CORRELATION OF ANA IMMUNOFLUOROSCENT TITRE AND PATTERN DISTRIBUTION WITH CLINICAL FEATURES: JOURNEY BEYOND SLE

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Article Info: Received 4 February 2019; Accepted 26 February, 2019
Cite this article as: Prapanna, Dr. P., & Bharioke, Dr. N. (2019). CORRELATION OF ANA IMMUNOFLUOROSCENT TITRE AND PATTERN DISTRIBUTION WITH CLINICAL FEATURES: JOURNEY BEYOND SLE. International Journal of Medical and Biomedical Studies, 3(2).
DOI: https://doi.org/10.32553/ijmbs.v3i2.122
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Conflict of interest: No conflict of interest.

Abstract
This is a Retrospective study conducted at Pathology department Bombay Hospital Indore. 300 patients were tested for presence of ANA antibody using indirect immunofluorescent test (IMMUNOSHOP AESKU SLIDES) over the period of one year.

ANA testing by IIF is a highly valuable and time tested technique for diagnosis of autoimmune disorder. Results should be interpreted in the light of clinical and biochemical findings as normal individuals have positive results on traditional ANA testing. The most definitive result from ANA testing is a negative test. This result, especially when coupled with negative tests on an ANA profile, suggests strongly that ANA associated diseases are unlikely to be present. This imparts a high NPV to ANA IIF tests. Apart from the usually described clinical features this study highlights few of the uncommon isolated clinical features like cytopenias, myopathies and Pyrexia of unknown origin and utility of ANA IIF in establishing diagnosis.

We at our centre perform ANA profile of patients to further classify the disease which is beyond the scope of this article.

Keywords: ANA, Immunofluoroscent, Titre & SLE

Introduction:
Anti nuclear antibodies are directed against intracellular antigens (ANAs), are important in the diagnosis of systemic autoimmune rheumatic diseases (SARDs) like systemic lupus erythematosus (SLE), Sjögren’s syndrome (SJ), mixed connective tissue disease (MCTD), systemic sclerosis (SSc), idiopathic inflammatory myopathies (IIMs). ANA may connote a form of systemic autoimmunity that is expressed as a wide variety of complaints, even in the absence of a definite diagnosis of CTD. Common clinical features of autoimmune destruction include fever of unknown origin, nephropathy, and dryness of mouth, paraesthesias, muscle weakness, Raynauds phenomenon, muscle effusions and other multisystem involvement.

Detection of ANA by indirect immunofluorescent method is a reliable technique for diagnosing SARD. Apart from specific diagnosis there are certain uncommonly described features which
we highlighted in our study. These symptoms along with ANA testing can help in diagnosis of the patient. Such uncommon features are cytopenias of unexplained origin, high grade fever with raised TLC etc.

This study aims at defining the correlation of ANA immunofluoroscent staining pattern with clinical features; both common and uncommon in a tertiary care setting

**Material and Methods**

This is a Retrospective study conducted at Pathology department Bombay Hospital Indore. 300 patients were tested for presence of ANA antibody using indirect immunofluorescent test (IMMUNOSHOP AESKU SLIDES) over the period of one year. Immunofluorescence testing involves incubating dilutions of patient sera with a monolayer of fixed, permeabilized cells [F1]. Antibodies adherent to the cell monolayer are visualized with an anti-human immunoglobulin reagent that has been conjugated to a fluorescent tag.

Presence or absence of nuclear staining and the pattern of nuclear staining is assessed by fluorescence microscopy.

Patients clinical details were recorded and then correlated with ANA staining, pattern and titre.

**Results**

ANA was found to be positive in about 43 % of the total patients tested, more in females as compared to males. The most frequent pattern in descending order were Speckled followed by Homogenous, Nuclear membrane Nucleolar, Centromeric and one rare pattern also encountered that is mixed nucleolar speckled also called as SS PATTERN. Cytoplasmic pattern that were seen and described in our study were Golgi pattern and Ribosomal P pattern, in patient of myopathy.

![Figure 1: ANA staining patterns according to frequency](image)

**Discussion**

Anti nuclear antibodies are directed against intracellular antigens, nuclear constituents like...
double stranded DNA (dsDNA), nucleosome, and histone proteins, and ribonucleoprotein are common antigen targets. In our study a wide spectrum of clinical features were found and correlated well with the patterns and titres of ANA, which correlated well with other studies. Cytopenias accounts for significant number of cases and ANA should be kept in mind while dealing with a case of cytopenia of unknown origin.

