

Connective Tissue Disease Testing: New Reflexive Panel Simplifies Test Ordering

Connective Tissue Diseases (CTDs) are a class of autoimmune disorders including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Less common entities include Sjogren's syndrome, scleroderma, mixed connective tissue disease and polymyositis. Symptoms tend to be nonspecific such as joint pain, fatigue, fever, depression, etc. CTDs may have serious complications including damage to the joints, kidneys, cardiovascular and other organ systems. Proper disease management is dependent upon accurate diagnosis, which in turn is dependent upon accurate laboratory tests. Unfortunately, laboratory testing is not always straightforward and there are a multitude of potential tests to consider. The new Bronson Connective Tissue Disease Testing Panel (CTD Panel) can greatly simplify the process for the ordering physician. But first, some background information on connective tissue disease testing will clarify the use of the panel.

Antinuclear Antibody (ANA) Testing

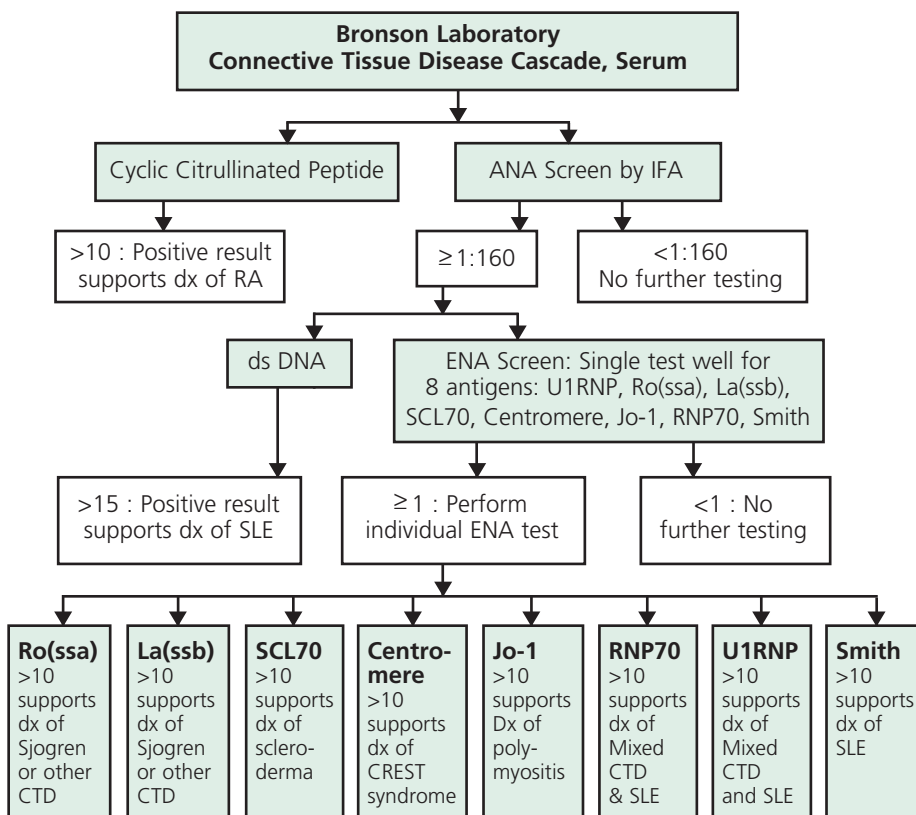
There are multiple techniques for ANA detection including automated solid state assays like enzyme linked immunosorbent assays (ELISA) and fluorescent multiplex immunoassays, but many labs, including ours, continue to utilize indirect immunofluorescence antinuclear antibody testing (IF-ANA) on intact Hep-2 cells. The problem with the IF-ANA is that it is an expensive, labor intensive process requiring an experienced technologist to read slides with an immunofluorescent microscope. The solid state assays are automated, but they contain a limited number of antigens, typically 8-10, and the antigens may lose sensitivity if they do not maintain their native 3-D conformation. The American College of Rheumatology considers IF-ANA as the gold standard because the cells have an estimated 100-150 target antigens and the greatest sensitivity for CTDs.

Strengths and Limitations of ANA Testing

The IF-ANA is highly sensitive, but not so specific. If the IF-ANA is negative there is a less than 0.14% chance of having SLE. A negative ANA, however, does not rule out other autoimmune diseases, rheumatoid arthritis (RA) for instance. The IF-ANA results are reported as a titer beginning with 1:40 and progressing to 1:80, 1:160, up to 1:2560. 32% of normal sera will be positive at 1:40 and it is considered a meaningless result, but at 1:160 only 5% of normal sera will be positive. Thus in the CTD Panel, only titers of 1:160 or higher will trigger additional testing. The ANA may be positive in a number of diseases and only about 30% will have a CTD such as SLE or RA. The other 70% may have various inflammatory diseases including thyroiditis, hepatitis or multiple sclerosis. Approximately 50% of patients with rheumatoid arthritis have a positive ANA. Because of the overlap in findings in SLE and RA, anti-cyclic citrullinated peptide (anti-CCP) antibodies are included as a first line test in our CTD-Panel. See accompanying article on anti-CCP.

