Anti-dense fine speckled (DFS) antibody: its staining pattern in indirect immunofluorescence and its clinical relevance

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Background: Dense fine speckled (DFS) pattern is defined by very intense, heterogeneous speckled staining of nucleoplasms of interphase HEp-2 cells and chromosomal areas of metaphase cells. The association of Anti-DFS70 and rheumatologic signs, symptoms, and diagnosis were evaluated.

Methods: One-hundred-eight anti-DFS70 positives who got consecutively admitted to the Rheumatology clinic between January and June 2020 were analyzed. The clinical and laboratory findings of positives for anti-DFS70 antibody were compared with those with DFS pattern ANA IFA staining rates. Also, anti-DFS70 positivity rates and their correlation with the DFS staining pattern were analyzed retrospectively in 1016 CTD patients.

Results: The most common complaint was joint pain seen in 77 (71.3%) and the most common laboratory abnormality was RF-positivity observed in 10/108 (9.3%) who had anti-DFS70 positivity. The most common ANA staining pattern was DFS (72/108; 66.7%); one-third had other than DFS. No statistical significance was found for the association of any of the rheumatological complaints and laboratory findings with the DFS staining pattern. ANA analysis was performed in a total of 964/1016 (94.88%) CTD patients and 44 (4.56%) of these positive for anti-DFS70. The correlation coefficient showed good correlations between the DFS pattern staining and anti-DFS70 antibody positivity ($r=+0.773$, $p<0.001$).

Conclusions: Anti-DFS70-positives have a low rate of CTD. A low anti-DFS70 positivity rate was observed in patients with CTD. As such, it can be considered that anti-DFS70 does not predict CTD or even excludes it.

Key words: Anti nuclear antibody, anti-DFS70, connective tissue disease, extractable nuclear antigen.

INTRODUCTION

Antinuclear antibodies (ANAs), which are synthesized against the nucleus, nucleolus, cytoplasm, and cell surface antigens, are used for diagnosis and follow-up of systemic connective tissue diseases (CTDs) [1]. Determining the presence of ANAs in the systemic circulation plays a key role in the diagnosis of many autoimmune diseases. The indirect immunofluorescent (IIF) staining method in human epithelial (HEp-2) cells is considered the gold standard screening method for the detection of ANA [2]. International reports have been published in order to provide standardization in the evaluation and naming of staining patterns in HEp-2 cells. Many staining patterns occurring in HEp-2 cells indicate autoantibodies against certain antinuclear antigens: for example, the

homogeneous staining pattern is often stimulating for anti-ds DNA, anti-Ro, and anti-histone. However, it remains confusing which autoantibody is associated with some staining patterns, such as speckled patterns [3]. In patients showing positive in the HEp-2 IIF ANA screening test, the Extractable Nuclear Antigens (ENAs) test can be performed with various methods to determine which antibody is positive in the patient. It is clinically valuable to detect ENAs, and anti-ENA antibodies developed against these ENAs in these patients. The staining pattern and intensity in the ANA test can predict the autoantibody positivity detected by the ENA immunoblot method. The DFS pattern frequently encountered in ANA screening tests is characterized by very intense, heterogeneous speckled staining of the nucleoplasms of interphase HEp-2 cells and chromosomal areas of

metaphase cells. The difference in size, brightness, and density of speckles across the interphase nucleoplasm is also an important distinguishing feature of this pattern [3]. The DFS pattern has been associated with the presence of autoantibodies (anti-DFS70) against a protein of 70 kd, called DFS70, lens epithelium-derived growth factor (LEDGF), or DNA binding transcription co-activator p75 [4]. The clinical significance of the anti-DFS70 antibody remains unclear. Anti-DFS70 antibodies were first demonstrated in patients with interstitial cystitis [5]. There are studies showing that these antibodies can be seen in various inflammatory diseases, especially atopic dermatitis, alopecia areata, asthma, various eye diseases, and cancers [6–11]. Studies have shown that anti-DFS70 antibodies are found to be positive at varying rates in healthy individuals [12,13]. In addition, there are studies suggesting that anti-DFS70 positivity removes the diagnosis of some systemic inflammatory diseases [14,15]. This study is aimed to evaluate the relationship of the ANA IIF staining patterns with patient's symptoms, laboratory findings, and final diagnosis in anti-DFS70 antibody-positive patients. Also, anti-DFS70 antibody positivity and its correlation with DFS staining pattern were analyzed retrospectively in CTD patients.

