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Review

The Role of Anti-DFS70 in the Diagnosis of Systemic Autoimmune Rheumatic Diseases

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Abstract: The diagnosis of systemic autoimmune rheumatic disease (SARD) or its exclusion is carried out taking into account the results of immunological studies, primarily antinuclear antibodies (ANA) and specific autoantibodies. Often, during ANA analysis via indirect immunofluorescence reaction on cellular and tissue substrates, a dense fine speckled 70 (DFS70) fluorescence pattern is observed. Studies on the diagnostic significance of antibodies to anti-DFS70 allow for optimizing the stepwise diagnosis of SARD. Currently, a two-step strategy for laboratory diagnostic investigation is recommended: in the first step, ANA screening is performed, and in the second step, patients with positive results undergo confirmatory tests to detect specific antibodies against individual nuclear antigens. The detection of anti-DFS70 in ANA-seropositive patients without clinical and/or other specific serological markers characteristic of a particular disease within the SARD group may be considered a negative prognostic marker. Also, in the process of decision making in clinical practice, we should remember that anti-DFS70 can be found in the blood of patients with a different, non-SARD pathology and that most people showing anti-DFS70 are healthy individuals.

Keywords: anti-DFS70; systemic autoimmune rheumatic disease; ANA; autoimmune serological markers

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1. Introduction

Systemic autoimmune rheumatic diseases (SARDs) are characterized by polyclonal activation of B cells and the formation of a broad spectrum of specific autoantibodies, which in turn trigger immune-inflammatory damage to tissues and internal organs. The main diagnostic laboratory markers of frequently encountered autoimmune diseases, such as systemic lupus erythematosus (SLE), systemic scleroderma (SSc), Sjögren's syndrome (SjS), polymyositis/dermatomyositis (PM/DM), and others, are antinuclear antibodies (ANA)—a heterogeneous group of autoantibodies directed to various components of the cell nucleus and cytoplasm. Due to the fact that modern diagnostic methods allow us to detect the autoantibodies to antigens located in various cell structures, including nuclear constituents, nuclear membrane, mitotic spindle apparatus, cytosol, cytoplasmic organelles, and cell membranes, a more accurate term for ANA is "anticellular antibodies" (anticell, AC), which is reflected in the modern nomenclature of immunofluorescence patterns recommended by the International Consensus on ANA Patterns—ICAP [1]. Positive results of the determination of ANA are among the diagnostic criteria for autoimmune diseases; they are utilized to assess disease activity, prognosis, and the characteristics of clinical-laboratory subtypes of the disease, and they serve as predictors of pathology development at the preclinical stage—all these factors underline the significance of this parameter in medicine. It is also important to mention the fact that the detection of autoantibodies may precede the clinical manifestation of the disease; for example, according to retrospective studies, elevated ANA levels were detected in the serum of 78% of systemic lupus erythematosus (SLE) patients up to 10 years prior to diagnosis [2,3]. Such patients

are typically under observation and are informed about potential symptoms that signify the possible onset of the disease.

2. Diagnosis of ANA and Autoantibodies in Clinical Practice

The gold standard for ANA determination is the indirect immunofluorescence assay (IFA) using Hep-2 substrate, as defined by the American College of Rheumatology ANA Task Force position statement in 2009 [4].

Currently, there are 30 different Hep-2 IFA patterns established, which, based on their fluorescence characteristics, are categorized into four groups: negative (AC-0), nuclear (AC-1–AC-14, AC-29), cytoplasmic (AC-15–AC-23), and mitotic patterns (AC-24–AC-28). Each pattern is assigned an alphanumeric anticell code [1,5–10] (Table 1).

Table 1. Types of Hep-2 IFA patterns *.

