Original Research Article

A study of correlation of antinuclear antibody immunofluorescence patterns with immune profile using line immunoassay in a tertiary care centre of western Uttar Pradesh

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Abstract

Connective tissue diseases (CTDs) such as systemic lupus erythematosus, systemic sclerosis, Sjogren's syndrome, and rheumatoid arthritis are systemic diseases which are often associated with a challenge in diagnosis. Antinuclear antibodies (ANA) are autoantibodies that are reactive with antigens in the nucleoplasm. The presence of ANA indicates the possibility of autoimmunity and indirect immunofluorescence (IIF) assay on Hep-2 cells and primate liver is the standard blood test to detect ANA. Our aim of this study is to understand a definite association between ANA patterns and specific antibodies by line immunoassay in the serum of the patients. A total of 360 serum samples from patient attending in the OPD and IPD clinics of the C.S.S.H., Subharti Medical College, Meerut were processed by biochip method. The serum samples which were found positive were confirmed by line immunoassay. In our study 68/360 (18.9%) cases were found to be positive by IIF method in a 1:100 dilution. The positive cases were further confirmed by ANA Profile method. Out of 68 samples positive by ANA-IIF tests, 62 samples gave concordant result with line immunoassay. ANA by IIF method therefore may be used as a cost effective and rapid screening method for patients with criterion for autoimmune diseases in daily clinical practice. These correlations are useful for the diagnosis of a specific rheumatic disease.

Keywords: Antinuclear antibodies (ANA), Line Immunoassay

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Introduction

A systemic autoimmune response is a common manifestation of the rheumatic connective tissue disease (CTD) and its hallmark is the presence of antinuclear antibodies (ANA).

Antinuclear antibodies (ANA) are autoantibodies that are reactive with antigens in the nucleoplasm. The presence of ANA indicates the possibility of autoimmunity.

Indirect immunofluorescence (IIF) assay on Hep-20-10 cells and primate liver is the standard blood test to detect ANA. The gold and recommended standard in the immunological diagnosis of systemic CTD is IIFA[1] based on the reaction of antigens with fluorescently labelled specific antibodies. Lower amounts of these antibodies can be seen in the normal population as well, a spurt in titers is seen in patients of CTD. Not only are these antibodies involved in the disease pathogenesis, but they also constitute the basis for diagnosis and treatment of CTD.

Their detection with high sensitivity and specificity is therefore of utmost importance. Various detection methods are in use and there is continuous pouring of newer techniques to facilitate diagnosis and therapeutic monitoring in CTD patients[2].

Material and methods

In the retrospective study in 3 years period, a total of 360 serum samples obtained from patients attending various OPD and IPD of C.S.S.H., Subharti Medical College, Meerut were processed by biochip method. Serum samples were processed in dilution of 1:100 using HEp -20-10 /Liver biochip (Monkey) (EUROIMMUN AG) and conjugated with specific antihuman IgG (EUROIMMUN AG)[3]. The fluorescence intensity was scored at 400X, semi-quantitatively from1+ to 4+ relative to the intensity of the positive control as (4+) and negative control (No fluorescence)[4]. The serum samples which were found positive on screening by ANA IIF were further confirmed by line immunoassay. To perform LIA, Serum was diluted using Dilution Buffer in 1:110 and left on the horizontal shaker for 30 min. After this, 3 x washing was done with the WASH SOLUTION for 5 min each. This was then followed by adding Conjugate to the strip for 30 min. Again the washing step was repeated. To the washed strip, Substrate was added and left for 10 min. Afterwards, the reaction was stopped by adding Stop Solution for 2 min. Then the strips were dried and evaluated by comparing with the intensity of Positive Control Line. The serum samples which were positive for ANA by IIF method were further processed for line immunoassay. Nylon strips coated with recombinant and purified antigens as discrete lines with plastic backing (EUROIMMUN AG) coated with antigens nRNP / Sm, Sm,

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SSA, Ro-52, SSB, Scl-70, PM-Scl, PCNA, Jo-1, CENP-B, dsDNA, nucleosomes, histones, ribosomal protein-P, anti-mitochondrial antibodies (AMA-M2) were used, along with a control band. The nylon strip was incubated with serum at a 1:100 dilution. The test strips, thus, processed at a 1: 10 dilutions were analyzed by comparing the intensity of the reaction with positive control line by image analysis [Figure 1].

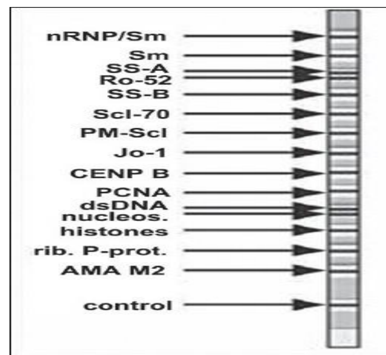

Fig 1: Incubated EUROLINE ANA profile showing the panel of auto antibodies tested using EuroLineScan

Results

In our study 68/360 (18.9%) cases were found to be positive by IIF method in a 1:100 dilution. The positive cases were further confirmed by ANA Profile method. All the 62 positive ANA-IIF tests showed 100% positivity with line immunoassay. Only 6 cases which were positive by IIF did not show positive results by line immunoassay.

