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Antinuclear Antibody: A Diagnostic Tool in Connective Tissue Disorders

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Abstract

Antinuclear antibodies (ANA) are autoantibodies that are produced and directed against cell nuclear contents that have the potential to bind and destroy them. These autoantibodies are detected in many rheumatologic and non-rheumatological disorders and in a small proportion of normal individuals. Thus, these form an important laboratory investigation for diagnosing and confirming various connective tissue disorders. For the detection of these autoantibodies, different methods have been used. This article highlights the history, various conditions with ANA positivity, different methods used for the detection of ANA and the drawbacks of ANA. The review article was done by referring various review articles, original articles, and literature from various databases.

Keywords: *Antinuclear Antibody, Connective Tissue Disorder, Diagnostics, Immunofluorescence.*

Introduction

In 1948, Hargraves and colleagues after observing the cell of a systemic lupus erythematosus (SLE) patient, first described the origins of ANA testing and named it the "L.E. cell.". The foundations for indirect immunofluorescence (IIF) testing were laid after investigations employing kidney cells of mice and rat as the IIF substrate [1]. This method continues to be used commonly to date.

In 1975, HEp-2 (human epithelial laryngeal carcinoma type 2) cells, due to their increased sensitivity, became the standard cell substrate for IIF [2]. In 2010, a study published by the American College of Rheumatology affirmed that the gold standard for ANA detection should be IIF using HEp-2 cells. However, it may be challenging to standardise HEp-2 IIF results because of variability in technique, HEp-2 preparation, and antibody expression [3].

Methodology

The review article was done by referring to various review articles, original articles and some books from various databases. The keywords used are antinuclear antibodies, connective tissue disorders, and immunofluorescence**.**

Discussion

Types of ANA

ANA are Classified Into Two Types

1. Autoantibodies Against DNA and Histones

These include the single- and double-stranded DNA (ssDNA and dsDNA) antibodies, which were first identified in 1957. Anti-histone antibodies, a marker of drug-induced SLE, were discovered in 1971 [4].

2. Autoantibodies to Extractable Nuclear Antigens (ENA)

In addition to DNA and histones, autoantibodies may also target other nuclear antigens. As they were originally extracted

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from the nuclei with saline, these nuclear antigens were named ENA. The first anti-ENA, the autoantibody to Smith antigen (Sm), discovered in 1966 is considered to be more specific for SLE. Following this further subtypes of ENA like ribonucleoproteins (RNP), SSA/Ro, or SSB/La, Scl-70, Jo-1 and PM1 were identified. Although most of these ENA are disease-specific, significant overlap still exists. Depending upon the type of underlying connective tissue disorder, the sensitivity and specificity also vary. Cytoplasmic antigens appear more diffuse under immunofluorescence with Hep2 cells as substrate.

ANA in Connective Tissue Disorders

Fluorescence Pattern

1. Nuclear Patterns

i. Fine speckled: The resting cells show fine or diffuse speckling throughout the nuclei, typically in a uniform pattern. Mitotic or dividing cells show negative staining.

ii. Course speckled: Mitotic cells show negative staining whereas resting cells show granular fluorescence throughout the nucleus.

iii. Homogeneous: The condensed chromatin of the mitotic cells exhibits solid, uniform fluorescence which is often more pronounced than in the resting cell nuclei. In the homogeneous pattern, the resting cells exhibit uniform, diffuse fluorescence of the entire nucleus.

iv. Nucleolar: The mitotic cells show diffuse cytoplasmic staining. The nucleolar pattern is associated with homogenous or speckled staining of the nucleoli, along with weak speckled or homogenous staining of the nucleoplasm.

v. Peripheral/rim, nuclear dots: The nuclear dot pattern is associated with negative metaphase mitotic cells and with few discrete speckles in the resting cell nuclei.

vi. Centromeric: In mitotic cells, these discrete speckles become closely associated which is often described as a “metaphase bar”. In the centromere pattern, resting cells show approximately 40-60 discrete speckles distributed throughout the nuclei.

vii. Others: nuclear membrane, PCNA (proliferating cell nuclear antigen), diffuse grainy.

