Prevalence of anti-DFS70 antibodies in patients with and without systemic autoimmune rheumatic diseases

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Abstract

Objective

Autoantibodies to the dense fine speckled 70 (DFS70) antigen are common among antinuclear antibodies (ANA) positive healthy individuals (HI). We assessed the prevalence of anti-DFS70 antibodies in patients with and without ANA-associated rheumatic diseases (AARDs) by two methods: chemiluminescent immunoassay (CIA) and an indirect immunofluorescence (IIF) assay based on immunoadsorption for DFS70.

Methods

Fifty-one ANA-positive sera samples from patients with confirmed clinical diagnosis of AARD, 92 samples from HI and 85 samples submitted to a reference laboratory for routine ANA testing were evaluated for the presence of anti-DFS70 antibodies. The samples were evaluated by QUANTA Flash DFS70 CIA using BIO-FLASH instrument and by NOVA Lite selected HEp-2 kit on NOVA View – an automated IIF system. Sera with DFS positive pattern were pre-absorbed with highly purified human DFS70 antigen, and then tested again.

Results

Twenty-four samples (10.5%) tested by QUANTA Flash DFS70 CIA were positive for anti-DFS70 antibodies. The prevalence of monospecific anti-DFS70 antibodies was significantly higher in healthy subjects than in patients with AARDs (10.9% vs. 1.9%, p=0.02). The frequency of anti-DFS70 antibodies in samples submitted for routine ANA testing was 15.2%. A very good agreement was found between CIA and the DFS pattern identified by the automated HEp-2 IIF (kappa=0.97). In 80% of the samples obtained from patients without AARDs, immunoadsorption effectively inhibited the anti-DFS70 antibodies.

Conclusion

The data confirm that mono-specific anti-DFS70 antibodies are a strong discriminator between ANA positive HI and AARD patients, and their evaluation should be included in ANA testing algorithms.

Key words

antinuclear antibodies (ANA), anti-dense fine speckled 70 antibodies, ANA-associated rheumatic diseases, chemiluminescent immunoassay, indirect immunofluorescence, immunoadsorption, autoimmunity, autoantibodies

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Introduction

ANA-associated rheumatic diseases (AARDs) constitute a diverse group of disorders characterised by the presence of autoantibodies against intracellular antigens, especially antinuclear antibodies (ANAs) (1). Positive ANA is one of the classification criteria for systemic lupus erythematosus (SLE), Sjögren's syndrome (SjS) and systemic sclerosis (SSc) (2-4). Moreover, the existence of ANA might predate the onset and prognosis (5, 6) of several autoimmune diseases including rheumatoid arthritis, myositis, SjS and SLE (7-9). These autoantibodies were traditionally detected by indirect immunofluorescence (IIF) assay on HEp-2 cells, and this method was recently proposed as a gold standard by the American College of Rheumatology (ACR) (10). However, approximately 20% of healthy individuals (HI) are found to be ANA positive, and in the majority of cases, this positivity is associated with anti-dense fine speckled 70 (anti-DFS70) antibodies (11). These antibodies are directed against the DNA binding transcription co-activator p75, also known as lens epithelium derived growth factor (LEDGF) (12-14). Different studies have confirmed that isolated anti-DFS70 antibodies were extremely rare in patients with AARDs and therefore their presence may exclude the diagnosis of current AARDs and future development of AARDs in ANA positive healthy subjects (15-20). However they have been reported in myositis with or without other myositis specific antibodies (21). Anti-DFS70 antibodies show a typical DFS pattern on HEp-2 cells and should be confirmed by specific immunoassays (22-24). Different technologies such as chemiluminescent immunoassay (CIA) (17), Immunoblot (19), and ELISA (25, 26) have been used for the detection of anti-DFS70 antibodies. Recently, a novel IIF immunoadsorption method has been developed, in which the anti-DFS70 antibodies are blocked from binding to their natural antigen on HEp-2 cells (27). This immunoadsorption method overcomes a significant limitation of the ANA HEp-2 assay associated with the presence of anti-DFS70 antibodies and significantly increases the specificity of the ANA test for AARDs. In the present study we assessed the prevalence of anti-DFS70 antibodies in patients with and without AARDs and compared two methods for the detection of these antibodies: IIF immunoadsorption and CIA.