Second Line Testing: Determining the Specificity of a Positive ANA

If an ANA is positive, it is crucial to determine if there is a specific antibody involved that correlates with a specific disease state. The CTD panel reflexes to testing for anti-double stranded DNA and a screen for 8 extractable nuclear antigens (ENAs). See Flow Chart. If the ENA screen is positive then each of the 8 components is tested individually. For example, a positive ANA followed by positive results for double stranded-DNA, positive ENA screen and positive Smith antibodies has a very high positive predictive value for SLE. In addition, if the anti-Ro is positive in SLE, there is increased risk of nephritis and vasculitis. In a similar situation, a positive ANA may lead to the identification of (*continued*)



Anti-CCP Antibody Testing for Rheumatoid Arthritis

Setting and History

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, tenderness, and destruction of synovial joints culminating in severe disability. RA is one of the most common systemic autoimmune diseases affecting up to 1% of the world population.¹ The American College of Rheumatology criteria for the classification of RA includes: 1) joint involvement, 2) serology with rheumatoid factor (RF) or Anti-CCP, 3) acute-phase reactants CRP or ESR, and 4) duration of symptoms for ≥ 6 weeks.² Historically the only serological test routinely used for detection of RA was RF IgM-RF. Indeed, 50-90% of patients with RA have RF antibodies, however the specificity is poor (68-90%) as RF is found in other autoimmune diseases as well as many infectious diseases and even in some healthy individuals.³ Better markers with higher specificity for RA were sought, and more recently antiperinuclear autoantibodies (APF) and antikeratin autoantibodies (AKA) have been demonstrated in patients with RA. These antibodies were demonstrated via immunofluorescence and hence testing for them never became popular. Since then, though, it has been discovered that APF and AKA react with the same antigen, a citrullinated amino acid. Multiple studies have now demonstrated that testing for anti-cyclic citrullinated peptide (anti-CCP) antibodies shows at least equal sensitivity to IgM-RF, but with superior specificity in the detection of early RA.^{4,5} The testing platform used here in our Bronson Laboratory (Phadia, EliA, ImmunoCAP 250) has demonstrated a sensitivity of 68% with a specificity of greater than 96%.^{3,5}

Anti-CCP Antibodies

Citrullination is the post-translational conversion of arginine to citrulline by the enzyme peptidyl arginine deaminase (PAD). PAD activation is assisted by calcium ions and is normally present as an inactive intracellular enzyme. PAD is thought to leak out of dying cells into synovial joints in patients with RA during apoptosis (cell death). Subsequently, this activated PAD causes citrullination of extracellular arginine and in the synovium behaves as an antigenic stimulant inducing the production of anti-CCP antibodies. Testing via enzyme linked immunosorbent assay (EliA) for these

anti-CCP autoantibodies has been shown to be both sensitive and highly specific for RA.^{3,5,6}

The first generation anti-CCP test platform was technically complex and thus was not widely used. However, the second generation platform has continued to show superior performance over the original test and is less complex. This has led to its adoption in most laboratories where it is offered, including here in our Bronson Laboratory (Phadia, EliA, ImmunoCAP 250).

Clinical Use of Anti-CCP

Detection of anti-CCP antibodies holds both diagnostic and therapeutic significance. Anti-CCP antibodies have been detected in the serum of patients with RA from 1.5 up to 9 years before the onset of disease.⁷ Additional clinical studies have revealed RA patients with anti-CCP antibodies may develop a more erosive form of the disease than those without anti-CCP antibodies.⁸ The use of anti-CCP antibodies allows a more specific diagnosis of RA with a sensitivity that is at least equal to that of the earlier IgM-RF testing. As the new classification criteria indicate, testing for anti-CCP antibodies should be incorporated into the work up of a patient with early signs and symptoms of RA.

Specimen Collection, Reference Values and Interpretation

The procedure can be performed with serum or plasma (0.5 mL) (Phadia, EliA, ImmunoCAP 250).

U/mL
< 7 U (negative)
7-10 U (equivocal)
>10 U (positive)

A positive result indicates a high likelihood of RA with a low false positive rate. Rarely, anti-CCP antibodies can be found in patients with other autoimmune or connective tissue diseases.⁹

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Connective Tissue Disease Testing

(continued) anticentromere antibody indicating the patient very likely has CREST syndrome or if the SCL-70 is positive, then the diagnosis is likely to be scleroderma. In summary, the CTD-Panel with reflex testing can provide not only a screen for connective tissue diseases, but may provide specific diagnostic and prognostic information.

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