2. Cytoplasmic Patterns: mitochondrial-like, speckled, Golgi apparatus, lysosomal-like, ribosomal-like, cytoskeletal filaments (actin, vimentin, cytokeratin)

3. Mitotic Patterns: centrosomes, mitotic spindle, CENP-F (centromere protein), NuMA (nuclear mitotic apparatus), midbody.

Among these, the more commonly observed and of clinical importance are homogenous, speckled, peripheral and nucleolar patterns [5]. Along with any of these fluorescence patterns, the intensity of staining with a qualitative scale of values from + to ++++ should also be reported as fluorescence intensity and is generally proportional to antibody concentration and thus predicts the severity of the CTD. Common IF-ANA patterns associated with specific diseases are shown in Table 1 [6]. Figure 1 gives examples of immunofluorescence mixed patterns on hep 2 ANA. [7] Figure 2 shows the algorithm for the use of ANAs in the diagnosis of connective tissue disorders.

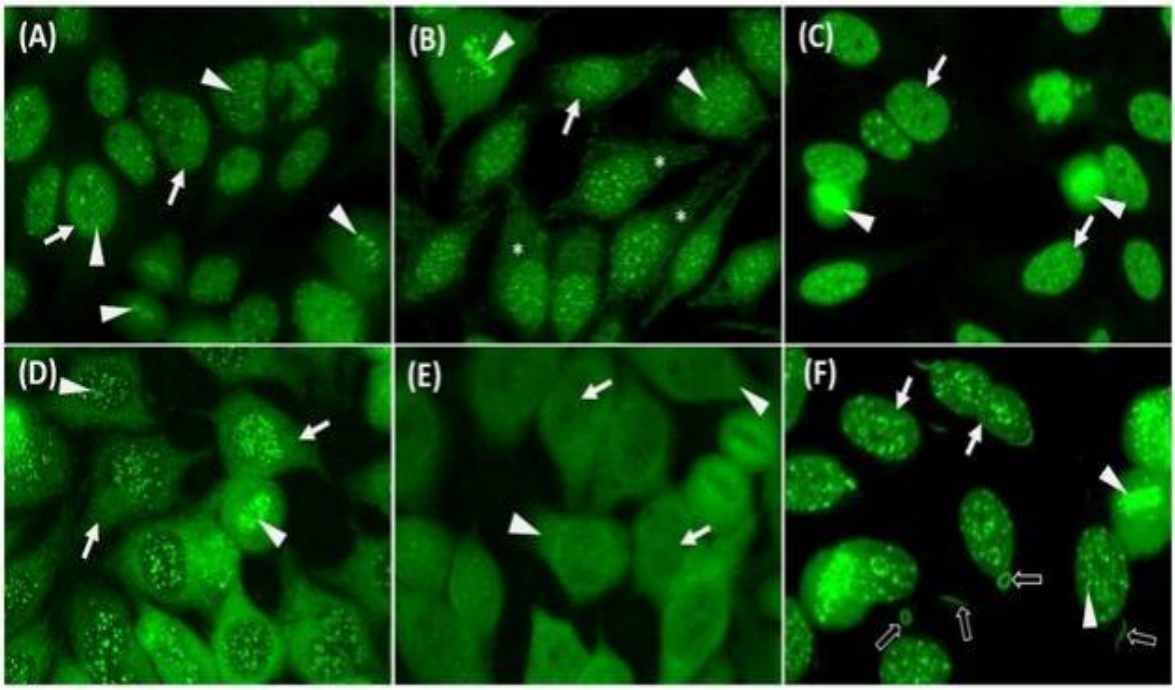

Figure 1. Showing Examples of Immunofluorescence Mixed Patterns on Hep-2 ANA Images (A) Mixed Centromere (Arrowhead)and Nuclear fine Speckled Patterns (Arrow); (B) Mixed Centromere (Arrowhead), Nuclear fine Speckled (Arrow) and Cytoplasmic AMA Patterns (Asterisk); (C) Mixed Nuclear Homogenous (arrowhead) and Nuclear Large/Coarse Speckled Patterns (Arrow); (D) Mixed Centromere (arrowhead)and Cytoplasmic Speckled Patterns (arrow); € Mixed Nuclear Fine Speckled (arrow) and Cytoplasmic Speckled Patterns (arrowhead); (F) Mixed Nuclear Fine Speckled (Arrow), Centromere (Arrowhead)and Cytoplasmic rods and Rings (Black Arrow) Patterns [7]