Materials and methods

Serum samples

We evaluated the presence of anti-DFS70 antibodies in different groups of samples (Table I). One group included sera samples from 51 patients with confirmed clinical diagnoses of AARDs. All patients were treated in the Zabludowicz Center for Autoimmune Diseases during 2015-2016. All the sera were tested by IIF on HEp-2 cells and were found to be positive for ANA. AARDs among this group included SLE (n=33), primary SjS (n=10), dermatomyositis/polymyositis (n=6) and mixed connective tissue disease (MCTD) (n=2). The second group consisted of 85 serum samples submitted to our laboratory for routine ANA testing. The medical records of the patients in this group were analysed following the laboratory evaluation. The third group of samples was obtained from 92 HI.

The study fulfilled the ethical guidelines of the most recent declaration of Helsinki and received approval by the local ethical committees (Edinburgh, 2000).

Diagnostic assays

QUANTA Flash DFS70 CIA

All samples were performed by QUANTA Flash DFS70 CIA on the BIO-FLASH instrument (Inova Diagnostics, San Diego, USA) in order to evaluate the presence of anti-DFS70 antibodies (18). The BIO-FLASH® instrument is a fully automated chemiluminescent immuno-analyzer, and the principles of this system has been recently described elsewhere (28). The cut-off was defined as 20 chemiluminescence units (CU).

Immunofluorescence assays (IIF) and Immunoadsorption

All samples were tested by NOVA Lite HEp-2 ANA and NOVA Lite HEp-2 Select on the NOVA View instrument, an automated fluorescent microscope

Table I. Prevalence of anti-DFS70 antibodies in different patient groups measured by chemiluminescence immunoassay (CIA).

Clinical characteristics	Total number of patients (n=228)	Numbers of anti-DFS70 positive patients (n=24)
ANA referral cohort	**85**	**13**
Infections	35	3
Malignancy	14	2
IBD	3	1
Asthma	4	0
Atopic dermatitis	3	3
Thromboembolism	4	0
CVID	2	0
Haematological disorders	5	2
Neurological disorders	10	2
Thyroid disorders	5	0
AARD	**51**	**1**
Healthy individuals	**92**	**10**

AARD: ANA-associated rheumatic disease; IBD: inflammatory bowel disease.

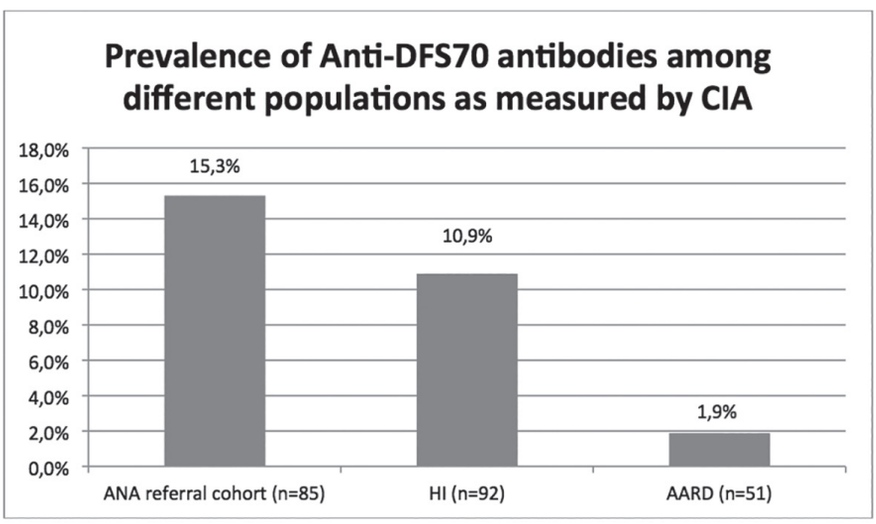


Fig. 1. Prevalence of the Anti-DFS70 antibodies among samples referred for routine ANA testing (ANA referral cohort), healthy individuals (HI) and patients with ANA-associated autoimmune rheumatic disorders (AARD) measured by CIA (n=228).

(Inova Diagnostics, San Diego, CA, USA). Samples were tested with and without DFS70 inhibition. The principles of this assay have been recently summarised elsewhere (27). The interpretation of ANA testing was based on pattern recognition and measuring light intensity units (LIUs) before and after immunoadsorption. Mono-specificity of anti-DFS70 antibodies was defined by successful and complete inhibition of ANA reactivity by the DFS70 antigen in the HEp-2 Select sample buffer.

