**CLINICAL VIGNETTE**

# A Rare Diagnosis of ANA Negative Lupus

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***Introduction***

Antinuclear antibody (ANA) negative lupus is considered a rare rheumatology diagnosis. The ANA test is a highly sensitive but non-specific serologic test used to screen for systemic lupus erythematosus (SLE) and other ANA-associated rheumatic diseases. ANA testing by indirect immunofluorescence is considered the gold standard with 94% sensitivity for SLE.[1] As a result, a negative ANA is often perceived as "ruling out" lupus and makes other ANA-associated rheumatic diseases less likely. The following case and discussion demonstrate that lupus can still be present, even with a negative ANA.

***Case Presentation***

A 33-year-old female with a history of migraines presented to rheumatology with intermittent rashes. She reports a new red rash on her arm for a few weeks, followed by subjective fevers and diffuse myalgias and arthralgias. The rash progressed to include her face, neck and arms and occurred intermittently. She noted associated photosensitivity, but denied Raynaud's phenomenon, oral ulcers, hair loss, chest pain or shortness of breath.

On examination she was afebrile with normal vital signs. She had malar erythema that spared the nasolabial folds, with clear oropharynx, and no conjunctival injection, or palatal or nasal ulcerations. Her lungs were clear and heart had a regular rate and rhythm with no murmurs. Joint exam was notable for tenderness and mild swelling in multiple proximal interphalangeal joints (PIP) bilaterally. She had scattered erythema on the dorsum of both hands which spared the PIP and DIP joints. Review of prior labs noted history of mild leukopenia. Additional labs including serologies were offered. CBC was notable for a white blood cell count of 2.78/µL and an absolute neutrophil count of 970/µL with no lymphopenia, anemia, or thrombocytopenia. Chemistries showed a normal creatinine, electrolytes and liver enzymes. Serologies included Antinuclear antibody (ANA), double stranded DNA antibody (dsDNA), Anti-SSA and Anti-SSB antibodies, smith/RNP antibodies, as well as antiphospholipid antibodies were all negative. Rheumatoid Factor and cyclic citrullinated peptide antibodies were also negative. She had a low C3 complement of 71 mg/dL (normal 86-175), with normal C4 of 17 mg/dL (14-46). Urinalysis and spot urine protein to creatinine ratio were normal. Creatinine kinase and thyroid stimulating hormone were also normal.

On a follow-up one month later, she reported the development of left-sided cervical lymphadenopathy with subjective low-grade fevers and unintentional weight loss of 10 pounds. Neck ultrasound showed sub-centimeter cervical lymphadenopathy and she was referred to Hematology/Oncology. Infectious studies including HIV, Hepatitis C, CMV and EBV serologies were all negative. FNA of the enlarged cervical lymph node showed minimal lymphoid tissue with normal flow cytometry. Core biopsy was not possible due to the size of the lymph node. CT imaging of the chest, abdomen, and pelvis did not demonstrate any other lymphadenopathy. Given her ongoing leukopenia and weight loss, a bone marrow biopsy was performed showing hypocellular marrow with multilineage maturation and myeloid left shift, with negative flow cytometry. Her lymphadenopathy was considered likely reactive in the setting of an otherwise negative evaluation. ANA serology was repeated and was again negative.

Three months later, the patient presented to the Emergency Department with inspirational chest pain. CT chest angiogram was normal and negative for pulmonary embolism, pleural effusion and aortic dissection. ECG and troponin were negative, and she was given a diagnosis of pleurisy. With her ongoing joint pain, rashes, reactive lymphadenopathy, and new pleurisy, a short course of steroids was started at her next rheumatology appointment, with significant symptom improvement.

Based on Systemic Lupus International Collaborating Clinics (SLICC) criteria, the patient met criteria for the diagnosis of SLE despite her negative ANA antibodies.[2] She met four clinical criteria, including acute cutaneous lupus rash (malar erythema), synovitis in two or more joints, serositis from her previous episode of pleurisy, and leukopenia. She met one immunologic criteria of low complement. Given a high clinical suspicion for systemic lupus, hydroxychloroquine was initiated. After 6 months of therapy there was no clear improvement in her rashes, joint pains or pleurisy. A trial of azathioprine was started, but patient was unable to tolerate due to gastrointestinal side effects. Methotrexate was discussed, but was avoided as patient was planning for future pregnancy. During this time her symptoms had been intermittently treated with prednisone as needed. Colchicine had also been given to treat episodes of pleurisy with some benefit. Given ongoing disease activity, monthly belimumab infusions were initiated. Six months into therapy the patient has tolerated the medication and has

reported significant improvement in her fatigue, rashes and pleurisy.