Table 1. Common IF-ANA Patterns Associated with Specific Diseases

ANA pattern	**Antigen**	**Associated diseases**
Speckled	ENA, RNP, Sm, SSA/Ro, SSB/La, Scl-70, Jo-1, ribosomal-P	SLE, Mixed CTD, SSc, Primary Sjogren's syndrome, PM
Homogenous	dsDNA, Histones	SLE, Drug induced SLE
Peripheral(rim)	RNP, Sm, SSA/Ro	SLE, SSc
Nucleolar	Scl, Anti-PM, anti-U3-RNP, anti-RNA polymerase I-III	SSc, PM
Centromeric	CENP, A-E	Limited SSc

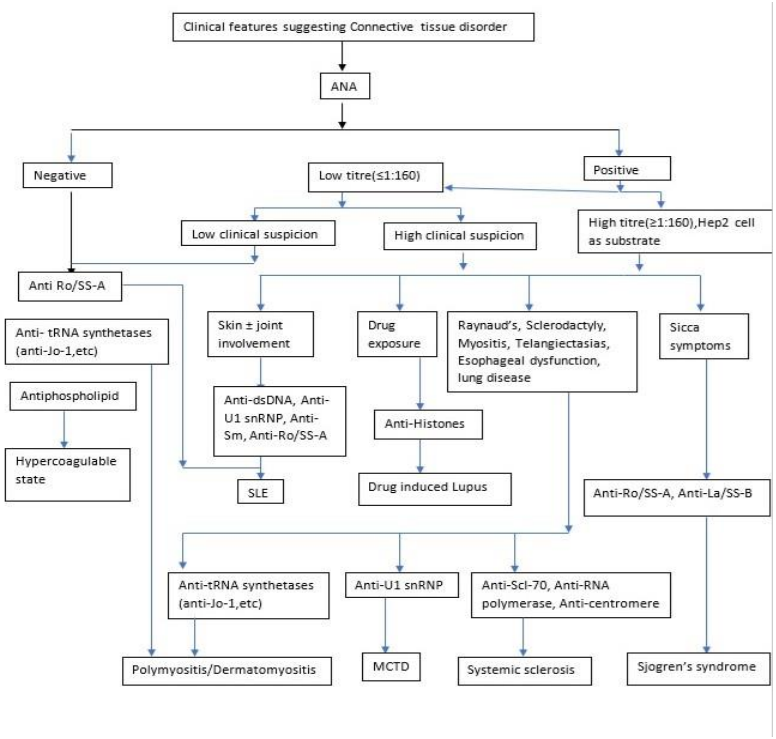

Figure 2. Showing Algorithm for the Use of ANAs in the Diagnosis of Connective Tissue Disorders

Disorders Commonly Associated with ANA [8]

1. Systemic Lupus Erythematosus

The presence of ANAs in the serum of almost every patient during the disease course at some time or another is the serologic hallmark of SLE.

ANA Titre

ANA positivity at a titre ≥1/80 is now mandatory as an entry criterion in the 2019 SLE EULAR/ACR classification criteria [9].

Anti-DNA Antibodies

These are the anti - single-stranded DNA antibodies (ssDNA-antibodies) that act on purine and pyrimidine bases of denatured DNA and dsDNA antibodies which in turn target the ribose phosphate backbone of native DNA. The presence of anti-dsDNA antibodies in higher titres is relatively specific for SLE, although several disorders are associated with anti-ssDNA antibodies. These appear in approximately 73% of patients during the disease course at some time or the other. Low levels of these antibodies are seen in individuals with Sjogren Syndrome (SS) and Rheumatoid arthritis (RA) and sometimes also in normal persons. In SLE, these antibodies correlate with the presence of nephritis, disease pathology and disease activity. Based on the study conducted by Pitta Villasboa, et al., the prevalence of nuclear antibodies in normal persons was 11.3%. The highest frequency was seen in women, the most common titres were 1:80 and 1: 160 and nuclear dense fine speckled was the most common pattern observed [10].

