EJR European Journal of Rheumatology

DOI: 10.5152/eurjrheum.2015.0003

Original Investigation

Frequency of dense fine speckled pattern in immunofluorescence screening test

Aslı Gamze Şener, İlhan Afşar

Abstract

Objective: The presence of antinuclear antibodies (ANA), directed against intracellular antigens, is a distinctive feature of systemic autoimmune rheumatic diseases (SARDs). The standard test for antinuclear antibody screening is the indirect immunofluorescence (IIF). Anti-dense fine speckled 70 (anti-DFS70) antibodies were initially identified as an ANA IIF pattern from a patient with interstitial cystitis, but they were later associated with various other conditions. The objective of the study was to determine the frequency of anti-DFS70 antibodies in a cohort of patients undergoing routine ANA testing.

Material and Methods: From January 2011 to January 2012, a total of 5800 serum samples were screened for ANA by IIF (Euroimmune AG, Lübeck, Germany). DFS pattern was searched.

Results: ANA were present in 1302 (22.4%) of all patients. There were 16 (1.2%) anti-DFS70 antibody-positive patients. The number of females and males who have anti-DFS70 antibody was eleven and five, respectively. All of the samples presented a titer of ≥1/320. There was one patient with SARD from the rheumatology department. Another 15 patients were from gastroenterology, endocrinology, and general internal medicine.

Conclusion: Although a distinctive clinical association has not been reported, anti-DFS70 have been proposed as a significant biomarker for the exclusion of SARD. The present study is a preliminary study. There is a need for a reliable assay to ensure reactivity to DFS70 and screening large populations.

Keywords: Anti-DFS70 antibodies, antinuclear antibodies, immunofluorescence method

Introduction

The presence of antinuclear antibodies (ANAs), directed against intracellular antigens, is a distinctive feature of systemic autoimmune rheumatic diseases (SARDs). The standard test for antinuclear antibody screening is the indirect immunofluorescence (IIF). The typical dense fine speckles IIF staining pattern is recognized as uniformly distributed fine speckles throughout interphase nuclei and on metaphase chromatin (1). Anti-dense fine speckled 70 (anti-DFS70) antibodies were initially identified as an ANA IIF pattern from a patient with interstitial cystitis, but they were later associated with various other conditions (1). The low prevalence of anti-DFS70 autoantibodies in patients with SARD represents a potentially important biomarker to discriminate SARD from ANA-positive healthy individuals and/or other inflammatory conditions. It was suggested that the presence of isolated anti-DFS70 antibodies could be used to exclude the diagnosis of SARD (2, 3). The objective of the study was to determine the frequency of anti-DFS70 antibodies in a cohort of patients undergoing routine ANA testing. It was a retrospective study without modification in the follow-up of patients.

Material and Methods

From January 2011 to January 2012, a total of 5800 serum samples from a spectrum of hospital departments were screened for ANA by IIF. The patterns were searched by using the HEp-2010/Liver (Monkey) IIF kit (Euroimmun AG, Germany). The serum samples were processed in a dilution of 1:100 and conjugated with specific antihuman IgG (Euroimmune AG, Lübeck, Germany). The fluorescence intensity was scored at ×400, semi-quantitatively from 1+ to 4+ relative to the intensity of the positive (4+) and negative control. The test result was discarded if the positive control sample failed to show the precise results. The serum samples, which were positive for DFS pattern, were re-evaluated in dilutions of 1:320 and 1:1000 by IIF. In addition, these samples were further processed by line immunoassay (Euroimmune AG, Lübeck, Germany) for examining its concomitance with various antigens. Nylon strips coated with recombinant and purified antigens as discrete lines with plastic backing coated with antigens nRNP/Sm, Sm, SSA, Ro-52, SSB, Scl-70, PM-Scl, PCNA, Jo-1, CENP-B, dsDNA, nucleosomes, histones, ribosomal protein-P, and anti-mitochondrial antibodies (AMA-M2) were used, along with a control band.

