

Fluorescent Antinuclear Antibody and Anti-Double-Stranded DNA Antibody Testing: A Four Years Experience

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Abstract

Fluorescent antinuclear antibody test (FANA) and anti-double stranded deoxytribonucleic acid (dsDNA) antibody testing is an integral part of the evaluation of the patients who are suspected of having connective tissue disease. We tested 2,140 serum samples for FANA and 1,460 serum samples for anti-dsDNA antibodies. Of 2,140 serum samples tested for FANA, 492 (23%) yielded a positive result (titre of 1:80 or greater) and of 1,460 serum samples tested for anti-ds DNA, 69 (4.7%) yielded positive results. Highest number (n=27) of serum samples positive for anti-dsDNA antibodies were found in serum samples that were positive for FANA test at a titre of 1:1280 or greater. In conclusion, FANA test can be used as an initial screening test for connective tissue/autoimmune disorders (JPMA 47:226,1997).

Introduction

Indirect Immunofluorescent Antinuclear Antibody (FANA) test is used to screen for patients with suspected connective tissue diseases like systemic lupus erythematosus (SLE), scleroderma, Sjogren's syndrome, mixed connective tissue disease (MCTD). The FANA results are positive in over 90% patients with connective tissue disease, suggesting it as a sensitive test and supporting its role as the laboratory "gold standard" for assistance in the diagnosis of connective tissue disorders¹. DNA was the first specific autoantigen identified in SLE^{2,3}. These antibodies are rarely found in diseases other than SLE, and their titres often fluctuate in accord with the changes in disease activity⁴⁻⁶. Thus, analysis of anti-dsDNA antibodies has become an important laboratory test for the evaluation of patients with SLE. FANA, together with anti-dsDNA antibody test also helps in differentiating drug-induced lupus from other forms of lupus^{7,8}. The present study reports our experience of FANA and anti-ds DNA antibody testing for over four years and general aspects of these parameters are discussed.

Material and Methods

A total of 2,140 serum samples were analyzed for FANA test and anti-dsDNA antibodies from January, 1992 to March, 1996. Of 2,140 serum samples, 1,460 were tested for both FANA and anti-dsDNA antibodies, whereas 680 serum samples were analyzed for FANA test alone.

FANA Testing

The FANA test for antinuclear antibodies was performed using ANAJ Hep-2 Test System (Zeus Scientific Inc., USA). Zeus, ANA/Hep- 2 Test System use ZORBA-NS sample diluent formulated to reduce non-specific staining. Polyclonal goat anti-human fluorescein- conjugated immunoglobulin was used as second antibody. These assays were performed according to the manufacturer's instructions. Initial screening of serum samples was done at 1:40 dilution. Test slides were read by using fluorescence microscope x10 for positivity and x25 for pattern identification. A titre of 1:40 was considered insignificant because of the frequency of such low titres in the presumed normal population.

Anti-dsDNA Antibody Testing

For most part of our study, we used the indirect immunofluorescence assay to detect anti-dsDNA antibodies. Cnithidia lucilae substrate and polyclonal goat anti-human fluoresccin-conj ugated immunoglobulin as a second antibody (Zeus Scientific Inc., USA). Manufacturer's instructions were followed for the procedure. Serum samples were screened at 1:10 dilution. Results were recorded as negative or positive with titres of 1:10 to greater than or equal to 1:160. No fuaher dilutions were performed for samples positive at 1:160 dilution. Recently, we have started radioimmunoassay for the detection of anti-dsDNA antibodies (DPC, Diagnostic Products Corporation, CA, USA). 125I- labelled recombinant DNA binds to anti-dsDNA in the patient sample. The bound fraction is then counted on a gamma counter (Gamma 4000, Beckman, USA) and patient sample concentrations are read from a calibration curve.

Results

FANA Testing

Of 2,140 serum samples tested for FANA, 492 (23%) yielded a positive result (titre of 1:80 or greater), while in 1,648 (77%) no immunofluorescence was detected (Table I).

Table I. Frequency of FANA test (N=2,140) and anti-dsDNA antibody test (n=1,460) results.

Test	Serum samples n	Negative		Positive	
		n	(%)	n	(%)
FANA	2,140	1,648	(77)	492	(23)*
Anti-dsDNA	1,460	1,391	(95.2)	69	(4.7)

FANA, indicates indirect immunofluorescent antinuclear antibody. dsDNA, indicates double-stranded DNA.

*Titre of $\geq 1:80$

A titre of 1:40 was detected in 119(5.56%). Of the total positive for immunofluorescence (n=492), majority (128) of serum samples showed a litre of 1:640 with a highest titre of 1:10,240 (Table II).

Table II. Frequency of FANA test results (n=2,140).

