Rheumatology International (2019) 39:1423–1429
https://doi.org/10.1007/s00296-019-04299-4

Rheumatology
INTERNATIONAL

BIOMARKERS

Clinical use of anti-DFS70 autoantibodies

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Received: 24 February 2019 / Accepted: 2 April 2019 / Published online: 10 April 2019

Abstract

The dense fine speckled (DFS) nuclear pattern is one of the most common indirect immunofluorescence (IIF) patterns detected during routine anti-nuclear antibody (ANA) screening. There is a negative association between anti-DFS70 status and systemic autoimmune rheumatic disease (SARD), especially in the absence of concomitant SARD-specific autoantibodies. The purpose of this study was to determine the need for confirming anti-DFS70 status when a DFS pattern is observed in IIF-ANA. The frequency of anti-DFS70 detection on Western blot and the positive rate of connective tissue disease (CTD)-related autoantibody screening with a fluorescence-based enzyme immunoassay was evaluated in DFS ($n = 182$) and non-DFS ($n = 359$) groups. Specific autoantibodies against 15 autoantigens were identified by line immunoassay. We evaluated the frequency of cases of DFS mistaken for non-DFS and non-DFS cases mistaken for DFS, as well as the clinical impacts of these misinterpretations. Among cases of IIF-ANA with an observable DFS pattern, 68.1% had only anti-DFS70 without CTD-related autoantibodies, 20.3% were false positive for IIF-ANA, and the remaining 11.5% had CTD-related autoantibodies independent of anti-DFS70 status. These results indicated that CTD-related autoantibodies may be present with or without anti-DFS70 even if a DFS pattern is observed in IIF-ANA. Among patients who are ANA negative or have a low probability of SARD, an anti-DFS70 confirmation test has no clinical benefit and cannot replace specific tests for detecting CTD-related autoantibodies. Specific tests to detect CTD-related autoantibodies should be performed instead of anti-DFS70 confirmation tests when a DFS pattern is observed in IIF-ANA.

Keywords Dense fine speckled 70 protein · Indirect immunofluorescence · Anti-nuclear antibody

Introduction

The indirect immunofluorescence (IIF) assay using HEp-2 cell substrate is the most commonly used assay for evaluating anti-nuclear antibodies (ANA) status, and has been the gold standard screening assay for decades [1–3]. The dense fine speckled (DFS) nuclear pattern is one of the most commonly detected IIF patterns in routine ANA screening in clinical laboratories and was recently classified as the AC-02 competency-level recognition pattern by the International Consensus on ANA Pattern (ICAP) committee [1, 3–5]. The DFS pattern is defined by dense and heterogeneous fine speckles throughout the interphase nucleus and on the metaphase chromosomal plate [1, 4, 5].

The nuclear target antigen of autoantibodies producing the DFS pattern was initially termed DFS70 after a 70-kDa protein was detected by Western blot (WB). The primary autoantigen was eventually identified as lens epithelium-derived growth factor (LEDGF), also known as DNA-binding transcription coactivator p75, by analysis of protein sequence databases [1, 6–8]. The mechanism underlying the appearance and the clinical impact of anti-DFS70 is not yet clear. However, it is well known that the DFS pattern on IIF-ANA and/or anti-DFS70 is present at a higher frequency in apparently healthy individuals compared to patients with systemic autoimmune rheumatic

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diseases (SARD) [1, 9]. Conversely, there is a negative association between anti-DFS70 and SARD, especially in the absence of concomitant SARD-specific autoantibodies [6]. Therefore, many previous reports have recommended that the presence of anti-DFS70 be confirmed by a specific method for anti-DFS70 when the DFS pattern is observed in IIF-ANA [3, 10–12]. Several anti-DFS70 specific commercial assays are currently available, including immunoblotting (IB) [13], enzyme-linked immunosorbent assay, chemiluminescent assay (CIA) [9, 14], HEp-2 cell IIF using immunoadsorption of anti-DFS70, and HEp-2 cell IIF using a DFS70/LEDGF knock-out cell line [9, 15]. However, there is a wide range of variability between IIF-ANA suspicion and confirmation by specific anti-DFS70 assays [15].

