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SHORT COMMUNICATION

Evaluation of Samples with DFS Staining Pattern Detected by Indirect Immunofluorescence Assay

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SUMMARY

Background: **In this study, we aimed to determine the presence of anti DFS70 antibody by a specific IB method in samples showing the DFS pattern and to determine the distribution of DFS pattern in different patient groups.**
Methods: **2,401 serum samples, which were received for ANA screening, were tested by IIF method at Akdeniz University Hospital Diagnostic Laboratory. Out of 139 samples with DFS pattern, 75 samples were tested for the presence of anti DFS70 antibody by IB and were included in the study. Patients' clinical diagnoses were obtained retrospectively from medical records.**
Results: **63 (84%) of 75 samples, which showed DFS patern by IIF, were found to have anti DFS70 antibody by IB. Five of these patients were diagnosed with SARD while the rest (58) had diseases other than SARD.**
Conclusions: **DFS pattern detected by IIF and isolated anti DFS70 antibody positivity detected by IB show high concordance. However IIF results should be confirmed because of the patterns that can be misidentified as DFS pattern. The presence of anti-DFS70 antibodies, which help to exclude SARD, prevent further unnecessary referral demands.**
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KEY WORDS

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INTRODUCTION

Autoantibodies that are detected in systemic autoimmune rheumatic diseases (SARD) are mostly directed against nuclear antigens and are named as anti nuclear antibodies (ANAs). Diseases with the existence of ANAs lie in a wide spectrum of diseases and are referred as ANA-associated rheumatic diseases (AARD) [1]. The indirect immunofluorescent antibody (IIF) method in which Hep-2 cells are used for ANA screening is the gold standard method [2]. One of the most frequently seen patterns in screening by IIF is dense fine speckled (DFS) staining pattern. In this pattern, staining appears in the metaphase and interphase cell nuclei, in which the size and brightness of the granules are typically different from each other [3].

The DFS pattern was first associated with interstitial cystitis in 1994 by Ochs et al. [4] and later an association with different diseases was found [5]. It was named DFS70 because it showed reactivation against a 70 kD protein by Western blot. Sequence analysis of DFS70 antigen revealed that it was similar to Lens Epithelium Derived Growth Factor (LEDGF) [6]. Because of high false positive rates of DFS pattern, it was suggested that the IIF method was not sufficient and it should be confirmed by methods such as CIA, DIA/LIA, and ELISA [7].

Negative correlations between isolated anti-DFS70 antibodies and AARD were reported previously [8,9]. Conrad et al. [7] found that detection of isolated anti-DFS70 antibodies reduced the need for further testing for AARD significantly and no healthy individuals with positive anti-DFS70 antibodies developed AARD within four years. Isolated anti-DFS70 antibodies were found in less than 1% of patients with AARDs. They were most frequently (66.7%) seen in Vogt-Harada syndrome and less frequently in non-systemic autoimmune diseases such as atopic dermatitis, alopesia areata, interstitial cystitis, asthma, chronic inflammatory diseases, and in cancers. They were shown to be found at variable rates between 4 - 33% in healthy individuals [4,5,10, 11].

In the present study, we aimed to determine the presence of anti-DFS70 antibodies by a specific IB method in samples showing DFS70 staining pattern which were detected by IIF method and to determine the distribution of DFS pattern in different patient groups.

MATERIALS AND METHODS

2,401 serum samples submitted for ANA screening from different clinics between Aug-2016 and Nov-2016 were investigated using IIF method in which Hep 2010 cells were used as substrate (Euroimmun Lübeck, Germany) at Akdeniz University Hospital Diagnostic Laboratory. Screening dilution was 1:100. DFS staining pattern is defined as dense, fine speckled staining of nucleoplasms of Hep-2 cells and chromosome plate of metaphase cells showing heterogeneity in size, brightness, and density of speckles.

Among 139 patients with DFS pattern, 75 who were tested for the presence with anti DFS70 antibody (Euroline ANA Profile 3 plus DFS70, Euroimmun Lübeck, Germany) by IB method were included in the study. IB method was performed according to the manufacturer's instructions. Antibodies to nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, and AMA M2 were determined in addition to anti-DFS70 antibodies. Clinical diagnoses of patients were analysed retrospectively by clinical chart review of medical records.

RESULTS

1,853 (77.2%) of 2,401 samples which were screened for ANA by IIF method had negative results for ANA, whereas 548 (22.8%) were found positive. 139 (25.4%) of the positive samples presented the DFS pattern and 75 (13.7%) of these patients who were tested for the presence of anti DFS70 antibody by IB method were evaluated. 63 (84%) patients had anti-DFS70 antibody, and anti-DFS70 antibody was the only antibody present in 54 (72%) of these patients.

