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DFS70 antibodies – biomarkers for the exclusion of ANA-associated autoimmune rheumatic diseases

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Abstract: Despite the progress in the establishment of specific autoantibody assays, screening for antinuclear antibodies (ANA) by indirect immunofluorescence on HEp-2 cells for quality-oriented laboratory diagnosis of ANA associated rheumatic diseases (AARD) remains indispensable. Research results on the relevance of the dense fine speckled (DFS) pattern and DFS70 antibodies disclosed novel possibilities to optimize the serological stepwise diagnostics of AARD. The DFS pattern on HEp-2 cells is well differentiated from the classic "homogeneous" ANA pattern associated with dsDNA antibodies. In DFS pattern positive sera the most important detectable ANA specificity is the DFS70 antibody (synonym LEDGF antibody). This antibody is also the most frequent ANA specificity in ANA positive healthy persons. The prevalence of DFS70 antibodies in AARD patients is significantly lower compared with the prevalence in ANA-positive healthy individuals. There is a negative association between DFS70 antibodies and AARD, especially if no concomitant AARD-specific autoantibodies are found. Isolated DFS70 antibodies are detectable in <1% of AARD, but are detectable in 5%–11% of healthy individuals. In the presence of an isolated DFS70 antibody, the posttest probability for AARD is reduced significantly. DFS70 antibodies are valuable novel biomarkers for the improved interpretation of positive ANA but without detectable AARD associated autoantibodies and should be integrated in modified test algorithms to avoid unnecessary referrals and examinations of ANA-positive subjects.

Keywords: antinuclear antibodies (ANA); ANA-associated autoimmune rheumatic diseases; DFS70 antibodies.

Introduction

The determination of antinuclear antibodies (ANA) by way of indirect immunofluorescence (IIF) with HEp2-cells has been the standard for decades in diagnostic laboratories in connection with suspected ANA-associated rheumatic diseases (AARD), such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), Sjögren's syndrome (SjS), mixed collagenosis or autoimmune myositis (AIM) [1, 2]. With a negative ANA finding, AARD can be ruled out with relative certainty, with the exception of AIM. However, ANA are not specific to AARD, because they are present also in other autoimmune diseases (e.g., autoimmune liver diseases), non-autoimmune diseases (e.g., tumors) and even in healthy individuals (depending on age and gender) [3–5]. Key to the diagnosis are ANA titers and ANA pattern, as well as the underlying ANA specificity, which is mostly determined on the basis of the diagnostic question and the present ANA pattern by means of specific immunoassays [6, 7]. The classification of ANA pattern is handled in various ways by routine laboratories, but the most common methods involve differentiation for granular (fine or coarse granular), homogeneous, nucleolar and centromere pattern. Generally, the term "homogeneous pattern" is used to combine all pattern that exhibit a chromatin staining of mitotic cells [8]. However, studies in recent years have shown that homogeneous patterns should be divided into at least two patterns of different diagnostic relevance (Figure 1): the classic, dsDNA-antibody associated homogeneous pattern and the dense fine speckled (DFS) pattern associated with DFS70 antibodies [9, 10].