Requests for IIF-ANA tests in clinical laboratories are becoming more frequent in various departments, including dermatology [6]. Therefore, the incidence of detecting the DFS pattern has increased compared to prior years when it was primarily ordered by rheumatologists. Confirmation for specificity of the DFS pattern detected on IIF-ANA screening tests has resulted in unnecessary follow-up testing, because most patients with a DFS pattern are unlikely to have SARD. Thus, the purpose of this study was to evaluate the need for additional confirmatory tests to identify the presence of anti-DFS70 in cases where the DFS pattern is observed on routine IIF-ANA screening in a university hospital laboratory. We evaluated the frequencies of anti-DFS70 by WB in both DFS samples and non-DFS sample with IIF patterns other than DFS. Cases of DFS mistaken for non-DFS and non-DFS mistaken for DFS were analyzed, with an emphasis on the clinical impacts of these misinterpretations.

Materials and methods

Patients

A total of 541 IIF-ANA positive sera samples were obtained from patients who underwent routinely requested ANA screening in the clinical laboratory of the Department of Laboratory Medicine, Gangdong KyungHee University Hospital, Seoul, South Korea, between June 2016 and September 2017. All IIF-ANA-positive sera were classified into either a 'non-DFS group' or 'DFS group' according to IIF-ANA patterns. The DFS group ($n = 182$) was defined by a DFS pattern on IIF-ANA, while the non-DFS group ($n = 359$) was defined by an IIF pattern other than DFS on IIF-ANA.

This study was performed according to the Declaration of Helsinki and approved by the Gangdong KyungHee University Hospital Ethics Committee (IRB No. KHNMC 2016-10-018).

IIF-ANA assay

The IIF-ANA screening test was performed using the PhD system (Bio-Rad Laboratories, Hercules, CA, USA) with Kallestad HEp-2 slides (Bio-Rad Laboratories, Redmond, WA, USA) according to the manufacturer's guidelines. Sera were considered positive for ANAs if IIF staining was observed at a serum dilution of 1:40 by the manufacturer's protocol. Reading and interpretation of IIF patterns were performed by two experts in the Department of Laboratory Medicine according to the ICAP standards (www.ANApatterns.org).

Western blot (WB) assay

WB was performed in the DFS group ($n = 182$) and the non-DFS group ($n = 359$) to confirm the presence of anti-DFS70. WB was performed using HeLa whole-cell lysates as previously described [16, 17]. WB positivity for anti-DFS70 was defined as obvious detection of a band in the 70–75 kDa region.

Connective tissue disease antibody (CTD Ab) screen using fluorescence enzyme immunoassay (FEIA)

The EliA CTD Screen assay (Thermo Fisher Scientific, Freiburg, Germany) is based on FEIA and is a sandwich immunoassay. We performed the EliA CTD Screen assay using the Phadia 100 system (Thermo Fisher Scientific) to identify CTD-related autoantibodies, especially in the DFS group. Each well of the EliA CTD Screen was coated with the following CTD-specific antigens: U1RNP (RNP70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70, Jo-1, fibrillarin, RNA Pol III, ribosomal P-protein, PM-Scl, PCNA, Mi-2, Sm, and native purified DNA. The cut-off ratios for positive, equivocal, and negative were > 1.0, 0.7–1.0, and < 0.7, respectively.

Identification of specific autoantibodies by line immunoassay (LIA)

Identification of specific autoantibodies was performed to identify the cause of IIF-ANA misinterpretation in 28 anti-DFS70 positive cases among the non-DFS group and 21 CTD Ab screen positive cases among the DFS group. LIA was performed using the Euroimmun EUROLINE ANA profile 3 (IgG) assay (Euroimmun AG, Lübeck, Germany), which detects autoantibodies against the following 15 autoantigens: nRNP, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP-B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-protein, and AMA

M2. Test strips coated with antigens were incubated with 1:101 diluted sera followed by addition of alkaline phosphatase-labeled anti-human IgG and substrate solutions for detection of bound autoantibodies. Using a flatbed scanner and EUROLineScan software (Euroimmun AG), semiquantitative results (negative, 1 +, 2 +, and + 3) were obtained automatically according to reaction intensity.