Anti-sn RNP Antibodies

Small nuclear ribonucleoprotein (sn-RNP) antibodies target the RNAs and proteins involved in pre-mRNA splicing. U1, U2, U4/U6, U7, U11, and U12 snRNPs are part of one group, with U representing uridine-rich RNA. Disease activity, arthralgias, arthritis, Raynaud's phenomenon, myositis, oesophageal hypomotility, infrequent nephritis, sclerodactyly, and interstitial pneumonitis are all associated with these. On the other hand, Sm

protein is the target of anti-Sm antibodies. These are quite particular, and their presence is associated with both disease flare-ups and milder central nervous system and renal diseases.

Anti-histone antibodies: These antibodies target numerous proteins designated as H1, H2A, H2B, H3, and H4 proteins, which are among the proteins that make up nucleosomes. These are present in the majority of drug-induced lupus cases, as well as a minor proportion of cases of RA, primary biliary cirrhosis, scleroderma, Epstein-Barr virus infection, and several cancers. They are less frequently seen in drug-induced lupus secondary to minocycline or propylthiouracil [11].

Anti-Ro (SSA) and Anti-La (SSB) Antibodies

These are directed towards ribonucleoprotein particles of two different types. Anti-Ro is due to photosensitivity, lung disease, lymphopenia and in some cases of nephritis whereas anti-La is seen in late-onset SLE, secondary SS, neonatal lupus erythematosus and protection from anti-Ro associated nephritis.

2. Systemic Sclerosis (SSc)

This disorder's serologic characteristic is the existence of antibodies that are specific for nucleolar antigens. In more than 90 per cent of patients' sera, ANAs are positive when tested on the HEp-2 cell line. Antibodies against centromere proteins are found in 22-36 percent of patients, and anti-topoisomerase I (Scl-70) antibodies are found in 22-40 percent of cases.

Anti-Centromere (Kinetochore) Antibodies

This includes the antibodies that are targeted against components of the mitotic spindle apparatus. CENP-A, CENP-C, and CENP-D are the three proteins that have been denoted as target antigens. Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasias, and limited cutaneous involvement are all linked to their presence. In some cases of diffuse cutaneous illness, primary biliary cirrhosis, and Hashimoto's thyroiditis, the incidence may vary.

Anti-Topoisomerase I (Scl-70)

These target DNA topoisomerase I enzyme components. These antibodies are associated with heart involvement, pulmonary fibrosis, digital pitting scars, and generalised skin involvement in SSc patients. These are crucial in classifying patients with SSc into limited and diffuse subtypes in addition to anti-centromere antibodies.

RNA Polymerase III Antibodies

These are associated with an increased risk of cancer, particularly of the lung and may predict diffuse cutaneous SSc, including higher skin score, tendon friction rubs, and renal crisis [12].

3. Sjogren's Syndrome

Individuals with SS are ANA-positive in around 95% of cases. Ro and La antigens are the main autoantigens that are targeted.

Anti-Ro Antibodies

These can be found in 40 to 95 per cent of people. Extra-glandular symptoms such as vasculitis, glandular dysfunctions, low haemoglobin, neurological involvement, platelet count, total leukocyte, and rheumatoid factor are associated with it.

Anti-La Antibodies

These can be found in up to 87 per cent of SS patient's serum. The existence of these is linked with extra-glandular symptoms such as vasculitis, cerebral involvement, glandular malfunction, low haemoglobin, total leukocyte count and platelet count, and also rheumatoid arthritis, which are similar to manifestations of anti-Ro antibodies.

4. Inflammatory myositis

Up to 90% of patients with this condition may have auto-antibodies directed against cellular antigens known as myositis-specific antigens, despite the fact that 40–80% of them have a positive ANA (Myositis Specific Autoantibody). Patients with inflammatory myositis and other conditions that share symptoms with myositis have them in their serum.

Anti-Synthetase Antibodies

Antibodies against synthetases are incredibly disease- and antigen-specific. These comprise five distinct aminoacyl-transfer RNA synthetases that target the synthetases for histidine, threonine, alanine, glycine, and isoleucine, respectively: anti-Jo1, PL-7, PL-12, EJ, and OJ. Of these, 30% of individuals with inflammatory myositis have anti-Jo-1 antibodies, which are more prevalent in those with polymyositis. These antibodies are the hallmark of the "anti-synthetase syndrome", which encompasses arthritic, interstitial lung disease, facial telangiectasia, Raynaud's phenomenon, sclerodactyly, calcinosis, and Sicca syndrome.