Group	Code	Pattern	Antigen Association	Pathology
negative	AC-0	-	-	-
Nuclear	AC-1	Nuclear homogeneous	dsDNA, nucleosomes, histones	SLE, chronic autoimmune hepatitis or juvenile idiopathic arthritis
	AC-2	Nuclear dense fine speckled	DFS70/LEDGF	Commonly found as high titer Hep-2 IIFA-positive in apparently healthy individuals or in patients who do not have a systemic autoimmune rheumatic disease
	AC-3	Centromere	CENP-A/B (C)	SSc, limited cutaneous SSc
	AC-4	Nuclear fine speckled	SS-A/Ro, SS-B/La, Mi-2, TIF1γ, TIF1β, Ku	SjS, SLE, subacute cutaneous lupus erythematosus, neonatal lupus erythematosus, congenital heart block, DM, SSc, and SSc-AIM overlap syndrome
	AC-5	Nuclear large/coarse speckled	hnRNP, U1RNP, Sm, RNA polymerase III	SLE, SSc, mixed connective tissue disease, SSc-AIM overlap syndrome, and undifferentiated connective tissue disease
	AC-6	Multiple nuclear dots	Sp-100, PML proteins, MJ/NXP-2	PBC, AIM (DM)
	AC-7	Few nuclear dots	p80-coilin, SMN	low positive predictive value for any disease
	AC-8	Homogeneous nucleolar	PM/Scl-75, PM/Scl-100, Th/To, B23/nucleophosmin, nucleolin, No55/SC65	SSc, SSc-AIM overlap syndrome
	AC-9	Clumpy nucleolar	U3-snoRNP/fibrillarin	SSc
	AC-10	Punctate nucleolar	RNA polymerase I, hUBF/NOR-90	SSc, Raynaud's phenomenon, SjS, and cancer
	AC-11	Smooth nuclear envelope	lamins A, B, C, or lamin-associated proteins	autoimmune-cytopenias, autoimmune liver diseases, linear scleroderma, APS
	AC-12	Punctate nuclear envelope	nuclear pore complex proteins	PBC
	AC-13	PCNA-like	PCNA	SLE, SSc, AIM, RA, HCV
	AC-14	CENP-F-like	CENP-F	neoplasms, Crohn's disease, autoimmune liver disease, SjS, graft-versus-host disease
	AC-29	DNA topoisomerase I (topo I)-like	Scl-70-like, Scl-86, DNA Topo I	SSc

Table 1. *Cont.*

Group	Code	Pattern	Antigen Association	Pathology
Cytoplasmic	AC-15	Cytoplasmic fibrillar linear	actin, non-muscle myosin	Autoimmune hepatitis type 1, chronic HCV infection, and celiac disease
	AC-16	Cytoplasmic fibrillar filamentous	vimentin, cytokeratins, tropomyosin	is not typically found in SARD
	AC-17	Cytoplasmic fibrillar segmental	alpha-actinin, vinculin	-
	AC-18	Cytoplasmic fibrillar linear	actin, non-muscle myosin	Autoimmune hepatitis type 1, chronic HCV infection, and celiac disease (IgA isotype)
	AC-19	Cytoplasmic dense fine speckled	PL-7, PL-12, ribosomal P proteins	SLE, anti-synthetase syndrome, interstitial lung disease, polyarthritis, Raynaud's phenomenon, and mechanic's hands
	AC-20	Cytoplasmic fine speckled	Jo-1/histidyl-tRNA synthetase	anti-synthetase syndrome, interstitial lung disease, polyarthritis, Raynaud's phenomenon, and mechanic's hands
	AC-21	Cytoplasmic reticular/AMA	PDC-E2/M2, BCOADC-E2, OGDC-E2, E1α subunit of PDC, E3BP/protein X	PBC, SSc, including PBC-SSc overlap syndrome and PBC-SjS overlap syndrome
	AC-22	Polar/Golgi-like	giantin/macrogolgin, golgin-95/GM130, golgin-160, golgin-97, golgin-245	-
	AC-23	Rods and rings	IMPDH2	HCV patients after treatment with pegylated interferon-α/ribavirin combination therapy
Mitotic	AC-24	Centrosome	pericentrin, ninein, Cep250, Cep110	Raynaud's phenomenon, localized scleroderma, SSc, SLE and RA
	AC-25	Spindle fibers	HsEg5	-
	AC-26	NuMA-like	NuMA	SjS, SLE, undifferentiated connective tissue disease, limited SSc, or RA
	AC-27	Intercellular bridge	-	-
	AC-28	Mitotic chromosomal	modified histone H3, MCA-1	-

