The Prevalence and Determinants of Anti-DFS70 Autoantibodies in an International Inception Cohort of Systemic Lupus Erythematosus Patients

Running Head: Anti-Dense Fine Speckled70 Autoantibodies in SLE

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Manuscript Word Count: 3702

Summary Word Count: 211

Number of Tables: 1

Number of Figures: 0

Number of Supplemental Tables: 3

SUMMARY

Autoantibodies to dense fine speckles 70 (DFS70) are purported to rule out the diagnosis of SLE when they occur in the absence of other SLE-related autoantibodies. This study is the first to report the prevalence of anti-DFS70 in an early, multi-national inception SLE cohort and examine demographic, clinical, and autoantibody associations. Patients were enrolled in the Systemic Lupus International Collaborating Clinics (SLICC) inception cohort within 15 months of diagnosis. The association between anti-DFS70 and multiple parameters in 1137 patients was assessed using univariate and multivariate logistic regression. The frequency of anti-DFS70 was 7.1% (95%CI: 5.7-8.8%), while only 1.1% (95%CI: 0.6-1.9%) were monospecific for anti-DFS70. In multivariate analysis, patients with musculoskeletal activity (Odds Ratio (OR) 1.24 [95% CI: 1.10, 1.41]) or with anti-β2 glycoprotein 1 (OR 2.17 [95% CI: 1.22, 3.87]) were more likely and patients with anti-dsDNA (OR 0.53 [95% CI: 0.31, 0.92]) or anti-SSB/La (OR 0.25 [95% CI: 0.08, 0.81]) were less likely to have anti-DFS70. In this study, the prevalence of anti-DFS70 was higher than the range previously published for adult SLE (7.1 vs 0 - 2.8%) and was associated with musculoskeletal activity and anti-β2 glycoprotein 1 autoantibodies. However, 'monospecific" anti-DFS70 autoantibodies were rare (1.1%) and therefore may be helpful to discriminate between ANA positive healthy individuals and SLE.

INTRODUCTION

Autoantibodies directed against nuclear and other intracellular autoantigens (anti-nuclear antibodies: ANA) are commonly used biomarkers for the diagnosis and classification of systemic lupus erythematosus (SLE) and other ANA-associated rheumatic diseases (AARD)[1,2]. Despite lacking specificity, the American College of Rheumatology (ACR) has identified the indirect immunofluorescence (IIF) ANA test as the diagnostic screening immunoassay of choice for AARD[3]. Of interest, autoantibodies directed against the dense fine speckles 70 (DFS70) nuclear antigen represent an apparent ANA paradox because they are reported to be less frequently in patients with SLE and other AARD than in apparently healthy individuals or those who do not meet diagnostic criteria for AARD{Mahler, 2012 24623 /id;Fitch-Rogalsky, 2014 26100 /id;Bizzaro, 2015 28210 /id;Nilsson, 2015 28173 /id;Conrad, 2016 29863 /id}. Thus, studies to date showing a lower prevalence of anti-DFS70 antibodies in adult patients with SLE compared to healthy adults (0-2.8% versus 2.0 -23.2% when anti-DFS70 is assessed by chemiluminescence immunoassay [CIA]) suggested that these autoantibodies may serve as biomarkers that differentiate ANA positive individuals without an AARD from patients with AARD (Supplemental Table 1). A summation of the published studies on the frequency of anti-DFS autoantibodies in various adult SLE cohorts is shown in Supplemental Table 2.

In previous studies, when anti-DFS70 antibodies were present in an SLE patient, at least one other autoantibody usually accompanied them [4,7,10-12]. Only 1 adult SLE patient reported in the literature (<0.4% of SLE) was identified to have anti-DFS70 antibodies in isolation (or monospecific anti-DFS70 antibodies) [4]. On the other hand, the frequency of monospecific anti-DFS70 autoantibodies in ANA positive apparently healthy patients was reported to be as high as

18.8% when assessed by CIA [5]. Hence, the presence of monospecific anti-DFS70 antibodies was proposed as an exclusion criterion for the diagnosis and classification of SLE [13].