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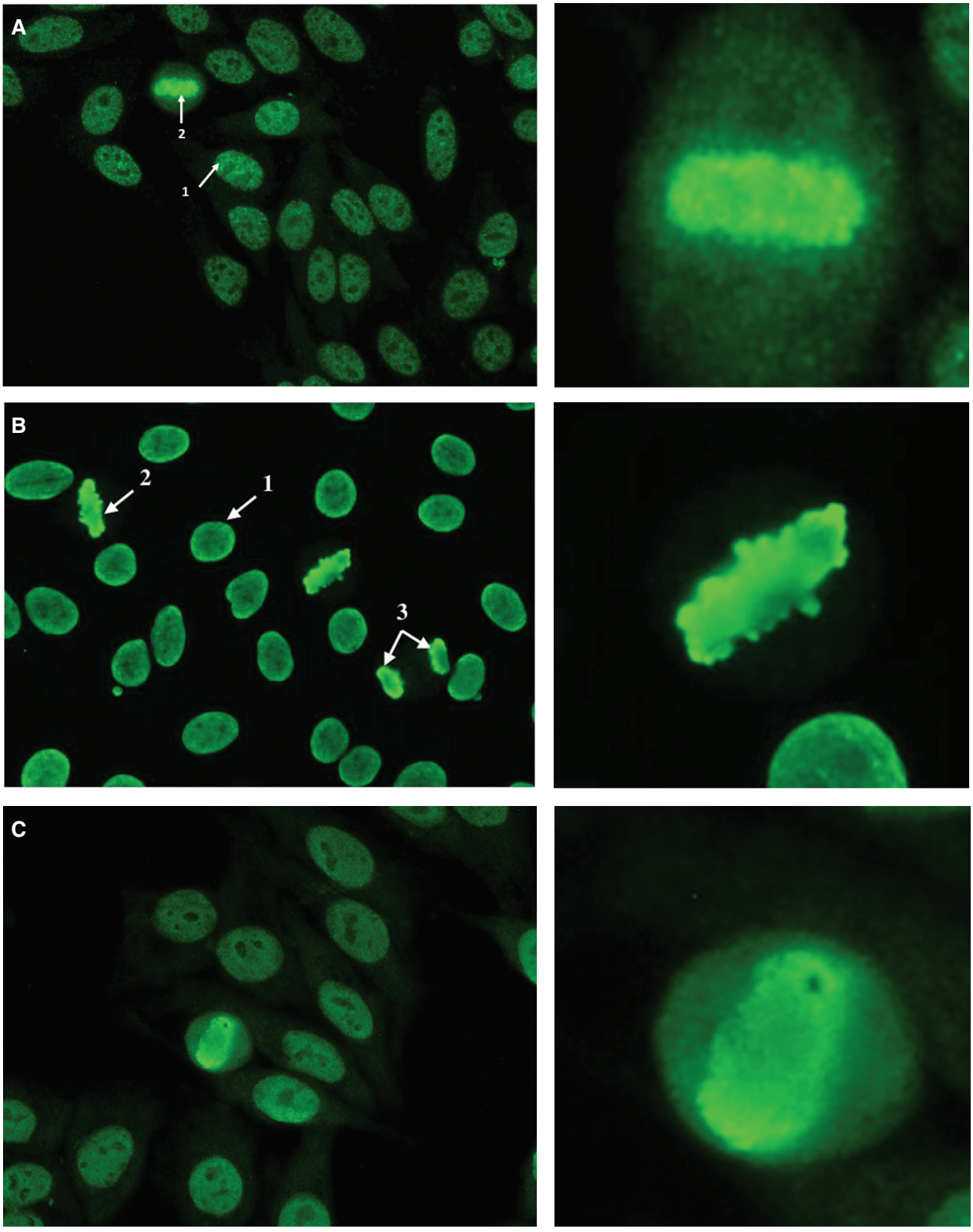

Figure 1: Differentiation of "homogeneous" ANA patterns on HEp-2 cells (left: overview; right: metaphase cells).
(A) Dense fine speckled pattern of the interphase nuclei (1) as well as of the metaphase chromatin (2) of a DFS70 antibody; (B) classic homogeneous ANA pattern of a dsDNA antibody with homogeneous-peripheral staining of the interphase nuclei (1) as well as of the chromatin in all mitotic phases (2,3); (C) "quasi-homogeneous" ANA pattern with homogeneous fine-granular staining of the interphase nuclei as well as of the metaphase chromatin of an autoantibody of unknown specificity.

History: DFS pattern and ANA specificity

The DFS pattern, which can be differentiated from other nuclear pattern relatively well, is characterized by irregularly distributed, fine-granular fluorescence of the nuclei in the interphase and of the metaphase chromatin (Figure 1). Although frequently observed in routine diagnostics, it was not until 1994 that it was described as a separate pattern of an autoantibody associated with interstitial cystitis [11]. The nuclear target antigen was called DFS70, according to the reactivity of the autoantibody with a 70 kDa protein in Western blot [11, 12]. Analyses of protein sequence databases have shown that the DFS70 antigen is identical to a protein designated as transcriptional coactivator p75 (synonym: "lens epithelium-derived growth factor", LEDGF) [12]. That protein (LEDGF/p75) was characterized by three groups of researchers independently: as transcriptional coactivator p75 [13] and as a growth factor for lens epithelial cells [14, 15]. The term LEDGF, however, is inaccurate, because the DFS70 antigen occurs in most tissue and does not have any direct involvement in the development of the lens epithelium. In 1997, LEDGF was identified as an autoantigen of an ANA specificity described as Sa-antibody [16]. Autoantibodies against LEDGF were also discovered through serological screening of recombinant cDNA libraries without knowledge of the ANA pattern [17, 18]. For example, LEDGF antibodies were characterized as one reactivity of anti-retinal antibodies in patients with atypical retinal degeneration [18].

