Frequency of ANA/DFS70 autoantibodies in Colombian patients with undifferentiated connective tissue disease

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SUMMARY

The objective was to describe the clinical characteristics and the frequency of the ANA/DFS70 autoantibodies in patients affected by undifferentiated connective tissue disease (UCTD) in a tertiary hospital in Colombia.

This descriptive cross-sectional study enrolled patients who fulfilled the classification criteria for UCTD. ANA-HEp-2 test and the modified assay for ANA/DFS70 autoantibodies were performed through the indirect immunofluorescence technique. Erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, and the antibodies to anti-extractable nuclear antigens, DNA, phospholipids (IgG, IgM, IgA), and cyclic citrullinated peptide were also evaluated.

Fifty-three patients were studied; 42/53 (79%) tested positive for ANA and 5/42 (11.9%) for ANA/DFS70 antibodies with a dense fine speckled fluorescent pattern (AC-2) in ANA HEp-2 test that was confirmed by a modified HEp-2-DFS70 assay. Patients had arthralgia (87%, n=47), non-erosive arthritis (66%, n=34), xerostomia (64%, n=34), xerophthalmia (42%, n=22), and Raynaud's phenomenon (17%, n=9). Arthralgia, xerophthalmia, xeroderma, and absence of disease evolution to a specific disease over five years were more frequent in patients with a positive result for the anti-DFS70 antibodies.

The ANA/DFS70 autoantibodies were more frequent in patients with UCTD compared to other rheumatic diseases for which they were initially evaluated. More studies are required to support the predictive role of this antibody to the absence of progression to a well-defined connective tissue disease.

Key words: *UCTD, ANA, anti-nuclear antibodies, anti-DFS70 antibodies.*

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INTRODUCTION

Autoimmune diseases have a broad phenotypic spectrum, with significant variability in symptoms, signs, and findings in the immunological tests. Some patients can show symptoms and signs of autoimmunity associated to abnormal laboratory findings, without fulfilling the specific criteria for a well-defined connective-tissue disease (CTD) as systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), polymyositis/dermatomyositis (PM/DM), or rheumatoid arthritis (RA). Therefore, these patients are diagnosed as undifferentiated connective tissue disease (UCTD) (1-5). Although the pathogenesis of UCTD is unknown, some clinical characteristics can be present such as arthralgia (37-80%), non-erosive arthritis (14-70%), Raynaud's phenomenon (45-60%), leukopenia (11-42%), xerostomia (7-40%), xerophthalmia (8-36%), skin rash, and ulcers. Serological profile mainly includes positive results for antinuclear antibodies (ANA) (58-100%), anti-Ro (8-30%), and anti-RNP (10-30%) antibodies, and less frequently for anti-DNA or anti-phospholipid antibodies. One-third of patients with UCTD eventually develop a well-defined CTD, typically within five years from the onset, where the antigenic specificities of the antibodies provide the main determinant of the evolution towards a definite CTD; however, to determine disease duration in these patients is difficult (6, 7).

Recently, some ANA-positive patients with

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UCTD were found to have a dense fine speckled (DFS) immunofluorescent pattern in the nucleoplasm of the interphase cells. This pattern typically excludes the nucleoli and brightly stains the chromosomes in the cell mitotic phase; it is classified as AC-2, and usually targets a 70 kDa protein (DFS70), commonly known as the lens epithelium-derived growth factor p75 (LEDGF/p75) (8-10). The presence of the anti-DFS70 antibody was initially documented in patients with different non-rheumatic inflammatory conditions and in apparently healthy individuals and was therefore associated with a low probability of CTD. In patients with CTD, anti-DSF70 antibody is usually accompanied by other specific autoantibodies such as anti-DNA and anti-Scl-70 (11-14). The prevalence of ANA/DFS70 antibodies in a population with UCTD ranges from 8-40%, and it is considered a potential negative predictor for the risk of progressing from UCTD to CTD (12, 15, 16). Published reports of the prevalence of ANA/DFS70 antibodies mainly include the Asian, European, and North American general populations, with a single report in the Hispanic general population and a few in Europeans with UCTD (10, 16-20). No previous reports of the evaluation of ANA/DFS70 antibodies in the Latin American population with UCTD have been published. Here, we describe the clinical features, laboratory findings, and the frequency of this antibody in patients with UCTD treated in the rheumatology and immunology department of the hospital in which the author works.