To date, there have been no studies examining the frequency of anti-DFS70 antibodies in an inception cohort of adult onset SLE. The ability to reliably discriminate SLE from ANA-positive individuals without AARD at onset of signs and symptoms could be an important tool that has diagnostic, socioeconomic and other implications, which include avoiding additional unnecessary investigations and undue anxiety among patients and physicians [14]. Evidence that autoantibodies can be detected years before the clinical onset of SLE [15,16] also raises the possibility that anti-DFS70 antibodies can be used as a prognostic discriminator for ANA positive individuals. The present study is the first to determine the frequency of anti-DFS70 antibodies and associations with demographic, clinical and serological features in a large international cohort of newly diagnosed SLE patients.

MATERIALS AND METHODS

Study Design and Setting

This study was performed using data and patient sera collected through the Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) inception cohort [17,18]. Between 1999 and 2012, investigators from 32 centers in 11 countries enrolled patients fulfilling the ACR Classification Criteria for definite SLE [19] within 15 months of diagnosis. The study was approved by the Institutional Review Board at each participating site and was performed in compliance with the Helsinki Declaration.

Clinically defined samples

Demographic and clinical data were collected at enrollment and included age, gender, race/ethnicity, study site, post-secondary education, history of smoking and alcohol consumption, presence of hypertension and nephritis, fulfillment of ACR Classification Criteria, disease activity, and medication use (Supplemental Table 3). Hypertension was defined as being on an anti-hypertensive medication or systolic blood pressure > 140 or diastolic blood pressure > 90; nephritis was based on renal biopsy or fulfillment of the renal item in the ACR Classification Criteria[19]; disease activity was measured by the SLE Disease Activity Index 2000 [SLEDAI-2K] [20].

Indirect Immunofluorescence assay (IIF)

The serum (earliest available on enrollment of each patient) was analyzed by Mitogen Advanced Diagnostic Laboratory (University of Calgary, Calgary, Alberta, Canada). Aliquots of the anonymized SLE sera obtained from a central biobank were stored at -80ºC until required for immunoassays. The IIF immunoassay was initially performed at a screening dilution of 1/160 using the HEp-2000 cell substrate kit (ImmunoConcepts, Sacramento, CA) and fluorescein (FITC) conjugated to anti-human IgG (H + L) according to the manufacturer's instructions. Taking differences in the performance of HEp-2 ANA IIF assays from different manufacturers into consideration [21], ANA were also performed on serum samples with discrepant IIF and DFS70 CIA results using HEp-2 IIF kits from another manufacturer (Inova Diagnostics Inc., San Diego, CA) and results determined by automated IIF platform (NovaView, Inova Diagnostics Inc.) according to the manufacturer's instructions. IIF results were read by technologists with >10 years of experience at Mitogen Advanced Diagnostics as previously described [22].

Chemiluminescence anti-DFS70 antibody assays

All samples were tested for the presence of anti-DFS70 antibodies by CIA (QUANTA Flash, Inova Diagnostics Inc.). The assay used purified full length human recombinant DFS70 coated onto paramagnetic beads contained in a reagent cartridge which was loaded onto the analyzer (BioFlash, Inova Diagnostics Inc.). The principles and protocols of the assay have been previously described [4]. The established cut-off for positive anti-DFS70 antibodies was established by Receiver Operator Characteristic (ROC) by the manufacturer (Inova Diagnostics) and validated by the diagnostic laboratory (Mitogen) and set at >20 chemiluminescent units (CU). Monospecific anti-DFS70 autoantibodies was defined as the presence of anti-DFS70 antibodies in the absence of anti-double-stranded DNA (dsDNA) and other autoantibodies detected in the extractable nuclear antigen (ENA) panel described below. Confirmation of IIF DFS70 reactivity was by an absorption immunoassay (HEp-2 Select: Inova Diagnostics Inc.) wherein anti-DFS70 reactivity is specifically removed from positive sera using methods as previously described [23].

Detection of anti-dsDNA and other autoantibodies

Anti-dsDNA antibodies were quantified using a CIA (Inova Diagnostics Inc.) using the same approach as described above for anti-DFS70 antibodies. Antibodies to PCNA, ribosomal-P, Ro52/TRIM21, SSA/Ro60, SSB/La, Sm, and U1-RNP were detected using the ENA FIDIS Connective Profile kit 13 addressable laser bead immunoassay array (ALBIA: TheraDiag, Paris, France) using a Luminex 200 flow luminometer (Luminex, Austin, TX). All assays were performed according to the manufacturer's instructions. Briefly, anti-human IgG coupled to

phycoerythrin was added to samples and was read on the Luminex 200 system using the MLX-Booster software. Samples were classified as negative at <30 arbitrary units (AU)/mL, weakly positive 30-49 AU/mL, moderately positive 50-80AU/mL, and strongly positive >81AU/mL. Other autoantibodies such as IgG anti-cardiolipin, IgG anti-β2 glycoprotein 1, and lupus anticoagulant were measured in a central laboratory as previously described [24].