Statistical analysis

Categorical data are presented as counts and corresponding rates. Continuous data are presented as means with the standard deviations (SD) or median with the range as appropriate. Normality test was done using z score (− 1.96 to 1.96) from skewness and kurtosis statistics.

Results

Analysis of the non-DFS group with IIF-ANA positivity other than the DFS pattern

The clinical characteristics and requesting departments of the non-DFS group ($n = 359$) are summarized in Table 1. Participants had a mean age of 49.2 ± 19.1 (range 5–100) years and 76.0% of the participants were female. The most frequently detected IIF-ANA pattern in the non-DFS group was Homogenous (25.1%), followed by Fine Speckled (19.5%), Mixed pattern (13.9%), and Coarse Speckled (13.1%). In the non-DFS group, the overall positive rate of the CTD Ab screen was 43.7% (157/359), and the prevalence of anti-DFS70 positivity was 7.8% (28/359). Among the IIF-ANA patterns observed in the non-DFS group, the pattern in which anti-DFS70 was most frequently detected was the few nuclear dots (FND) pattern (8/10, 80.0%), followed by other (1/5, 20%), Cytoplasmic (3/32, 9.4%), Homogenous

Table 1 Analysis of the non-DFS group with indirect immunofluorescence-antinuclear antibody positivity other than a dense fine speckled pattern

	No. (%)	SARDs (%)	Anti-DFS70 (%)	CTD Ab screen+ (%)
Distribution of department				
Rheumatology	95 (26.5%)	55 (57.9%)	7 (7.4%)	48 (50.5%)
Internal medicine[a]	58 (16.1%)	10 (17.2%)	3 (5.2%)	27 (46.6%)
Other	206 (57.4%)	2 (1.0%)	18 (8.7%)	82 (39.8%)
Total	359 (100%)	67 (18.7%)	28 (7.8%)	157 (43.7%)
IIF-ANA pattern				
Mixed pattern[b]	50 (13.9%)	15 (30.0%)	3 (6.0%)	29 (58.0%)
H	90 (25.1%)	17 (18.9%)	7 (7.8%)	22 (24.4%)
FS	70 (19.5%)	14 (20.0%)	3 (4.3%)	36 (51.4%)
CS	47 (13.1%)	12 (25.5%)	3 (6.4%)	32 (68.1%)
Cytoplasmic[c]	32 (8.9%)	1 (3.1%)	3 (9.4%)	9 (28.1%)
Nucleolar	32 (8.9%)	4 (12.5%)	0	7 (21.9%)
Centromere	19 (5.3%)	2 (10.5%)	0	18 (94.7%)
FND	10 (2.8%)	0	8 (80.0%)	1 (10.0%)
MND	4 (1.1%)	2 (50.0%)	0	2 (50.0%)
Others	5 (1.4%)	0	1 (20.0%)	1 (20.0%)
Anti-DFS70/CTD Ab screen				
+/+	7 (1.9%)	3 (42.9%)		
+/−	21 (5.8%)	0		
−/+	150 (41.8%)	44 (29.3%)		
−/−	181 (50.4%)	20 (11.0%)		

DFS dense fine speckled, *SD* standard deviation, *SARDs* systemic autoimmune rheumatic diseases, *CTD* connective tissue diseases, *Ab* antibody, *H* homogenous, *FS* fine speckled, *CS* coarse speckled, *FND* few nuclear dots, *MND* multiple nuclear dots

[a]Department of Internal Medicine other than Rheumatology

[b]Most commonly observed pattern in mixed pattern was cytoplasmic ($n = 33$), followed by H ($n = 26$), centromere ($n = 13$), nucleolar ($n = 12$), FS ($n = 10$), CS ($n = 6$), MND ($n = 3$), and FND ($n = 1$). Mixed patterns including DFS pattern were excluded in this category

[c]Most commonly detected cytoplasmic pattern was cytoplasmic reticular ($n = 15$) followed by cytoplasmic fibrillar filamentous ($n = 8$) and cytoplasmic fibrillar linear ($n = 4$)

(7/90, 7.8%), Coarse Speckled (3/47, 6.4%), Mixed pattern (3/50, 6.0%), and Fine Speckled (3/70, 4.3%). The distributions of SARD patients and CTD Ab screening positive rates according to IIF-ANA pattern are summarized in Table 1.