MATERIALS AND METHODS

This descriptive cross-sectional study was carried out in the Rheumatology and Immunology Service of a high complexity university hospital in Bogotá, Colombia, between January 2013 and January 2018. The study aimed to describe the demographic, clinical and laboratory characteristics of patients who fulfilled the classification criteria for UCTD (21) and had an evolution of disease of more than one year. The ANA testing was performed through the indirect immunofluorescence assay (IIFA) using the AESKUSLIDES® ANA-Hep-2 Test Kits (AESKU.DIAGNOSTICS, Germany). Samples were considered positive if they exhibited a dense fine speckled pattern in the nucleoplasm of interphase cells (AC-2) and excluded nucleoli with brightly stained chromosomes in mitotic cells. Samples were screened at a dilution of 1/80, and the positive ones were tittered. The positive samples were evaluated using the modified HEp-2 ELITE DFS70-KO Substrate (IMMCO Diagnostics 1108, Buffalo, NY). This kit uses *psip1*$^{-/-}$ HEp-2 cells, which do not express the 70 kDa protein, thus preventing the binding of the anti-DSF70 autoantibodies. These modified cells are suitable for the detection of all nuclear autoantibodies but DFS70. The respective positive and negative controls for each test were run in parallel. Antibodies against the extractable nuclear antigens (ENA) were tested by the enzyme-linked immunosorbent assay (ELISA) technique using kits from Sera Quest (Secaucus, NJ) including anti-SSA (Q01-250®), anti-SSB (Q01-260®), anti-RNP (Q01-240®), and anti-Sm (Q01-230®). Levels of C-reactive protein (CRP) were determined by a chemiluminescent technique (Abbot 6K26-30®, Chicago, IL, USA). The erythrocyte sedimentation rate (ESR) was determined according to the Westergren method using a VES-MATIC Cube 30® instrument (Diesse Diagnostica Senese, Italy). The anti-cyclic citrullinated peptide antibodies (ACPA) and the anti-dsDNA IgG antibodies were tested by ELISA (QUANTA Lite™ CCP IgG 3.0 and HA dsDNA, respectively; INOVA Diagnostics, San Diego, CA). The rheumatoid factor (RF) was evaluated by turbidimetry using the SPAPLUS® Analyzer (Binding Site Group Ltd, United Kingdom). The profile of the anti-phospholipid antibodies included the testing by ELISA of the anti-cardiolipin IgG, IgM, and IgA antibodies (QUANTA Lite™ Generic Assay GA4016® and ACA IgA, respectively; INOVA Diagnostics), and the anti-beta 2 glycoprotein IgG and IgM antibodies (QUANTA Lite™ GA4041 INOVA Diagnostics).

Ethics

All patients gave informed consent to the study, according to the Helsinki Declaration. All procedures performed in this study were in accordance with the ethical standards of the Research Ethics Committee of Hospital Militar Central (Approval No. 2018-026) and followed the 1964 Helsinki Declaration and its later amendments as well as national ethical standards (Resolution 8430 of 1993). The Research Ethics Committee of the institution reviewed and approved the study and the use of the medical records, under the data privacy laws for clinical studies and scientific publications.

Statistics

The analysis of data included the description of qualitative variables by frequencies and percentages and a summary of the quantitative variables by measures of central tendency (averages) and dispersion. Serological assays were performed by two different evaluators who were blinded, and the patients' medical records remained anonymous.

