Lupus: Open Access

OPEN ACCESS Freely available online

Commentary

Does the Dense Fine Speckled Pattern in an ANA Test Actually Tell Us Anything Important Clinically?

Mia C. Lundgren*, John T. Crosson

Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN, USA

ABSTRACT

Antinuclear Antibody (ANA) testing is used as a screening tool for Systemic Autoimmune Rheumatic Disease (SARD). While most patterns identified have known disease associations, the dense fine speckled (ANA-DFS) pattern has no confirmed clinical correlations. For this reason, the ANA-DFS pattern has generated confusion and frustration for both patient and provider. Previous studies have observed a higher frequency of this pattern in healthy individuals compared to patients with SARD. Additional studies have shown associations with other diseases including, but not limited to, interstitial cystitis, skin disease, thyroid disease, ocular disease, prostate cancer, and fibromyalgia. In our recent retrospective study, the objective was to determine the prevalence of SARD among 425 patients with the ANA-DFS pattern and to determine the prevalence of non-SARD along with possible clinical associations. The prevalence of SARD was 24%, a much higher frequency compared to prior studies. The most common non-SARD were atopic disorders (21.2%), fibromyalgia/chronic pain syndrome/chronic fatigue syndrome (17.6%), and skin disorders (16.7%). Given that over 75% of the patients with the ANA-DFS pattern from our study were noted to have an important inflammatory disorder requiring treatment and that almost 25% had a disorder requiring management by Rheumatology, the ANA-DFS pattern appears to be an indicator of a proinflammatory microenvironment.

Keywords: Antinuclear antibodies; Dense fine speckled; Lens epithelium-derived growth factor; DFS70; Systemic autoimmune rheumatic disease.

DESCRIPTION

In the field of Antinuclear Antibody (ANA) testing, the significance of the ANA Dense Fine Speckled (ANA-DFS) pattern, as well as the role of anti-DFS70/LEDGF antibodies, is currently one of the most discussed topics. ANA testing is an important screening test in the diagnostic workup for Systemic Autoimmune Rheumatic Diseases (SARD), such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Sjögren's syndrome, systemic sclerosis, dermatomyositis/polymyositis, and mixed connective tissue disease. The gold standard for ANA detection is Indirect Immuno Fluorescence (IIF) microscopy, which uses Human Epithelial type-2 (HEp-2) cells as a substrate to highlight specific antibody-antigen interactions in different parts of the cell. These interactions, or patterns, were further defined and categorized at the International Consensus on Antinuclear Antibody Pattern (ICAP) in 2014 which led to the identification of 29 distinct patterns [1]. Certain patterns, such as the homogeneous pattern, have known antigen and disease associations. To date, the only confirmed Antigen associated with the ANA-DFS pattern is DFS70/LEDGF. This protein is expressed in many cell types, including lens epithelial cells, keratinocytes, fibroblasts, and cancer cells. It may play a role in cell survival and in the protection against environmental stress through the activation of stress-related proteins. However, the role of anti-DFS70/LEDGF antibodies remains largely unknown.

There are no specific confirmed clinical associations with the ANA-DFS pattern, though it has been reported to be rare in SLE, Sjögren's syndrome, and systemic sclerosis [1]. Since its first association with interstitial cystitis in 1994, there have been additional reports in the literature linking the ANA-DFS pattern to other non-SARD diseases, including atopic dermatitis, asthma, psoriasis, alopecia areata, Vogt-Harada syndrome, Hashimoto's thyroiditis, ocular disease, prostate cancer, chronic fatigue syndrome, and fibromyalgia [2-7]. Recently, there has been increased interest in the potential use of anti-DFS70/LEDGF as a marker to exclude a diagnosis of SARD, an idea supported by prior studies that demonstrated the rare prevalence of anti-DFS70/LEDGF in SARD patients compared to healthy individuals [8, 9]. In our research article recently published by the Journal of Immunological Methods, we sought to determine the prevalence of SARD among a population of patients with the ANA-DFS pattern and to discern

Correspondence to: Mia C. Lundgren, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN, USA, E-mail: lund1111@umn.edu

Received: January 15, 2021; **Accepted:** January 29, 2021; **Published:** February 05, 2021

Citation: Lundgren MC, Crosson JT (2021) Does the Dense Fine Speckled Pattern in an ANA Test Actually Tell Us Anything Important Clinically. Lupus: Open Access. 6:S1:001.

whether there were other diseases associated with this particular ANA pattern [10].