MATERIAL AND METHOD

One hundred nineteen anti-DFS70 antibody-positive patients detected by IIF over the age of 18 who applied consecutively to the Kayseri City Education and Research Hospital, Rheumatology outpatient clinic between 1 January 2020 and 30 June 2020 were included in the study. After the staining patterns and titers of the ANA screening tests, if the patient was classified with rheumatologic disease, the patients' final diagnoses were noted. Rheumatological complaints such as the presence of joint pain, morning stiffness, low back pain and its inflammatory character, presence of oral and/or genital aphthae, photosensitivity, Raynaud phenomenon, sicca symptoms, livedoid eruptions, neuropathic complaints, miscarriages were noted. In addition, patients with leukopenia, lymphopenia, anemia, thrombocytopenia, and proteinuria, which may be associated with these rheumatological symptoms, along with erythrocyte sedimentation rate (ESR) and

C-reactive protein (CRP) values were also noted. Patients whose detailed history, physical examination, and laboratory findings could not be reached were excluded from the study. Patients with any autoantibody positivity in the ANA profile except anti-DFS70 autoantibody were also excluded from the study. Other exclusion criteria were the presence or history of solid or hematological malignancies, anti-neutrophil cytoplasmic antibody (ANCA) positivity, autoimmune diseases such as primary biliary cholangitis, Hashimoto thyroiditis, Graves' disease, multiple sclerosis, or other demyelinating diseases. The clinical and laboratory findings of people positive for anti-DFS70 antibodies were compared with the rates of those with DFS pattern ANA IFA staining. In addition, anti-DFS70 antibody positivity rates were analyzed retrospectively in 1016 CTD patients [RA, Sjögren's Syndrome (SjS), undifferentiated connective tissue disease (UDCTD), systemic lupus erythematosus (SLE), systemic sclerosis, idiopathic inflammatory myositis (IIM)] who were admitted to the relevant outpatient clinics between the same dates.

ANA screening test method: DFS and the other staining patterns were identified by IIF on HEp-20-10 / Liver (Monkey) cell substrates (EUROStar III plus (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). Fluorescein isothiocyanate (FITC) conjugated to anti-human IgG. With the manufacturer's recommendations, sera were diluted 1/100 for ANA. Reading and interpretation of IIF preparations were done by experienced microbiologists.

Anti-DFS70/LEDGF antibody (and other antibodies) detecting method in ANA profile: The EUROLINE ANA Profile 3 plus DFS70 (IgG) (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) test kit used for the detection of antibodies against DFS70 antigen offer in a single reaction and evaluation of the test results EUROLIneScan software was used. In the first reaction step, diluted patient samples were incubated with immunoblot strips. In the cases of positive samples, a second incubation was performed using an enzyme-labeled anti-human IgG (enzyme conjugate) that catalyzes a color reaction to detect specific IgG antibodies (also IgA and IgM) that bind to the respective antigenic site. For automated incubation, EUROBlotMaster compact tabletop device was used and the Euro01 AAK EL30 program was selected.

Statistical analysis

For frequencies, percentage rates and total percentage rates were used. The continuous variables of these Anti-DFS70 positive patients in the ANA IIF test were expressed as mean $\pm$ standard deviation. Continuous variables were evaluated in terms of normal distribution by the Kolmogorov-Smirnov test. These variables in a normal distribution were evaluated with the test of differences between two independent groups. The non-normal distribution of continuous variables was assessed with the Mann-Whitney U test. Differences between categorical variables were evaluated using Chi-Square and Fisher's Exact Chi-Square tests. Correlation between two categorical data was evaluated using Spearman's Test. The results between > 0.8 were defined as excellent, between 0.6 and 0.8 as good, between 0.4 and 0.6 as moderate, and < 0.4 as the bad correlation between two values. $p < 0.05$ was accepted as statistical significance. All statistical analyzes were performed using the SPSS 22.0 (SPSS Inc.) computer program.

Our study was conducted in accordance with the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects.

