A CME-CERTIFIED SUPPLEMENT TO RHEUMATOLOGY NEWS®

CLINICAL UPDATE

Diagnosis of Lupus in the New Age of Biomarkers

New and Emerging Biomarker Technology in the Diagnosis of Lupus

Joseph M. Ahearn, MD
Director, Autoimmunity Institute, Director, Lupus Center of Excellence, Allegheny Health Network
*Dr Ahearn is a consultant for Exagen Diagnostics.**

The author would like to thank Global Academy for Medical Education and Charles Bankhead for assistance with the preparation of this supplement.

Recent advances in cellular and molecular biology have provided new insights into the etiology and pathogenesis of diseases. This progress has catalyzed a new era of biomarker discovery that will likely play a major role in the diagnosis and clinical management of many diseases that continue to challenge even the most astute physicians. There is no greater need for improved diagnostic biomarkers than in the field of systemic lupus erythematosus (SLE), which continues to be frequently misdiagnosed, even by expert rheumatologists.[1] Improved accuracy of lupus diagnosis is essential to optimize therapeutic intervention and ensure treatment of the right patient with the right drug at the right time. Correct diagnosis of lupus versus lupuslike mimics is also critical for enrollment of subjects into lupus clinical trials that might be tainted even by a small number of misdiagnosed patients.

Historical "Gold Standards"

Assays for antinuclear antibody (ANA) and anti-double-stranded DNA antibody (anti-dsDNA) have been the basis of diagnostic laboratory testing for SLE for decades, with little advance. (In fact, efforts to increase the speed of ANA testing while reducing cost have led to a deterioration in the utility of currently available assays for ANA.[2]) Despite this controversy, both tests remain widely used, and both will likely retain a role in the clinical management of patients with lupus for the foreseeable future. The ANA assay is highly sensitive for SLE but lacks specificity. A negative test for ANA will exclude SLE with about 95% accuracy. As a result, the test remains useful as a general screen for SLE. In contrast, the anti-dsDNA assay has high specificity, such that the likelihood of SLE is 95% or greater if a patient tests positive. However, the test lacks sensitivity, and more than 50% of the patients with SLE will test negative at a single point in time.

Combining ANA and anti-dsDNA assays is a reasonable approach but still less than ideal. A patient who tests positive by ANA and anti-dsDNA assays almost certainly has SLE. However, in the majority (>50%) of cases, a patient with SLE will have a negative test for anti-dsDNA. A positive ANA and negative anti-dsDNA result does not distinguish a patient with lupus from a patient with another disease or even from a healthy individual, yet such individuals are often misdiagnosed and labeled as having lupus.

Harnessing the Complement System

Rheumatology researchers and clinicians have long recognized that the complement system is more intimately involved in the pathogenesis of SLE than virtually any other disease.[3] Many rheumatologists routinely monitor serum levels of C3 and C4 to assess disease activity in patients with SLE and as a diagnostic aid, even though the proteins have not been formally incorporated into classification systems for SLE. However, assays for serum C3 and C4 have never been validated as diagnostic biomarkers for lupus despite their clinical use for decades.

Laboratory tests for serum C3 and C4 measure the parent molecules or substrates of complement activation as opposed to the products. This is one of the drawbacks of C3 and C4 as lupus biomarkers because the acute-phase response during inflammation can increase C3 and C4 synthesis, offsetting or balancing activation. In addition, partial deficiencies of C4 occur in the general population and even more frequently in patients with SLE, resulting in below-normal C4 levels, which might not be distinguishable from lower C4 levels associated with complement activation or SLE flare.[4,5]

Over the past several years, investigation of the diagnostic potential of the complement system has shifted away from the parent protein and toward exploration of soluble complement activation products, including C3a, C5a, and C4d. Although promising, the results of these efforts to date have been insufficient to supplant measurement of the native C3 and C4 proteins.[3] The long history of clinical use of C3 and C4 for the clinical management of patients with lupus, the suboptimal utility of the assays, and advances in our understanding of the complement system have led to investigation of cell-bound complement activation products (CB-CAPs) as potential biomarkers for a lupus diagnosis.