Aside from a series of physiological functions, such as cell protection against stress-induced apoptosis, LEDGF/p75 is a cofactor of HIV replication by interacting with the viral integrase [19]. However, the role of this autoantigen in connection with the induction of a specific immune response is still unclear. Even though the correct designation of the protein is PSIP1 ("PC4 and SFRS1 interacting protein 1"), LEDGF/p75 is still commonly used as the autoantigen for DFS70 antibodies. Synonyms for DFS70 antibodies, therefore, are LEDGF or LEDGF/p75 antibodies. The epitope of the DFS70 antibodies has been localized by means of recombinant proteins and is located in the C-terminal region of the protein between amino acids 349 and 435 [20].

Different requesters

In the past, the ANA-HEp-2 test was requested and ordered exclusively by rheumatologists and immunologists in connection with AARD diagnostics. Over the years, however, the ANA test enjoyed increasing popularity, and today is ordered by many clinicians from a variety of disciplines. These include general practitioners, hematologists, nephrologists, oncologists, cardiologists, gynecologists, neurologists and gastroenterologists. Even though its clinical significance is controversial or ambiguous in many respects, the test can often yield an important indication of an autoimmune process. However, given the reduced pre-test probability, one must expect a greatly diminished post-test probability for AARD [1]. A precise pattern differentiation allows for conclusions about the underlying, diagnostically-relevant ANA specificities and thus increases the significance of a broad ANA screening through the targeted selection of subsequent specific immunoassays (e.g., determination of DFS70 antibodies in presence of the DFS pattern).

Clinical relevance of DFS70 antibodies

Despite numerous studies, the clinical relevance of the DFS70 antibodies remains largely unclear. Early studies revealed an association with interstitial cystitis, atopic dermatitis and various other inflammatory diseases, as well as prostate cancer [11, 12, 17, 21, 22]. However, none of these associations has been verified unambiguously and become part of routine diagnostics so far. Table 1 summarizes the most important studies on DFS70 antibodies. Findings to date indicate a possible association with eye diseases (cataract, atypical retinal degeneration, Vogt-Koyanagi-Harada syndrome), but this would have to be corroborated by further studies. The studies also show that the prevalence of DFS70 antibodies can vary substantially within a certain disease cohort (e.g., atopic dermatitis). This has varied causes, which are to be found in the heterogeneity of the epitope specificity of anti-DFS70 reactivities [2], as well as in different study designs (source and preparation of the autoantigen, DFS70 antibody detection method, inclusion and exclusion criteria for specific disease entities). It cannot be ruled out that there are different populations of DFS70 antibodies of different clinical and pathogenetic relevance. For example, LEDGF antibodies with a cytotoxic effect on lens epithelial cells have been described in the sera of patients with atypical dermatitis, which might be significant to the cataract development in these patients [23]. The high prevalence of DFS70 antibodies in ANA-positive, healthy individuals indicates that DFS70 antibodies likely represent a

Table 1: Results of studies on the prevalence of DFS70 antibodies in healthy individuals and patients with autoimmune and non-autoimmune diseases.