Anti-SRP (Signal Recognition Particle) Antibodies

The cytoplasmic proteins involved in protein translocation across the endoplasmic reticulum are the target of these antibodies. These are observed in 4% of myositis patients and are associated with an immediate onset, a severe disease, relative therapeutic resistance, and a higher mortality rate.

Anti-Mi-2 Antibodies

These are most commonly found in patients with dermatomyositis, and they are linked to the "V" and "shawl" symptoms. Anti-mi2 antibodies are associated with a better prognosis.

Anti-PM-Scl Antibodies

These are related to skin lesions, arthritis, calcinosis, dermatomyositis, mechanic's hands, and eczema in 50 per cent of individuals with overlap with scleroderma.

Anti-snRNP Antibodies

Anti-U1 snRNP antibodies are the most common antibodies, and they are frequently linked to SLE-myositis overlap, myositis-scleroderma overlap, mixed connective tissue disease, and undifferentiated connective tissue disease.

Anti – MDA 5 Antibodies

It is an autoantibody particular to myositis. Clinical signs that these antibodies are present in a patient include vasculopathy, soft erythematous papules, skin ulcerations in nail folds, and the extensor aspects of joints. Patients who carry these antibodies are more likely to experience fever, and polyarthritis, and are at a higher risk of developing rapidly worsening lung conditions [13].

Anti – TIF1 – Gamma antibodies: (anti-Transcription Intermediary Factor – Gamma)

It is one of the most commonly detected myositis-specific antibodies (MSAs). It is also called anti-p155/140, TRIM33, Ret-fused gene 7 and PTC 7 and ectodermin. The most characteristic clinical feature of anti–TIF1 gamma autoantibodies is their greater chances of malignancies such as genitourinary malignancies, especially ovarian cancer, and colon. Other commonly detected malignancies include nasopharyngeal, breast, lung, gastric, pancreatic carcinomas and lymphomas. Additionally, patients with these antibodies frequently experience severe and widespread skin complaints. Arthritis, interstitial lung disease, and Raynaud's phenomenon are relatively uncommon.

5. Antiphospholipid Antibody Syndrome:

The antiphospholipid antibody is helpful in determining whether or not someone has antiphospholipid antibody syndrome. This antibody is found in approximately 40-50 percent of people with this condition. If ANA is present in a patient with this illness, it's more likely that it's caused by SLE.

ANA and Pregnancy

In women with autoimmune illnesses, a history of recurrent pregnancy loss is linked to reactivity against antinuclear antibodies as well as the existence of antithyroglobulin antibodies [14].

In multiple studies, it was found that patients with both explained and unexplained pregnancy losses had a significant frequency of low-titre ANA in their sera [15].

Table 2 shows the sensitivity and specificity of ANA.

Table 2. Sensitivity and Specificity of ANA

Autoantibodies	Associated connective tissue disorder	Sensitivity (%)	Specificity (%)
ANA	SLE	93	57
	Sjogren's syndrome	48	52
	Systemic sclerosis	85	54
	Polymyositis/dermatomyositis	61	63
	Raynaud phenomenon	64	41
Specific ANA			
Anti – dsDNA	SLE	57	97
Anti – Sm	SLE	25-30	High
Anti – SSA/Ro	Sjogren's syndrome, subacute cutaneous SLE, Neonatal lupus syndrome	8-70	87
Anti – SSB/La	Sjogren's syndrome, subacute cutaneous SLE, Neonatal lupus syndrome	16-40	94
Anti – U3-RNP	SS	12	96
Anticentromere	Limited cutaneous SS	65	99.9
Scl-70	SS	20	100
Jo-1	Polymyositis	30	95

Anti - Neutrophil Cytoplasmic Antibodies

Autoantibodies that cross-react with antigens in neutrophils and monocytes are known as anti-neutrophil cytoplasmic antibodies. These autoantibodies were first described in a few patients with necrotizing

glomerulonephritis and systemic vasculitis by Davies et.al in the year 1982. Later, two indirect immunofluorescence patterns, diffuse granular cytoplasmic staining pattern (C-ANCA) and perinuclear staining pattern (P-ANCA) on ethanol fixed neutrophils were described. Proteinase 3 (PR3) and myeloperoxidase (MPO) are the primary antigens for C-ANCA and P-ANCA, respectively [16].