RESULTS

Data of 119 consecutive patients whose anti-DFS70 antibody test on IIF was positive applied between the specified dates were screened. Five patients' data could not be reached. These patients were also excluded from the analysis because of Hashimoto's thyroiditis in 3 patients, history of breast cancer in two patients, and borderline p-ANCA positivity in one patient. ANA staining patterns, symptoms, and laboratory parameters of the remaining 108 Anti-DFS70 positive patients were analyzed. One hundred and two (94.44%) patients were women and the mean age of the patients was 43.15 ± 12.30 . Forty-four (40.74%) patients had at least one comorbid disease, and among these, the highest rate of comorbidity was hypertension in 10 (9.26%) patients. The demographic characteristics, mean values of ESR, and CRP of the patients are summarized in Table 1.

The most common ANA staining pattern in anti-DFS70 positive patients was the DFS pattern with a rate of 72/108 (66.7%). One of three patients (36/108) showed a staining pattern other than DFS. The least observed pattern was the cytoplasmic granular pattern (1/108; 0.9%). Table 2 summarizes the ANA staining patterns and titers of the patients.

Table 1

Demographic findings of anti-DFS70 antibody positive patients

	Anti-DFS70 antibody positives (n=108)
Age, years, mean $\pm$ S.D.	43.15 $\pm$ 12.30
Female, no. (%)	102 (94.4)
CRP (mg/L), mean $\pm$ S.E.M.	5.38 $\pm$ 4.98
ESR (mm/h), mean $\pm$ S.E.M.	13.08 $\pm$ 12.0
Co-morbidities	No (%)
Hypertension	10 (9.26)
DM, Insulin resistance	8 (7.4)
Hyperlipidemia	8 (7.4)
Asthma	3 (2.78)
BAD, Depression	3 (2.78)
ASHD	2 (1.85)
COPD	1 (0.93)
Others	7 (6.48)
No comorbidity	64 (59.26)

Abbreviations: DFS: Dense fine speckled, S.D.: standard deviation, CRP: C-reactive protein, S.E.M.: Standard error of the mean, ESR: Erythrocyte sedimentation rate, DM: Diabetes mellitus, BAD: Bipolar affective disorder, ASHD: Atherosclerotic heart disease, COPD: Chronic obstructive pulmonary disease.

Table 2

Staining patterns and titers of anti-DFS70 antibody positives

Staining Pattern	No (%)	Titer				
		1/80, n	1/160, n	1/320, n	1/1000, n	1/10000, n
DFS	72 (66.67)	10	30	29	2	1
Speckled	24 (22.22)	6	7	7	1	3
Homogeneous	4 (3.7)	1	2	0	0	1
Nucleolar	3 (2.78)	1	1	0	0	1
Nuclear membrane	2 (1.85)	1	0	0	0	1
A Few Nuclear Dots	2 (1.85)	0	0	1	0	1
Cytoplasmic Granular	1 (0.93)	0	0	1	0	0

Abbreviations: DFS: Dense Fine Speckled

The most common rheumatological complaint was joint pain seen in 77 (71.30%) of the patients and inflammatory joint pain accompanied by more than 30 minutes of morning stiffness was present in almost a quarter of these patients (19/77; 24.68%). Of these 77 patients with joint pain, 45 had staining in the DFS pattern ($p = 0.006$). However, among 19 patients with inflammatory joint pain, the DFS staining pattern was present in 13 patients ($p = 0.858$). None of the patients had a history of genital aphtha and uveitis. No statistical significance was found for the association of any of the remaining rheumatological complaints with the DFS70 staining pattern. The most common laboratory abnormality was rheumatoid factor (RF) positivity observed in 10/108 (9.3%) patients, while the second was anti-cyclic citrulline peptide (anti-CCP) positivity observed in 9/108 (8.3%) patients. Serology of none of these RF positive patients did not indicate hepatitis B and hepatitis C disease. ANA staining pattern in IIF was in the form of DFS in 7 (70%) of the RF positives and 7 of the anti-CCP positives (77.78) (p values: 0.559 and 0.715, respectively). Three (2.8%) patients had leukopenia and all of them accompanied lymphopenia. One of these patients was diagnosed with SjS, one was diagnosed with UDCTD, and the last patient was not diagnosed with any rheumatologic disease. None of the 3 patients with thrombocytopenia was diagnosed with rheumatologic disease, only one was followed up with severe thrombocytopenia and a diagnosis of immune thrombocytopenic purpura. One of 5 patients with proteinuria had seronegative RA and one had immunoglobulin A nephropathy, and the remaining 3 patients were not diagnosed with rheumatologic disease. Antiphospholipid antibody tests were not positive in any of the four women

with multiple miscarriages. As seen in Table 3b, no statistical significance was found for any of the laboratory tests with the DFS pattern.