The IFA technique is based on a simple immunological principle that involve incubation of patient sera with substrate (cell line or tissue section) fixed to a glass slide. At present, the HEp-2 cell line is the preferred substrate for the detection and quantification of ANAs, because the cell nuclei are large and express a wide variety of antigens associated with SARDs. Serial dilutions of positive samples are tested to obtain an endpoint titer. Fluoroscent microscopy for ANA detection introduced in 1950 by Holman, Kinkel and Friou, still remains gold standard for diagnosis.

ANA PATTERN

Recent recommendations for assessing ANAs from the International Consensus on ANA Patterns (ICAP) have been published. The experts for ICAP recommended that patterns be categorized in three major groups, namely, nuclear, cytoplasmic, and mitotic patterns.

The ICAP committee also described 28 distinct patterns on the HEp-2 substrate and provided each pattern an anticell (AC) number of 1 through 28, with an assignment of competent or expert level. Of the 28 patterns, the homogeneous, speckled, centromere, discrete nuclear dots, nucleolar, and dense fine speckled nuclear patterns (AC-1 to AC-14) are considered true ANA patterns and belong to the competent level.

For the patterns associated with cytoplasmic staining (AC-15 to AC-23), the ICAP classification tree indicates the fibrillar, speckled, reticular/antimitochondrial antibody (AMA), and polar/Golgi-like patterns, as well as rods and rings, as belonging to the competent level. All of the patterns associated with the mitotic category were assigned expert level recognition. In our study we found following patterns and their associations as shown in table.

Possible antigens based on ANA staining patterns

![Possible antigens based on ANA staining patterns](image)

Figure 3:
In the homogeneous staining pattern, the entire nucleus is diffusely stained, (Titre: 1:1280)

**Figure 4:**

**Figure 5:** Speckled pattern showing, fine or coarse speckles are seen throughout the nucleus (mark showing negative stained chromatin plate) Titre: 1:1280

**Figure 6:** The nucleolar pattern refers to homogeneous or speckled staining of the nucleolus; Titre 1: 150

**Figure 7:** The centromere pattern refers to the presence of 30 to 60 uniform speckles distributed throughout the nucleus of resting cells. In mitotic cells, the speckles localize to the chromosomes at the metaphase plate (Titre: 1: 320)

**Figure 8:**

ANA TITRE: Titre refers to serial dilution of sera which in our study was used as: 1:80,160,320,640,1280.

SIGNIFICANCE OF ANA POSITIVITY: both sides of the coin:

First and foremost fact to keep in mind with ANA testing is that it is a diagnostic test. Once the diagnosis is established reassessment of the patient’s ANA status is not required. ANA positivity does not mean that patient has autoimmune disease as ANA positivity is quiet common in general population in low titres. This imparts a low positive predictive value to the test. This can be overcome by increasing serial
ANA titre in general population is usually 1: 80 and as dilutions are increased to 1:160 or 320 the chances of false positivity decreases, as low as 5%.\(^5,6\)

Furthermore, ANA pattern or titre do not provide any information regarding disease activity so repeat testing is not indicated.\(^7\) In patients with established diagnoses of autoimmune diseases, however, ANA positivity can subdivide patients with regard to prognosis and response to therapy. In juvenile chronic arthritis, where ANA positivity is associated with an increased risk of uveitis, autoimmune hepatitis where ANA positivity defines a disease subtype.\(^8\) In patients with scleroderma, the presence of a centromere pattern of staining may suggest the CREST syndrome, while a diffuse or nucleolar pattern of staining would be more consistent with diffuse cutaneous scleroderma. This distinction may be important, since the incidence of major end organ complications such as interstitial lung disease is lower in CREST syndrome.

In contrast, scleroderma patients with antitopoisomerase (Scl-70) antibodies (one of the causes of a diffuse pattern of ANA staining) have increased scleroderma lung disease.

CONCLUSION:

ANA testing by IIF is a highly valuable and time tested technique for diagnosis of autoimmune disorder. Results should be interpreted in the light of clinical and biochemical findings, as normal individuals have positive results on traditional ANA testing. The most definitive result from ANA testing is a negative test. This result, especially when coupled with negative tests on an ANA profile, suggests strongly that ANA associated diseases are unlikely to be present. This imparts a high NPV to ANA IIF tests. Apart from the usually described clinical features this study highlights few of the uncommon isolated clinical features like cytopenias, myopathies and Pyrexia of unknown origin and utility of ANA IIF in establishing diagnosis.

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