* information from site: https://www.anapatterns.org/index.php (accessed on 13 July 2023). AIH—autoimmune hepatitis; AIM—autoimmune myositis; APS—antiphospholipid syndrome; BCOADC-E2—E2 component of branched chain 2-oxo acid dehydrogenase complex; CENP—centromere protein; Cep110—centrosome-associated protein 110; Cep250—centrosome-associated protein 250; DFS70—dense fine speckled 70; dsDNA—anti-double stranded deoxyribonucleic acid; E1α subunit of PDC—E1α subunit of Pyruvate Dehydrogenase Complex; E3BP—E3 binding protein; HCV—hepatitis C; hnRNP—heterogeneous nuclear ribonucleoproteins; IMPDH2—Inosine 5'-Monophosphate Dehydrogenase 2; LEDGF—lens epithelium-derived growth factor; MCA-1—mitotic chromosomal autoantigen1; NuMA—nuclear mitotic apparatus; OGDC-E2—E2 component of 2-oxoglutarate dehydrogenase complex; PBC—primary biliary cholangitis; PCNA—proliferating cell nuclear antigen; PDC-E2/M2—E2/M2 subunits of pyruvate dehydrogenase complex; SMN—survival of motor neuron complex; SS-A/Ro—Sjögren's-syndrome-related antigen A; SS-B/La—Sjögrens's syndrome-associated antigens B; RA—rheumatoid arthritis; RNA—ribonucleic acid; TIF1γ—transcriptional intermediary factor 1 γ; TIF1β—transcriptional intermediary factor 1 β; U1RNP—U1 ribonucleoprotein.

A two-step strategy is recommend for laboratory diagnostic investigation [11]: in the first step, ANA screening (HEp-2 IFA) is performed, and in the second step, patients with positive results undergo confirmatory tests to detect specific antibodies to individual nuclear antigens (referees to IgG) using techniques such as enzyme-linked immunosorbent assay, immunoblot, chemiluminescent immunoassay, multiplex technologies, and others.

Antibodies specific to certain SARDs are presented in Table 2 [12,13].

Table 2. Autoantibodies specific to certain SARDS.

Disease	Autoantibodies	Autoantibodies as a Diagnostic Criterion
autoimmune myositis, PM/DM	Anti-ARS, anti-Jo-1, anti-PL-7, anti-PL-12, anti-OJ, anti-EJ, anti-KS, anti-Ha, anti-Zo, Anti-SRP, Anti-Mi2, Anti-MDA5, Anti-TIF1, Anti-NXP2, Anti-HMGCR, Anti-SAE	anti-Jo-1 [14]
Systemic sclerosis	Anti-centromere, Anti-topoisomerase I, Anti-RNA polymerase, Anti-U3 RNP, Anti-Th/To, Anti-U11/U12 RNP, Anti-PDGFR, Anti-M_3R, Anti-ICAM-1, Anti-AT1R, Anti-ETAR	anti-centromere antibody, anti-scl70 antibody, and anti-RNAP III [15]
SLE	Anti-dsDNA, Anti-Nucleosome, Anti-Sm, Anti-RNP, Anti Ro/SSa, Anti La/SSB, Anti-Phospholipid, Anti-C1q, Anti-Ribosomal P, Anti-NMDAR,	Anti-dsDNA, Anti-Sm [16]
MCTD	anti-U1-RNP	anti-U1-RNP [17]