Considering the final classification of the patients, the most common diagnosis was RA, seen in 13 (12.04%) patients [eight (7.41%) was seropositive]. There was staining in the DFS pattern at a rate of 6/8 (75%) in seropositive patients and 8/13 (61.54%) in total RA patients (p : 0.757 and 0.716, respectively). Ten patients (9.26%) had UDCTD, 6 (5.56%) had SjS, 4 (3.70%) had ankylosing spondylitis, 3 (2.77%) had psoriatic arthritis, and 2 had familial Mediterranean fever (The p values for their relationship with the DFS pattern are 0.296; 0.655; 0.593; 0.708; and 0.551, respectively). The final diagnosis of the patients and their correlations between DFS70 patterns were detailed in Table 3c.

Data of 1016 patients diagnosed with CTD were analyzed. The mean age of these patients was 50.53 ± 13.97 and 815 (80.22%) of them were women. ANA analysis was performed in a total of 964 (94.88%) patients and 527 (54.67%) tests were found to be positive. The staining pattern of 41 patients was in the form of DFS (7.78% among positive ANA screening tests and 4.25% among all patients tested for ANA). Also, 46 patients had anti-DFS70 positivity in the ANA profile (8.73% among positive ANA test and 4.77% among all patients ANA tested). The most common anti-DFS70 positivity among patients with CTD was UDCTD with a rate of 14/147 (9.52%). While 12 of these patients had a positive DFS staining pattern, two had different patterns. On the other hand, of the 14 UDCTD patients with DFS patterns, 12 had anti-DFS70 antibodies, while one did not have any auto-antibodies and the other had anti-PM/SCL

antibodies [correlation coefficient (r) = 0.842 and p <0.001]. Looking at the analysis of RA, the most common CTD, ANA was tested in 547 (91.32%) of 599 RA patients and 132 (24.13%) of them were positive. DFS pattern was present in 17 (3.11%) of these RA patients. 13/17 (81.25%) patients had anti-DFS70 antibody, but two patients had anti-Ro/SS-A one had anti-RNP and one had no antibody. Among 16 patients who were anti-DFS70 positive, the ANA staining pattern was DFS in 13 patients (r = 0.782, p <0.001).

DFS pattern was seen in 9 (3.11%) of SjS patients. In eight of these patients (88.89%) had anti-DFS70 antibody and the remaining one had no antibody in the ANA panel. Additionally, 5 of these anti-DFS70 positives were also positive for anti-Ro / SS-A and the remaining 3 patients were only anti-DFS70 positive, but the diagnosis was made by minor salivary gland biopsy in addition to xerosis/xerophthalmia. Among 10 SjS patients who were anti-DFS70 positive, the ANA staining pattern

was DFS in 8 patients (r = 0.830, p <0.001). There were no patients with DFS patterns in 96 SLE patients, but 4 (4.17%) were positive for anti-DFS70. None of these patients had anti-DFS70 positivity alone; anti-dsDNA was additionally positive in all of them. One patient had both anti-SSa and anti-Sm positivity, in addition to anti-DFS70 and anti-dsDNA. In another patient, the antibody profile was very diverse; anti-DFS70, anti-dsDNA, anti-Sm, anti-histone, anti-Ro52, anti-La, anti-centromere. Considering all CTD patients, 34 (82.93%) of 41 patients with DFS patterns had anti-DFS70, while the remaining 7 patients had either non-DFS70 autoantibodies or no antibodies. On the other hand, 12 (26.01%) of 46 anti-DFS70 positive patients had non-DFS staining pattern (r = 0.773, p <0.001). Table 4 shows the rates of ANA, DFS pattern, and anti-DFS70 positivity in CTDs. In addition, the correlations of anti-DFS70 antibody with DFS staining pattern, specific to diseases and cumulatively, are also specified in Table 4.