The combined WB and CTD Ab screen test results were as follows: anti-DFS70−/CTD Ab+ 150 (41.8%), anti-DFS70−/CTD Ab− 181 (50.4%), anti-DFS70+/CTD Ab+7 (1.9%), and anti-DFS70+/CTD Ab− 21 (5.8%) (Table 1). In the 28 cases with positive anti-DFS70 despite a non-DFS interpretation, the most frequently observed IIF-ANA pattern was the FND pattern (9/28, 32.1%) followed by Homogenous (8/28, 28.6%), Coarse Speckled (4/28, 14.3%), Anti-mitochondrial antibody (4/28, 14.3%), and Fine Speckled (3/28, 10.7%) (Table 2). Detailed analysis of these 28 cases identified three SARD patients out of seven that were anti-DFS70+/CTD Ab+. The remaining four cases are summarized in Table 2. The most frequently observed IIF-ANA patterns in the seven anti-DFS70+/CTD Ab+ cases were Coarse Speckled ($n=3$) and Fine Speckled ($n=2$). Likewise, the most frequently identified CTD-related autoantibodies were against Ro52, followed by SS-A, RNP, dsDNA, and histone.

In the 21 cases classified as anti-DFS70+/CTD Ab−, the most frequently observed IIF-ANA pattern was FND ($n=8$), followed by Homogenous ($n=7$). In 9 out of these 21 cases, various CTD-related autoantibodies were identified by line immunoassay despite negative results on the CTD Ab screen. The specificities of the identified autoantibodies are summarized in Table 2. The remaining 12 out of 21 cases showed isolated anti-DFS70, which was compatible with WB and CTD Ab screen test results. No SARD patients were identified among the 21 anti-DFS70+/CTD Ab− cases, although nine of these cases were positive for CTD-related autoantibodies.

Analysis of the DFS group with the DFS pattern on IIF-ANA

The requesting departments of the DFS group ($n=182$) are summarized in Table 3. Participants had a median age of 31 (range 3–84) years and 73.6% of the participants were female. In the DFS group, the prevalence of anti-DFS70 was 75.3% (137/182) and the frequency of CTD Ab screen positivity was 11.5% (21/182). Among 137 cases with anti-DFS70, we found that the 'only anti-DFS70 without CTD-related autoantibodies' presented as anti-DFS70+/CTD Ab− in 68.1% of cases (124/182), while 'co-occurrence of

Table 2 Analysis of 28 anti-DFS70-positive cases among the non-DFS group

ANA pattern	No.	Identified autoantibodies	Diagnosis
Anti-DFS70+/CTD Ab screen+ ($n=7$)			
CS	1	DFS70+SS-A+Ro52+Sm+RNP	SLE
CS+H	1	DFS70+SS-A+Ro52+Histone	Sjogren's syndrome
CS	1	DFS70+RNP+PCNA	Overlap syndrome
FS	1	DFS70+SS-A+Ro52	Raynaud syndrome
FS	1	DFS70+Ro52	Ankylosing spondylitis
AMA	1	DFS70+SS-A+Ro52+dsDNA+Histone	Autoimmune hepatitis
FND	1	DFS70+dsDNA	Fibromyalgia
Anti-DFS70+/CTD Ab screen− ($n=21$)			
H	7	4 DFS70	3 Alopecia, 1 Raynaud syndrome
		1 DFS70+Histone	1 Alopecia
		1 DFS70+Ro52+SS-B	1 Alopecia
		1 DFS70+Scl70	1 Alopecia
FND	7	6 DFS70	5 Alopecia, 1 Dermatitis
		1 DFS70+Ro52	1 Toxic hepatitis
FND+AMA	1	1 DFS70+M2	1 Alopecia
FS	1	1 DFS70+Scl70	1 Ankylosing spondylitis
CS	1	1 DFS70+PM-Scl	1 Alopecia
CENP-F	1	1 DFS70	1 Lung cancer
AMA	1	1 DFS70+Histone	1 OA
AMA+MND	1	1 DFS70+M2	1 Alopecia
CFF	1	1 DFS70	1 OA

