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Biochemistry, Antinuclear Antibodies (ANA)

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Introduction

The antinuclear antibody (ANA) is a defining feature of the connective tissue autoimmune disorders. ANAs are a class of antibodies that bind to cellular components in the nucleus including proteins, DNA, RNA, and nucleic acid-protein complexes.[1] First described in 1948, ANA identification has been the foundation of diagnosis for connective tissue autoimmune disorders including systemic lupus erythematosus (SLE), Sjogren's syndrome, and polymyositis/dermatomyositis.[2] Although 20-30% of the average population has detectable levels of ANAs, increased titers are characteristic of individuals with connective tissue disorders.[3] Thus, the sensitivity and specificity of methods used to detect ANAs are key to diagnosis.

Fundamentals

Antinuclear antibodies (ANA) refer to an autoantibody directed at material within the nucleus of a cell. ANAs classify typically into two groups, antibodies to nuclear material, and antibodies to DNA and histones. Antibodies to DNA and histones include anti-dsDNA antibodies and anti-histone antibodies. The remaining category includes an additional targeted nuclear antigen. The first to be identified in this category was the anti-Smith antibody.[3] Others include anti-SSA/Ro, anti-SSB/La, anti-U3-RNP, anticentromere, Scl-70, and Jo-1.

Issues of Concern

Given that ANAs are present in up to 30% of the average healthy population, there are inherent issues with using them to diagnose autoimmune connective tissue disorders. Physicians must consider positive results with existing clinical manifestations to establish a diagnosis. Furthermore, initial immunofluorescence on HEp-2 cells subjectively depends on the laboratory manufacturing the substrate cells and the skill of the individual reading the result.[4] Each laboratory must establish their own scale to define a positive result.

Molecular

ANAs bind to a variety of molecular compounds with the nucleus of the cell including nucleic material and proteins. Antibodies may bind to double-stranded DNA, and studies suggest that antibodies form during incomplete removal of cellular material during apoptosis.[5] Additionally, anti-Sm antibodies bind to the Smith protein, a protein contained within small nuclear ribonucleoprotein (snRNP) particles.[6] Scl-70 antibodies interfere with DNA replication by binding to Topoisomerase I, and anti-centromere antibodies affect cell division by binding to centromeres during interphase. Antibodies to Jo-1 prevent the binding of histidine to tRNA during protein synthesis by targeting histidyl-tRNA synthetase.[7] Additionally, antibodies may target the Ro/SSA antigen, an amino acid sequence that binds to double-stranded DNA and single-stranded DNA. The suspected mechanism is that they may bind to viral DNA of the Ebstein Bar Virus with molecular mimicry later causing autoimmune disease. Unlike Ro, La/SSB is a protein found

primarily in the nucleus. But similar to Ro, La is also known to bind to nuclear material from EBV.[8]

Testing

The origins of ANA testing were first described in 1948 when Hargraves and colleagues observed the cell of a patient with systemic lupus erythematosus (SLE) and termed it the "L.E. cell."[9]. Following experiments used mice and rat kidney cells as the substrate for indirect immunofluorescence (IIF) and established the foundations for IIF testing.[1] The patient's serum antibodies interact with substrate cells and form fluorescent patterns. This method has been the most commonly used since.

In 1975, HEp-2 (human epithelial laryngeal carcinoma type 2) cells became the standard cell substrate due to their increased sensitivity. And in 2010, the American College of Rheumatology published a study affirming that indirect immunofluorescence (IIF) with HEp-2 (human epithelial laryngeal carcinoma type 2) cells should be the gold standard for detecting ANAs. However, due variables in technique, HEp-2 preparation, and antibody expression, HEp-2 IIF results may be difficult to standardize.[10]

IIF patterns correlate to specific ANA subtypes, and pattern recognition is a useful tool in ANA testing. Homogenous fluorescence typically suggests antibodies directed at dsDNA, histones, or nucleosomes.[1] A membranous pattern may show antibodies to membrane proteins. Antibodies directed to other nuclear antigens correlate with speckled fluorescent patterns. Anti-Smith antibodies fluoresce in a course speckled pattern. Anti-SSA/Ro and anti-SSB/La form a fine speckled pattern. Discrete speckles represent antibodies targeted to the centromeres in cells undergoing interphase. Nucleolar speckles have an association with antibodies directed at DNA topoisomerase (Scl-70). And the speckled cytoplasmic pattern suggests antibodies to aminoacyl-tRNA synthetase (Jo-1).[1]

