**CLINICAL VIGNETTE**

# What is in an A.N.A. (Anti-Nuclear Antibody)?

Olga Popel, MD, MBA and Mark Hall, MD

A 25-year-old nulliparous Caucasian female sought evaluation from a gastroenterologist due to unexplained post-prandial bloating, nausea, and constipation. An anti-nuclear antibody (ANA) laboratory test was ordered as part of her evaluation and returned positive with a 1:640 titer in a homogeneous pattern. She was referred to rheumatology for further evaluation. Her review of systems was remarkable for chronic fatigue reportedly due to poor sleep quality, anxiety followed regularly with a therapist, and facial acne. She otherwise felt healthy and denied swollen/tender joints, myalgias, weakness, ocular or oral sicca symptoms, oral ulcerations, skin rashes, alopecia, recurrent fevers, weight changes, gastroesophageal reflux disease, Reynaud's, shortness of breath, cough, or frequent infections. Surgical or social history were negative other than rare social alcohol consumption. Medications included topical gel for acne and an oral contraceptive. Family history was negative for systemic inflammatory rheumatic disease (SIRD) other than her paternal grandmother with Crohns' disease.

Office vital signs were normal except for an elevated BMI of 28. Her exam was essentially normal, without lymphadenopathy, malar or other suggestive rashes, scleral injection or discoloration, oral or nasal ulcerations, alopecia, abnormal heart or cardiac sounds, tenderness or distension of the abdomen, palpable organomegaly, synovitis or effusions in examined joints, diminished or asymmetrical pulses, or focal neurological deficits.

Her subsequent evaluation revealed a negative serological profile including Celiac/Hashimoto's disease antibodies, anti-Smith, Sm/RNP, SSA (Ro), SSB (La), and dsDNA. Thyroid testing, complete metabolic panel, muscle enzymes, complete blood count with differential, serum complements 3 and 4, and urinalysis were within normal limits. C-reactive protein and erythrocyte sedimentation rates were also within normal limits. Screening tests for syphilis and viral hepatitis were negative.

***Discussion***

A positive anti-nuclear antibody blood test is frequently a source of consternation, wonder, and dread for the ordering clinician. In seemingly cruel irony, it is one of the easiest tests to order and one of the hardest to interpret as positivity does not automatically correspond to the active presence or future development of a SIRD. It is often a source of anxiety to patients who, after searching the internet, self-confer a diagnosis of lupus, scleroderma, or another incurable disease. A positive ANA requires consideration of titer, pattern, and clinical context as well as acknowledgement of its diagnostic limitations.

The discovery and subsequent broad utilization of the ANA is based upon on its close association with systemic lupus erythematosus (SLE). Important foundational work by Hargraves et al in 1948 led to the discovery of "L.E. cells" derived from bone marrow samples in lupus patients.[1] Around ten years later, Kunkel and Holman discovered antibodies to deoxyribonucleoprotein.[2] These and other breakthroughs led to the widespread testing we see today. Though multiple laboratory techniques are available, not all are equally reliable which is why the joint American College of Rheumatology/European Alliance of Associations for Rheumatology 2019 systemic lupus erythematosus diagnostic guidelines recommend use of "immunofluorescence on HEp-2 cells or another solid-phase ANA screening immunoassay with at least equivalent performance".[3]

Once ANA positivity is confirmed utilizing a high-quality assay, attention must turn to the next most crucial point: titer. The systemic lupus erythematosus diagnostic criteria demand a titer of 1:80 or higher. This is largely due to the high frequency of low positive titers in the healthy population. Tan et al estimated up to 30% of the healthy population will have a titer of 1:40.[4] Therefore, titers <1:80 are commonly considered negative or clinically insignificant. However, as titers increase there are statistically fewer false or clinically insignificant positives. The same cohort study reports only about 5% of healthy individuals have an ANA of 1:160. Therefore, ANA positivity and elevated titers are still insufficient to determine the actual presence of SIRD.

Following considerations of assay method and titer, attention should turn to pattern. The visual patterns of staining have associations with various autoimmune diseases.[5] For example, the patterns associated with SLE include homogeneous, nucleolar, or speckled. Speckled or homogeneous patterns may be associated with Sjogrens Syndrome. We expect most patients with mixed connective tissue disease to have a nuclear speckled pattern in conjunction with a strongly detectable RNP antibody. Other pattern associations exist for systemic sclerosis and others. With the discovery of an extensive number of highly specific antibody tests, clinician's reliance upon ANA patterns has somewhat diminished.

Even when ANA-associated SIRD is excluded, the clinician is challenged by increasing ANA positivity in many non-rheumatic autoimmune diseases, certain malignancies, and infections. Autoimmune thyroid disease, vitiligo, and diabetes mellitus type 1 are common examples, although many others are reported. Chronic viral hepatitis is an important infectious example. In a cohort of women with breast masses, Nisihara et al reported 44.4% ANA positivity in those found to have breast cancer compared to 15.7% in those with benign breast lesions (P-value = 0.03).[6] This underscores the need for a detailed review of systems, physical exam, and consideration of chronic medical conditions.

The development of new diagnostic tools will alter testing. One recent tool is the ant-DFS70 laboratory test. The dense fine speckled (DFS) ANA pattern is the most frequent Hep-2 cell pattern seen in ANA positive healthy individuals. The more specific antibody to DFS70 is detectable in only 1% of individuals with SIRD, is yet seen in up to 22% of healthy individuals.[7] Its negative association with SIRD is even stronger when other ANA-associated serologies are absent. As awareness spreads, it may become more widely utilized to reduce unnecessary referrals and healthcare costs.[7]

There are multiple published algorithms and recommendations to guide appropriate use of ANA blood testing. However, no single universally implemented schema is used in clinical practice. Practice variation remains even amongst rheumatologists. Routine testing in asymptomatic patients is discouraged. Testing in patients with isolated highly non-specific symptoms such as fatigue usually warrant evaluation for more common non-rheumatic causes prior to considering serological testing for ANA-associated diseases. However, in any patient with a positive ANA with clinically significant titer, it is reasonable to refer to rheumatology for diagnostic assistance. Preliminary laboratory testing often includes screening for cytopenias, proteinuria/hematuria, and systemic inflammation markers. Selection of other tests is often informed by a detailed review of systems, physical exam, and expertise of the ordering clinician.

In the above case, the patient received reassurance that there was no ANA-associated rheumatic disease and encouraged to continue her evaluation for gastrointestinal complaints. She will follow-up in six to twelve months to reassess signs/symptoms of an ANA-associated SIRD.

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