Serum Biomarker Panel Testing for Systemic Lupus Erythematosus and Other Connective Tissue Diseases

Policy Number: 2.04.123
Origination: 8/2015
Last Review: 8/2018
Next Review: 8/2019

Policy
Blue Cross and Blue Shield of Kansas City (Blue KC) will not provide coverage for Serum Biomarker Panel Testing for Systemic Lupus Erythematosus and Other Connective Tissue Diseases. This is considered investigational.

When Policy Topic is covered
n/a

When Policy Topic is not covered
Serum biomarker panel testing with proprietary algorithms and/or index scores for the diagnosis of systemic lupus erythematosus and other connective tissue diseases is considered investigational.

Description of Procedure or Service

<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
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</thead>
</table>
| Individuals:  
  • With signs and/or symptoms of systemic lupus erythematosus | Interventions of interest are:  
  • Serum biomarker panel testing | Comparators of interest are:  
  • Established systemic lupus erythematosus classification systems  
  • Individual serum biomarker tests | Relevant outcomes include:  
  • Test accuracy  
  • Symptoms  
  • Quality of life |
| Individuals:  
  • With signs and/or symptoms of connective tissue disease (besides systemic lupus erythematosus) | Interventions of interest are:  
  • Serum biomarker panel testing | Comparators of interest are:  
  • Clinical diagnosis  
  • Individual serum biomarker tests | Relevant outcomes include:  
  • Test accuracy  
  • Symptoms  
  • Quality of life |

Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disease that can be difficult to diagnose because patients often present with diverse,
nonspecific symptoms, and commonly used laboratory tests are not highly accurate. Currently, the diagnosis of SLE depends on a combination of clinical signs and symptoms and individual laboratory tests. More accurate laboratory tests for SLE could facilitate diagnosis of the disease in many patients. Recently, laboratory-developed, diagnostic panel tests with proprietary algorithms and/or index scores for the diagnosis of SLE have become commercially available.

For individuals with signs and/or symptoms of SLE who receive serum biomarker panel testing, the evidence includes several diagnostic accuracy studies. Relevant outcomes are overall survival, test accuracy, and symptoms. One study evaluated a panel similar to a commercially available test; it found that the panel test had somewhat higher specificity and lower sensitivity than the most commonly currently used biomarkers. The clinical significance of this degree of difference in diagnostic accuracy is unclear. There is also uncertainty about how the use of a serum biomarker panel test for SLE would change patient management. The evidence is insufficient to determine the effects of the technology on health outcomes.

**Background**

**CONNECTIVE TISSUE DISEASES**

**Systemic Lupus Erythematosus**

Systemic lupus erythematosus (SLE) is an autoimmune connective tissue disease (CTD) that affects approximately 1.5 million individuals in the United States. It is one of several types of lupus, the others being cutaneous and drug-induced lupus. About 90% of lupus patients are women between the ages of 15 and 45 years. SLE causes inflammation and can affect any part of the body, most commonly the skin, heart, joints, lungs, blood vessels, liver, kidneys, and nervous system. Although generally not fatal, SLE can increase mortality, most commonly from cardiovascular disease due to accelerated atherosclerosis. SLE can also lead to kidney failure, which may reduce survival. The survival rate in the United States is approximately 95% at 5 years and 78% at 20 years. The morbidity associated with SLE is substantial. Symptoms such as joint and muscle pain can impact quality of life and functional status. SLE also increases patients’ risk of infection, cancer, avascular necrosis (bone death), and pregnancy complications (eg, preeclampsia, preterm birth). The course of the disease is variable, and patients generally experience periods of mild-to-severe illness (called flares) and remission.

**Other Connective Tissue Diseases**

Several other CTDs may require a differential diagnosis from SLE (eg, rheumatoid arthritis, Sjögren syndrome, antiphospholipid syndrome, and polymyositis.