Table 3

a: Clinical complaints, b: laboratory parameters, c: final diagnosis of the anti-DFS70 antibody positive patients and their correlations with DFS staining pattern

a)

Clinical Complaint	No (%)	DFS-Pattern, no (%)*	<i>p value</i>
Joint Pain	77 (71.3)	45 (58.44)	.006
<i>Inflam. character</i>	19 (17.6)	13 (68.42)	.858
Xeroftalmia	32 (29.63)	22 (68.75)	.766
Inflammatory back pain	31 (28.7)	21 (67.74)	.880
Xerosis	27 (25)	19 (70.37)	.637
Arthritis	18 (16.67)	13 (72.22)	.584
Oral Aphthae	12 (11.11)	10 (83.33)	.194
Photosensitivity	8 (7.41)	5 (62.5)	.795
Thromboembolism	7 (6.48)	6 (85.71)	.420
Heel Pain	7 (6.48)	4 (57.14)	.684
Proteinuria	5 (4.63)	4 (80)	.663
<i>Nephrotic Proteinuria</i>	1 (0.93)	1 (100)	.667
Pleuritic Chest Pain	5 (4.63)	5 (100)	.167
Hematuria	4 (3.7)	1 (25)	.107
Miscarriages	4 (3.7)	3 (75)	.593
Livedo Reticularis	4 (3.7)	3 (75)	.593
Abdominal pain attacks	3 (2.77)	3 (100)	.549
Neurological involvement	2 (1.85)	2 (100)	.458
Raynoud Phenomenon	2 (1.85)	0	.109
Dactylitis	2 (1.85)	1 (50)	.558
Fever	1 (0.93)	1 (100)	.667
Erythema Nodosum	1 (0.93)	1 (100)	.667

(Table 3 continued)

Genital Aphthae / Scar	0	0	<i>null</i>
Uveitis	0	0	<i>null</i>

b)

Laboratory Finding	No (%)	DFS-Pattern, no (%)*	<i>p value</i>
RF	10 (9.26)	7 (70)	.559
Anti-CCP	9 (8.33)	7 (77.78)	.715
Hypocomplementemia	7 (6.48)	6 (85.71)	.420
Proteinuria	5 (4.63)	4 (80)	.663
Neuropathy in ENMG	4 (3.70)	3 (75)	.593
Leukopenia	3 (2.77)	2 (66.67)	.708
Lymphopenia	3 (2.77)	2 (66.67)	.708
Thrombocytopenia	3 (2.77)	1 (33.33)	.257
Elevated CRP	29 (26.85)	19 (65.52)	.878
Elevated ESR	24 (22.22)	13 (54.17)	.141

c)

Final Diagnosis	No (%)	DFS-Pattern, no (%)*	<i>p value</i>
RA	13 (12.04)	8 (61.54)	.757
Seropositive RA	8 (7.41)	6 (75)	.716
UDCTD	10 (9.26)	5 (50)	.296
SjS	6 (5.56)	4 (66.67)	.655
AS	4 (3.7)	3 (75)	.593
PsA	3 (2.77)	2 (66.67)	.708
FMF	2 (1.85)	2 (100)	.551
Sarkoidoz	1 (0.93)	0	.333
Takayasu	1 (0.93)	1 (100)	.667
Localised Scleroderma (Morphea)	1 (0.93)	0	.333
IgA nephropathy	1 (0.93)	1 (100)	.477

* Percentages and numbers represent proportions within clinical data or diagnosis in the corresponding row.

Abbreviations: DFS: Dense fine speckled, RF: Rheumatoid Factor, Anti-CCP: Anti-cyclic citrullinated peptide, ENMG: Electroneuromyography, RA: Rheumatoid arthritis, UDCTD: Undifferentiated connective tissue disease, SjS: Sjögren's Syndrome, AS: Ankylosing Spondylitis, PsA: Psoriatic arthritis, FMF: Familial Mediterranean fever, IgA: Immunoglobulin A

Table 4

Anti-DFS70 antibody positivity and DFS pattern staining rate and the correlation of DFS pattern and anti-DFS70 antibody positivity rate in CTD patients

	Gender (Female), n, %	Age (mean $\pm$ S.D.), years	ANA Positivity, n, %	DFS Pattern in IIF*, n, %	Anti-DFS70 Positivity**, n, %	Spearman's Rank Correlation Coefficient (r)**	<i>p value</i>
RA (n:599) *	435 (72.62)	53.07 $\pm$ 14.08	132 (24.13)*	17 (3.11)	16 (2.93)	.782	< .001
UDCTD (n: 147)	139 (94.56)	48.13 $\pm$ 12.96	147 (100)	14 (9.52)	14 (9.52)	.842	< .001