Our retrospective study included 425 patients from our university health care system with a positive ANA-DFS pattern consecutively from August 2017 to September 2018. Patient sera underwent ANA testing by IIF on HEp-2 cell substrates (Euroimmun, Germany). The study included 335 women (78.8%) and 90 men. The mean age for women was 44.0 years (range 3-92); men 43.7 (4-85). Family Practice was most frequently the ordering provider (45%), and the most common indications for ordering the ANA test were joint pain, fatigue, and a rash or skin lesion. Almost half (49%) were referred to Rheumatology. There were 102 patients with SARD (24%). The SARD patients had a higher frequency of joint pain, referrals to Rheumatology, and abnormal follow-up immunological tests. The prevalence of SARD increased with increasing titer, and the most common SARD were seronegative or seropositive RA and UCTD (Table 1). The frequency of SLE was highest in the highest titer group. The most common non-SARD diseases were atopic disorders (21.2%), some combination of fibromyalgia, chronic pain syndrome, and chronic fatigue syndrome (17.6%), and skin disorders (16.7%) (Table 2).

Table 2: Prevalence of SARD diseases in ANA-DFS positive patients by titer a,b.

Non-SARD Diagnosis	Titer		
	1:40 to 1:80 n=76 (%)	1:160 to 1:320 n=165 (%)	1:640 to >1:1280 n=82 (%)
Undetermined	12 (15.8)	20 (12.1)	23 (28)
Skin disorders	13 (17.1)	22 (13.3)	4 (4.9)
Fibromyalgia	5 (6.6)	22 (13.3)	11 (13.4)
Osteoarthritis	5 (6.6)	17 (10.3)	3 (3.7)
Chronic pain syndrome	3 (3.9)	6 (3.6)	3 (3.7)
Raynaud's	2 (2.6)	9 (5.5)	1 (1.2)
Atopic disorders	2 (2.6)	13 (7.8)	3 (3.7)
Hashimoto's thyroiditis	3 (3.9)	3 (1.8)	3 (3.7)

Abbreviations: SARD, systemic autoimmune rheumatic disease; ANA, antinuclear antibody; DFS, dense fine speckled.
a Results from this table exclude patients with a past medical history of SARD.
b Total percentage is <100% in each titer group, because not all diagnoses are represented.

Table 1: Prevalence of SARD diseases in ANA-DFS positive patients by titer a,b.

SARD n=102	Titer		
	1:40 to 1:80 n=17 (%)	1:160 to 1:320 n=55 (%)	1:640 to > 1:1280 n=30 (%)
SLE/cutaneous lupus	2 (11.8)	6 (10.9)	5 (16.7)
Seropositive RA	5 (29.4)	11 (20)	5 (16.7)
Seronegative RA	8 (47.1)	18 (32.7)	6 (20)
Juvenile RA	0 (0)	8 (14.5)	5 (16.7)
Sjögren's syndrome	1 (5.9)	10 (18.2)	2 (6.7)
Ankylosing spondylitis	0 (0)	5 (9.1)	1 (3.3)
Psoriatic arthritis	1 (5.9)	7 (12.7)	1 (3.3)
Systemic sclerosis/scleroderma	0 (0)	1 (1.8)	0 (0)
Dermatomyositis/polymyositis	0 (0)	2 (3.6)	3 (10)
Mixed connective tissue disease	0 (0)	0 (0)	1 (3.3)
Undifferentiated connective tissue disease	3 (17.6)	7 (12.7)	5 (16.7)

Abbreviations: SARD, systemic autoimmune rheumatic disease; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.
a Results from this table take into account a past medical history of SARD in addition to final diagnosis.
b Total percentage is >100% in some titer groups, because some patients had >1 SARD.

Given the high frequency of symptomatic patients and disease processes with an immunologic basis (including SARD), we argue that the ANA-DFS pattern may actually be an indicator of a proinflammatory microenvironment rather than a marker associated with healthy individuals or one that potentially excludes a SARD diagnosis. Previous studies have observed a higher prevalence of the ANA-DFS pattern in healthy individuals compared to SARD patients [8, 9]. However, on closer review of the study by Mariz et al, the frequency of ANA-positivity overall in the healthy population was only 12.9%, and the ANA-DFS pattern was observed in only 39 of 118 ANA-positive individuals (33.1%). Other studies have shown a low prevalence of anti-DFS70/LEDGF auto reactivity in patients with SARD, particularly isolated auto reactivity, compared to healthy individuals [8-11]. ICAP has acknowledged this negative association between anti-DFS70/LEDGF and SARD but states there must be isolated auto reactivity without the presence of other common extractable nuclear antigens in order to be valid [12]. More recent studies have observed higher frequencies of anti-DFS70/LEDGF auto reactivity among SARD patients, suggesting anti-DFS70/LEDGF may not be a good exclusion marker for SARD [13]. Because our study was retrospective, determination of anti-DFS70/LEDGF was not available for any of the patients.