At the same time, the screening determination of ANA using the HEp-2 IFA method has high sensitivity (93%) but low specificity (57%) and positive predictive value (3%) for diagnosing SARDs. The reduction in pre- and post-test probability of SARD presence among ANA-positive individuals is associated with the detection of ANA across a sufficiently broad spectrum of pathological conditions, not always directly linked to autoimmune activation of pathologies (juvenile arthritis, autoimmune hepatitis, primary biliary cholangitis, inflammatory bowel diseases, vasculitis, fibromyalgia, multiple sclerosis, thyroid disorders, chronic infections, malignancies), alongside an increase in the number of tests ordered by medical practitioners from various specialties other than rheumatology —therapists, dermatologists, nephrologists, oncologists, cardiologists, neurologists, gastroenterologists, otolaryngologists, ophthalmologists, hematologists, and gynecologists [18,19]. Moreover, it is important to remember that among patients who have experienced severe SARS-CoV-2 infection, ANA is "false positive" in up to 35% of cases [20,21].

3. Diagnostics of DFS70 Pattern

The DFS70 pattern and autoantibodies were initially described by Ochs R.L. et al. in 1994 [22], and its presence was described in patients with interstitial cystitis. This pattern is characterized by a heterogeneous dense fine speckled staining of the nucleoplasm of interphase cell nuclei and chromatin in the mitotic zone. The nuclear target antigen was named DFS70 based on the reactivity of the autoantibodies with a 70 kDa protein in Western blotting. Later, it was established that the DFS70 antigen is identical to a protein known as a transcriptional coactivator p75 or lens epithelium-derived growth factor, LEDGF [23], which has functions as a transcriptional coactivator p75 and a growth factor for lens epithelial cells; however, the use of the synonym LEDGF/p75 in routine practice is not entirely accurate, as the direct influence of the DFS70 antigen on lens development has not been established. Nevertheless, in scientific literature, there is an equivalence between the terms anti-DFS70, anti-LEDGF, and LEDGF/p75. Anti-DFS70 antibodies are primarily of the immunoglobulin of G class, but in certain atopic conditions, immunoglobulin of E class antibodies is also found.

To confirm the presence of antibodies to DFS70 in ANA-positive sera, methods such as solid-phase enzyme-linked immunosorbent assay, immunoblotting, chemiluminescent immunoassay, and HEp-2 IFA with selective antibody adsorption using a knockout DFS70/LEDGF cell line are currently used. A one-step analysis of DFS70 antibodies using the IFA method on HEp-2/DFS70 cells eliminates the need for additional confirmatory tests when investigating these antibodies, so using a substrate (HEp-2 ELITE/DFS70-KO) composed of a mixture of standard HEp-2 cells and genetically engineered DFS70-Ko

HEp-2 cells that do not express the DFS70/LEDGF/p75 antigen, and this prevents the binding of DFS antibodies to the target antigen, allowing for a clear differentiation between DFS and classical types of nuclear staining.

4. Assessment of Detecting DFS70 Antibodies in Clinical Practice

It has been established that up to 20% of healthy individuals can be seropositive for ANA in HEp-2 IFA, which in turn, in half of the cases, is due to the presence of DFS70/LEDGF/p75 antibodies (pattern AC-2 according to the nomenclature of ANA patterns as agreed upon by the International Consensus on Patterns—ICAP) [11,24]. The relatively high rate of false-positive results for the ANA test (referring to situations where subsequent autoimmune diseases do not develop) among healthy people and patients with non-autoimmune diseases often raises concerns and alertness for both patients themselves and primary care physicians, creating an unnecessary burden on the healthcare system as it leads to the performance of additional, including expensive investigations.