Disease	DFS70-antibody positive, %	Determination method	References
Various inflammatory diseases			
Alopecia areata	22/111 (20)	IIF, IB, EIA	[22]
Asthma	8/50 (16)	IIF, IB	[12]
	1/25 (4)	CIA	[24]
Atopic dermatitis	19/64 (29.7)	IIF, IB	[12]
	15/21 (71.4)	EIA	[23]
	3/29 (10.3)	EIA, IB	[16]
	0/16 (0)	CIA	[24]
Atopic dermatitis with cataract	8/8 (100)	EIA	[23]
Atypical retinal degeneration	3/3 (100)	IB	[18]
Behçet's disease	11/32 (34.4)	EIA	[17]
Hashimoto's thyroiditis	4/67 (6%)	CIA	[24]
Chronic fatigue syndrome	2/60 (3.3)	IIF, IB	[12]
	90/226 (40)	EIA, IB	[16]
Interstitial cystitis	9/103 (8.7)	IIF, IB	[12]
	2/40 (5)	CIA	[24]
Psoriasis	1/22 (4.5)	IIF, IB	[12]
Vogt-Koyanagi-Harada syndrome	24/36 (66.7)	EIA	[15]
Sympathetic ophthalmia	5/7 (71.4)	EIA	[17]
Sarcoidosis	4/16 (25)	EIA	[17]
Systemic autoimmune diseases			
Systemic lupus erythematosus	0/36 (0)	IIF, IB	[12]
	1/55 (2)	IIF, IB, EIA	[25]
	7/251 (2.8)	CIA	[24]
	7/124 (6)	IIF, IB	[26]
Systemic sclerosis	1/40 (25)	IIF, IB	[12]
	0/50 (0)	IIF, IB	[25]
	0/29 (0)	CIA	[24]
	1/164 (0.6)	IIF, IB	[26]
Sjögren's syndrome	2/29 (6.9)	IIF, IB	[12]
	3/30 (6.7)	IIF, IB, EIA	[25]
	8/71 (11)	IIF, IB	[26]
Autoimmune myositis	0.25 (0)	IIF, IB, EIA	[25]
	7/116 (6.4)	EIA (MBL)	[27]
	4/80 (5)	IIF, IB	[26]
Mixed collagenoses	0/8 (0)	IIF, IB	[26]
Rheumatoid arthritis	1/39 (2.6)	CIA	[26]
	0/13	IIF, IB	[26]
Tumors			
Prostate cancer	38/206 (18.4)	EIA	[21]
Various tumors	6/334 (1.6)	IIF	[28]
Colon and breast cancer	0/40 (40)	CIA	[24]
Healthy individuals			
	0/39 (0)	IIF, IB	[12]
	8/37 (21.6)	EIA	[17]
	64/597 (11)	IIF, IB, EIA	[25]
	35/650 (5.4)	EIA	[23]
	11/124 (8.9)	CIA	[24]
	8/105 (8)	IIF, IB, EIA	[22]

CIA, chemiluminescence immunoassay (QUANTA Flash® DFS70, Inova Diagnostics Inc., San Diego, CA, USA); IB, immunoblot; IIF, indirect immunofluorescence (HEp-2 cell test); EIA, enzyme immunoassay.

paraphenomenon (e.g., as a response to increased stress-induced LEDGF expression) in such individuals, as well as in a number of ANA-positive patients with various conditions (see Table 1).

DFS70 antibodies in apparently healthy individuals

The prevalences in healthy individuals, analyzed by various methods, fluctuate between 0% and 22% (Table 1); the causes are essentially found in the methodology (autoantigen, assay configuration, calculation of the limit titer) [25]. Comparable studies indicate a prevalence between 5% and 11% [10, 22–25]. Thus, DFS70 antibodies are the most frequent ANA specificities that can be detected in healthy individuals. In a large cohort of medical staff, DFS70 antibodies were found in 11% (64/597) through a combination of the HEp-2 cell test (DFS pattern) and immunoblots with recombinant LEDGF [25]. The DFS70 antibodies were exclusively of the IgG type and represented 54% of the ANA in this population. A survey of the 52 persons who were positive for DFS70 antibodies did not yield any indication of existing AARD. Interestingly, the prevalence of DFS70 antibodies is significantly higher in the group of those under 35 years of age than it is for those older than 35. Such age dependence of DFS patterns was also demonstrated in a population study of 918 healthy individuals [10]. After excluding AARD, serious infections and tumors based on case history, this population was used as an AARD comparison group. More than 90% of the ANA with the DFS pattern were observed in people younger than 50 years. Our own findings obtained via chemiluminescence immunoassay (CIA) testing of 300 blood donors also show such age dependence of DFS70 reactivities. While 7.3% of those under 40 years were positive for DFS70 antibodies, this was true only of 3.4% among those over 40 years. The DFS70 antibodies found in healthy individuals exhibit, almost without exception, the typical DFS pattern on HEp-2 cells [24, 25]. It is interesting to note that the 70% of the DFS pattern are in the medium- to high-titer range (from 1:320), while the fine granular ANA pattern, the most frequent pattern in healthy individuals, shows up predominantly in the low-titer range (<1:320) [10].