While IIF remains the primary initial testing method, other methods including enzyme-linked immunosorbent assays (ELISA) and immunoassays offer confirmatory testing for specific ANAs. ELISAs offer quantitative screening for specific ANAs and have proven to perform comparably to IIF, and commercial panels for antibodies to SSA/Ro, SSB/La, Sm, Scl-70, Jo-1, and centromeres are available.[11]. In addition, multiplex immunoassays offer similar benefits and use a series of known antigen-coated beads, and when combined with patient serum, indicate their specific antibody targets.

Clinical Significance

Systemic autoimmune disorders affect 3-5% of the general population, and ANAs are one of the few specific markers of disease.[10] Therefore, ANA testing is often the first step in the diagnosis of systemic autoimmune connective tissue disorders. Laboratories report the staining pattern and the titer of ANA as an indication for further testing. While the presence of ANAs and their subtypes increase the likelihood of a systemic autoimmune disorder, they do not necessarily confirm that an individual has or will develop a rheumatic disease. Nevertheless, there are notable clinical correlations between the ANA subtypes and autoimmune connective tissue disorders.

Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disorder that affects nearly every system in the body. Individuals have variations in disease presentation where one system often deteriorates significantly more than the others. Clinical manifestations may include fatigue, arthritis, vasculitis, nephritis, pleuritis, and myocarditis. Clinical manifestations and immunologic criteria are both required to establish a definitive diagnosis of SLE. Immunologic criteria include abnormal ANA titers in the absence of drugs and the presence of anti-dsDNA or anti-Sm antibodies.[12]

Scleroderma

Scleroderma, or systemic sclerosis, involves progressive fibrosis of the skin and organs. Scleroderma presents either as a limited form and a diffuse cutaneous form. The diagnostic basis is via a combination of clinical symptoms and increased ANA titers. Scl-70 is highly correlated with scleroderma, while anti-centromere antibodies are moderately correlated.

Myositis

Polymyositis (PM) and dermatomyositis (DM) are a group of inflammatory disorders that primarily affect the proximal muscles and cause inflammation. The primary clinical manifestations of PM is the gradual weakening of the proximal muscles. Likewise, DM presents with gradually increasing proximal weakness, but cutaneous symptoms such as facial erythema, poikiloderma in sun-exposed areas, and Gottron's papules on the extensor surfaces of the hands are also present. General ANA testing is used in the diagnosis of PM and DM, while anti-Jo1 antibodies are associated with 30% of patients with PM/DM.[3]

Sjogren's

Sjogren's syndrome is a chronic autoimmune pathology that destroys the exocrine glands including the lacrimal and salivary glands. Diagnosis centers on clinical manifestations and serologic testing. Clinical manifestations of Sjogren's syndrome include chronic dry eye due, dry mouth, Raynaud's phenomenon, arthritis, and bronchitis. When there is a suspicion for Sjogren's syndrome, testing for anti-Ro/SSA and anti-La/SSB titers are the protocol.[8]

Psoriatic Arthritis

Psoriatic arthritis is a subtype of inflammatory arthritis seen in association with associated with psoriasis (a member of the spondyloarthritis family). This condition can present a diagnostic challenge for clinicians as the clinical presentation can be relatively subtle and under-appreciated. There are several case reports regarding the potential long-term consequences and/or associated conditions that may have an association with psoriatic arthritis -- these associations can ultimately compromise the patient's outcome.[13] ANA testing has long been thought to be a diagnostic tool and potentially detectable serum marker to enhance clinical recognition of the condition. A 2015 study found an increase in the serum detection of ANA antibodies in patients with psoriatic arthritis compared to healthy controls.[14] Of note, the cohort under investigation excluded other confounding conditions (e.g., rheumatoid arthritis, etc.) which are known to result in an elevated serum ANA level.

Sacroiliitis

Other inflammatory conditions known to have diagnostic serum laboratory patterns can benefit from a negative serum ANA test result. For example, in cases of true ankylosing spondylitis (and sacroiliitis), HLA-B27 will often be positive, while ANA and rheumatoid factor would be expected to be negative upon serum testing.[15]

Questions

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