Among healthy individuals, the frequency of detecting isolated anti-DFS70 antibodies (i.e., in the absence of other specific antibodies for SARDs autoantibodies) ranges from 2% to 21.6% (with an average of 6.8%), in ANA-positive donors, this frequency varies from 23.8% to 57% (with an average of 43.9%) [23–26]. Furthermore, these antibodies are often found at high titers (frequently reaching levels of 1:5120). However, it is worth noting a considerable range of results in these studies; in Dellavance A. et al.'s work, it is reported that a total of 30,728 serum samples were screened for HEp-2 IFA ANA, and the frequency of anti-DFS70 antibodies was 16.6 [27], while Bizzaro N. et al. indicated that a total of 21,516 serum samples were screened for ANA, and the frequency of anti-DFS70 antibodies was only 0.8 [28]. In this population, a wide range of anti-DFS70 titers is observed, and the frequency of their detection is influenced by factors such as gender (more frequent in women), age, geographical region, and the method of determination [26]; however, these results need to be studied on larger samples to identify clinically significant results. The observed differences in the prevalence of anti-DFS70 antibodies are likely due to differences in analysis methods and the selection of the study population. There have also been mixed results in studies that explored the relationship between the frequency of detecting anti-DFS70 in healthy individuals and age: one study found higher occurrence in individuals under 35 years old among 597 healthy hospital workers [29], other researchers showed that the frequency of anti-DFS70 occurrence is 32% in individuals aged from 18 to 30, which increases to 42% in the 31–40 age group, decreases to 36% in the 41–50 age group, and drops to 10% in those over 50 [9]. At the same time, another study found isolated anti-DFS70 in only 2.1% of healthy children [30]. Prospective studies have also been conducted to monitor the health status and antibody titer dynamics in healthy individuals with confirmed presence of anti-DFS70; for example, a four-year observational study did not register any cases of SARD among 41 healthy individuals with permanent high levels of isolated anti-DFS70 and no other autoantibodies in their blood serum [9]. In a 10-year follow-up study by Gundín S. et al. of 181 patients with positive anti-DFS70, antibody results showed that none of them developed SARD during the observation period [31].

According to the results of various studies, a negative association has been observed between isolated anti-DFS70 antibodies and SLE and other SARDs [32], in which these autoantibodies are found in isolation in less than 1% of patients (except for this figure in children with SLE, where it was 1.8%—though these data are based on the results of just one study) (Table 3) [33]. It can be stated that we observe a lower frequency of anti-DFS70 occurrence in this patient cohort compared to healthy individuals.

With the increasing frequency of ANA detection testing, the amount of information regarding the interpretation of the detected DFS70 pattern also increases (Table 4).

Table 3. Frequency of anti-DFS70 detection in patients with immune-inflammatory rheumatic diseases.

Indicator/Diseases	SLE	SLE among Children	SSc	SjS	PM/DM	MCTD
Number of studies	9	1	7	7	4	1
Number of patients	1434	331	536	144	231	8
Frequency of anti-DFS70 detection, Me (min–max)	2.7% (0–5.7%)	5.7%	1.5% (0–5.7%)	9.7% (0–26.6%)	3.5% (0–6.4%)	0
Frequency of isolated anti-DFS70 detection, Me (min–max)	0.7% (0–0.7%)	1.8%	0 (0–2.4%)	1% (0–1.4%)	0.9% (0–2.5%)	0

MCTD—mixed connective tissue disease; Me—Median.

Table 4. The analysis of anti-DFS70 prevalence in patients with various diseases.

Author	Number of Cases	Conclusion
Santler B. [34]	150	The antibody DFS70 is associated with atopic dermatitis and may be responsible for misdiagnosis of SARD
Alev Cetin Duran [35]	281	Autoantibodies to DFS70 can be linked to organ-specific autoimmune diseases, allergic conditions, and hematological disorders
Yingxin Dai [36]	1256	Antibodies to DFS70 are predominant in Chinese ethnic patients with SLE
Consuelo Romero-Sánchez [37]	530	Autoantibodies to ANA/DFS70 were present in Colombian SARD patients with low frequency and were more common in healthy individuals
D. Rincón-Riaño [38]	53	Autoantibodies to ANA/DFS70 were more frequent in patients with undifferentiated connective tissue disease compared to other rheumatic diseases for which they were initially evaluated
Mirjam Freudenhammer [39]	308	Among ANA-positive children, monospecific antibodies to DFS70 can help distinguish SARD-related conditions from non-SARD-related states
Fulya Ilhan [40]	876	Low frequency of detection of anti-DFS70 and observation of centriolar pattern staining in patients with Behcet's disease
Dandan Chen [41]	955	Antibodies to DFS70 were not associated with the development of lupus nephritis in SLE patients but were linked to antibodies to dsDNA, proliferative lupus nephritis, and acute renal failure. This suggests their potential to serve as a non-histological biomarker for lupus nephritis subclass and activity status
Louisa-Marie Mockenhaupt [42]	460	Autoantibodies to DFS70 appear to be more prevalent in patients with connective tissue diseases compared to healthy individuals and therefore are not a good exclusion criterion
Alev Çetin Duran [43]	5710	Autoantibodies against DFS70 may be associated with rheumatic diseases not related to SARD and can be diagnosed in many diseases (dermatological, gastrointestinal, hematological, thyroid diseases) related to other systems
Samet Karahan [44]	1124	It can be considered that anti-DFS70 does not predict systemic connective tissue disease or even exclude it
Claudia A. Seelig [45]	1243	In patients exclusively having anti-DFS70 antibodies, the odds ratio for the absence of SARD approaches clinically significant values