CB-CAPs were recognized as a potential source of lupus biomarkers for several reasons, one of which was the observation that soluble CAPs might be rapidly hydrolyzed in the circulation or absorbed by cells and/or tissues, making them short lived. In addition, multiple types of hematopoietic cells express receptors for complement activation (split) products. In this regard, C4d has been identified on surfaces of normal erythrocytes, T and B lymphocytes, and reticulocytes.[6] In addition to potential as diagnostic biomarkers, the capacity of CAPs to bind covalently to cell surfaces suggested that CB-CAPs might be a fertile source of biomarkers for disease stratification based on the biology of distinct circulating cell types. Over the past decade, a series of investigations has demonstrated that patients with SLE have substantially higher levels of erythrocyte-bound, lymphocyte-bound, platelet-bound, and reticulocyte-bound CAPs than do healthy individuals or patients with other inflammatory, autoimmune, and rheumatologic diseases.[6-9]

Diagnostic Panel

Given the complexity of SLE, a single test is unlikely to provide results that a clinician can use with confidence for a definitive diagnosis. As such, CB-CAP assays have been shown to add significant value to accurate lupus diagnosis when combined with other tests such as the ANA and anti-dsDNA assays. The current panel, which will likely evolve with future study, includes ANA, anti-dsDNA, anti-mutated citrullinated vimentin (anti-MCV) antibody, and the CB-CAPs erythrocyte-bound C4d (E-C4d) and B-cell C4d (B-C4d). In a multicenter clinical trial conducted at 14 sites by investigators with expertise in lupus diagnosis, this panel demonstrated 80% sensitivity and greater than 80% specificity for a lupus diagnosis.[10]

From the most practical perspective, the CB-CAPs can prove especially useful to accurately rule in or rule out a diagnosis of lupus in patients who are ANA positive and anti-dsDNA negative, assays that are included in this panel. Moreover, a second-generation test panel has incorporated additional autoantibody tests for connective-tissue diseases, which can help distinguish patients with SLE from lupuslike conditions such as scleroderma and polymyositis.

CB-CAPs Beyond Lupus Diagnosis

Monitoring: The chronicity of SLE requires regular follow-up and adjustment of management strategies to control disease activity. In this regard, there is an urgent need to identify and validate lupus biomarkers for monitoring and predicting increasing disease activity and flares. Preliminary investigations of the reticulocyte-based RC4d and RC3d have demonstrated good correlation with disease activity and superior performance compared with measurements of serum C3 and C4.[11] A multicenter validation study was launched earlier this year to confirm the potential of CB-CAPs in monitoring SLE disease activity.

Stratification: Preliminary studies have identified platelets bearing C4d (PC4d) as a potential biomarker to identify patients with SLE who have an increased risk of acute ischemic stroke.[12]

Precision Medicine: Clinical care of patients with lupus is rapidly moving toward improved precision (personalized) medicine. It is generally held that not all patients benefit from the same therapeutic agents and not all biomarkers will be useful in all subsets of patients. Current efforts are under way to determine if CB-CAPs might serve as useful biomarkers to assess potential response to specific treatments such as those that interfere with complement activation during disease pathogenesis.

Original Release Date: November 2013
Most Recent Review Date: November 2013
Expiration Date: October 31, 2014
Estimated Time to Complete Activity: 0.5 hour
Medium or Combination of Media Used: Written Supplement
Method of Physician Participation: Journal Supplement
Hardware/Software Requirements: Windows operating system and high-speed Internet connection
Provider Contact Information: For questions about the CME activity content, please contact University of Louisville at cmepd@louisville.edu
Privacy Policy: All information provided by course participants is confidential and will not be shared with any other parties for any reason without permission.

Summary

CB-CAP assays have demonstrated potential for improving the diagnosis of SLE, as complementary tests to anti-dsDNA, ANA, and anti-MCV antibody. An assay panel containing tests for all of those markers has been validated by multicenter study and is available for use in clinical practice. Encouraged by the performance of CB-CAPs as a diagnostic aid, lupus researchers have begun exploring the potential of CB-CAPs for monitoring disease activity, detecting lupus flares, identifying patients at increased risk of thrombosis, and increasing therapeutic precision.