Detection of specific ANAs

The anti-dsDNA and anti-ENA assays (including the individual antigens RNP, Sm, Scl-70, Jo-1, Ro60, Ro52 and La) were performed by BioPlex 2200 ANA Screen system (29).

Statistical analysis

The data was statistically evaluated using the MedCalc Software (v. 16.4.3; MedCalc Software bvba, Ostend, Belgium). Cohen's kappa agreement tests were carried out to analyse the agreement between IIF and CIA, and *p*-value >0.05 was considered significant.

Results

According to the results of QUANTA Flash DFS70 CIA assay the overall prevalence of anti-DFS70 antibodies was 10.5% (n=24). The prevalence of anti-DSF70 antibodies was significantly higher in HI than in patients with AARD (10.9% *vs*. 1.9%, *p*=0.02; Fig. 1). The prevalence of anti-DFS70 antibodies in samples submitted for routine ANA testing was 15.3%. The retrospective analysis of clinical and serological features of these patients demonstrated that the patients suffered from different diseases, with infections and atopic dermatitis being the most common (3 of 13 patients with each disease). There was no AARD diagnosis among these anti-DFS70 positive patients and no other ANA were found. In addition, the antibody levels measured by CIA were significantly higher in healthy subjects compared to patients with AARDs (*p*=0.01; Fig. 2). In 80% of the samples from patients without AARDs, the immunoadsorption assay effectively inhibited the anti-DFS70 antibodies, reducing false positive ANA results (Fig. 3). A significant agreement was found between QUANTA Flash DFS70 CIA and the DFS pattern identified by automated NOVA Lite HEp-2 (kappa=0.97). Only one SLE patient had anti-DFS70 antibodies accompanied with anti Sm anti dsDNA antibodies and DFS pattern was not inhibited by NOVA Lite HEp-2 Select.

Discussion

The recognition of ANA pattern by IIF using mitotic-rich HEp-2 cell substrate has diagnostic significance and is usually included in an ANA testing routine. It has been well demonstrated that the nuclear homogeneous and coarse speckled patterns are commonly caused by autoantibodies that strongly associate with AARDs. Another standard typical pattern is nuclear dense fine speckled (DFS) pattern that is recognised as uniformly distributed fine speckles throughout the interphase nucleus and on metaphase chromatin. The accurate identification of the DFS pattern may be difficult and may sometimes result in misinterpretation, especially when discriminating between DFS and mixed homogeneous and speckled or quasi-homogeneous patterns (19, 25, 30). Moreover, the DFS pattern may be associated with the presence of anti-

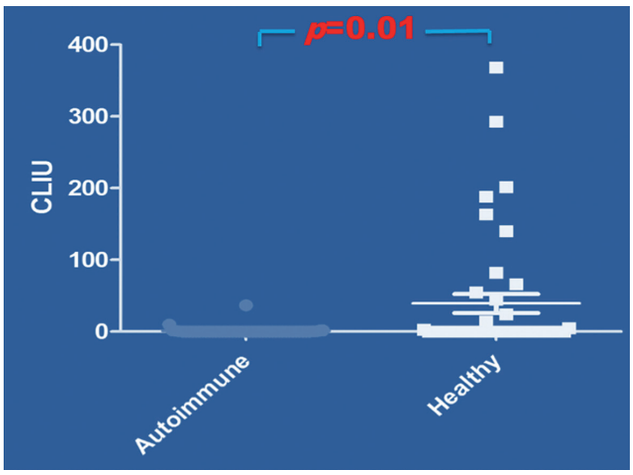
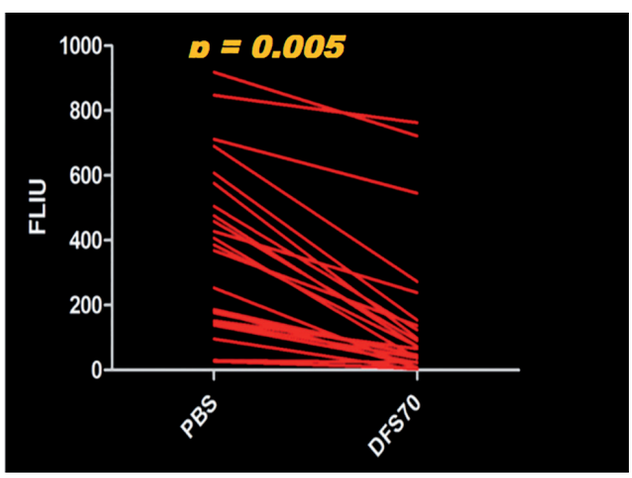


Fig. 2. Level of Anti-DFS70 antibodies in patients with systemic autoimmune rheumatic disorders and healthy subjects measured by CIA. Significantly higher level of anti-DFS70 antibodies was observed in healthy individuals.