There were 28 cases with anti-DFS70: 9 FND (32.1%), 8 H (28.6%), 4 CS (14.3%), 4 AMA (14.3%), 3 FS (10.7%), and 3 other (10.7%)

RNP ribonucleoprotein, *SLE* systemic lupus erythematosus, *PCNA* proliferating cell nuclear antigen, *AMA* anti-mitochondrial antibody (cytoplasmic reticular), *CENP-F* centromere protein-F, *OA* osteoarthrosis, *CFF* cytoplasmic fibrillar filamentous

Table 3 Number of cases with anti-DFS70 and CTD Ab screen positivity according to clinical department distribution analysis of DFS group with dense fine speckled pattern on indirect immunofluorescence-anti-nuclear antibody in DFS group ($n = 182$)

Distribution of department	No. (%)	SARDs (%)	Anti-DFS70 (%)	CTD Ab screen positivity (%)
Department				
Rheumatology	42 (23.1%)	7 (16.7%)	30 (71.4%)	7 (16.7%)
Internal medicine[a]	9 (4.9%)	2 (22.2%)	4 (44.4%)	1 (11.1%)
Other	131 (72.0%)	0	103 (78.6%)	13 (9.9%)
Total	182 (100%)	9 (4.9%)	137 (75.3%)	21 (11.5%)

[a]Department of Internal Medicine other than Rheumatology

anti-DFS70 and CTD-related autoantibodies' presented as anti-DFS70+/CTD Ab+ in 7.2% of cases (13/182) (Table 4). The most commonly identified autoantibody in the 13 cases identified as anti-DFS70+/CTD Ab+ was against Ro52, followed by SS-A, SS-B, dsDNA, and histone. Unexpectedly, no SARD cases were identified in the 13 anti-DFS70+/CTD Ab+ cases despite the presence of CTD-related autoantibodies. The IIF-ANA titer results for the 137 cases with positive anti-DFS70 were as follows: 1:40 in 52 cases, 1:80 in 45, 1:160 in 21, 1:320 in 15, 1:640 in three, and 1:1280 in one case.

Finally, there were 45 cases without anti-DFS70 despite being interpreted as DFS pattern on IIF-ANA. Among these 45 cases, 37 had an anti-DFS70−/CTD Ab− result and were determined to be ANA negative. The IIF-ANA titer results for these 37 cases were as follows: 1:160 in three cases, 1:80 in nine, and 1:40 in 25 cases, with most patients having a titer < 1:160. The remaining eight cases had an anti-DFS70−/CTD Ab+ result and were determined to have only CTD-related autoantibodies. The most commonly identified autoantibody in the remaining eight cases was against histone, followed by autoantibodies against SS-A, dsDNA, and Ro-52. Among these eight cases, there were three cases of SARD.

Discussion

The presented study has demonstrated that, among the DFS group ($n = 182$) with an identifiable DFS pattern on IIF-ANA, 37 (20.3%) were actually ANA negative and 124 (68.1%) had isolated anti-DFS70 positivity. In other words, in cases of an identifiable DFS pattern in IIF-ANA, most DFS cases (88.4% = 20.3% + 68.1%) have only anti-DFS70

Table 4 Distribution of combined anti-DFS70/CTD Ab screen results in DFS group ($n = 182$)