Titres	Serum Samples		Significance
	n	(%)	
Negative	1,529	(71.44)	Negative
1:40	119	(5.56)	Negative*
1:80	100	(4.67)	Borderline
1:160	89	(4.15)	Borderline
1:320	88	(4.11)	Significant
1:640	128	(5.90)	Significant
1:1,280	49	(2.20)	Significant
1:2,560	22	(1.02)	Significant
1:5,120	14	(0.65)	Significant
1:10,240	2	(0.09)	Significant

*A 1:40 titre was considered not significant because of the frequency of such low titres in the presumed normal population.

The most common patterns of fluorescence detected were speckled and homogenous.

Anti-dsDNA Testing

Of 1,460 serum samples tested for anti-dsDNA antibodies, 69 (4.7%) yielded positive results and 1,391 (95.2%) were negative (Table I).

Comparison of FANA Titre and Anti-dsDNA Antibody results Of the total 69 serum samples positive for anti-dsDNA antibody, 6 (8.7%) were positive with negative FANA test (Table III),

Table III. Frequency of positive results for anti-dsDNA antibody (N=69) with FANA test titre results.

FANA titres	anti-dsDNA antibody	
	n	(%)
Negative	6	(8.7)
1:40	5	(7.2)
1:80	8	(11.5)
1:160	6	(8.7)
1:320	9	(13.0)
1:640	8	(11.5)
≥1:1,280	27	(39.1)

5(7.2%) were positive with FANA test at titre 1:40.

A total of 27 (39.1%) anti-dsDNA antibodies were detected at a FANA titre of 1:1,280 or greater.

Discussion

Antinuclear antibodies (ANAs) are found in different individuals, including healthy blood donors and patients with a variety of connective tissue disease. The study of autoantibodies is important in patient care and research in rheumatic/connective tissue diseases. The first understanding of ANAs began in 1948 when Hargraves and colleagues⁹, demonstrated that the concentrated bone marrow of patients with SLE contained "L.E." cells. Later, Holman and Kunkel¹⁰ demonstrated that the basis for LE cells was antibodies to deoxyribonucleoprotein, which acted as opsonins for directed phagocytosis by polymorphonuclear leukocytes of antibody-sensitized nuclei. The LE cell phenomenon was widely used as a diagnostic tool until the middle of the 1970s when its use began to be supported by more specific test for individual ANAs (like antibodies to extractable nuclear antigens). The second major development in the study of ANAs occurred in 1957, when Friou used indirect immunofluorescence to the study of ANAs popularly known as FANA (fluorescence antinuclear antibody test). This technique is much more sensitive than the LE cell test, and since it detects ANAs in a broad variety of other disease and has become the method of choice for initial screening purposes in systemic connective tissue disorders. With the advent of new techniques and our understanding of the molecular biology of ANAs and their antigens, it is now possible to define the exact specification of these ANAs and their antigens, it is now possible to define the exact specification of these ANAs and help in the diagnosis of underlying rheumatic/connective tissue disorder. With all these developments, FANA test still holds to be the important screening tool for the initial evaluation of patients who are suspected of having connective tissue disease. Like all other laboratory parameters, FANA test has its own limitations and pitfalls. DNA was the first specific autoantigen identified in SLE¹⁴. Antibodies to dsDNA are quite specific for SLE. These antibodies recognized the native, double-stranded DNA and bind the deoxyribose phosphate back-bone of DNA. A number of assay systems are available for the detection of these antibodies. One of the common method is the indirect immunofluorescence technique using *Critidia lucillae* substrate¹⁵⁻¹⁶. However, there are radioimmunoassay (RIA)¹⁷ and ELISA¹⁸ also available for this purpose.

It is concluded that FANA and anti-dsDNA antibody testing is useful for the evaluation of patients who are suspected of having connective tissue disease. We found 23% serum samples positive for FANA test. This test may be used as an initial screening tool when a connective tissue disease is suspected. Further investigations like anti-dsDNA antibody or antibodies to extractable nuclear antigens (ENA) may then follow. Whether to evaluate further the FANA test negative specimen for antibodies to other extractable nuclear antigens is slightly controversial. Rare cases of SLE patients have negative FANA test^{19,20}. FANA test is unable to detect anti-Ro antibodies, antibodies to exclusively single-stranded DNA or cytoplasmic ribonucleic protein (RNPs) such as the Jo-1 antigen^{21,22}. Some of the published data indicate that further evaluation of FANA test negative serum samples is generally unjustifiable^{23,24}. We however, believe that clinical findings remain the cornerstones for the diagnosis of rheumatic/connective tissue disease and selected tests must be used to refine the pretest assessments of disease probability.

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