***Discussion***

Antinuclear antibodies are autoantibodies against nuclear auto-antigens. These antibodies are a defining feature of lupus and are also seen in other rheumatic diseases including Sjogren's, mixed connective tissue disease, and systemic sclerosis. They play an important role in classification criteria for lupus, and are used by the American College or Rheumatology and the Systemic Lupus International Collaborating Clinics to classify this disease.[3]

While ANA negative systemic lupus erythematosus is a rare presentation of the autoimmune disease, it is well documented in the literature despite the emphasis on presence of the auto-antibody. It is important to consider the reasons why a negative ANA test may be reported. It is necessary to understand how the ANA test is performed and recorded.

The gold standard for antinuclear antibody testing is indirect immunofluorescence (IIF), which uses human epithelial type 2 (Hep-2) cells.[4] The ANA test is considered positive when the presence of an indirect immunofluorescence staining is noted within the nucleus, hence the name anti-nuclear antibody. The detection of indirect immunofluorescence staining is reported as a titer, the detection of the antibody at various dilutions. The higher the dilution when the indirect immunofluorescence is detected, the higher the listed titer. High titer ANA results demonstrate positive staining despite multiple dilutions, due to a stronger ANA signal.

The staining pattern within the nucleus is also reported using this method. The types of patterns include homogenous, nucleolar, centromere, and speckled, as well as others which are noted along with the titer. Some patterns may be associated with certain ANA spectrum disorders. Homogenous pattern is associated with lupus, whereas the centromere pattern is associated with systemic sclerosis.[4]

Because ANA testing by indirect immunofluorescence test requires a laboratory technician to read and report the dilution and staining pattern, there can be variability with reported laboratory values on repeat testing. This is one reason why ANA testing is can vary in positivity and titer values when repeatedly tested.

While nuclear staining is traditionally required for a positive ANA test, there can be other patterns of indirect immuno-fluorescence staining that are not isolated to the nucleus. These other patterns can be isolated in the cytoplasm and mitotic cell patterns of the cell.[3] These patterns are not commonly reported or considered as ANA positive in many laboratories. This was demonstrated in a study by Choi et al, which found that of newly diagnosed lupus patients, 6.2% of them did not have any cellular antibody positivity, but 1.5% did have an isolated cytoplasmic and mitotic cell pattern. As a result, not including these staining patterns as positive results can misidentify patients with lupus antibodies that do not display clear nuclear patterns. In the future, patients that display these isolated cytoplasmic and mitotic cell patterns may be classified as having lupus, as classification criteria evolve to include patients that once would have been considered ANA negative.[3]

In patients where there is a high suspicion for lupus despite negative ANA testing, it is reasonable to test for other ANA sub-serologies including the anti-SSA and the anti-double stranded DNA antibodies. The presence of these antibodies may suggest systemic autoimmune disease despite the lack of ANA indirect immunofluorescence.[5] It is also noted that ANA negativity in lupus patients can be related to disease duration as well as disease activity and prior treatment. Patients early in their disease presentation may be ANA negative. Additionally, SLE patients with long standing disease or treated disease may no longer have ANA positivity due to disease remission.[5]

However, given that ANA negative lupus is rare, it is important that alternative diagnoses be considered. Other autoimmune diseases that should be ruled out include rheumatoid arthritis, undifferentiated and mixed connective tissue disease, Sjogren's, dermatomyositis, and adult-onset Still's disease. Additionally, evaluation for infectious etiologies such as HIV, Hepatitis B and C, EBV, CMV, and malignancies such as leukemia, lymphoma or myelodysplastic syndromes should be pursued.

***Conclusion***

ANA negative lupus is rare, through the well-documented presence gives us insight into the intricacies of ANA testing. Understanding ANA testing can assist interpretation of laboratory tests in the context of patients' clinical presentations.

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