While the significance of anti-DFS70/LEDGF antibodies is largely unknown, the ANA-DFS pattern may be indicative of an underlying antigen-antibody interaction that plays a role in or is a marker of either single or multiple inflammatory disorders. DFS70/LEDGF is the only target auto antigen identified to date. However, it is unlikely that the anti-DFS70/LEDGF antibody is the only antibody

responsible for the ANA-DFS pattern. Studies have shown that the ANA-DFS pattern is not specific for anti-DFS70/LEDGF. In one study, only 14% of 94 ANA-DFS positive individuals were positive by anti-DFS70/LEDGF ELISA, and, in other studies, only 50% of ANA-DFS positive patients were positive for anti-DFS70/LEDGF by chemiluminescent or immunoblot methods.[13-15] More research is necessary to identify additional antibody specificities.

Currently, we are pursuing further studies on an expanded data base of patients with the DFS pattern by IIF ANA. We are particularly looking at the incidence of inflammatory markers in all of these patients and also looking at the frequency of the DFS pattern among all the ANAs performed during the same time period. We hope, using standard statistical methods, to be able to more precisely define the clinical significance of the ANA-DFS pattern. When final control of the SARS-CoV2 pandemic allows us to do prospective studies, we plan, in collaboration with Euroimmun, to determine the frequency of antibodies to the DFS70/LEDGF antigen in patients with the ANA-DFS pattern. We also expect to be able to begin looking for other auto antigens whose antibodies produce the DFS pattern by IIF ANA.

REFERENCES

1. Chan EK, Damoiseaux J, Carballo OG, Conrad K, Francescantonio PL, Fritzler MJ, et al. Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014-2015. Front Immunol, 2015;6(2):410-412.
2. Ochs RL, Stein TW, Peebles CL, Gittes RF, Tan EM. Autoantibodies in interstitial cystitis. J Urol, 1994;151(3):587-92.
3. Ochs RL, Mahler M, Basu A, Rios-Colon L, Sanchez TW, Andrade EL, et al. The significance of autoantibodies to DFS70/LEDGFp75 in health and disease: integrating basic science with clinical understanding. Clin Exp Med.2016;16(3):273-93.
4. Ochs RL, Tan M, Chan EKL, Hui G, Si Y, Muro Y, et al. Autoantibodies to DFS 70 kd/transcription coactivator p75 in atopic dermatitis and other conditions. J Allergy Clin Immunol. 2000;105(6):1211-1220.
5. Takeichi T, Sugiura K, Muro Y, Matsumoto K, Ogawa Y, Tomita Y, et al. Overexpression of LEDGF/DFS70 induces IL-6 via p38 activation in HaCaT cells, similar to that seen in the psoriatic condition. J Invest Dermatol, 2010;130(12):2760-2767.
6. Okamoto M, Ogawa Y, Watanabe A, Aoki N, Muro Y, Tomita Y, et al. Autoantibodies to DFS70/LEDGF are increased in alopecia areata patients. J Autoimmun. 2004;23(3):257-266.
7. Jeong J, Kim DH, Park G, Kim SK, Park S. Clinical significance of anti-dense fine speckled 70 antibody in patients with fibromyalgia. Korean J Intern Med. 2019;34(2):426-433.
8. Mariz HA, Saito EI, Barbosa SH, Rodrigues SH, Dellavance A, Luis EC, et al. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum. 2011;63(1):191-200.
9. Mahler M, Parker T, Peebles CL, ANDRADE EL, SWART A, CARBONE Y, et al. Anti-DFS70/LEDGF antibodies are more prevalent in healthy individuals compared to patients with systemic autoimmune rheumatic diseases. J Rheumatol. 2012;39(11):2104-2110.
10. Lundgren MC, Sapkota S, Peterson DJ, and Crosson JT. The antinuclear antibody dense fine speckled pattern and possible clinical associations: An indication of a proinflammatory microenvironment. J Immunol Methods. 2021;488(15): 112901-112904.
11. Muro Y, Tomita Y, Morita Y, Sugiura K. High concomitance of disease marker autoantibodies in anti-DFS70/LEDGF autoantibody-positive patients with autoimmune rheumatic disease. Lupus. 2008; 17(3):171-176.
12. Damoiseaux J, Andrade CEL, Carballo OG, Conrad k, Fritzle JM, Chan EK, et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective. Ann Rheum Dis. 2019;78(7):879-889.
13. Yumuk Z, Demir M. Clinical value of anti-DFS70 antibodies in a cohort of patients undergoing routine antinuclear antibodies testing. J Immunol Methods. 2020;480(25):112750-112754.
14. Bizzaro NE, Tonutti E, Villalta D. Recognizing the dense fine speckled/lens epithelium-derived growth factor/p75 pattern on HEP-2 cells: not an easy task! Comment on the article by Mariz et al. Arthritis Rheum. 2011;63(12):4036-4038
15. Bizzaro N, Pesente F, Cucchiaro F, Infantino M, Tampoia M, Fabris M, et al. Anti-DFS70 antibodies detected by immunoblot methods: A reliable tool to confirm the dense fine speckles ANA pattern. J Immunol Methods. 2016;436(15):50-53.