Anti-DFS70/CTD Ab screen	No. (%)	SARDs (%)	Identified CTD-related autoantibodies (diagnosis)
+/+	13 (7.2%)	0	1 SS-A (Kikuchi disease)
			1 SS-A+Ro52 (AA)
			1 SS-A+Ro52+SS-B+M2+Jo1 (AS+TB)
			1 SS-A+SS-B+Scl (Moyamoya disease)
			1 Ro52+RNP+Histone (AT)
			4 Ro52 (AnA, Chronic cough, Low back pain, Wrist pain)
			1 dsDNA (ICH)
			1 dsDNA+Histone (OA)
			1 M2 (Urticaria)
			1 Negative (AU)
+/−	124 (68.1%)	4 (3.2%)	Not done
−/+	8 (4.4%)	3 (37.5%)	2 dsDNA+Histone (SLE, Dermatitis)
			2 SS-A+Ro52 (AnA, Urticaria)
			1 Histone+SS-A+SS-B (SPRA)
			1 Histone+SS-A+Ro52 (SLE)
			1 Histone (AnA)
			1 dsDNA (GB stone)
−/−	37 (20.3%)	2 (5.4%)	Not done

ICH intracranial hemorrhage, *AA* alopecia areata, *AS* ankylosing spondylitis, *TB* tuberculosis, *AU* alopecia universalis, *AT* alopecia totalis, *AnA* androgenic alopecia, *SPRA* seropositive rheumatoid arthritis, *GB* gall bladder

or no ANA, and these cases are much less likely to be SARD patients. In addition, despite being interpreted as a DFS pattern on IIF-ANA, 37 cases were identified as anti-DFS70−/CTD Ab−.

The reason for overestimating negative DFS patterns seems to be related to an increase in the false-positive rate due to low screening titer. According to the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR), one of the criteria for SLE is a positive ANA at a titer of 1:80 or greater [18]. However, we used a screening dilution of 1:40 (the manufacturer's recommendation) for IIF-ANA in this study, and it is well known that low positive titers of IIF-ANA are frequently nonspecific for CTD, with frequent false-positive results identified in healthy individuals. Consistently, the IIF-ANA titers for the DFS pattern in our 37 cases were low and only 3 out of 37 cases had a titer of 1:160, followed by 1:80 (nine cases) and 1:40 (25 cases).

With respect to the opposite cases, 21 (11.6%) in the DFS group were positive for CTD Ab screening, among which several CTD-related autoantibodies were identified. These results indicated that some CTD-related autoantibodies were missing despite being present in approximately 10% of the DFS group. Among these cases, 13 (7.2%) also had anti-DFS70, while the remaining eight (4.4%) were negative for anti-DFS70. There are several potential reasons why the IIF-ANA patterns corresponding to each CTD-related autoantibody were not observed in the 13 cases with anti-DFS70+/CTD Ab+, and also why they were interpreted as a DFS pattern despite having CTD-related autoantibodies. For example, the IIF-ANA patterns corresponding to CTD-related autoantibodies may have been missed, because those patterns were masked by the DFS pattern or their titer was below the limit of detection. In addition, interpretation of mixed pattern in IIF-ANA is difficult even for experts. The autoantibody most commonly identified in eight cases (4.4%) with anti-DFS70−/CTD Ab+ was directed against histone, which presents as a homogenous pattern on IIF-ANA and was, thus, highly likely to be misinterpreted as a DFS pattern. Thus, to demonstrate that patients with DFS pattern in IIF-ANA are unlikely to have SARDs, an anti-DFS70 confirmation test alone is insufficient, and demonstration of the absence of CTD-related autoantibodies is important [15].

