

BEST PRACTICES IN: Identification and Diagnosis of Connective Tissue Disease

Introduction: According to the Lupus Foundation of America (LFA), an estimated 1.5 million Americans have some form of lupus.¹ Systemic lupus erythematosus (SLE) accounts for approximately 70% of all lupus cases, and in 50% of these cases, a major organ (heart, lungs, kidneys, or brain) is affected.¹ Diagnosing SLE and/or Connective Tissue Disease (CTD) is challenging for both the generalist and specialist—these diseases have heterogeneous symptomatic presentations that gradually evolve over time, they share similar signs and symptoms, mimicking one another and making differential diagnosis extremely difficult; with unpredictable courses.²⁻⁴ SLE and/or CTDs are associated with a reduced quality of life (ranging from fatigue and joint pain for mild patients to death for severe patients) and financial consequences associated with doctor appointments, additional testing costs, lost work, and reduced productivity.

Diagnosis is currently based upon the patient's medical history, current symptoms, and laboratory tests; disease activity is measured using various available indices.³ Until recently, there has not been an easy-to-use, reliable assessment using biomarkers for SLE.³

Current Diagnostic Paradigm: Many patients are initially evaluated—and often initially diagnosed—in the primary care setting. Recent evidence demonstrated that nearly half of these patients are misdiagnosed—with potentially serious consequences—often based solely on a positive ANA.⁴ Patients misdiagnosed with SLE may be unnecessarily prescribed potentially toxic and harmful medications (such as high dose steroids), undergo referrals and unnecessary laboratory tests, and may experience difficulty obtaining health or life insurance because of the diagnosis.⁴ On the other hand, patients with a delayed or undiagnosed SLE diagnosis may continue to experience disease progression leading to serious tissue and irreversible organ damage (eg, renal failure or pulmonary fibrosis) that could otherwise have been prevented with prompt diagnosis and appropriate treatment.⁴

A majority of patients with autoimmune diseases such as SLE and CTD have autoantibodies to cellular constituents, the most common of which are antinuclear antibodies (ANAs).⁵ Standard laboratory studies for SLE include ANA and anti-double stranded DNA (anti-dsDNA) antibody tests.² However, neither of these serological markers are adequately sensitive and specific to diagnose SLE or CTD by themselves.² A recent analysis of 4754 adults ≥ 12 years in the National Health and Nutrition Examination Survey 1999-2004 found that 13.8% of the population—an estimated 32 million Americans—are ANA positive (ANA+) and the majority of them do not have lupus.⁵ Thus, while ANA has high sensitivity (~95%) but low specificity (~25%), ds-DNA has high specificity (>95%) but low sensitivity (~40%), indicating that most patients with lupus do not have a positive anti-dsDNA. There is need for an accurate diagnostic test that can identify CTD/SLE early in the disease process, with both high specificity and sensitivity, and that may help provide definitive, differential diagnoses of lupus and CTDs.

Emerging Alternative to Current Practice: AVISE SLE and AVISE SLE+ Connective Tissue: Avise SLE and Avise SLE+ Connective Tissue diagnostic assays are unique in that they utilize biologically relevant Cell-Bound Complement Activation Product (CB-CAPs) technology in the diagnosis of SLE/CTDs. The complement system has been linked more closely to SLE than to any other human disease, supporting its consideration for biomarkers for SLE.³ Because the CB-CAPs fragment is bonded to the surface of the red or white blood cell, CB-CAPs levels are more stable and consistent than soluble complement (C3 and C4). In addition, there is a correlation between CB-CAPs and the degree of complement activation.³ These properties make CB-CAPs ideally suited as biomarkers of SLE.

Avise SLE is the only SLE test that incorporates the validated CB-CAPs technology, EC4d and BC4d markers, in a diagnostic panel for SLE. In addition to ANA and anti-dsDNA, Avise SLE assesses for anti-mutated citrullinated vimentin (MCV) antibodies and 2 CB-CAPs—EC4d (C4d fragments bound to erythrocytes) and BC4d (C4d fragments bound to B cells). Additionally, reflex testing is done on all anti-dsDNA positive results via *Critidia Lucilia IFA* to help rule-out false positives and on ANA negative results by indirect immunofluorescence to help rule-out false negatives. CB-CAPs (EC4d and BC4d) are increased in SLE patients indicating a high affinity for the disease.³ These 2 CB-CAPs markers are more stable and consistent than soluble complement markers and have been shown to be significantly increased in SLE patients compared to patients with other diseases or healthy controls.³ **Avise SLE+ Connective Tissue** includes the 5 marker Avise SLE test along with 8 extractable nuclear auto-antibodies (ENAs), 3 additional rheumatoid arthritis auto-antibodies, and 4 anti-phospholipid syndrome auto-antibodies. As a result, Avise SLE+ Connective Tissue can help clinicians diagnose SLE/lupus, rheumatoid arthritis, scleroderma, CREST, dermatomyositis, Sjogren's syndrome, polymyositis, and mixed CTD, and aids in the diagnosis of undifferentiated CTD (UCTD) for patients with initial, yet unclassified symptoms.