66 (88%) of the patients were female while 9 (12%) were male. Patients' DFS70 results and distribution according to clinical diagnosis were shown in Table 1.

DISCUSSION

The DFS pattern is a commonly observed pattern in routine IIF-ANA screening. In this pattern, speckled staining occurs in the metaphase chromatin plate and interphase cell nuclei and, characteristically, the size and brightness of the speckles are heterogenous (Figure 1) [6]. The DFS pattern can be mistaken for other patterns such as the "homogenous + speckled combination pattern" and the "nuclear quasi-homogenous pattern" which was defined by Mariz et al. [12], as fine, grainy staining of the interphase nucleus and metaphase chromatins. False positive results can be obtained in the recognition of DFS pattern even with an experienced eye. It was reported that the quasi-homogeneous pattern and the homogeneous + speckled combination pattern were associated with SARD more frequently, whereas the DFS pattern tended to occur in healthy individuals [4,5, 10,11]. For this reason, DFS70 antibody positivity should be confirmed by a more specific method such as ELISA and IB in cases in which the DFS pattern is observed.

DFS70 antigen is thought to be a protein located in the cell nucleus of all organs and tissues and is over-expressed or altered during inflammation thereby stimulating the autoantibody response [13,14]. It was shown that anti DFS70 antibodies were common in healthy people and in patients with localized rheumatic diseases and there was a negative correlation between SARD and presence of isolated anti DFS70 antibodies [6,8,9,15]. In our study, 58 (77%) non-SARD patients with anti-DFS70 antibody had localized rheumatic symptoms and diseases such as arthritis, arthralgia, reynaud phenomenon, localized scleroderma or allergic diseases such as allergic rhinitis and asthma. Anti-DFS70 antibody rates ranged between 0 - 5.7% and isolated anti-DFS70 antibody positivity rates ranged from 0 - 1.8% in SLE patients in six different studies [5,8,9,16-18].

Only four (5%) of our patients with SARD had isolated anti-DFS70 antibody. Clinical diagnoses of our patients were SLE, Sjogren’s syndrome, rheumatoid arthritis and Behçet's disease. Muro et al. [8] found that anti DFS70 antibody positivity rate was 5.7% in 124 SLE

Table 1. DFS70 antibody results and diagnosis of the patients.

	Anti DFS70							Total
		Positive n (%)		Intermediate value n (%)		Negative n (%)		n (%)
			Isolated Anti DFS70					
SARD	SLE	1 (1)	1 (1)	RA	3 (4)	SLE	1 (1)	
	RA	1 (1)	1 (1)					
	SS	2 (3)	1 (1)					
	Behçet Diseases	1 (1)	1 (1)					
	Total	5 (7)	4 (5)		3 (4)		1 (1)	9 (12)
SARD off	Arthritis/ arthralgia	33 (44)	29 (39)	Arthritis/ arthralgia	1 (1)	Arthritis/ arthralgia	2 (3)	
	Allergic Diseases	7 (9)	6 (8)	Other	1 (1)	Allergic Diseases	2 (3)	
	Localised Scl	3 (4)	2 (3)			Other	2 (3)	
	Other	15 (20)	13 (17)					
	Total	58 (77)	50 (67)		2 (3)		6 (8)	66 (88)
Total		63 (84)	54 (72)		5 (7)		7 (9)	75 (100)

SLE - Systemic lupus erythematosis, RA - Rheumatoid arthritis, SS - Sjogren syndrome, SARD - Systemic autoimmune rheumatic diseases.