Statistical Evaluation

Statistical analysis was performed using Stata 14.1 (StataCorp, College Station, TX, USA). Univariate and multivariate logistic regression analyses were used to examine potential predictors of the odds of having anti-DFS70 antibodies. Potential predictors included: age at diagnosis, disease duration, gender, race/ethnicity, post-secondary education, smoking, alcohol use, hypertension, nephritis at enrolment, ACR Classification Criteria fulfilled (total and individual), SLEDAI-2K (global score and organ system scores), medications (steroids, anti-malarials, immunosuppressive agents), ANA, anti-dsDNA, ENA panel, IgG anti-cardiolipin, IgG anti-β2 glycoprotein 1, and lupus anticoagulant. For the most informative multivariate model, only the remaining statistically significant predictors at the 95% confidence interval (CI) were included, after eliminating all other potential predictors individually, starting with the least likely to be associated with the outcome.

Although we were interested in measuring regional variation, we did not include the different study sites in the primary model as it was correlated with some clinical and serological features. Hence, we have conducted a secondary analysis where the study site is included in addition to the other variables included in the primary model.

RESULTS

1137 patients were enrolled in the study with a mean age of 35.1 years at diagnosis, a mean duration of 0.47 years; 1022 (89.9%) were female, 511/1130 (45.2%) were of non-White ethnic background, and 724/1085 (66.7%) had obtained post-secondary education (Table 1). Three hundred and twelve of 1084 or approximately 29% of the cohort had nephritis at enrolment, the mean global SLEDAI-2K was 5.3, and 80.3% reported a history of steroid use, 73.6% antimalarial use, and 42.6% immunosuppressant use other than biologics.

The frequency of anti-DFS70 autoantibodies as detected by CIA was 7.1% or 81/1137 (95% CI: 5.7, 8.8%) but by contrast, 13/1137 or 1.1% (95% CI: 0.6, 1.9%) were monospecific positive for anti-DFS70 (Table 1). Approximately 94% (1066/1137) of the entire cohort had a positive ANA by IIF on HEp-2000 substrate; 6.2% (5/81) of the anti-DFS70 positive sera by CIA had a negative ANA by IIF and 6.3% (66/1056) of the anti-DFS70 negative sera had a negative ANA by IIF, both on HEp-2000 substrate. The anti-DFS IIF pattern was observed in 12.3% (10/81) of patients with an anti-DFS70 by CIA and in 0.7% (7/1056) of those without an anti-DFS70 by CIA. Only 15.4% (2/13) of the monospecific anti-DFS70 positive sera had a DFS IIF pattern. Of the 5 sera that were anti-DFS70 positive but ANA negative on HEp-2000 substrate and retested on HEp-2 cells from another manufacturer (see Methods), 4 were found to have a positive ANA. When the 10 sera that were positive for anti-DFS70 by CIA and had the typical IIF DFS pattern were tested by the IIF absorption immunoassay, one serum demonstrated complete abolition of the ANA IIF staining pattern. The IIF staining patterns after absorption included nuclear speckled, centromere, nuclear dots, chromosome coat protein, centrosome, and cytoplasmic speckled, indicating that the majority of these sera were not monospecific for anti-DFS70 autoantibodies. When testing for the conventional SLE-related autoantigen targets using

ALBIA, the most common autoantibodies in the DFS70 positive sera were SSA/Ro60 (34.6%), followed by Ro52/TRIM21 (27.2%) and dsDNA (26.3%).