(Table 4 continued)

SjS (n: 122)	113 (92.62)	47.82 ± 12.61	103 (84.42)	9 (7.38)	10 (8.20)	.830	< .001
SLE (n:96)	81 (84.38)	41.95 ± 12.95	95 (98.96)	0	4 (4.17)	<i>null</i>	<i>null</i>
SSc (n:49)	44 (89.80)	49.78 ± 11.56	47 (95.92)	1 (2.04)	2 (4.17)	.700	< .001
IIM (n:3)	3 (100)	56.67 ± 18.87	3 (100)	0	0	<i>null</i>	<i>null</i>
Total (n:1016)	815 (80.22)	50.53 ± 13.97	527 (54.67)	41 (4.25)	46 (4.77)	.773	< .001

* ANA tested in 547 /599 (91.32%) RA patients and 964/1016 (94.88%) in total

** The ratio is given among all patients undergoing ANA testing.

Abbreviations: DFS: Dense fine speckled, S.D.: Standard deviation, ANA: Anti nuclear antibody, IIF: Indirect immunofluorescent, RA: Rheumatoid arthritis, UDCTD: Undifferentiated connective tissue disease, SjS: Sjögren's Syndrome, SLE: Systemic lupus erythematosus, SSc: Systemic sclerosis, IIM: Idiopathic inflammatory myocytes.

DISCUSSION

The most important finding of our study was that the most common ANA staining pattern in patients with anti-DFS70 autoantibody positivity was dense fine speckled (granular) staining (72/108; 66.7%) and this staining pattern was not associated with any rheumatologic complaint, laboratory finding or disease classification. The staining pattern in the remaining patients was large speckled, fine speckled, homogeneous, nucleolar, few nuclear dots, nuclear membrane, and cytoplasmic granular patterns in order of frequency. Another considerable finding is that, although our study population was selected from patients in the rheumatology outpatient clinic, the rate of CTDs in patients with anti-DFS70 positivity was found to be as low as one-quarter. Furthermore, considering the patients with CTD, a low positivity rate of <5% of the DFS staining pattern and anti-DFS70 autoantibody was observed. Correlations of the anti-DFS70 antibody with the DFS staining pattern were found to have a good correlation cumulatively.

CTDs can be classified as RA, SjS, systemic sclerosis, SLE, IIM, antiphospholipid antibody syndrome (aPAS), mixed connective tissue disease (MCTD), and UDCTD [16]. These diseases can be found individually or may occur in the form of overlap or secondary to the other (such as SjS secondary to RA or aPAS secondary to SLE). ANA test is an important test used in the detection of these CTDs. ANAs can also occur in healthy subjects, especially at low dilutions, and the staining pattern under the IIF microscope can provide important information.

Depending on where the autoantigens are located in the cell, it has been described that staining patterns in IIF can be associated with a variety of diseases, for example, myositis autoantigens are most commonly found in the cytoplasm, while anti-dsDNA autoantibodies are often seen in homogeneous staining patterns [17]. In recent years, several studies have shown that a new auto-antibody called anti-DFS70 has been detected in healthy subjects or patients with various clinical conditions other than CTD. The DFS pattern, also available in different namings such as granular-chromosomal pattern, is characterized by the irregularly distributed, fine speckled (granular) fluorescence of interphase of nuclei and metaphase chromatin (chromosomal). Although the DFS staining pattern partially eliminates the suspicion of disease as CTD, it does not always correspond to the anti-DF70 antibody. Also, another important challenge is to separate the DFS pattern from both the homogeneous and speckled pattern [3]. It should be noted that DFS pattern and anti-DF70 autoantibodies may rarely be seen in SLE, SjS, and systemic sclerosis. Due to the fact that it requires experience to differentiate it from other speckled and homogeneous forms, and because it is rarely reported in diseases that may threaten life and organs, such as SLE, it is required to be reported by competent persons.