Department of Medical Microbiology, İzmir Katip Çelebi University Atatürk Training and Research Hospital, İzmir, Turkey

Address for Correspondence: Aslı Gamze Şener, İzmir Katip Çelebi Üniversitesi Atatürk Eğitim ve Araştırma Hastanesi, Medikal Mikrobiyoloji Anabilim Dalı, İzmir, Türkiye
E-mail: agsener@gmail.com

Submitted: 13.01.2015
Accepted: 02.03.2015
Available Online Date: 20.05.2015

Results

ANAs were present in 1302 (22.4%) patients. Anti-DFS70 antibody was detected in 16 (1.2%) patients. The number of females and males who have anti-DFS70 antibody was eleven and five, respectively. All of the samples presented with a titer of ≥1/320. DFS samples had no positivity on line immunoassay. There was one patient with SARD from the rheumatology department. Another 15 patients were from gastroenterology, endocrinology, and general internal medicine (Table 1).

Table 1. Departments and diagnosis of the patients that DFS-positive

Patient	Department	Diagnosis	Dilution
1	Gastroenterology	Toxic hepatitis	1:320
2	Gastroenterology	Chronic hepatitis B	1:320
3	Endocrinology	Thyroiditis	1:320
4	Endocrinology	Thyroiditis	1:320
5	Endocrinology	Diabetes	1:320
6	Endocrinology	Thyroiditis	1:320
7	General int. medicine	Haemolytic anemia	1:1000
8	General int. medicine	Anemia	1:1000
9	General int. medicine	Anemia+dermatitis	1:320
10	General int. medicine	Diabetes+dermatitis	1:320
11	General int. medicine	Cystitis	1:320
12	General int. medicine	Behcet disease	1:320
13	General int. medicine	Fibromyalgia	1:320
14	General int. medicine	COPD	1:320
15	General int. medicine	CTD	1:320

COPD: Chronic obstructive pulmonary disease; CTD: Connective tissue disease

Discussion

Although a distinctive clinical association has not been reported, anti-DFS70 has been proposed as a significant biomarker for the exclusion of SARD. A suggestion is that samples with a DFS staining pattern identified by IIF should be tested for anti-DFS70 antibodies using a specific immunoassay (2, 3). The detection of anti-DFS70 as detected by IIF may be problematic because these sera may be accompanied by other antibodies such as anti-dsDNA, anti-SS-A/Ro, or anti-Sm, which may mask the DFS IIF staining pattern (4). Muro et al. (2) examined the presence of various disease-marker autoantibodies in anti-DFS70antibody-positive patients with autoimmune rheumatic disease. They examined serum samples from 500 patients with various types of autoimmune rheumatic disease for anti-DFS70 antibodies by indirect immunofluorescence and immunoblotting. Twenty-two patients were positive for anti-DFS70 antibodies. They reported that eighteen patients also had disease-marker autoantibodies including anti-double stranded DNA, anti-cardiolipin, anti-SS-A, or other antibodies. In another study (1), the coexistence of other autoantibodies was similar: 5/7 anti-DFS70 positive SLE patients were positive for anti-dsDNA, and one for anti-Sm antibodies. Only 1/7 SLE patients with anti-DFS70 antibodies had no additional detectable autoantibodies. According to these data, anti-DFS70 antibodies are rarely observed in SARD and if observed, they are usually accompanied by additional SARD related autoantibodies.

Dellavance et al. (5) examined serum samples for the DFS pattern within a 2-year period. Positive samples with consistent clinical information were studied further by IIF with isotype-specific conjugate and immunoblotting. Among 13.641 ANA-positive samples, 5081 (37%) presented the DFS pattern. According to the study, anti-LEDGF/p75 antibodies were a common finding among ANA-positive individuals with no evidence of rheumatic autoimmune disease, and should be regarded as a low specificity finding even when in moderate or high titer. Mariz et al. (6) informed that anti-DFS antibodies are more prevalent in healthy individuals than in patients with SARD and that anti-DFS-positive individuals did not develop SARD after a clinical follow-up of 4 years. Similarly, another study confirms previous observations that anti-DFS70 antibodies are significantly more prevalent in healthy individuals compared to patients with SARD and other conditions (1). Watanabe et al. (7) analyzed the sera of 597 healthy hospital workers for ANAs and for anti-DFS70 antibodies by IIF with HEp-2 cells as a substrate and by immunoblotting using DFS70 recombinant protein and whole HeLa cell extract. ANAs were present in 20% of all individuals by IIF. They informed that anti-DFS70 antibody positivity is rare in patients with systemic autoimmune diseases, introducing the anti-DFS70 antibody examination as a screening test for ANA-positive persons that could be used to rule out systemic autoimmune diseases, resulting in considerable cost-saving potential.