DFS70 antibodies in ANA-positive routine cohorts

The prevalence of the DFS pattern in ANA screenings on HEp-2 cells is between 0.8% and 1.6% [24, 28, 29]. In the study carried out by Dellavance et al. [9], it is actually as high as 16.5% due to the methodology and population. In over 90% of cases with the DFS pattern, DFS70 antibodies were also detected by means of immunoblot, CIA or ELISA [9, 24, 30]. The clinical spectrum for DSF70-antibody positive patients is very heterogeneous and, for example, comprises non-specific rheumatic symptoms, autoimmune diseases, infections, tumors and atopic diseases [9]. The most common diagnoses were arthralgias (20%) and Hashimoto's thyroiditis (16%). Interestingly, the diseases associated with DFS70 antibodies in previous studies were found in only one case (interstitial cystitis) and three cases (atopic dermatitis). AARD were diagnosed in 7/80 DFS70-antibody-positive patients (five SLE, two SSc); unfortunately, there was no information about AARD-associated ANA specificities [9].

Table 2: Prevalence of DFS70 antibodies in ANA-associated rheumatic diseases (AARD)[a].

Disease	DFS70-Ab positive	DFS70-Ab monospecific[b] positive
Systemic lupus erythematosus	15/466 (3.2%)	2/466 (0.4%)
Systemic sclerosis	2/283 (0.7%)	1/283 (0.35%)
Sjögren's syndrome	13/130 (10%)	1/71 (1.4%)
Autoimmune myositis	11/196 (5.6%)	6/196 (3.1%)
AARD total	41/1075 (3.8%)	10/1016 (1%)

[a]summary of the results of five studies [9, 24–27]. [b]DFS70-antibody positive in the absence of the autoantibodies relevant to the corresponding AARD diagnosis (valuation only with clear indications).

DFS70 antibodies in patients with ANA-associated rheumatic disease

An increasing number of studies in recent years have demonstrated a negative association of DFS70 antibodies with AARD, particularly when the antibodies do not occur in connection with clinically relevant autoantibodies (e.g., antibodies against dsDNA, Sm, U1-RNP, ribosomal P proteins, DNA topoisomerase I, centromere proteins, Ro52, Ro60, La/SS-B, Ku, Mi-2, Jo1). In the case of an isolated DFS70 antibody, the posttest probability for AARD is reduced significantly [30, 31].

The prevalence of DFS70 antibodies in AARD patients is significantly lower compared to the prevalence in healthy individuals [24, 25]. This becomes particularly evident in comparison with ANA-positive, healthy individuals. A meta-analysis of studies comprising 1075 AARD patients [9, 24–27] revealed a DFS70 antibody prevalence

of 3.8% (Table 2). If only the DFS70 antibodies that were measured as positive in the absence of AARD-relevant autoantibodies are analyzed, the detection frequency is at only 1%, and in SLE patients even as low as 0.4%. Since the present studies have not yet determined all myositis-specific antibodies (e.g., Mi-2, NXP-2, MDA5, TIF1y, HMGCR, SAE, PL7, PL12), the proportion of DFS70 antibodies that can be detected monospecifically in myositis patients, and thus in the overall AARD population, should be substantially lower than 1% [32]. The significance of DFS70 antibodies as a criterion to exclude AARD is also confirmed by follow-up studies on DFS70 antibodies of positive, healthy individuals, who did not develop any AARD in the period under observation of 4 years [10].

The findings of studies to date on the clinical association of DFS70 antibodies have a potential effect on the classification criteria for SLE over the long term, where one criterion is a positive ANA finding [33, 34]. Based on existing data, it would be recommended to exclude ANA with a DFS pattern as a criterion when detecting DFS70 antibodies. The testing for DFS70 antibodies may also have possible health-economic effects, because these antibodies may be used to avoid unnecessary referrals and follow-up tests for asymptomatic, ANA-positive patients.