C-ANCA is described as diffuse, granular staining of the cytoplasm of neutrophils and monocytes, but not lymphocytes, often with central or interlobular accentuation of fluorescence intensity, while P-ANCA is described as perinuclear staining pattern with or without nuclear involvement [17].

C-ANCA (atypical), with "flat" neutrophil cytoplasmic fluorescence, and without central accentuation, rarely have PR3 specificity but is often due to minor antigens or unidentified specificities.

Atypical ANCA patterns are uncommon and often comprise a mix of cytoplasmic and perinuclear fluorescence with multiple antigen specificities.

These antibodies are found mainly in vasculitic conditions such as microscopic polyangiitis, Wegener's granulomatosis and Churg Strauss Syndrome and also non vasculitic conditions such as inflammatory bowel disease, rheumatoid arthritis, sclerosing cholangitis, autoimmune liver diseases, malignancies and infections.

Conditions other than autoimmune connective tissue diseases with antinuclear antibody positivity are given in Table 3 [18].

Table 3. Conditions Other than Autoimmune Connective Tissue Diseases with Antinuclear Antibody Positivity

Liver disease	Autoimmune hepatitis, Chronic hepatitis, Primary biliary cirrhosis
Infections	Parasitic diseases, Syphilis, Tuberculosis, Viral hepatitis
Skin diseases	Lichen planus, Psoriasis
Malignancies	Breast cancer, Hodgkin's lymphoma, Leukemia, Prostate cancer
Other autoimmune diseases	Addison's disease, Autoimmune anemia, Hashimoto's thyroiditis, Type 1 diabetes

Negative ANA

Patients usually present with features similar to that of subacute lupus erythematosus. True negative ANA is very rare with the advent of human tissue culture cells as the substrate as opposed to using rodent tissue substrate. A previously positive ANA test can become negative during disease remission or following treatment. In ANA-negative patients, where the suspicion of SLE remains high, the clinician should discuss the ANA assay used with the laboratory because of the varying reproducibility of ANA tests and their standardization, as well as the increasing use of quantitative, automated, high-volume solid phase assays. Over 60 percent of the patients have anti-Ro antibodies and approximately one-third have the anti-La antibody. 25 percent have antibodies to ssDNA. Approximately 10 percent of the patients eventually over a period of 10 years become positive for antinuclear factor [19, 20].

Clinical Utility of ANA

ANA encompasses several autoantigen specificities. Though these autoantibodies

possess unique rheumatologic associations, the specificity of these associations has decreased over recent years due to increased assay sensitivity resulting in the detection of these specificities in rheumatic and non-rheumatic diseases. Thus, ANAs have only an adjunct role in the diagnosis of rheumatic diseases but still aid in the clinical evaluation of the patients in the context of their particular disease.

Screening and Profile Assay for ANA Detection [21]

Table 4 shows qualitative and quantitative tests for ANA detection.

Table 4. Qualitative and Quantitative Tests for ANA Detection

Quantitative	Qualitative
ELISA, Multiplex immunoassay, Farr assay, Flowcytometry, Passive haemagglutination (semiquantitative)	Immunofluorescence using Hep2, Line blot immunoassay, Dot blot immunoassay

1. ANA by Indirect Immunofluorescence on Hep 2 Cells

The IIF HEp-2 test was phased out in favour of newer technologies for ANA detection over the last decade, and some large laboratories transitioned to automated high-throughput immunoassay systems. However, in 2010, a position paper was published stating that IIF on HEp-2 cells should remain the "gold standard" for the detection of ANA, causing the IIF ANA test to resurface. However, due to differences in the substrate and fixation procedure across manufacturers, as well as the characteristics of the secondary antibody utilised, standardisation of this assay is problematic. Even in the presence of large titres of antibodies directed to SS-A, Ro52, Jo-1, and other antigens, IIF detection of ANA can result in false negative results. Furthermore, the difficulty of obtaining considerable diversity in staining patterns on the ANA HEp-2 IIF substrates using slides from various manufacturers has led to the development of an IIF pattern nomenclature. Figure 3 shows immunofluorescence testing for identifying the presence of ANAs. [22]