Validation of Avise SLE: The CAPITAL Validation Study: The performance of Avise SLE was validated in 2 prospective multi-centered studies involving 692 patients. The CAPITAL

validation study, published in *Arthritis & Rheumatism*, enrolled 593 adults in 15 lupus centers across the United States, including 210 patients with SLE, 178 with other rheumatic diseases, and 205 healthy subjects.² All study participants had their blood drawn once: one 10mL EDTA blood tube for FACS analysis (EC4d, ECR1, BC4d, PC4d) and one 5mL SST tube for serum isolation. Anti-dsDNA, ANA levels and anti-MCV were all measured using FDA cleared kits; an in-house developed and validated FACS assay was used to measure EC4d, BC4d, PC4d, and ECR1. Avise SLE is the only clinically validated SLE diagnostic.

The Avise SLE assay panel combining anti-dsDNA, ANA, and anti-MCV antibodies with cell surface EC4d and BC4d was both sensitive and specific for SLE—and required only 1 blood draw.² An index score (combining the weighted sum of ANA, EC4d, BC4d with anti-MCV) of >0 was 71.6% sensitive and 90.1% specific for SLE.² Combining the index score with anti-DNA led to a 50.5% improvement in clinical sensitivity (from 29.5% with anti-DNA alone to 80% combined) that outweighed the 9.6% loss in specificity (from 96.1% to 86.5%).² Overall, the Avise SLE assay was almost 30% more specific (86.6%) than ANA alone (57.7%) and 50% more sensitive (78%) than anti-dsDNA alone (28%).^{2,6}

Susan Manzi, MD

Richard H. Haddad, MD

Integrating the Avise SLE into Practice: The Avise SLE+ Connective Tissue uses a single blood draw (10mL EDTA,

5mL Serum) and delivers up to 20 marker results all via an easy-to-interpret single test requisition. Unlike other diagnostic tests for SLE and CTD, the Avise SLE assay does not require a patient to be in an active disease state/flare to register a test result. Physicians use pre-provided kits that are then shipped out that same day using pre-paid overnight delivery to Exagen's CLIA registered, CAP accredited laboratory, and results are returned within 5 business days. In contrast to other assays, which provide the clinician with raw laboratory results and require them to make their own interpretations, the Avise SLE provides clinicians with a single test report that includes not only the specific analyte results, but also an "overall result assessment." Included in this assessment is the actual "index value", the interpretation of the patient's results, and titer levels for each of the 20 markers ordered. An index value greater than 0 is suggestive of SLE; higher index values are highly suggestive of SLE. Physicians receive more information with the single Avise SLE+ Connective Tissue test result to help determine a differential, definitive and earlier diagnosis, minimizing the risk (and consequences) of delayed or misdiagnosis. This minimizes the need for ongoing laboratory tests over numerous years before obtaining an accurate diagnosis, which is medically, emotionally, and financially better for the patient.

Clinical Implications: The diagnosis of SLE or CTD is challenging—patients rarely present with all the symptoms at one time, and symptoms are nonspecific and heterogenous. The comprehensive Avise SLE and SLE+ Connective Tissue tests are easy to use (1 blood draw) and interpret based on the most current CB-CAPs technology, enabling physicians to provide a prompt and accurate diagnosis for patients with suspected disease.

Dr. Susan Manzi is co-director of the Lupus Center of Excellence and serves as chair of the Department of Medicine of West Penn Allegheny Health System in Pittsburgh, Pennsylvania. She is a vice chair and professor at Temple University in Philadelphia, Pennsylvania.

Dr. Richard H. Haddad is an internist and rheumatologist at Allegra Arthritis Associates in Red Bank, New Jersey. He is a clinical assistant professor of Medicine at UMDNJ/Robert Wood Johnson Cooper Hospital in Camden, New Jersey, faculty director of the A.J. Reginato Musculoskeletal Ultrasound course at Cooper Hospital in Camden, New Jersey, and director of the Albert Einstein College of Medicine Musculoskeletal Ultrasound Course in New York, New York.

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