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 and investigation. 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. Improved accuracy of lupus diagnosis is essential to optimize therapeutic intervention and ensure treatment of the right patient at the right time. Correct diagnosis of lupus versus lupuslike mimics is also critical to enrollment of subjects into 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 change in approach, even though the proteins have not been formally incorporated as a general screen for SLE. In contrast, the anti-dsDNA assay has 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 time point.

Combining ANA and anti-dsDNA assays is a reasonable approach but still not 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 will test negative by both tests if ANA is a general screen for SLE. In contrast, the anti-dsDNA assay has 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 time point.

Harnessing the Complement System

Rheumatologists and clinicians have long recognized the critical role that complement in the progression of SLE. 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 C3 and C4 occur in the general population and even more frequently in patients with SLE, resulting in below-normal C4 levels, which might confound a differential diagnosis. CB-CAPs have been identified in 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. These include C3a, C4a, C5a, and C5b-9, which might assist in distinguishing acute from chronic disease. CB-CAPs are 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 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 reticuloocytes. 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 reticuloocyte-bound C4d and anti-dsDNA than do healthy individuals or patients with other inflammatory, auto-immune, and rheumatologic diseases.5,6

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 test 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.7 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 auto-antibody 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 chronology 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 reticuloocyte-bound RC4d and RC3d have demonstrated good correlation with disease activity and superior performance compared with traditional measurements of serum C3 and C4 levels. 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 thrombosis.8

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 patients would benefit the most from specific therapeutic interventions. Without useful biomarkers 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 complement tests to anti-dsDNA, ANA, and anti-MCV antibody. An array of 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 to treat SLE by monitoring disease activity, detecting lupus flares, identifying patients at increased risk of thrombosis, and increasing therapeutic precision.

References

2. Foderer ML, Bond MG. Nucleosome antibody test: Last or last gap. Arthritis Rheum. 2011;63:19-22;3. Liu CC, Manzi S, Kau AH, Navratil JS, Ahearn JM. New improvements in complement assays for systemic lupus erythematosus: From benchtop to bedside. Rheum Dis Clin North Am. 2016;42:161-172.4. Liu CC, Manzi S, Kau AH, Ahearn JM. Cell-bound complement activation products (CB-CAPs) are useful in the diagnosis of SLE with high sensitivity and specificity. 2016;43:577-581.6. Liu CC, Kau AH, Manzi S, Ahearn JM. In systemic lupus erythematosus, 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 reticuloocytes. 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 reticuloocyte-bound C4d and anti-dsDNA than do healthy individuals or patients with other inflammatory, auto-immune, and rheumatologic diseases.

Dr. Ahearn is a consultant for Exagen Diagnostics. © The University of Louisville Continuing Medical Education. This supplement is produced by Global Academy for Medical Education, LLC. Neither the editors of AARC, the American Association for Respiratory Care, nor the Regents of the University of Louisville, the University of Louisville School of Medicine, or the ACCME have a financial interest in the content. The opinions expressed in this supplement are those of the faculty and do not necessarily reflect the views of the editors or any other participants for any reason without permission.

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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: November 30, 2015
Estimated Time to Complete This Activity: 0.5 hour

To get instant CME credits online, go to http://tinyurl.com/mrecos9z. 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

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>
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<th>Somewhat Agree</th>
<th>Disagree</th>
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Please elaborate on your answer.

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

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Please elaborate on your answer.

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

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Please elaborate on your answer.

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How certain are you that you will implement this change?

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

__________________________________________

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__________________________________________

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.

__________________________________________

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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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