References: **1.** Narain S, Richards HB, Satoh M, et al. Diagnostic accuracy for lupus and other systemic autoimmune diseases in the community setting. *Arch Intern Med*. 2004;164:2435-2441. **2.** Fritzler MJ. The antinuclear antibody test: Last or lasting gasp. *Arthritis Rheum*. 2011;63:19-22. **3.** Liu CC, Manzi S, Kao AH, Navratil JS, Ahearn JM. Cell-bound complement biomarkers for systemic lupus erythematosus: From benchtop to bedside. *Rheum Dis Clin North Am*. 2010;36:161-172. **4.** Liu CC, Ahearn JM, Manzi S. Complement as a source of biomarkers in systemic lupus erythematosus: Past, present, and future. *Curr Rheumatol Rep*. 2004;6:85-88. **5.** Liu CC, Manzi S, Danchenko N, Ahearn JM. New advances in measurement of complement activation: Lessons of systemic lupus erythematosus. *Curr Rheumatol Rep*. 2004;6:375-381. **6.** Liu CC, Kao AH, Manzi S, Ahearn JM. Biomarkers in systemic lupus erythematosus: Challenges and prospects for the future. *Ther Adv Musculoskel Dis*. 2013;5:210-233. **7.** Manzi S, Navratil JS, Ruffing MJ, et al. Measurement of erythrocyte C4d and complement receptor 1 in systemic lupus erythematosus. *Arthritis Rheum*. 2004;50:3596-3604. **8.** Liu CC, Kao AH, Hawkins DM, et al. Lymphocyte-bound complement activation products as biomarkers for diagnosis of systemic lupus erythematosus. *Clin Transl Sci*. 2009;2:300-308. **9.** Liu CC, Manzi S, Kao AH, Ruffing MJ, Navratil JS, Ahearn JM. Reticulocytes bearing C4d as biomarkers of disease activity for systemic lupus erythematosus. *Arthritis Rheum*. 2005;52:3087-3099. **10.** Kalunian KC, Chatham WW, Massarotti EM, et al. Measurements of cell-bound complement activation products enhances diagnostic performance in systemic lupus erythematosus. *Arthritis Rheum*. 2012;64:4040-4047. **11.** Kao AH, Navratil JS, Ruffing MJ, et al. Erythrocyte C3d and C4d for monitoring disease activity in systemic lupus erythematosus. *Arthritis Rheum*. 2010;62:837-844. **12.** Kao AH, McBurney CA, Saltar A, et al. Relation of C4d with all-cause mortality and ischemic stroke in patients with systemic lupus erythematosus. *Trans Stroke Res*. In press.

Accreditation: This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the University of Louisville School of Medicine and Global Academy for Medical Education, LLC. The University of Louisville School of Medicine is accredited by the ACCME to provide continuing education for physicians.

Designation Statement: The University of Louisville Continuing Medical Education designates this enduring material supplement for a maximum of 0.5 *AMA PRA Category 1 Credit(s)*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Target Audience: This educational activity is designed for rheumatologists, internists, primary care physicians, physician assistants, nurse practitioners, and other healthcare providers who treat patients with SLE and other connective tissue diseases.

Educational Needs: Systemic lupus erythematosus (SLE) affects 1.5 million Americans, according to the Lupus Foundation of America. These patients have a substantially increased risk of morbidity and mortality as compared with the general population. Advances in diagnosis and treatment of SLE have led to a dramatic increase in 5-year survival, from about 50% during the 1950s to more than 90% today. However, much work remains to reduce the morbidity burden imposed by SLE. A great need exists for earlier and more accurate diagnosis. Studies suggest a misdiagnosis rate as high as 50%. In particular, clinicians and their patients could benefit greatly from more accurate biomarker-based laboratory tests for SLE. Tests for antinuclear antibodies represent only a general screen, and the widely used assay for anti-double-stranded DNA antibodies fails to provide definitive diagnoses for a substantial proportion of patients.

Learning Objectives: Upon completing this educational activity, participants should be able to:

- Identify conventional and emerging assays and biomarkers used to diagnose systemic lupus erythematosus (SLE)
- Understand the limitations of conventional diagnostic tests for SLE
- Describe the principles underlying the use of cell-bound complement activation products (CB-CAPs) to diagnose SLE
- Appreciate the need for multiple biomarkers and assays to obtain information necessary to make an accurate diagnosis of SLE.