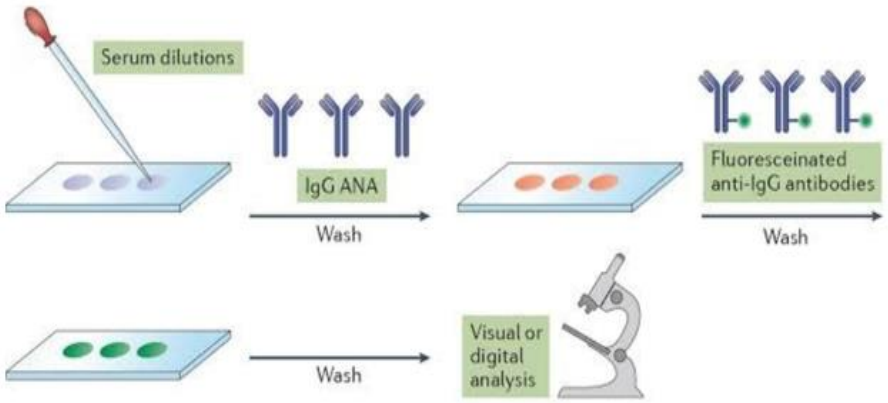

Figure 3. Showing Immunofluorescence Testing for Identifying the Presence of ANAs. The Experimental Procedure of an Indirect Immunofluorescence Assay (IFA) is Illustrated. A Slide with Tissue Culture Cells is Exposed to Dilutions of Serum. Following the Washing Steps to Remove Unbound Antibodies, the Slide is Incubated with a Fluoresceinated anti-IgG Reagent. Following Another Washing Step, Fluorescence Microscopy is Performed. At Present, in Most Laboratories, a Technician Visually Inspects the Slide to Determine the Presence and Pattern of Fluorescence. In

Determining the Positivity of a Sample, the Dilution in which Fluorescence is Still Visible is Assessed. This End-point Titre Provides a Quantitative Measure of the Amount of Antinuclear Antibodies (ANAs) Present. In Addition to Visual Inspection, the Presence of Fluorescence can be Determined from Digital Images [22]

2. ANA Screening ELISA

In ANA testing, two different types of ELISA procedures are used. These assays are: generic and antigen-specific.

Generic assays, like IF-ANA, detect ANA with broad specificity, whereas antigen-specific assays detect ANA and react with a single autoantigen, such as dsDNA, SS-A, SS-B, Scl-70, Sm, and so on. Multiple antigens are coated on microtitre plates in antigen-specific assays, commonly a combination of SSA, SSB, Sm, and U1-RNP, with many also including Jo-1 and Scl70. Because this new test is both extremely specific and sensitive, it cuts down on the time it takes to screen a large number of patient samples. As a result, ELISA is quickly becoming the most used technology for routine screening as well as detecting particular ANA. However ELISA techniques have been found to miss low titre ANA and sera with specific ANA. Figure 4 shows a schematic representation of the ANA ELISA method [23].