Table 4. *Cont.*

Author	Number of Cases	Conclusion
Gali Aljadeff [46]		Injecting anti-DFS70 to mice slowed the progression of glomerulonephritis in mice with SLE and increased survival time. Circulating autoantibodies to DFS70 may play a protective role against kidney damage in lupus nephritis
Greisha L. Ortiz-Hernandez [26]		"Monospecific" autoantibodies to DFS70/LEDGF (detectable only by ANA in serum) were not associated with SARD and were found in healthy individuals and some patients with inflammatory conditions not related to SARD
Teck Choon Tan [47]	645	Anti-DFS70 was not associated with the absence of SARD
Verónica Romero-Álvarez [48]	240	The presence of ANA DFS70 has been confirmed only in systemically healthy individuals
Ora Shovman [49]	228	The prevalence of monospecific antibodies to DFS70 was significantly higher in healthy subjects than in patients with rheumatic diseases
Maria Infantino [50]	91	The high prevalence of antibodies to DFS70 in patients with undifferentiated connective tissue disease suggests a potential role for these autoantibodies as markers in the evolution towards differentiation. Undifferentiated connective tissue disease has a high risk of transforming into a differentiated form over time.
Michael Mahler [32]	3263	"Monospecific" antibodies to DFS70/LEDGF can serve as biomarkers for differentiating SARD from non-SARD individuals.(The prevalence of antibodies to DFS70/LEDGF was significantly higher in healthy individuals compared to patients with SARD.)
Cristian C. Aragón [51]	127	Autoantibodies to DFS70 can be considered biomarkers for differentiating patients with SLE from ANA-positive individuals without autoimmune diseases. (i.e., antibody = absence of autoimmune disease)
Zeki Yumuk [52]	3432	The DFS pattern cannot exclude the presence of SARD, but the likelihood is lower than with other patterns
John B. Carter [53]	6511	Recognition of isolated anti-DFS70 ANA allows patients to be reassured that SARD is unlikely to happen
D. Kiefer [54]	270	It has been found that antibodies to DFS70 are rarely present in patients with connective tissue disease, with positive ANA, but the diagnosis of a systemic disease cannot be reliably excluded based solely on the presence of antibodies to anti-DFS70
Maria Infantino [55]	768	Monospecific antibodies to DFS70 can be a useful biomarker for distinguishing individuals with SARD from non-SARD individuals with a positive ANA.
M. Y. Choi [56]	1137	"Monospecific" autoantibodies to DFS70 can be useful for distinguishing between ANA-positive healthy individuals and those with SLE
Simón Gundín [34]	181	None of the included patients with a positive result for monospecific antibodies to DFS70 developed SARD during a 10-year observation period
Makoto Miyara [57]	100	While antibodies to DFS70 cannot exclude the presence of SARD, the probability of pathology development is significantly low
Y. Muro [58]	500	Patients having only antibodies to DFS70 are rarely diagnosed with autoimmune rheumatic disease
Jisoo Jeong [59]	75	Antibodies against DFS70 may serve as a useful biomarker for differentiating fibromyalgia and other autoimmune diseases