Although anti-DF70 antibodies are frequently reported in routine clinical practice today, they were first defined in interstitial cystitis patients in 1994 [5]. Subsequently, it was observed that these anti-DF70 autoantibodies were highly concentrated in atopic dermatitis

patients, and because these autoantibodies were autoreactive against a 70 kD protein in the Western immunoblot, they were named anti-DFS70 [8]. Potential protective effects of lens epithelium-derived growth factor (LEDGF) antigen, one of the first names of DFS antigen, have been revealed. Therefore, it has been shown that anti-DFS70 (also known as anti-LEDGF) antibodies against LEDGF can be observed more frequently in patients with Vogt-Koyanagi-Harada syndrome [11]. However, another finding of the same study is that the prevalence of anti-DFS70 autoantibodies in patients with Behçet's Syndrome and sarcoidosis with ocular involvement was almost the same as in healthy controls [11]. The high rate of anti-DFS70 antibody observed in prostate cancer in some studies could not be confirmed in others (6,7). In addition, anti-DFS70 antibody positivity was not observed in any of 20 breast cancer and 20 colon cancer patients in a study in which the anti-DFS70 antibody ratio was compared to healthy individuals with patients with systemic autoimmune rheumatic diseases [18].

The range of anti-DFS70 positivity among patients with CTD varies between 0–28.6%, depending on the methods of detection of ANAs, the ethnicity of the patients, the name, and the choice of classification criteria of the disease. According to our findings, the most common anti-DFS70 positive disease among CTD patients was UDCTD with a rate of close to 10%. Patients who do not meet the criteria for classifying a particular disease as symptoms and laboratory findings, although suggestive of a CTD, are often considered UCTD. Arthritis/arthralgia with Raynaud's phenomenon, nonspecific rash, or dry mouth and eyes are common features in these patients. There are no clear documented criteria for classifying these patients; however, although these patients often have positive ANAs, they do not exhibit a specific autoantibody profile [19]. Infantino *et al.* found that the anti-DFS70 antibody was positive in 14 (13.2%) of 91 patients with long-term UDCTD [20]. In these UDCTD patients, anti-ENA and anti-DNA autoantibodies were observed equally in patients with and without anti-DFS70 antibodies. The clinical features of both groups were similar. Anti-DFS70 antibodies were observed more frequently in young patients. In conclusion, the authors considered that these autoantibodies have a potential role as a marker in the evolution of

UDCTD to CTD, due to the high prevalence of anti-DFS70 antibodies in UDCTD patients.

In a recent study conducted in Spain, the anti-DFS70 positivity rate in RA patients was 1.66% when all patients were taken into account, while the positivity rate was 12.9% among only ANA-positive patients [21]. In another study from Japan, the anti-DFS70 positivity rate was found to be 18.1% using Enzyme-linked immunosorbent assay and IIF methods in RA patients [22]. In the same study, the anti-DFS70 positivity rate in other CTDs and healthy people were also analyzed and the lowest positivity rate was observed in DM-PM patients (3.0%). In addition, as a result, the researchers reported that the anti-DFS70 positivity rate of healthy individuals and CTD patients was similar. In our study, the data related to RA were partially similar to the data of Spanish researchers mentioned above; while the rate among ANA positives was 16/132 (12.12%), the rate in all RA patients was 16/547 (2.93%).

According to the data of an article published years ago by Muro *et al.*, anti-DFS70 positivity was found in 8 (11.27%) of 71 SjS patients [23]. According to a study in which Watanabe *et al.* studied in the Japanese race, anti-DFS70 antibody was found positive in only 2 (6.7%) of 30 SjS patients (3 males and 27 females), and both of these patients were female [24]. The 7.38% anti-DFS70 autoantibody positivity observed in patients with SjS in our study seems to be consistent with these data. In a multicentric study by Choi *et al.*, anti-DFS70 positivity was found in 81 (7.1%) of 1137 patients classified as SLE according to the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria. However, among them, the proportion of only anti-DFS70 positive (monospecific anti-DFS70) patients was 13 (1.1%) [25]. In our study, there were no monospecific-positive patients in SLE patients and anti-DFS70 positivity was slightly lower than the Choi *et al.*'s (4.17% vs 7.12%). Furthermore, we think that there was no staining in the DFS pattern in the SLE patients in our study because none of our patients were monospecific-positive. We think that the reason for this issue is that the physician evaluating the IIF reports only that antibody if there is another dominant antibody other than anti-DFS70.