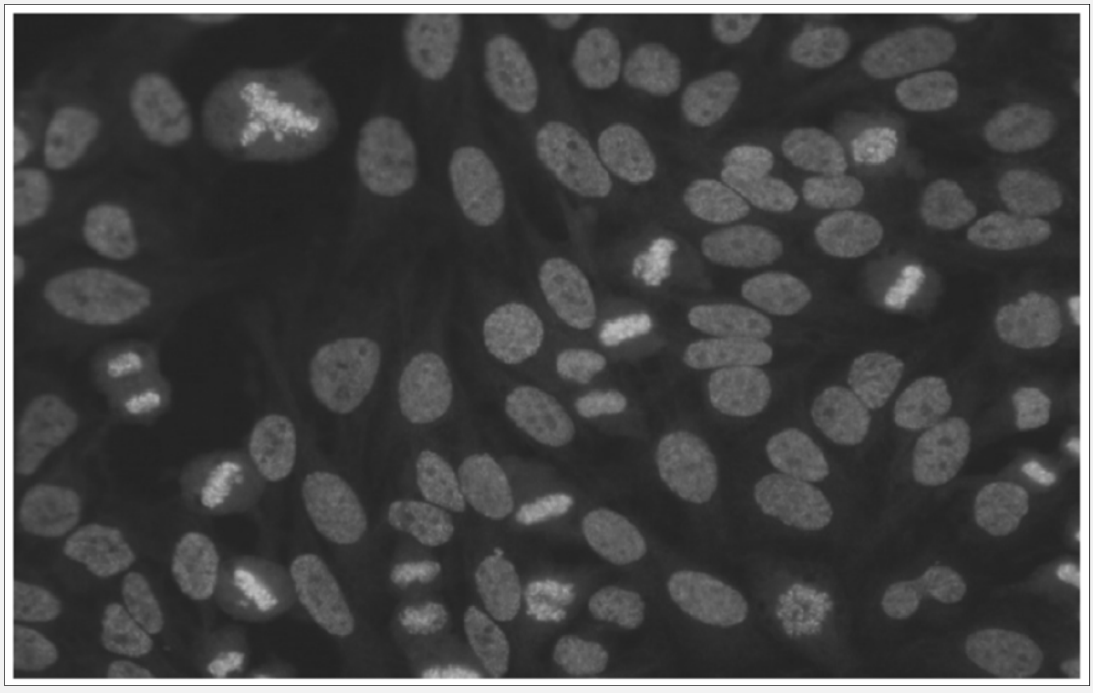

Figure 1. Fluorescent microscope appearance of the DSF70 pattern detected by IIF method.

patients but none of the patients had isolated anti DFS70 antibody. Additionally, they did not detect anti-DFS70 antibody in thirteen RA patients. In the light of these findings, we suggest that isolated anti-DFS70 antibody positivity can be used to exclude SARD especially in patients where SLE is not suspected.

In our study, we determined anti-DFS70 antibodies by IB method in 63 (84%) of 75 patients who had DFS pattern. In our previous study, Mutlu et al. [19] found anti-DFS70 antibody positivity in 62 (83.8%) of 74 DFS pattern positive patients by ELISA. Şener et al. [20] detected DFS pattern in 16 of 1,302 patients, but none of them were found to have anti-DFS70 antibody by LIA. Anti-DFS70 antibody positivity rates differ in samples with DFS pattern in different studies. This difference was thought to be because of the errors in recognition of the pattern and antigenic differences in the kits used. In two studies performed in our laboratory, samples were evaluated by two independent observers and our results were similar. In our line-blot assay, we used a recombinant protein (*E. coli*) of 1 - 530 amino acids in length as antigen. This protein contains an epitope, that is located in the C-terminal sequence (between amino acids 347 - 429) and involves an immunodominant epitope for DFS70 [14,21]. The results obtained with different immunoblot methods involving the truncated state of the recombinant protein or the full-length DFS70 antigenic sequence showed no difference in diagnostic accuracy [22]. In our study, anti-DFS70 antibody was not detected in 13 (17.3%) cases with DFS pattern by line-blot method. This was similar to the results of our previous study (16.2%) in which two different methods such as ELISA and line blot were used. On the other hand, Basu et al. [23] showed another 75 kDa DFS protein, which had the same reactivity as DFS70, was against the methyl CpG binding protein 2 (MeCP2) and was localized with DFS70 in the nucleus. For this reason, we thought that different antibodies might be responsible from the similar staining in thirteen patients with DFS pattern and without anti-DFS70 antibody.

In our study, it is determined that 5 (7%) patients had intermediate value and 7 (9%) patients had negative result for anti-DFS70 antibody. Two of the intermediate patients had no other positivity, whereas histone, dsDNA, Sm, nRNP, SS-A antibodies were also intermediate in three of them. One of the samples with an intermediate result was weakly positive at 1/100 while the remaining four samples were positive at 1/100. We can suggest that positivities at low titre by IIF can yield uncertain results and also by IB.

CONCLUSION

DFS pattern by IIF method and isolated anti-DFS70 positivity by IB method is highly parallel, but it would be useful to confirm the IIF result due to the different patterns such as "quasi-homogeneous" and “homogeneous + speckled combination” pattern that can lead to misidentification. Isolated anti-DFS70 antibody positivity will exclude SARD and avoid unnecessary additional tests. Diagnostic confirmatory tests including DFS70 antibody positivity should be implemented in routine use.

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No support was received for this project.

Declaration of Interest:
The authors declare no conflicts of interest or funding to disclose.

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