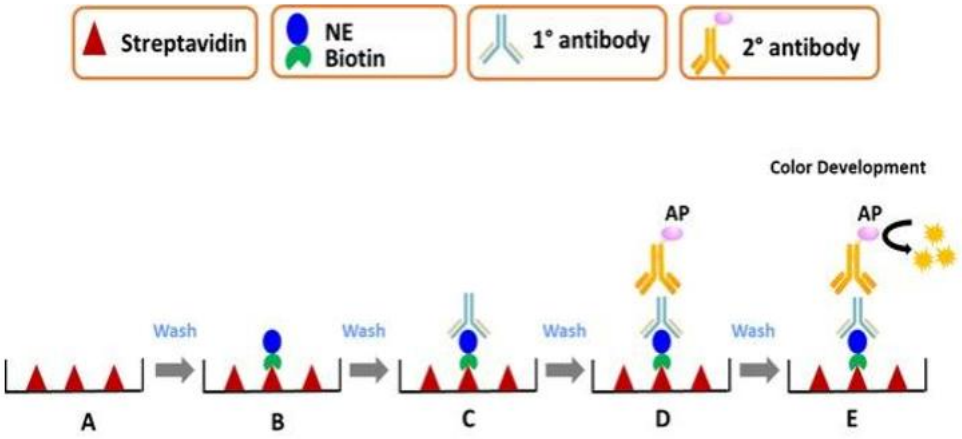

Figure 4. Showing a Schematic Representation of the ANA ELISA Method. A. Coating of Streptavidin on ELISA Plate. B. Addition of Biotinylated-Nuclear Extract (Bio-NE) to the ELISA Plate. C. Addition of Primary Antibodies to the ELISA Plate. D. Addition of Secondary Antibodies (AP-conjugated) to the ELISA Plate. E. Addition of Substrate for Color Development Monitored by Spectrophotometer [23]

3. Line Immunoassay

Line immunoassays are available in a variety of formats and are often used to confirm autoantibodies detected by the HEp-2 ANA IIF or other screening immunoassays. Line immunoassays have some limitations, including a lack of sensitivity and specificity for particular autoantibodies, despite their convenience of use. However, in terms of specificity and sensitivity, line blots are comparable to ELISA, and automated interpretation is also conceivable.

4. Dot Blot

The dot blot method is a qualitative assay that uses nitrocellulose strips to blot pure antigens at pre-located locations. The antigen sources are bovine and rabbit thymus (for SSA, Sm, and Scl-70) or calf spleen and rabbit thymus (for SSb and Sm/RNP). Following a 50-fold dilution of patient serum, the strips are treated with an alkaline phosphatase protein A conjugate.

5 bromo-4-chloro-3 indolylphosphate/nitroblue tetrazolium is used to stain the test strips at the end. A blue spot is stained on the positive strip. The dot blot test is

useful in terms of time management because it takes just 30 minutes to complete, is simple to perform, and is reasonably inexpensive. However, blotting RNP antigens in combination with other antigens has a significant disadvantage.

5. Multiplex Bead-Based Assay

The recently developed multiplex immunoassay (MIA) allows for the simultaneous detection of multiple unique ANAs as distinct entities. ALBIA (addressable laser bead immunoassays) is a term used to describe multiplex assays based on the Luminex technology that uses addressable laser beads.

6. Flowcytometry

In recent years, flow cytometry using autoantigen-coated fluorescent beads has grown in popularity. Fluorescent beads-based approaches, also known as Reflex ANA, are said to have several benefits, including simultaneous antigen recognition testing, automation, cost-effectiveness, and high sensitivity. However, the method's most significant flaw is that it only produces a single conclusion for each analysis.

7. Antigen Microarray

Antigen microarray is not commonly used at the moment, but it could be a great advancement for measuring multiple ANA at the same time. Pre-synthesized antigens are printed on polystyrene and incubated with serum samples before being incubated with horseradish peroxidase-conjugated secondary antibodies and chemiluminescent substrates. A charge-coupled device camera-based chip reader records the light signals produced. Calibration curves are used to quantify antibodies. The results are essentially comparable to those obtained using current clinical laboratory procedures.

Drawbacks of ANA

Although the indirect fluorescent assay has been designated as the gold standard for ANA testing by an American College of Rheumatology expert panel, the method has several known flaws, including interobserver variability and differences in sensitivity and specificity of the test based on the substrate used (eg. Different HEp2 kits). Although a positive ANA test result can reflect an interaction between serum antibodies and up to 150 different nuclear antigens, only a small percentage of these antigens are well-known and have established associations with disease, and many of the remaining antigen-antibody reactions may be clinically insignificant. Different techniques, such as ELISA and multiplex bead assay, are employed in other frequently used commercially available ANA kits, and studies have shown that ANA test results are influenced by these approaches.

Conclusion

Antinuclear antibodies (ANA) are autoantibodies that are produced and directed against cell nuclear contents that have the potential to bind and destroy them and are detected in many rheumatologic and non-rheumatological disorders, and a small proportion of normal individuals. Thus, these form an important laboratory investigation for diagnosing and confirming various connective tissue disorders. For the detection of these autoantibodies, different methods have been used. This review highlights the history, various conditions with ANA positivity, different methods used for the detection of ANA and the drawbacks of ANA and serves as a refresher to the physician.

Conflicts of Interest

None.

Acknowledgements

None.

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