Recommendations for routine diagnostics

DFS70-antibody detection methods

DFS70 antibodies can be analyzed by means of different methods. These include ELISA, immunoblots, line or dot immunoassays, as well as CIA [24, 30, 33, 35]. Only one enzyme immunoassay is currently available commercially (DFS70/LEDGF ELISA Kit, MBL Medical & Biological Labs. Co., Ltd, Nagano, Japan) for research purposes, and one CIA for diagnostic purposes (QUANTA Flash® DFS70, Inova Diagnostics, San Diego, CA, USA). Another way of detecting DFS70 antibodies is based on IIF using HEp-2 cells following immunoadsorption of DFS70 antibodies (NOVA Lite® HEp-2 Select, Inova Diagnostics, San Diego, CA, USA). This method uses a sample buffer that contains a recombinant DFS70 antigen [36]. This helps prevent the DFS70 antibodies from attaching to the DFS70 binding sites in the HEp-2 cell. An advantage of this method is that mixed patterns of DFS and other patterns (e.g., centromere patterns) can be identified.

As already described, the results, when using different assays, can lead to different outcomes [22, 25, 37], which is caused, among other things, by different antigen sources in combination with differing fine specificity [12, 17, 18, 38]. This becomes especially apparent in the prevalence of DFS70 antibodies in connection with atopic diseases (Table 1). Confirmation of clinical relevance in these diseases and various eye diseases may require different assays. However, when it comes to the differential diagnostic relevance regarding the exclusion of AARD in ANA-positive patients, this is not relevant. The DFS70 antibodies that can be detected in healthy individuals and in AARD do not seem to exhibit any relevant epitope differences [20]. A quantitative comparison between DSF70 ELISA (MBL) and CIA (Inova) also yielded an excellent correlation of these two methods, despite differences in terms of the antigen [24].

ANA screening and DFS pattern

The fine granular and the DFS are the most common pattern in the routine screening of ANA detectable on HEp-2 cells. While the fine granular pattern is predominantly low-titer, the DFS antibodies are also found frequently in medium and higher titers, regardless of the clinical background [9, 10, 21, 29]. Information about the association of this pattern with DFS70 antibodies fluctuates between 11% [39] and over 90% [9, 20, 30]. In our own studies, we have found a prevalence of DFS70 antibodies detectable via CIA in 37% of samples with DFS pattern from a titer level of 1:640. Some of these differences can be traced back to variable detectability when using different HEp-2 cell assays and to problems in the interpretation of DFS70 patterns [39]. It is, indeed, very subjective to differentiate DFS patterns in low titers from DFS-like, e.g., “quasi-homogeneous”, patterns (see Figure 1). When there are also AARD-associated ANA specificities (especially dsDNA antibodies), the DFS patterns can be masked or overlooked. What is most common with AARD is that DFS70 antibodies are associated with Ro/SS-A antibodies [29], but this does not have any influence on the DFS pattern of the mitotic chromatin. This is why DFS or DFS-like patterns are more commonly found with Sjögren’s syndrome than with other AARD. As well, an association between coilin and DFS70 antibodies has been discovered [25, 40]. The typical coilin dots in the interphase nuclei are combined with the DFS pattern in these cases [40]. In summary, one can recommend that, for the purposes of ANA diagnostics, all sera showing chromatin staining in the absence of dsDNA antibodies should be tested for DSF70 antibodies.