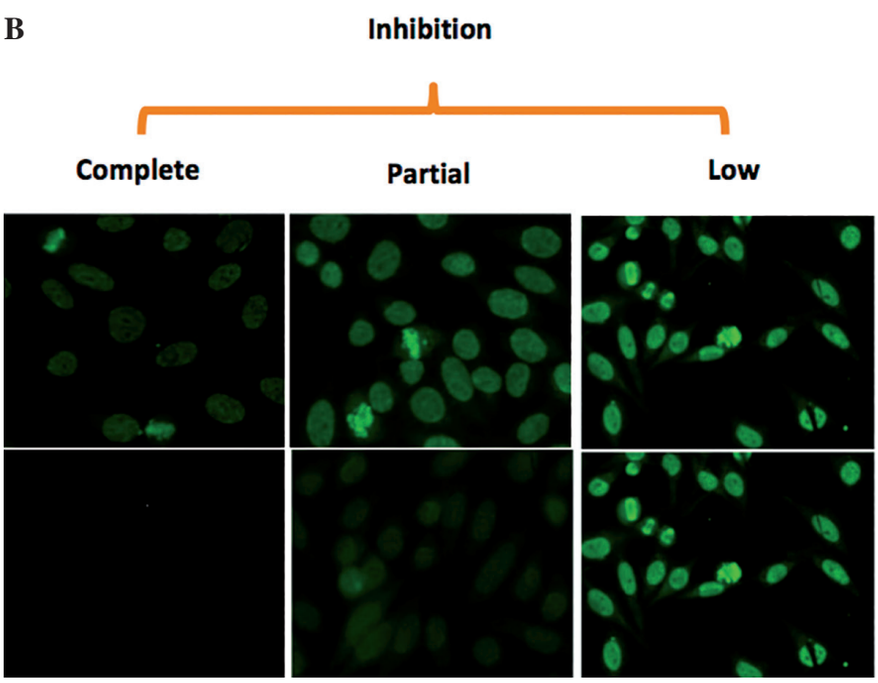


Fig. 3. Characterisation of monospecific anti-DFS70 antibodies by HEp-2 Select assay.
A. Twenty four serum samples were tested in presence and in absence of recombinant human DFS70 antigen. The light intensity units (LIUs) were evaluated by NOVA View automated fluorescence microscope. Significant reduction of LIU was observed after adsorption with DFS70 antigen.
B. Representative IIF pictures for different levels of inhibition of with DFS70 antigen.

DFS70 antibodies but specific ANAs including dsDNA and ENA might lead to similar pattern recognition (15). In light of this, samples with DFS staining pattern identified by IIF should be tested for anti-DFS70 antibodies by a specific assay (*i.e.* ELISA or CIA) (17, 24, 26, 31). Additionally, screening for disease specific antibodies is recommended especially in patients with high clinical suspicion for AARDs.

In the present study we assessed the frequency of anti-DSF70 antibodies in samples obtained from AARDs patients, HI and patients undergoing routine ANA screening due to different reasons. Our cohorts were tested by two different assays: CIA and IIF immunoadsorbtion. Similar to previous investigations, the prevalence of anti-DSF70 antibodies tested by CIA was significantly higher in HI than in patients with AARDs (10.9% *vs.* 1.9%) (31, 32). Only one SLE patient had anti-DFS70 antibodies and they were accompanied with anti-Sm and anti-dsDNA antibodies. This prevalence was relatively low but close to the mean prevalence of anti-DFS70 antibodies accompanied by AARD specific markers (3.8±2.9%) in 1234 AARDs patients from a recent meta-analysis of five studies (32). We did not find isolated anti-DFS70 antibodies in our AARDs cohort, in concordance with this meta-analysis which found solitary anti-DFS70 in a small proportion of AARDs patients (0.7±0.9%). The prevalence of anti-DFS70 antibodies in healthy individuals was consistent with the data reported in the literature (31, 32).