There were no significant differences in age, disease duration, gender, race/ethnicity, education level, smoking, alcohol consumption, presence of hypertension, nephritis, mean number of ACR Classification Criteria fulfilled, the global SLEDAI-2K, and medication usage (including duration of current and previous doses of immunosuppressive) between the anti-DFS70 negative and positive patients (Table 1). The anti-DFS70 negative patients had a lower mean score on the musculoskeletal items of the SLEDAI-2K (i.e. arthritis and myositis) compared the anti-DFS70 positive patients (0.8 vs 1.5). The anti-DFS70 negative patients had a higher frequency of anti-dsDNA (40.1% vs. 26.3%), anti-SSA/Ro60 (46.3% vs. 34.6%), anti-SSB/La (16.0% vs. 4.9%), anti-U1-RNP (31.5% vs. 21.0%), but a lower frequency of anti-$\beta2$ glycoprotein 1 (14.3% vs. 24.7%). Also, the percentage of patients from the United States was lower among the anti-DFS70 negative patients (25.2% vs. 46.9%).

In the primary multivariate analysis, patients with musculoskeletal activity (based on the SLEDAI-2K) (Odds Ratio (OR) 1.24 [95% CI: 1.10, 1.41]) or with anti-$\beta2$ glycoprotein 1 (OR 2.17 [95% CI: 1.22, 3.87]) were more likely to have anti-DFS70 antibodies, while those with anti-dsDNA (OR 0.53 [95% CI: 0.31, 0.92]) or anti-SSB/La (OR 0.25 [95% CI: 0.08, 0.81]) were less likely to have anti-DFS 70 antibodies (Table 1).

In a secondary multivariate analysis examining factors associated with anti-DFS 70 antibodies, which included study site location as a predictor (in addition to the variables specified in the primary model), patients from Canada (OR 0.39 [95% CI: 0.20, 0.73]) or Europe (OR 0.47 [95% CI: 0.25, 0.88) were less likely to have anti-DFS70 antibody than patients residing in other countries and patients of African descent (OR 0.36 [95% CI: 0.14, 0.91]) were

also less likely to have anti-DFS70 antibody than those who were not of African descent. As in the primary multivariate model, patients with musculoskeletal activity (OR 1.23 [95% CI: 1.08, 1.40]) or with anti-β2 glycoprotein 1 autoantibodies (OR 2.24 [95% CI: 1.25, 4.02]) were more likely and those with anti-SSB/La (OR 0.23 [95% 0.07, 0.74]) were less likely to have anti-DFS70 autoantibodies. In contrast to the primary model, those with neurological activity (based on the SLEDAI-2K) were also more likely to have anti-DFS 70 (OR 1.13 [95% CI: 1.0, 1.27]) and anti-ds DNA was no longer associated with the outcome.

DISCUSSION

Our study is the largest published to date on the prevalence of anti-DFS70 antibodies in a newly diagnosed, multi-national and multi-ethnic SLE cohort. Anti-DFS70 antibodies were first reported in a patient with interstitial cystitis [25] and were later found to be associated with other conditions including atopic dermatitis, Vogt Koyanagi Harada syndrome [26], and prostate cancer [27]. In these early studies, anti-DFS70 autoantibodies were initially identified by IIF screening on HEp-2 cells and then confirmed by immunoblotting. The DFS70 nomenclature refers to the IIF staining pattern characterized by uniformly distributed fine speckles throughout the interphase nucleus and on metaphase chromatin along with the autoantigen target having a molecular mass of 70kDa in immunoblots [26]. DFS70, also termed lens epithelium derived growth factor (LEDGF), has a number of functions, including serving as a cofactor for human immunodeficiency virus replication [28], but is also a stress oncoprotein as evidenced by its overexpression in diverse human malignancies [29], its association with cell aggression and survival, enhancement of resistance to cellular stress {28034} and its role as the DNA-binding transcription coactivator p75 [31].

The present study used a CIA to examine the frequency of anti-DFS70 and determine associations between the presence of this autoantibody and demographic, clinical, and serological characteristics. We report that the prevalence of anti-DFS70 antibodies detected by CIA was higher than that in other previously published adult SLE cohorts (7.1% versus 0-2.8%). The frequency of monospecific anti-DFS70 autoantibodies as detected by CIA was also higher than previously reported (1.1% versus 0-0.4%), but is still relatively rare. However, among the 10 sera that were anti-DFS 70 positive by both CIA and IIF, when an absorption immunoassay that specifically removed anti-DFS70 reactivity was used [23], only one serum was found to be monospecific because anti-DFS70 sera positive by IIF and negative for immunoassays for SLE-related autoantibodies had other autoantibodies directed to a variety of intracellular targets. Further, we observed that those with higher musculoskeletal scores on the SLEDAI-2K and with anti-β2 glycoprotein 1 were more likely to have anti-DFS70 antibodies by CIA whereas those with anti-dsDNA and anti-SSB/La were less likely to have anti-DFS70 antibodies.