RESULTS

Fifty-three patients were enrolled in the study. Their clinical and laboratory characteristics are summarized in Table I. The patients fulfilled the classification criteria proposed for UCTD (21) (Table II). Of the 53 patients diagnosed with UCTD, 42 (79%) had positive ANA, and 5/42 were positive for the ANA/DFS70 antibody with a dense fine speckled fluorescent pattern (AC-2) in the ANA Hep-2 test. All positive samples with an AC-2 pattern resulted positive also by the confirmatory test using the modified HEp-2. Table III shows the fluorescence patterns of ANA and and the type of anti ENA antibodies observed in the patients. The 5 patients positive for ANA/DFS70 antibodies were women, with an average age of 54 years (±18.1 years), and an average disease duration of 9.4 years (±2.9 years). Only 1/5 patients had a high titer of ANA/DSF70 antibodies (1/640), whereas all other patients presented a title of 1/160. In this group, anti-Sm antibodies were seen

Table I - Clinical characteristics of the population with UCTD.

Population, n=53		
Age (years)	**Female**	**Male**
Average=44.2 (±18.1)	44 (±12.6)	44 (±18.6)
Sex		
	87% (46)	13% (7)
Occupation		
Employee	47.1% (25)	
Unemployed	37.7% (20)	
Student	15% (8)	
Medical history		
No antecedents	75.4% (40)	
Smoking	18.8% (10)	
Hypothyroidism	16.9% (9)	
Family history of autoimmune disease	11.3% (6)	
Clinical features		
Arthralgia	66% (35)	
• DFS70 (+)	9.4% (5)	
Xerostomia	64% (34)	
• DFS70 (+)	1.8% (1)	
Arthritis	45% (24)	
• DFS70 (+)	5.6% (3)	
Xerophthalmia	42% (22)	
• DFS70 (+)	5.6% (3)	
Raynaud's phenomenon	17% (9)	
• DFS70 (+)	3.7% (3)	
Thrombophlebitis	11.3% (6)	
Photosensitivity	11.3% (6)	
Mononeuritis	1.8% (1)	
Interstitial lung disease	1.8% (1)	
Serositis	1.8% (1)	
Aphthosis	0% (0)	
Laboratory tests		
ANA	79% (42)	
CRP	30.1% (16)	
ESR	20.7% (11)	
Altered blood count	18.8% (10)	
Lymphopenia	9.4% (5)	
• DFS70 (+)	1.8% (1)	
Thrombocytopenia	5.6% (3)	
• DFS70 (+)	1.8% (1)	
Leukopenia	2.7% (2)	
• DFS70 (+)	1.8% (1)	
Positive anti-cardiolipin/anti-B2 glycoprotein antibodies	0% (0)	
Positive rheumatoid factor	0% (0)	
Positive ACPA	0% (0)	

ANA, antinuclear antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ACPA, anti-cyclic citrullinated peptide antibody; DFS70, dense fine speckled 70 kDa.

Table II - Preliminary classification criteria suggested for UCTD.

1. Signs and symptoms of a CTD without fulfilling the complete criteria for a defined CTD* for at least three years**
2. Presence of antinuclear antibodies evaluated on two different occasions***

*Using the established classification criteria as described in the following references: for SLE (23), MCTD (24), SSc (25), PM/DM (26), AR (27), SS (28).
**If the length of the disease is less than three years, the patient can be diagnosed with an early UCTD.
***The presence of ANA is not required to be classified as UCTD.
Taken from reference (22).

in 2/5 patients, anti-RNP in 2/5 patients and the coexistence of them in another patient. RF and ACPA were negative, CRP and ESR were normal, and the profile for the antiphospholipid syndrome was negative. Otherwise, only one patient presented hemolytic anemia and leukopenia, with decreased neutrophils and lymphocytes. All presented arthralgia, and 3/5 arthritis, xerophthalmia and pulmonary interstitial disease, 2/5 Raynaud's phenomenon. In addition, in 2/5 aphthous stomatitis was presented. None presented photosensitivity, serositis or thrombophlebitis. The persistence of ANA/DFS70 over time was evaluated, finding that at 5 years all patients continued to have a diagnosis of UCTD and only 1 of 5 differentiated into autoimmune disease (SLE) at 9 years. However, the comparison with patients without ANA/DFS70 resulted not significant (p=0.587). Table IV summarizes the laboratory results of these five patients with positive results for ANA/DFS70 antibodies.