The frequency of anti-DFS70 antibodies in samples submitted to a reference laboratory from an internal medicine department for routine ANA testing was relatively higher than in earlier reports and reached 15.2% (n=13). The retrospective analysis of clinical and serological features of these patients demonstrated that the patients suffered from different diseases, with infections and atopic dermatitis being the most common (3 of 13 patients with each disease). There was no AARD diagnosis among these anti-DFS70 positive patients and no other ANA were found. According to different studies,

the prevalence of the DFS IIF pattern is different and the presence anti-DFS70 antibodies in routine samples fluctuates significantly between 0.8 and 12.3% depending on study cohorts and diagnostic test performance (18, 31-34). For instance, one previous study informed that only 0.8% of 21,512 samples screened for ANA in a clinical laboratory displayed the typical DFS IIF pattern (25). However, an additional study from the same group noted that 86% of the sera exhibiting DFS pattern failed to recognised DFS70 antigen in ELISA (33). Similarly a low prevalence (1.62%) of DFS IIF pattern was found in 3263 routine serum samples, but in all samples this pattern was attributed to the presence of anti-DFS70 antibodies when evaluated by ELISA and/or CIA (18). Another investigation showed that anti-DFS antibodies were present in as much as 12.3% of consecutive samples tested for ANA (34). This controversial data was mainly associated with intra laboratory discrepancies and a reliable uniform assay is required for the detection of anti-DFS70 antibodies. The novel IIF immunoadsorption has been developed that enables identification of IIF pattern following the prior elimination of anti-DFS70 antibodies from the Hep-2 cell substrate and therefore significantly increases the specificity of this assay (27). The use of this original method for diagnostic aims and its efficiency have been already demonstrated in several studies (27, 35). Thus, the development and evaluation of IIF immunoadsorption were done in a multicenter study that collected 99 sera with DFS pattern from three different laboratories. Analysis of these sera using IIF and CIA revealed that anti DFS-70 antibodies were positive in 73.7% of these samples when screened by CIA, and a good quantitative correlation was found between CIA and IIF. IIF immunoadsorption showed a negative staining with no specific pattern in 64.8% of the samples that were positive for anti-DFS70 antibodies when tested by CIA. Moreover, adsorption of anti-DFS70 antibodies prior to ANA IIF test eliminated the masking effect of these antibodies on other clinically relevant patterns in mixed IIF staining patterns and therefore clinically relevant patterns were clearly observed. Our results were compatible with those described above and showed that complete effective inhibition of the anti-DFS70 antibodies was detected in 80% of the samples obtained from patients without AARDs. In addition, the DFS pattern was not inhibited by adsorption procedure in the sera sample from the single SLE patient who had disease specific antibodies.

Numerous studies have addressed themselves to evaluate the clinical value of anti DFS-70 antibodies in routine laboratory practice (26, 36, 37). These studies reinforced the conclusion of studies conducted on healthy subjects and patients with AARDs: ANA IIF specificity associated with isolated anti DFS70 antibodies decreases likelihood of AARDs. Therefore, these antibodies may represent attractive clinical biomarkers to potentially exclude a diagnosis of AARD and should be included in the diagnostic algorithms for routine laboratories. Recently, a new ANA algorithm was proposed in one preliminary study that included testing for anti-DFS70 antibodies using CIA as specific assay (38). Comparison between the conventional and modified algorithms was performed in 181 ANA positive patients with suspicion of AARD who were in follow-up for ten years. The implementation of this new ANA workup algorithm resulted in considerable cost-saving potential with significant reduction of both unnecessary follow-up testing for AARDs specific antibodies including anti-ENA and anti-dsDNA antibodies and outpatient clinic visits. Furthermore, a recent publication assessing the clinical and financial efficacy of different methods of ANA testing (39) demonstrated significantly higher sensitivity of single analyte ANA testing compared to line blot testing. This finding accentuated the significance of clinically guided laboratory testing and the importance of refining the current ANA workup algorithm in order to reduce unnecessary costs for health systems, as well as limit redundant tests and potential distress for patients.

Conclusion

Our data confirm that mono-specific anti-DFS70 antibodies are a strong discriminator between ANA positive healthy individuals and AARD patients. In addition, anti-DFS70 antibodies are very common in ANA routine samples. Consequently, the detection of anti-DFS70 antibodies should be included in ANA testing algorithms to aid in the interpretation of ANA positivity without underlying AARD. In addition, anti-DFS70 antibodies should be considered for future revisions of the disease classification criteria.

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