Jointly sponsored by University of Louisville Continuing Medical Education & Professional Development; Global Academy for Medical Education

Supported by an educational grant from eXagen Diagnostics

*For additional disclosure information, go to the Web posting at globalacademycme.com/rheumatology

To get instant CME credit online, go to http://bit.ly/lupus2013

globalacademycme.com/rheumatology

Diagnosis of Lupus in the New Age of Biomarkers

New and Emerging Biomarker Technology in the Diagnosis of Lupus

CME Post-Test Answer Sheet and Evaluation Form

Release Date of Activity: November 2013 • **Expiration Date of Activity for AMA PRA Credit:** October 31, 2014

Estimated Time to Complete This Activity: 0.5 hour

To get instant CME credits online, go to **http://bit.ly/lupus2013**. Upon successful completion of the online test and evaluation form, you will be directed to a Web page that will allow you to receive your certificate of credit via e-mail. Please add cmepd@louisville.edu to your e-mail "safe" list. If you have any questions or difficulties, please contact the **University of Louisville School of Medicine Continuing Medical Education (CME & PD)** office at cmepd@louisville.edu.

CME Questions

Instructions: For each question or incomplete statement, choose the answer or completion that is correct. Circle the most appropriate response.

1. **Complete the statement.**
   Advances in cellular and molecular biology have:
   A. Eliminated most problems associated with misdiagnosis of SLE
   B. Eliminated overdiagnosis of SLE
   C. Eliminated underdiagnosis of SLE
   D. Failed to produce a biomarker or assay that permits diagnosis of SLE with reasonable certainty

2. **The historical laboratory standard for diagnosis of SLE has been**
   A. Antinuclear antibodies (ANA)
   B. Anti-dsDNA
   C. ANA and anti-dsDNA
   D. Cell bound complement-activation products

3. **A negative ANA test will:**
   A. Exclude a diagnosis of SLE with about 95% certainty
   B. Diagnose SLE with about 95% certainty
   C. Lacks both sensitivity and specificity for SLE
   D. None of the above

4. **The soluble complement-activation product Cd4 has been identified on:**
   A. Reticulocytes
   B. Erythrocytes
   C. T and B lymphocytes
   D. All of the above

EVALUATION FORM

We would appreciate your answering the following questions in order to help us plan for other activities of this type. All information is confidential. *Please print.*

Name: _____

Specialty: _____

Degree: ☐ MD ☐ DO ☐ PharmD ☐ RPh ☐ NP ☐ RN ☐ BS ☐ PA

☐ Other _____

Affiliation: _____

Address: _____

City: _____ State: _____ ZIP: _____

Telephone: _____ Fax: _____

E-mail: _____

Signature: _____

CME CREDIT VERIFICATION

I verify that I have spent _____ hour(s)/_____ minutes of actual time working on this CME activity. No more than 2.0 CME credit(s) will be issued for this activity.

COURSE EVALUATION: GAPS

This activity was created to address the professional practice gaps listed below. Please respond regarding how much you agree or disagree that the following gaps were met:

- Utilizing new treatment targets being researched for systemic lupus erythematosus (SLE).
- Using updated diagnostic testing methods for SLE.
- Utilizing adequate tools to diagnose SLE.

Did participating in this educational activity change your KNOWLEDGE in the professional practice gaps that are listed on the left?

Strongly Agree	Agree	Somewhat Agree	Disagree	Strongly Disagree
1	2	3	4	5

Please elaborate on your answer. _____

_____

_____

Did participating in this educational activity change your COMPETENCE in the professional practice gaps that are listed on the left?

Strongly Agree	Agree	Somewhat Agree	Disagree	Strongly Disagree
1	2	3	4	5

Please elaborate on your answer. _____

_____

_____

Did participating in this educational activity change your PERFORMANCE in the professional practice gaps that are listed on the left?

Strongly Agree	Agree	Somewhat Agree	Disagree	Strongly Disagree
1	2	3	4	5

Please elaborate on your answer. _____

_____

_____

Please identify a change that you will implement into practice as a result of participating in this educational activity (eg, new protocols, different medications).

_____

_____

How certain are you that you will implement this change?

Strongly Agree	Agree	Somewhat Agree	Disagree	Strongly Disagree
1	2	3	4	5

What topics do you want to hear more about, and what issue(s) in your practice will they address? _____

_____

_____

Were the patient recommendations based on acceptable practices in medicine? ❍ Yes ❍ No

If no, please explain which recommendation(s) was (were) not based on acceptable practices in medicine. _____

_____

_____

Do you think the articles were without commercial bias? ❍ Yes ❍ No

If no, please list the article(s) that was (were) biased. _____

_____

The University of Louisville thanks you for your participation in this CME activity. All information provided improves the scope and purpose of our programs and your patients' care.