In previous reports, the presence of monospecific anti-DFS70 antibodies has been proposed as a criterion to exclude the diagnosis of SLE and other AARD (reviewed in ([32]). The reasons for this apparent autoantibody paradox are still unclear but may be related to demographic, genetic, ethnic and environmental factors, concurrent medical therapies, or even misdiagnosis [4,13]. In a recent North American study of 743 children with AARD and related conditions, the overall frequency of anti-DFS70 antibodies in SLE children was 5.7% and monospecific anti-DFS70 antibodies were observed in 1.8% [11]. Of note, none of the American SLE children had anti-DFS70 antibodies compared to >10% of the Canadian SLE children, raising the possibility that variability in age, geographic, ethnic/racial, environmental and other factors may be involved in the B cell anti-DFS70 autoantibody response. In contrast, in the

present study we observed that adult patients from Canada were less likely to have anti-DFS70 autoantibodies; patients from Europe or of Black descent were also less likely to have anti-DFS 70. Although we were unable to verify the findings, another study reported a higher frequency of anti-DFS70 antibody in females [33] and in younger subjects [33,34]. More studies are needed to better understand the demographic, geographical and racial/ethnic factors involved.

Our finding that patients with higher musculoskeletal SLEDAI-2K scores were more likely to have anti-DFS70 autoantibodies whereas those with anti-dsDNA were less likely to have anti-DFS70 autoantibodies suggests that SLE patients with anti-DFS70 antibodies tend to have milder disease. Like certain other autoantibodies [35], anti-DFS70 antibody may have a protective effect where the titers of the autoantibody might paradoxically increase with disease remission [4,36]. A study of 251 adult SLE patients also reported lower disease activity in the anti-DFS70 antibody positive SLE patients [4] and in another report, increasing anti-DFS70 antibody levels were associated with disease remission in patients with amyopathic dermatomyositis complicated by interstitial lung disease[36]. However, it seems unlikely that lower disease activity is an explanation for the higher frequency of anti-DFS70 observed in our inception cohort. SLE patients at disease diagnosis would be expected to have more active disease than those with more established treated disease. Hence, the frequency of anti-DFS70 would be expected to be lower at disease onset and may increase with disease duration. Although we did not observe that anti-DFS70 antibodies were less prevalent in patients with other indicators of active disease, such as a higher global SLEDAI-2K score or nephritis, it would be of interest to study the inception cohort longitudinally to determine if anti-DFS70 antibodies change with disease activity, damage accrual, and/or therapeutic interventions.

To our knowledge, an association between anti-DFS70 antibody and anti-β2GP1 has not been previously published although anecdotal evidence suggested there was no association between the two autoantibodies [37]. In the report by Muro et al. [10], anti-cardiolipin autoantibodies were observed in 2/7 anti-DFS70 positive SLE patients and 1/7 had a lupus anticoagulant. Although the study design was challenged [37], a more recent report of 421 patients with anti-DFS70 antibodies with various conditions (12.4% had SLE), thrombosis and/or obstetric complications occurred at a higher prevalence compared to healthy blood donors (13.1 % vs. 3.0%) leading the authors to conclude that anti-DFS70 antibodies may be associated with a hypercoagulable state [38].

It is likely that technical aspects of anti-DFS70 autoantibody detection may also account for differences observed in different studies [4,13] (Supplemental Table 1). We used a state of the art, validated CIA that utilizes highly purified human DFS-70 [4]. It is now recognized that the accurate detection of the DFS ANA IIF pattern, particularly in sera with mixed IIF patterns composed of DFS and other clinically relevant ANA patterns, poses a significant technical challenge [9,39]. For example, in our study only 12.3% of those with anti-DFS70 by CIA were classified as having a typical anti-DFS pattern by IIF. The failure to detect anti-DFS accurately in the SLE serum is likely due to a masking effect of other autoantibodies on the DFS IIF pattern where one dominant pattern may obscure identification of other patterns [6]. However, even among the monospecific anti-DFS70 antibody patients (13 of 1137; 1.1%), only 15.4% (2/13) had a DFS IIF pattern. In addition, when the five samples with a negative HEp-2000 ANA IIF test but a positive anti-DFS70 by CIA were retested using another manufacturer's HEp-2 substrate, we found that all except 1 of these sera had a positive ANA test. This apparent discrepancy is in keeping with a recent report that substantial variation in ANA positivity in SLE

