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, with 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 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, 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 these assays, despite their broad clinical use for decades.) 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 of the high sensitivity, both tests remain widely used, and both will likely remain a vital component of the clinical management of patients with lupus for the foreseeable future. The ANA assay is sensitive for some patients with SLE and other connective tissue diseases. The ANA test is most specific for patients with SLE and other connective tissue diseases, such as Sjögren syndrome. Also, the ANA test is not sensitive for some patients with SLE, particularly those with late-stage disease. The ANA test is highly sensitive for SLE but lacks specificity. 

Combining ANA and anti-dsDNA assays is a reasonable approach but still less than ideal. A patient who tests positive for ANA and anti-dsDNA assays almost certainly has SLE. However, in the majority (>50%) of cases, a patient with SLE will test negative for both tests. As a result, a negative 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

Rheumatologists and researchers have long recognized the importance of the complement system in the pathogenesis of SLE; however, the chronicity of SLE requires regular follow-up to adjust disease activity and to detect disease progression. Many rheumatologists routinely monitor serum levels of C3 and C4 and 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 be distinguished from normal low levels associated with complement activation or SLE flare.3,4

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, C4a, and C5a. By using these markers as biomarkers for lupus, researchers have begun to validate assays for detecting 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 CB-CAPs might be rapidly hydrolyzed in the circulation or absorbed by cells and/or tissues, making them short-lived. In addition, multiple types of hemopoietic 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.5 In addition to potential as diagnostic biomarkers, the capacity of CB-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 C4d than healthy individuals or patients with other inflammatory, autoimmune, and rheumatologic diseases.6,7

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.8 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 C4-erythrocyte-bound C4d (E-C4d) and B-cell C4d (B-C4d). In a multicenter clinical trial conducted at 16 sites by investigators with expertise in lupus diagnosis, this panel demonstrated 80% sensitivity and greater than 80% specificity for a lupus diagnosis.

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 RBC4d and RCD4 have demonstrated good correlation with disease activity and superior performance compared with routine laboratory measurements of serum C3 and C4. In addition, 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 platelet-bearing C4d (PC4d) as a potential biomarker to identify patients with SLE who have an increased risk of acute ischemic stroke.2,9

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 which biomarkers will be useful to assess potential response to specific treatments such as those that interfere with complement activation during disease pathogenesis.

Summary

CB-CAP assays have demonstrated potential for improving the diagnosis of SLE, as well as being of interest as indicators of lupus disease activity.CB-CAP assays have demonstrated potential for improving the diagnosis of SLE, as well as being of interest as indicators of lupus disease activity. Assays that detect soluble and cell-bound CB-CAPs have shown improved accuracy for lupus diagnosis, this panel demonstrated 80% sensitivity and greater than 80% specificity for a lupus diagnosis. 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.

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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

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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?

<table>
<thead>
<tr>
<th>Strongly Agree</th>
<th>Agree</th>
<th>Somewhat Agree</th>
<th>Disagree</th>
<th>Strongly Disagree</th>
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<td>1</td>
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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?

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<thead>
<tr>
<th>Strongly Agree</th>
<th>Agree</th>
<th>Somewhat Agree</th>
<th>Disagree</th>
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Please elaborate on your answer. ____________________________________________

___________________________________________________________

How certain are you that you will implement this change?

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<thead>
<tr>
<th>Strongly Agree</th>
<th>Agree</th>
<th>Somewhat Agree</th>
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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.

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