.5 (0-240) months in the AS group, and 28.5 (0-228) months in the USpA group. Time delay in diagnosis was reported as 4.3 years in the study of Bodur et al.⁵.

Genetic susceptibility and environment are considered to be factors in the etiopathogenesis of SpA. Also a strong relationship with HLA B-27 has been definitively shown. In Western populations, HLA B-27 positivity rates were 90-95% among AS patients and 50-75% in other diseases belonging to the SpA group⁶. Interestingly low HLA B-27 positivity rates in both of our groups indicated that the genetic characteristics of the disease were not only defined by HLA B-27 in our population. It was reported in the TRASD Turkey data that HLA B-27 frequency was 73.7% in AS patients⁴. In the study reported from Iran, HLA B-27 positivity rate was 73.4% in AS patients⁷. It was reported in studies performed in Europe that the HLA B-27 frequency of USpA patients was 70%⁸. This ratio was reported as 54% in another study from Brazil⁹. These data indicate that HLA B-27 positivity rates might differ between populations in SpA patients, and the contributions of other genetic and environmental factors might be prominent. Therefore, delayed diagnosis might be observed in patients negative for HLA B-27. Moreover, the possibility of USpA patients belonging to disease categories with lower HLA B-27 levels such as reactive arthritis, and psoriatic arthritis might also support this low rate. Large-scale studies investigating the presence of other genetic factors which affect disease development will shed light on this issue in populations with low HLA B-27 positivity rates. Küçükşahin et al. reported in their study that the ERAP1 (rs26653) gene polymorphism might be a risk factor in the pathogenesis of AS and IBD¹⁰.

Recently, ACPA tests have also been inves-

tigated in many other diseases. In the present study, patients in the AS and USpA groups were compared with the ones in the healthy control and RA control groups. As expected, anti-CCP positivity rates in the RA group were found to be significantly higher than in all other groups. In a large meta-analysis, sensitivity rates of anti-CCP were 75.2% in RA, and 61% in early RA¹¹.

In the present study, distributions of anti-CCP were statistically similar in the AS and USpA groups, and anti-CCP positivity rate was significantly higher than in the healthy control group. Anti-CCP positivity was determined as 17% in a study conducted on SLE patients¹². The positivity was observed especially with erosive arthritis, so it was thought that accompanying anti-CCP might indicate a more severe disease course as it was observed in clinical progression of RA. However, it was also emphasized that an overlap syndrome (SLE/RA) should also be considered in these patients. Orozco C et al. reported that some anti-CCP positive cases might be found in undifferentiated arthritis (UA) cases, and they may differentiate into RA during follow-up periods¹³. Similar findings were also reported in patients with psoriatic arthritis and primary SS (10% anti-CCP positivity)¹⁴. In another study, Kim JO et al. reported that anti-CCP antibodies were occasionally present in AS, and their presence may be helpful as a serum marker in predicting peripheral arthritis¹⁵.

Singh Sangha M. et al. performed in their case-series based review that there is high titers of anti-CCP positivity in patients who had sacroiliitis and reactive arthritis without peripheral small joint involvement after *E. coli* infection¹⁶.

It has been reported in the literature that anti-CCP prevalence may be also increased in patients with non-rheumatologic diseases such as active tuberculosis. Variable values as high as 32-29% or as low as (7%) rates have been determined in different studies¹⁷. Anti-CCP was also rarely determined in serums of patients with Hepatitis C virus infection. In a study performed on 257 patients with α 1-antitrypsin deficiency and 113 patients with chronic obstructive pulmonary disease, anti-CCP antibody positivity rates were 3% and 5%, respectively¹⁸. These data may indicate that the presence of anti-CCP may suggest some granulomatous diseases, chronic infections or cross-reactivity. However, anti-CCP determination was not useful in any differential

diagnostic step for these diseases. Ioan-Facsinay A. et al. showed the presence of anti-CCP antibodies in 91% of RA patients, in 19% of their first-degree relatives, and in 9% of healthy controls¹⁹. Finally, in a recent study reported by Yamazaki et al, PsA patients reported a significantly higher prevalence of positive anti-CCP antibodies among SpA patients, and a higher rate of positive anti-CCP antibodies in hyperostosis, and osteitis syndrome (SAPHO) and USpA²⁰. In our study, anti-CCP positivity was higher in the AS group than to the USpA group, but anti-CCP positivity was statistically similar.

Anti-CCP was negative in all patients in the healthy group without any chronic diseases, and this indicated that the rate of anti-CCP positivity within healthy populations might be different between different communities. Large scale frequency studies are required for certain results.

As mentioned above, it has been shown previously that anti-CCP positivity might be related to erosive joint damage in RA, SLE and primary SS patients. In the present study, there was no significant relationship between anti-CCP and BASDAI and ASDAS scores in the SpA group (AS and USpA) and it was determined that anti-CCP positivity was higher in SpA patients than in healthy controls, although it was not correlated with disease activity. It should be kept in the mind that in patients with musculoskeletal complaints, positivity of anti-CCP does not rule out a diagnosis of SpA. As far as we know, this is one of the rare studies investigating the frequency of anti-CCP in patients with AS and USpA. Determination of anti-CCP positivity will contribute to large scale prospective studies, which will investigate its effects on diagnosis and disease progression and will be performed in these diseases of which there is growing awareness in the community.

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