DISCUSSION

The complexity in the diagnosis of UCTD is due to the different symptoms, signs, and results of clinical and immunological tests. The name of this disease has varied from incomplete lupus and early or incomplete CTD to UCTD, and therefore, it was necessary to differentiate it from mixed diseases

Table III - Patterns of ANA/anti-DNA and ENA in patients with UCTD.

ANA-IIFA			ENA		
Fine speckled	15	28.3%		**Total**	**%**
Negative	11	21%	RNP	13	31%
Coarse speckled	9	16%	Ro	13	31%
Nucleolar	8	15%	Sm	10	23%
Dense fine speckled	5	9.4%	La	7	17%
Cytoplasmic	3	5.7%	DFS70	5	11.9%
Centromere	2	3.8%	dsDNA	1	2.3%
Homogeneous	0	0			
Total	53	100%			

ANA, anti-nuclear antibodies; ENA, anti-extractable nuclear antigen antibodies; Sm, anti-Smith antibody; Ro, anti-Ro antibody; La, anti-La antibody; dsDNA, anti-double stranded DNA antibody; RNP, anti-ribonucleoprotein antibody; DFS70, anti-dense fine speckled 70 kDa antibody; IIFA, indirect immunofluorescence assay (screening dilution 1:80).

Table IV - Serological characterization of patients with UCTD and positive ANA/DFS70.

DFS70 (+) n=5	Dilution	Pattern	Anti ENA Ab	Anti-DNA	AAP	ESR/CRP
1	1/160	DFS (AC-2)	Anti-Sm	N	P	N
2	1/640	DFS (AC-2)	Anti-Sm/RNP	N	N	N
3	1/160	DFS (AC-2)	N	P	N	N
4	1/160	DFS (AC-2)	Anti-RNP	N	N	N
5	1/160	DFS (AC-2)	N	N	N	N

AC-2-DFS, dense fine speckled; Anti ENA Ab, anti-extractable nuclear antigen antibodies; AAP, antiphospholipid antibodies (anti-cardiolipin IgG, IgM, IgA; and anti-beta 2 glycoprotein IgG, IgM antibodies); N, negative; P, positive; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

or overlap syndromes (22, 23). The first description of UCTD, made in 1969, already suggested three possible outcomes for this condition: development of a CTD, intermittent undifferentiated, and persistence of the undifferentiated condition (24). Years later, Alarcón et al. and other authors reported that about 60% of patients would remain with a diagnosis of UCTD. These individuals, similarly, to the patients of the present study, are characterized by positive ANA and symptoms as arthralgia, arthritis, Raynaud's phenomenon, and sicca syndrome. Of these individuals, only one-third, approximately, will evolve to a CTD within the next five years, being SLE or SS the most frequent (2, 6, 7, 25-27).

