Antinuclear Antibody Testing Clinician Handout

SYNOPSIS AND RELEVANCE

Laboratory testing for antinuclear antibodies (ANAs) can be complicated by different methodologies as well as the availability of numerous available individual antigen-specific tests. Adherence to the strategies in this module will:

1. Ensure that an ANA test is ordered before any subsequent antigen-specific testing, and only in the setting of clinical suspicion for a rheumatologic disease.
2. Understand the general differences in basic ANA testing methodology

BACKGROUND

Antinuclear antibody (ANA) testing is familiar to the clinical lab and is an important initial test when the patient has a suspected systemic autoimmune rheumatic disease, such as systemic lupus erythematosus (SLE). A positive antinuclear antibody (ANA) can be due to autoantibodies against many different detected antigens, such as double-stranded DNA (dsDNA), DNA histone complexes, u1RNP, Sm, La, and fibrillin, to name a few. Although the negative predictive value of positive ANA tests is quite high, the positive predictive value is relatively low, as up to 30% of the healthy population can demonstrate a positive ANA.[1-3] Additionally, the availability of individual antibody testing, such as anti-dsDNA, anti-Sm, and anti-La, complicates the testing menu for the serologic diagnosis of rheumatologic diseases. In 2013, the American College of Rheumatology partnered with the Choosing Wisely campaign to help guide the use of ANA testing. They recommended not ordering individual autoantibody panels or more specific ANA subserologies unless: 1) an ANA screening test is positive, AND 2) there is clinical suspicion and evidence of an immune-mediated or rheumatologic disease.[4]

In addition to the guidelines for judicious test utilization, different methodologies are available for ANA testing, and can generate different results, especially when testing is done on different analytical platforms. There are two major methodologies for ANA screening. The traditional method, which is currently generally considered the reference method, is the use of indirect immunofluorescence (IFA).[3] With this methodology, patient serum is incubated with HEp-2 cells, a human cell line with a large nucleus that makes it easy to visualize various staining patterns. After incubation, the cells are incubated with a fluorescent-conjugated antibody against human IgG. Specific positive staining patterns, whether homogeneous, speckled, centromeric, nucleolar, or a combination of these, can be reported. This can be helpful to guide the next steps, although the association between staining pattern and disease specific autoantibodies is relatively weak. Of note, standardization of such patterns is difficult, although the International Consensus on Antinuclear Antibody Patterns (ICAP) has attempted to achieve consensus, and labs should be familiar with these guidelines if reporting ANA results using IFA.[5-8]

The second, more recent methodology for detecting ANAs is the use of solid phase immunoassays. With this methodology, cell extracts, or more commonly a panel of recombinant antigens, are prepared and immobilized on a solid surface to which patient serum is added. Based on the serum binding to one or more of these specific antigens, the exact autoantibody in the patient's serum is identified.

Overall, the advantage of the IFA methodology is that it allows for a large number of autoantibodies to be detected (given the presence of over 150 antigens) and is therefore more sensitive. The disadvantages are, however, that it is labor intensive, requires trained personnel to interpret staining patterns, lacks reference sera for staining patterns, needs to be titered to enable autoantibody semiquantitative reporting , and does not identify specific autoantibodies (subserologies).[3] Solid phase immunoassays, on the other hand, have high throughput, detect specific autoantibodies, and are semiquantitative, but lack sensitivity, since the antigens tested are limited to those immobilized on the solid surface. Of note, although both methods are useful in many scenarios, the American College of Rheumatology considers HEp-2 based IFA testing to be the gold standard for the detection of ANAs.

With IFA-based ANA testing, the titer reported is generally the dilution prior to endpoint, which is the point at which less than half of cells show fluorescence. Many large center studies of healthy volunteers have demonstrated positive ANAs at relatively low-level titers among healthy volunteers[1,9]; in one study, for example, approximately 32% of the population aged 20-60 had a positive ANA at a 1:40 titer; however, only 5% of the population had a positive ANA at a 1:160 titer.[10] A general suggestion has been to use an initial screening dilution that results in only 5% positivity in normal controls as the cutoff for a positive reaction. However, even at 5%, there is a high tendency towards false positives, so pre-test probability should be high before ANA testing is performed (as recommended by the Choosing Wisely campaign); otherwise, a lengthy, non-diagnostic/specific, and potentially expensive workup may ensue.

For laboratories utilizing IFA as their initial screen, if a patient has clinical suspicion for an autoimmune disease and the testing is positive, additional testing for specific autoantibodies using solid phase immunoassay may be helpful to

further elucidate the diagnosis. The exact identification of these subserologies can help hone in on specific disease states; for example, whereas antibodies against dsDNA are generally associated with SLE, antibodies against Ro and La are associated more strongly with Sjogren's syndrome. This "reflex" testing can be all-inclusive or can specifically target certain autoantibodies based on the original IFA staining pattern. One must be circumspect in interpreting negative IFA results, since some antibodies, including anti-La/SS-B and anti-Jo-1, which exhibits cytoplasmic staining, may be undetectable or unreported in HEp-2 cells, limiting its sensitivity.[3]

Once a patient has been diagnosed with an ANA-associated autoimmune disease, further ANA titers after the diagnosis is established are generally not helpful.

INSIGHTS

1. ANA testing should not be performed unless there is clinical suspicion for a rheumatologic disease.
2. Antigen-specific testing generally should not be performed unless the patient has a positive ANA screen.
3. ANA screening can be performed either by IFA or solid phase immunoassay; both have their advantages and disadvantages from a clinical and laboratory perspective.
4. Certain reflex testing may be useful subsequent testing after an initial positive ANA screen to establish the exact autoantibody present.

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