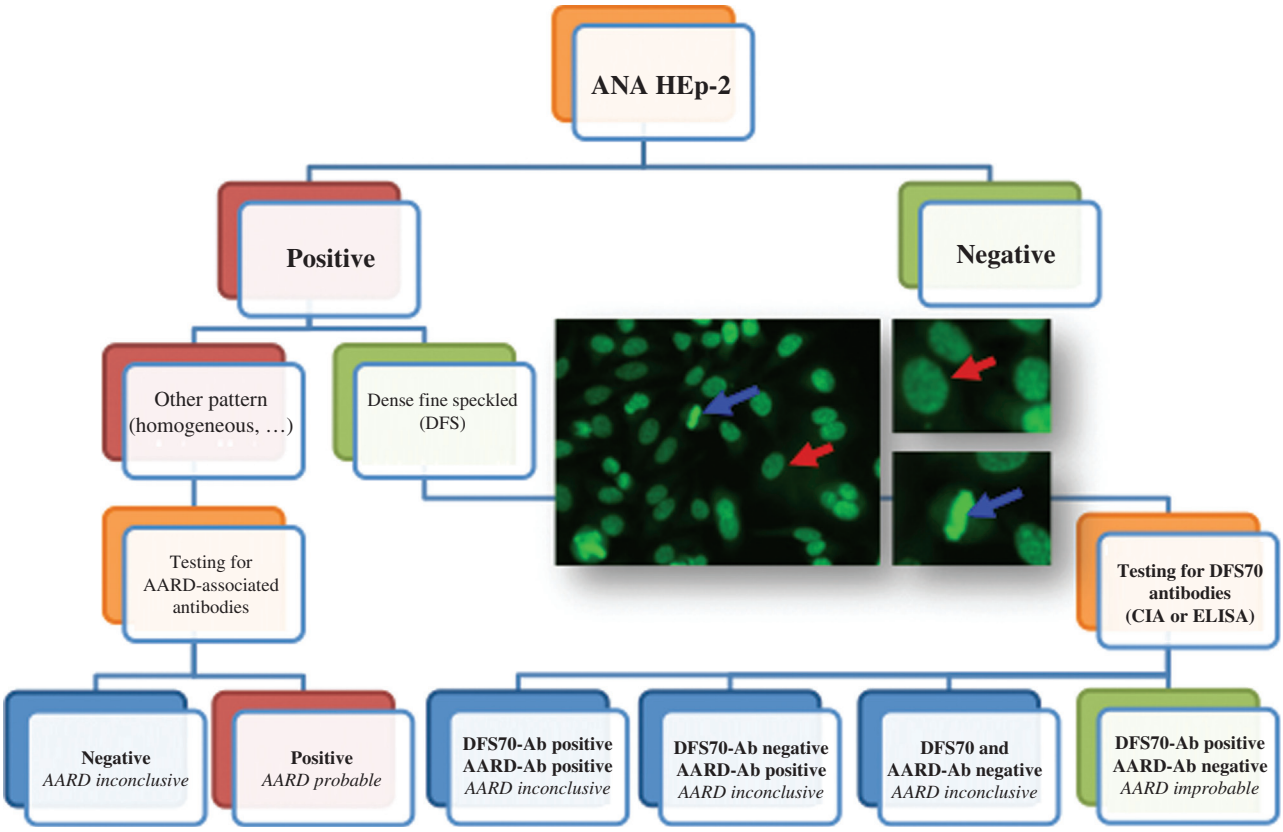

Figure 2: Recommended testing algorithm for the routine diagnosis of ANA-associated rheumatic diseases (AARD), including DFS pattern and DFS70 antibodies (modified according to [35]).
Due to the negative association of the DFS70 antibodies with AARD, the DFS pattern should be differentiated of other ANA pattern (homogeneous, fine granular, nucleolar, centromeric). The following tests are carried out as a function of the IIF pattern on HEp-2 cells (DSF pattern, other ANA pattern, cytoplasmic pattern) and of the clinical question. Patients with negative AARD-associated antibodies and positive DFS70-antibody results have a (very) low probability for the presence or development of AARD.

Diagnostic strategy

Despite all progress in establishing specific autoantibody assays, the ANA screening by means of IIF on HEp-2 cells is still indispensable for quality-oriented laboratory diagnostics of AARD [1, 41, 42]. The findings on the relevance of DFS patterns and DFS70 antibodies open up new possibilities for optimizing serological stepwise diagnostics in connection with suspected AARD. ANA are considered reliable screening parameters for AARD, and are even used as classification criteria for SLE, but they can also lead to misdiagnoses and, in the event of unclear or strange clinical symptoms, can cause unnecessary concern among physicians and patients. Therefore, DFS70 antibodies are valuable new biomarkers for an improved interpretation of positive ANA in connection with negative findings for AARD-associated autoantibodies, and should be integrated with modified test algorithms (Figure 2) in order to avoid unnecessary referrals and follow-up tests on ANA-positive individuals.

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