cohorts is highly dependent on which ANA kit is chosen for the study [21]. Thus, it is becoming

clear that the detection of anti-DFS70 antibodies by IIF is dependent on the performance of

different manufacturer's kits and using IIF alone underestimates the frequency of this

autoantibody [9,37]. Accordingly, it is imperative that DFS-specific immunoassays such as

immunoblot [40], immunoabsorption [23], enzyme linked immunosorbent assay (ELISA) [41] and/or

CIA [6,23] should be used to confirm the presence of anti-DFS70 antibodies before definitive

results are reported. An advantage of our study, even though samples were retrieved from

different centers, is that all samples were processed in one laboratory with the same standardized

immunoassay and interpreted by a single highly trained technologist.

In addition, there is mounting evidence that anti-DFS detected by IIF may be attributable

to more than one target autoantigen (i.e. a molecular ligand of DFS70). This is potentially

reflected in our observation that 0.7% of sera negative for anti-DFS 70 by CIA were anti-DFS

positive by IIF but did not have detectable anti-DFS70 by CIA. Other antibodies such as those

directed to methyl CpG binding protein 2 (Mecp2) gave a staining pattern that is

indistinguishable from anti-DFS70 [40], although a study reported that Mecp2 did not react with

DFS sera as detected by IIF [42] and in our hands these sera also did not bind to Mecp2 (data not

shown). Hence, future studies will likely focus on other DFS70 ligands.

Our study is limited in that we did not use the anti-DFS CIA to study a comparator group

of ANA positive subjects who were healthy and did not have an AARD. Comparing the

prevalence of anti-DFS70 between these two groups is necessary to determine if anti-DFS70 can

be used to rule out SLE and other AARD in patients who are referred to a rheumatologist

because they have a positive ANA [5,14]. Furthermore, we did not perform statistical correction for

multiple comparisons, which is consistent with the exploratory and hypothesis generating aspects

of our study. However, even after Bonferroni adjustment in both multivariate models, positive

associations with the musculoskeletal component of the SLEDAI-2K (OR 1.24, adjusted CI:

1.06, 1.46 in the primary model; OR 1.23, adjusted CI: 1.03, 1.47 in the secondary) and with

anti-β2GP1 (OR 2.17, adjusted CI: 1.04, 4.53 in the primary model; OR 2.24, adjusted CI: 1.01,

4.99 in the secondary) remained statistically significant, as well as the negative association with

residing in Canada in the secondary model (OR 0.39, adjusted CI 0.16, 0.92). Thus, while other

effects included in these models may be subject to further confirmation, these three predictors

remain quite robust. On the other hand, our study has several advantages over previous reports as

it involved a highly characterized, multinational, multi-ethnic inception cohort with a larger

sample size and serum samples were analyzed in a single laboratory.

SUMMARY

The prevalence of anti-DFS70 in our inception SLE cohort was higher than the range previously

published for adult SLE (7.1% vs. 0-2.8%) and was associated with a higher musculoskeletal

SLEDAI-2K score and anti-β2 glycoprotein 1. It is, however, lower than in ANA positive

healthy adults (23.2%) as reported by other studies. Similarly, the frequency of 'monospecific'

anti-DFS70 autoantibodies (1.1%) was higher than in other adult SLE cohorts (0-0.4%), but it

was still substantially lower than in ANA positive healthy adults (18.8%) that was seen in prior

studies. Therefore, monospecific anti-DFS70 may provide a potentially useful way to

discriminate between SLE and ANA positive individuals that are either healthy or have other

non-AARD conditions. Further, if a patient has monospecific anti-DFS70 autoantibodies, in the

setting of possible SLE or other AARD, this should not be considered as a criterion for

classification or diagnostic purposes. From a technical perspective, reading and interpreting the

IIF DFS pattern can be challenging and, hence, autoantigen specific immunoassays that employ purified DFS70 or absorption immunoassays are highly recommended in the diagnostic laboratory setting. Additional studies of large cohorts of patients are required to confirm these and other clinical findings such as disease duration and the impact of therapeutic intervention on the B-cell anti-DFS70 response [37].

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