Table 4. *Cont.*

Author	Number of Cases	Conclusion
Mia C. Lundgren [60]	425	The ANA-DFS pattern can indicate a pro-inflammatory microenvironment, given the high frequency of symptomatic patients and pathological processes with an immunological basis (including SARD)
So Young Kang [61]	2654	The frequency of the DFS pattern was higher in seborrheic dermatitis (14.3%), herpes zoster (11.1%), rheumatoid arthritis (16.9%), systemic lupus erythematosus (15.4%), and Sjögren's syndrome (14.3%).A relatively high frequency of the DFS pattern was observed in autoimmune diseases.

At the moment, for some SARDs, we have a significant number of studies where large numbers of patient samples with detected anti-DFS70 antibodies have been analyzed. Perhaps the largest number of such studies relates to SLE.

Aleksandrova et al. examined the frequency of detecting anti-DFS70 antibodies in the sera of 45 healthy donors and 12 patients with SLE. Among ANA-positive individuals, 15.6% of healthy volunteers and 100% of SLE patients exhibited positive results. Classical ANA patterns with homogenous, speckled, mixed fluorescence types, and absence of antibodies to anti-DFS70 were observed in 100% of SLE patients and 6.7% of healthy individuals. Monospecific antibodies to anti-DFS70 without classical ANA patterns were detected in 8.9% of healthy individuals and were absent in SLE. Among ANA-positive healthy individuals, the frequency of isolated detection of antibodies to anti-DFS70 was 57%. The authors concluded that monospecific antibodies to anti-DFS70 serve as a negative serological marker for SLE [62]. However, in some studies, an assessment of their potential association with serological and clinical manifestations and disease activity has been conducted. So, in an early SLE (15 months from diagnosis) involving a multinational cohort of patients from 11 countries (n = 1137), anti-DFS70 antibodies were identified in 7.1% of cases, with isolated anti-DFS70 especially in the absence of antibodies against double-stranded DNA and other extractable nuclear antigens found in 1.1% of cases and multivariate analysis showed an association between anti-DFS70 and musculoskeletal manifestations of SLE, and concentration of antibody levels against β2-glycoprotein-1, as well as inverse correlation with anti-dsDNA and anti-La/SSB antibodies [56]. In contrast, Mahler et al. [22] did not find an association between anti-DFS70 and clinical or immunological manifestations of SLE. An analysis of six studies involving 1396 SLE patients showed a frequency of anti-DFS70 antibody occurrence of 2.7% when detecting anti-DFS70 antibodies in the absence of SLE-specific antibodies, and only 0.7% of patients had this combination. Therefore, the exclusive detection of anti-DFS70 antibodies can be considered an exclusion criterion for diagnosing SLE in ANA-positive patients with nonspecific symptoms such as arthralgia, weakness, or rash [24].

The data from a limited number of studies assessing anti-DFS70 antibodies in patients with SSc also indicate a low frequency of detecting this antibody; furthermore, the conclusion is drawn that the concurrent absence of SSc-specific antibodies in individuals with nonspecific symptoms and suspicion of SSc makes this scenario unlikely.

For SjS patients, a low frequency of mono-carriage of anti-DFS70 antibodies has also been established; this finding also allows for its utilization in routine practice as a negative predictor for disease development in this situation. However, a notable feature of SjS is the relatively high frequency of detecting anti-DFS70 antibodies alongside anti-Ro/SS-A antibodies.

For inflammatory myopathies (PM/DM and sporadic inclusion body myositis), the information is extremely limited; in the available studies, DFS70 antibodies were generally diagnosed in a low percentage of cases and were also more commonly associated with patients carrying myositis-specific autoantibodies.

For patients with undifferentiated connective tissue disease, according to the currently available data, a higher frequency of diagnosis of anti-DFS70 antibodies (10.8–12%) is characteristic [24] compared to other SARDs; however, considering the small number of studies, this idea should be examined in more comprehensive research. However, this is complicated by the low frequency of occurrence of this pathology.