When the ANA HEp-2 test became available in the 1960s, predominantly rheumatologists and clinical immunologists ordered the ANA test. With the emerging recognition that many other diseases with autoimmune features are also associated with ANAs, a broader range of clinical disciplines (such as primary care, dermatology, nephrology, gastroenterology, neurology, oncology, hematology, obstetrics, gynaecology, as well as cardiology) currently order the ANA test (8). In line with the previous study, our study revealed that anti-DFS positive patients who applied from various departments. There was no SARD diagnosis among anti-DFS70-positive patients. Accurate IIF pattern recognition, interpretation, and reporting of results to clinicians are of high importance because it could decrease the necessity of urgent referral of patients with a positive ANA for tertiary care consultation and evaluation. The accurate identification of the DFS IIF pattern may be challenging for routine diagnostic laboratories. The presence of ANA is considered reliable screening clinical indicators for SARD. However, not all sera demonstrating the DFS pattern are from healthy individuals and it remains unclear whether this IIF staining pattern is universally recognized in clinical diagnostic laboratories (8). Our data support previous observations that SARD is less prevalent in patients with anti-DFS70 antibodies than in patients with other patterns such as homogeneous, speckled, homogeneous and speckled, nucleolar, mixed pattern, and centromere. In our tertiary hospital, DFS pattern is determined by IIF and the clinician is acquainted with this pattern for evaluation. In addition, the test results and the significance of the findings need to be clearly explained to clinicians. The present study is a preliminary study. A significant limitation of our study is the lack of following-up patients. Similar studies with different study groups from

various countries are needed to investigate the prevalence of this antibody. Moreover, there is a need for a reliable assay to ensure reactivity to DFS70 and screening a large population.

Ethics Committee Approval: Ethics Committee Approval was not received due to the retrospective nature of the study.

Informed Consent: Informed consent was not received due to the retrospective nature of the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - A.G.S.; Design - A.G.S.; Supervision - A.G.S.; Materials - A.G.S.; Data Collection and/or Processing - I.A.; Analysis and/or Interpretation - A.G.S.; Literature Review - A.G.S.; Writer - A.G.S.; Critical Review - A.G.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The author declared that this study has received no financial support..

References

1. Mahler M, Parker T, Peebles CL, Andrade LE, Swart A, Carbone Y, et al. Anti DFS70/LEDGFantibodies are more prevalent in healthy individuals compared to patients with systemic autoimmune rheumatic diseases. J Rheumatol 2012; 39: 2104-10. **[CrossRef]**
2. Muro Y, Sugiura K, Morita Y, Tomita Y. High concomitance of disease marker autoantibodies in anti-DFS70/LEDGF autoantibody-positive patients with autoimmune rheumatic disease. Lupus 2008; 17: 171-6. **[CrossRef]**
3. Mahler M, Hanlyand JG, Fritzler MJ. Importance of the dense fine speckled pattern on HEp-2 cells and anti-DFS70 antibodies for the diagnosis of systemic autoimmune diseases. Autoimmunity Reviews 2011; 11: 642-5. **[CrossRef]**
4. Miyara M, Albesa R, Charuel JL, El Amri M, Fritzler MJ, Ghillani-Dalbin P, et al. Clinical phenotypes of patients with anti-DFS70/LEDGF antibodies in a routine ANA referral cohort. Clin Dev Immunol 2013; 2013: 703759. **[CrossRef]**
5. Dellavance A, Viana VS, Leon EP, Bonfa ES, Andrade LE, Leser PG. The clinical spectrum of antinuclear antibodies associated with the nuclear dense fine speckled immunofluorescence pattern. J Rheumatol 2005; 32: 2144-9.
6. Mariz HA, Sato EI, Barbosa SH, Rodrigues SH, Dellavance A, Andrade LE. Pattern on the antinuclear antibody-HEp-2 is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. Arthritis Rheum 2011; 63: 191-200. **[CrossRef]**
7. Watanabe A, Kodera M, Sugiura K, Usuda T, Tan EM, Takasaki Y, et al. Anti DFS antibodies in 597 healthy hospital workers. Arthritis Rheum 2004; 50: 892-900. **[CrossRef]**
8. Mahler M, Fritzler MJ. The clinical significance of the dense fine speckled immunofluorescence pattern on Hep-2 cells for the diagnosis of systemic autoimmune diseases. Clin Dev Immunol 2012; 2012: 494356. **[CrossRef]**