In Colombia, the only available data for UCTD derived from a retrospective analysis of a cohort of 94 patients who were followed for one year. Less than one-third of those patients evolved to a CTD, mainly SLE and SS, and in the five years following the diagnosis, they remained stable. Arthralgias, Raynaud's phenomenon, and fatigue were the most frequent symptoms. The ANA/DFS70 antibodies were not evaluated in this cohort (28). Given the absence of severe organ involvement, UCTD usually has a benign course; however, it is essential to identify patients at high risk of developing a CTD. To this purpose, different combinations of clinical and serological findings have been proposed (29). Within these possible predictors, the DFS pattern, together with a positive ANA/DFS70, has been proposed as a potential serological marker to help identify a patient with stable UCTD or a UCTD with a low risk to evolve into a CTD (16). The present study is consistent with other reports where the ANA/DFS70 antibodies were frequent in women with UCTD who exhibited few symptoms. In fact, a higher frequency of ANA/DFS70 (11.9%) was confirmed in our population with UCTD in comparison with other autoimmune diseases such as SLE (3%), in which it generally associates with positive anti-dsDNA, anti-SSA/Ro, or anti-Sm antibodies (15), or as AR (0%) (13, 30). Patients with UCTD are followed by the rheumatologist for several years; therefore, some cost-effectiveness studies have been carried out using algorithms that include the testing of ANA/DFS70 and the low probability of developing a CTD when the test is positive (Figure 1). This algorithm was applied in the study of Gundín et al., who followed 181 patients with UCTD for ten years; they found a reduction of up to 70% in the paraclinical tests and medical visits with a total saving of up to € 60.869,53 (31).

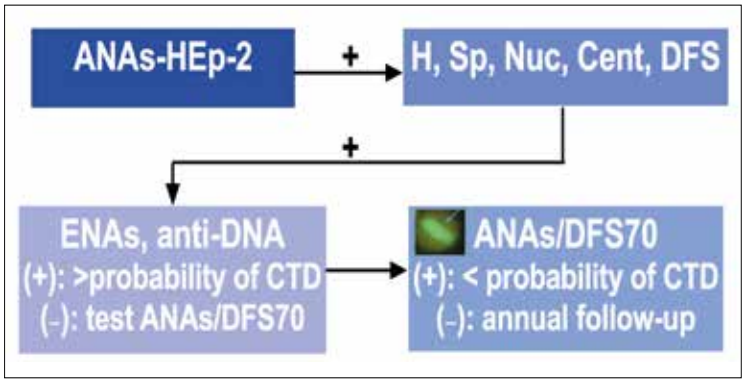

Figure 1 - Algorithm for patients with UCTD. Abbreviations refer to the different patterns found in the detection of ANA by IIFA on HEp-2 cells. H, homogeneous; Sp, speckled; Nuc, nucleolar; Cent, centromere; Dense fine sp, dense fine speckled; ANA, anti-nuclear antibodies; ENA, anti-extractable nuclear antigen antibodies; CTD, connective tissue disease.

The limitations of the present study include the low number of patients with UCTD and the difficulties in their adequate classification according to the proposed criteria. However, we consider essential to characterize the population with UCTD and the subset positive for ANA/DFS70 antibodies, for the long-term follow up. This is the first report of ANA/DFS70 antibodies in a group of patients with UCTD in Colombia and Latin America.

CONCLUSIONS

In recent years, research on patients with UCTD has increased, including studies about the relevance of identifying individuals at high risk of developing a well-defined CTD, and those who will remain with UCTD. Some clinical and laboratory characteristics, as the ANA/DFS70 antibodies, could help to differentiate these patients. The present study reports a higher

prevalence of the ANA/DSF70 antibodies in women with UCTD, with more than five years of disease duration, with a predominance of joint and dryness symptoms, and a lower frequency of hematological and neurological ones: these findings are in line with data from other studies. The ANA/DFS70 antibodies are more frequent in patients with UCTD than CTDs. Therefore, the ANA/DFS70 antibodies could be a useful diagnostic tool in the follow-up of patients because of their possible association with a lower probability of differentiation to a well-defined CTD. Testing of the ANA/DFS70 antibodies may be included in a routine algorithm for the study of patients with UCTD.

Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Diana Rincón-Riaño, Daniel Fernández-Ávila, Juan Manuel Bello, Diana Acero-Molina and Consuelo Romero-Sanchez. The first draft of the manuscript was written by Diana Rincón-Riaño, and all authors commented on previous versions of the manuscript. All co-authors take full responsibility for all aspects of the study and the final manuscript.

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