In the non-DFS group ($n = 359$), there were 28 cases (7.8%) in which anti-DFS70 was detected. In these cases, it appears that the DFS pattern was masked by other patterns or misinterpreted as another pattern. Furthermore, 7 out of these 28 cases also had CTD-related autoantibodies and their IIF-ANA results were mainly Coarse or Fine Speckled patterns. We interpreted this result to mean that the DFS pattern is frequently obscured by the Speckled pattern, similar to how the Speckled pattern was masked by the DFS pattern in 13 cases with anti-DFS70+/CTD Ab+ in the DFS group, or that anti-DFS70 may have been expressed at too low of a level for detection by fluorescence. In addition, the remaining 21 of 28 cases were determined to be isolated anti-DFS70, although some autoantibodies were identified in nine cases and were largely interpreted as either a Homogenous or FND pattern. However, the Homogenous pattern is more difficult to distinguish from the DFS pattern than other IIF-ANA patterns, and has a high probability of being misinterpreted. In cases where the FND pattern was due to anti-p80 coilin, we assumed that anti-DFS70 was present even if the DFS pattern was not recognized in IIF-ANA, since anti-p80 coilin autoantibodies frequently coexist with anti-DFS70 [19].

Approximately 8% (28/359) of the non-DFS group were positive for anti-DFS70, but the DFS pattern may not have been detected, because it was hidden by other patterns or misinterpreted as another pattern. However, failure to identify anti-DFS70 does not result in clinically serious consequences in non-DFS patients. Specifically, in the traditional diagnostic approach, a very sensitive test like IIF-ANA is used initially followed by a highly specific test in positive cases to identify true positive cases, which will identify CTD-related autoantibodies. Thus, the addition of a process to identify anti-DFS70 in the traditional diagnostic approach is unlikely to be clinically relevant, not only in cases of DFS but in non-DFS cases, as well.

The main limitation of this study was due to the current limitations associated with the IIF-ANA assay. Specifically, interpretations of IIF-ANA assays are subjective and depend on the observer's experience, and thus, there may be inter-laboratory variability. According to the results from an international Internet-bases survey, the unmixed DFS pattern is recognized with significantly lower accuracy (~50%; $p < 0.05$) [3]. In addition, different IIF-ANA results may be observed depending on the source of Hep-2 cell substrate. For example, while we observed that only 11.6% of the cases of an observed DFS pattern in this study actually had CTD-related autoantibodies, different results may have been obtained in situations where the DFS pattern was either over- or underestimated due to inter-observer variability. Another limitation of this study was the lack of a specific test for CTD-related autoantibodies when the CTD Ab screen was negative with the exception of the 21 cases that were anti-DFS70+/CTD Ab− in the non-DFS group. Surprisingly, the frequency of anti-DFS70−/CTD Ab− was very high in the non-DFS group, which we initially expected to have high levels of CTD-related autoantibodies. However, the purpose of this study was not to evaluate the diagnostic approach for IIF-ANA patterns other than DFS, but rather to evaluate whether confirmation of anti-DFS70 in a useful diagnostic approach in cases where the DFS pattern is observed on routine IIF-ANA. Therefore, we excluded a discussion about the

high frequency of anti-DFS70−/CTD Ab− in the non-DFS group. Finally, since the IIF-ANA screen was performed at a 1:40 dilution, detection of a high number of false positives was not unreasonable.

Clinical departments that request ANA screening tests are not limited to Rheumatology. Indeed, requests for ANA testing by various departments are increasing, especially in dermatology. As a result, the DFS pattern is being observed more frequently, but at a cost of a high number of false-positive results. In this study, among patients with a DFS pattern in IIF-ANA, 68.1% had only anti-DFS70 without CTD-related autoantibodies, 20.3% were false positive for IIF-ANA, and the remaining 11.5% had CTD-related autoantibodies independent of anti-DFS70 status. Performing an anti-DFS70 confirmation test on patients who are ANA negative or have a low probability of SARD offers no clinical benefit and cannot be used in place of specific tests for detecting CTD-related autoantibodies. Thus, specific tests for CTD-related autoantibodies are needed regardless of the process for confirming the presence of anti-DFS70 when a DFS pattern is observed in an IIF-ANA screen.

Acknowledgements This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science and Technology (Grant No. NRF-2016RIA2B4014121).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human specimens were in accordance with the ethical standards of the institutional research committee (IRB No: KHNMC 2016-10-018) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent The institutional research committee had waived the process of obtaining consents from all individual participants included in this study, because this research was a study using residual samples.

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