In a study aimed at determining the need to confirm the anti-DFS70 antibody when the DFS pattern is observed in IIF, Kang *et al.* analyzed the

frequency of patients of DFS pattern mistaken for non-DFS / non-DFS pattern mistaken for DFS and found that 68.1% of patients in the DFS pattern had only anti-DFS70 antibodies without CTD-associated autoantibodies [26]. In addition, 20.3% of patients had false positivity in IIF and the remaining more than 10% had CTD-associated autoantibodies independent of anti-DFS70 in this study in which 182 patients in DFS patterns were analyzed. In our study, 34 (82.93%) of 41 patients with DFS patterns had anti-DFS70 antibodies, regardless of the presence of an additional antibody other than anti-DFS70. Monospecific anti-DFS-70 antibody was present in 27/41 (65.85%) patients. On the other hand, 3 patients (7.32%) showed false positivity for DFS pattern IIF (one was classified as RA, one as UDCTD, one patient as SjS).

The strength of the present study was that patients with positive anti-DFS70 autoantibody may have staining patterns other than the DFS pattern, and the DFS staining pattern is not related to any rheumatological symptom, laboratory finding, and final rheumatologic diagnosis. Another strength is the evaluation of the DFS staining pattern in a high number of CTD patients and whether this is associated with anti-DFS70 autoantibody positivity. In addition, anti-DFS70 antibody positivity rates have been shown in various CTD patients with a high number of patients. One of the limitations of our study may be the number of physicians evaluating ANA staining patterns in more than one. Although there are expert microbiologists,

it will be more appropriate to make this subjective assessment by a single person. Another limitation of our study is that the anti-DFS70 autoantibody of the patients was not compared with healthy individuals. Due to the retrospective design of this study, there were not enough healthy control cases tested for anti-DFS70 at the relevant time interval. On the other side, the fact that ANA was not measured in all RA patients can be considered a limitation. Since it is not known whether these patients have additional CTDs or not, it will not be understood whether their accurate diagnosis is RA alone or not. Larger and prospective studies are needed to support these retrospective data and to identify whether anti-DFS70 antibodies are predictive or protective for CTD.

CONCLUSION

For years, anti-DFS70 antibodies have been thought to be clinically protective against some rheumatological diseases. Although the antibody profile of a patient with the DFS staining pattern in the IIF ANA screening test overlaps with the anti-DFS70, it may have different staining patterns. Even in rheumatology clinic patients who are positive for this antibody and consist of a selected patient group, the rate of CTD is low. Furthermore, a very low positivity rate of anti-DFS70 autoantibody was observed in patients with CTD. In the light of these data, we think that the anti-DFS70 antibody does not predict CTD or even excludes CTD.

Introducere. Aspectul DFS este heterogen, foarte intens și apare în nucleoplasma interfazică a celulelor Hep-2 și zonele cromozomiale ale metafazei. Scopul studiului a fost de a evalua asocierea dintre anticorpii anti-DFS70 și semnele, simptomele și diagnosticul reumatologic.

Metode. 180 de pacienți anti-DFS70 pozitiv internați consecutivi în perioada ianuarie–iunie 2020 au fost incluși în studiu. Aspectele clinice și paraclinice au fost evaluate. Asocierea dintre aspectul în imunofluorescență și pozitivitatea anti DFS-70 a fost evaluată retrospectiv pe 1 016 pacienți cu colagenoze.

Rezultate. Durerea articulară a fost cel mai frecvent simptom, întâlnit la 77 de pacienți (71.3%). Pacienții pozitivi pentru anticorpi anti-DFS-70 au avut în proporție de 9,3% și factor reumatoid. Aspectul în imunofluorescență al anticorpilor antinucleari a fost DFS (72/108 66,7%). Nu s-a găsit o asociere statistică între acuzele reumatologice și modificările paraclinice. Analiza factorului antinuclear a fost realizată la 964/1,016 pacienți cu CTD (colagenoze). 44 au fost pozitivi pentru anticorpii anti-DFS70. S-a observat o corelație puternică între aspectul DFS și prezența anticorpilor anti-DFS-70 ($r=+0,773$, $p<0,001$).

Concluzii. Prevalența anticorpilor anti-DFS-70 este mică la pacienții cu CTD: Așadar anticorpul anti-DFS-70 nu prezic diagnosticul de colagenoză, dar nici nu îl exclude.

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Funding: No specific funding was received from any funding bodies in the public, commercial or not-for-profit sectors.

Conflict of interest disclosure: There is no conflict of interest declared by the authors.

Acknowledgments: None.

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Received 26th June 2021