Table 1: Spectrum of ANA-IIF patterns observed in the study population

ANA patterns	Samples (n= 68, %)
Homogeneous	25, (36.8%)
Speckled	31 (45.6%)
Cytoplasm granular	5 (7.4%)
Nucleolar	4 (5.89%)
Centromere	3 (4.4%)

Values in paranthesis are in percentage

Table 2: Immunoassay details of samples with ANA-IIF homogenous pattern

Line immunoassay specificity	Samples (n= 24)
dsDNA	10
ds DNA, Nucleosomes, Histones	6
ds DNA, Nucleosomes, Histones, SSA, Ro-52	4
ds DNA, Nucleosomes, Histones, nRNP/Sm	3
ds DNA, RIB	1

Values in paranthesis are in percentage

Table 3: Immunoassay details of 28 samples with ANA-IIF speckled pattern

Line Immunoassay specificity	Samples (n= 28)
nRNP/Sm, SSA	18
SSA, Ro-52, SSB	8
nRNP/Sm, Ro-52	2

Values in parenthesis are in percentage

Table 4 :Immunoassay details of 10 samples with ANA-IIF nucleolar, cytoplasm granular and Centromere pattern which showed pattern on LIA

Pattern	Samples (n= 10)	Line Immunoassay specificity
Nucleolar	4	Scl-70 (2/4), PCNA (1/4), PM-Scl (1)
Cytoplasm granular	3	AMA-M2 (1/4), Jo-1 (2/4)
Centromere	3	Centromere protein- B

Table 5: Distribution of various rheumatic diseases in study population

Disease	Total (68)	Female (48)	Male (20)
Systemic lupus erythematosus (SLE)	34	27	7
Mixed connective tissue Disease (MCTD)	13	8	5
Progressive systemic sclerosis (PSS)	8	6	2
Sjogren’s syndrome (SS)	6	4	2
Myositis	4	0	4
Limited Systemic sclerosis	1	1	0
Primary biliary liver cirrhosis	1	1	0
Rheumatoid arthritis (RA)	1	1	0

Table 6: Autoantigens of cell nuclei and fluorescence pattern

Autoantigens of cell nuclei	Fluorescence pattern
Nucleosomes	Homogeneous (cell nuclei)
AMA-M2	Cytoplasm granular(Hep-2:coarse granular)
Jo-1	Cytoplasm granular(Hep-2: Fine granular)
SSA-native	Nucleoplasm granular (Hep-2:fine granular, nucleoli often accentuated) (Speckled/Speckled SSB)
Ro-52	Nucleoplasm granular (Hep-2:coarse granular) (Speckled/Speckled SSB)
Scl-70	Nucleoli positive (Nucleoli accentuated, nucleoplasm homogeneous)
PM-Sci	Nucleoli positive (Nucleoli homogeneous)
ds DNA	Homogeneous pattern

Discussion

The Indirect Immunofluorescence Assay (IFA) is among the most commonly used methods for ANA detection and is the gold standard. An Immunoblot is considered for the confirmation of particular antigen. Sixty-eight of the 360 antinuclear antibody tests performed on a Hep-20-10/Liver primate substrate were positive. The most common immunofluorescent patterns was Homogeneous (36.8%) followed by Nucleoplasm granular (45.6%) and Cytoplasm granular (7.4%), nucleoli positive (5.89%) and centromere (4.4%). ANA by IIF method therefore may be used as a comparatively cost effective and rapid screening method for patients with criterion for autoimmune diseases in daily clinical practice. These correlations are useful for the diagnosis of a specific autoimmune disorders. IIFA is used as a preliminary study to assess the presence of autoantibodies[5]. This method is time-consuming and requires experienced personnel[6]. The homogenous pattern was the most common ANA pattern, similar to the study conducted by Sabestian et al in which seen in homogenous pattern was found in 46 (45.5%) of the positive 101 samples[7] similar to our study. The most commonly utilized method is indirect immunofluorescence, which detects a variety of nuclear antigens, including DNA, RNA and proteins (generic ANA). ANAs detected by this technique are present not only in SEE but also in other auto immune conditions like Hashimoto's thyroiditis, rheumatoid arthritis and Sjogren's syndrome. The immuno-fluorescence test for ANA is sensitive for the detection of SLE, but it is 95% specific for SLE[8]. The interpretation of ANA by immunofluorescence assay which is the gold standard is limited by the cost, requirement of skills and proper handling of the specimen. The detection of ANA by IFA does not always yield the specific clinical diagnosis; most often specific antibody assay is required to diagnose the clinical symptoms[9]. Our study somehow, presents a contradiction and reflects a high positive coincidence percentage in accordance with ANA-IFA.

Conclusion

In conclusion, the most cost - effective ANA test by IIF method using biochip wells may be used for screening purposes for patients with or without any clinical criterion for autoimmune disease in daily clinical practice. Its fluorescent pattern could also predict the presence of certain specific antibodies in the sera. Only 6 cases were missed which showed good concordant with ANA IIF.

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