Also, when making decisions in clinical practice, it is important to remember that anti-DFS70 can be detected in the blood of patients with conditions other than SARDs. So, elevated levels of anti-DFS70 can be diagnosed in eye diseases (cataracts, atypical retinal degeneration, sympathetic ophthalmia, uveomeningeal syndrome (Vogt–Koyanagi–Harada syndrome), Behcet's disease, and others); in this situation, a protective role of the DFS70/LEDGF/p75 antigen towards eye structures (lens, retinal pigment epithelial cells) is assumed in response to stress or damage [24]. An association with conditions like interstitial cystitis, bronchial asthma, atopic dermatitis, alopecia areata, chronic fatigue syndrome, prostate cancer, and others has also been identified [22,63–65]. However, the determination of anti-DFS70 has not become a routine part of the diagnostic process for these conditions. There is a compelling assumption that this autoantigen may not be a growth factor but rather a protein responding to stress or damage, which is ubiquitously expressed in mammalian cells and tissues, with increased expression in cancer cells and tumors [66].

Therefore, at the present moment, it is recommended to adhere to the following algorithm for utilizing the anti-DFS70 test in the diagnosis of SARD.

- In the case of a positive test for ANA via the HEp-2 IFA method with a DFS70 fluorescence pattern, an anti-DFS70 test should be conducted;
- In the case of a positive test for ANA via the HEp-2 IFA method with a fluorescence pattern other than DFS70, it is recommended to conduct an analysis for specific autoantibodies, such as by immunoblotting;
- In the case of a negative ANA result via the HEp-2 IFA method in conjunction with the absence or presence of anti-DFS70, the likelihood of SARD is minimal;
- In the case of a positive ANA result via the HEp-2 IFA method in conjunction with the presence of anti-DFS70, the likelihood of SARD is moderate;
- In the case of a positive ANA result via the HEp-2 IFA method in conjunction with the absence of anti-DFS70, the likelihood of SARD is high.

Therefore, the detection of anti-DFS70 in ANF-positive patients without clinical and/or serological markers characteristic of a specific SARD can be considered a potential marker for excluding the diagnosis of SARD, especially in the early preclinical period. However, the duration of this period, primarily characterized by elevated ANA titers, is not precisely defined and can vary from a few months to several years until the influence of exogenous or endogenous triggering factors leads to the development of clinical symptoms. Detecting both predictors of high-risk development of specific SARD and "excluding" markers at an early stage remains a relevant task for practicing physicians.

5. Conclusions of This Review

At present, it is quite challenging to draw definitive conclusions regarding the diagnostic significance of testing for anti-DFS70. On the one hand, it can be stated that incorporating anti-DFS70 testing into clinical practice for patients with a positive ANA test leads to a reduction in the number of expensive diagnostic procedures aimed at excluding diseases from the SARD group. Such a tactic can help in interpreting a positive ANA result, particularly when it is associated with negative results for autoantibodies linked to SARD, thus preventing unjustified treatment and stress for patients. In modern clinical practice, the identification of monospecific antibodies to DFS70 in serum can be considered a potential criterion for excluding the diagnosis of SLE and other SARDs in ANA-positive patients without clinical signs of these conditions. At the same time, given the relatively low frequency of detecting anti-DFS70 in healthy donors, it can be assumed

that the determination of SARD-specific autoantibodies has greater diagnostic value in clinical conditions, although it is associated with higher economic costs.

Thus, to achieve a certain consensus on the use of anti-DFS70 as a clinically reliable biomarker for excluding SARDs, additional large-scale studies with substantial cohorts of both SARD patients and healthy donors are required, and these cohorts should be diverse in terms of race, gender, age, ethnic background, and geographic regions; moreover, the observation period for both patients and healthy donors should be sufficiently prolonged, and not momentary, considering the potential development of systemic pathology at a later stage. In these studies, multiple highly sensitive and specific research methods should be employed, and proposed ANA testing algorithms, including the determination of anti-DFS70, should be rigorously evaluated.

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