Rheumatoid arthritis is a chronic inflammatory peripheral polyarthritis. Rheumatoid arthritis can lead to deformity through stretching of tendons and ligaments and destruction of joints through erosion of cartilage and bone. Rheumatoid arthritis can also affect the skin, eyes, lungs, heart, and blood vessels.
Graves disease is an autoimmune disorder that leads to overactivity of the thyroid gland. The disease arises from thyroid-stimulating hormone receptor antibodies. It is the most common cause of hyperthyroidism. Blood tests may show raised thyroid-stimulating immunoglobulin antibodies.

Hashimoto disease, also known as chronic lymphocytic thyroiditis, is an autoimmune disorder and is the most common cause of hypothyroidism second to iodine insufficiency. It is characterized by an underactive thyroid gland and gradual thyroid failure. Diagnosis is confirmed with blood tests for thyroid stimulating hormone (T4) and antithyroid antibodies.

Sjögren syndrome is an autoimmune disorder characterized by dryness of the eyes and mouth due to diminished lacrimal and salivary gland function. Affected individuals may also have symptoms of fatigue, myalgia, and cognitive dysfunction, which may be difficult to distinguish clinically from fibromyalgia or medication side effects. Typical antibodies include antinuclear antibody (ANA), anti-Sjögren-syndrome related antigen, anti-Sjögren syndrome type B, or rheumatoid factor.

Antiphospholipid syndrome is a systemic autoimmune disorder characterized by venous or arterial thrombosis and/or pregnancy morbidity. Antiphospholipid antibodies are directed against phospholipid binding proteins.

Polymyositis and dermatomyositis are inflammatory myopathies characterized by muscle weakness and inflammation. Dermatomyositis may also have skin manifestations.

**Diagnosis**
Patients with SLE often present with nonspecific symptoms such as fever, fatigue, joint pain, and rash, which can make the disease difficult to diagnosis. In some patients, the diagnosis of SLE can be made with certainty (eg, when there are typical symptoms of rash and joint symptoms, and laboratory testing shows a high-titer abnormal ANA in a pattern specific for SLE). However, in many other patients, the symptom patterns of SLE are less clear, and ANA testing is equivocal; as a result, cascade testing with additional serologic tests may be ordered. In addition, ANA testing alone can result in false positives due to low specificity.

**Classifications**
The diagnosis of SLE has been based on a combination of clinical symptoms and laboratory results. In 1997 the American College of Rheumatology (ACR) updated 1982 criteria for the classification of SLE.(3,4)

The ACR classification criteria are as follows:

1. Malar rash
2. Discoid rash
3. Photosensitivity
4. Mouth or nose ulcers (usually painless)
5. Arthritis (nonerosive) in 2 or more peripheral joints, along with tenderness, swelling, or effusion
6. Serositis: pleuritis or pericarditis
7. Renal disorder: excessive protein in the urine, or cellular casts in the urine
8. Neurologic disorder: seizures and/or psychosis, in the absence of offending drugs or known metabolic derangements
9. Hematologic disorders: hemolytic anemia, leukopenia, lymphopenia, or thrombocytopenia
10. Immunologic disorder: antibodies to double-stranded DNA (anti-dsDNA), antibodies to Smith antigen (anti-Sm), positive antiphospholipid antibody, or false-positive serologic test for syphilis known to be positive for at least 6 months
11. ANA test in the absence of drugs known to induce it

These criteria were originally developed for research, but they have been widely adopted in clinical care. Individuals who meet 4 or more of the 11 criteria are diagnosed with SLE. If a patient meets fewer than four of criteria, lupus can still be diagnosed by clinical judgment; it is recommended that a rheumatologist confirm the diagnosis.(5) ANA testing is usually performed for patients who present with signs and symptoms involving two or more organ systems, and individuals who test positive are recommended for additional laboratory testing.(6) Assessments of ACR’s 1982 criteria have reported sensitivities ranging from 78% to 95% and specificities ranging from 89% to 100%, with lower accuracy in patients with mild disease.(6)

In 2012, the Systemic Lupus International Collaborating Clinics (SLICC), an international group of researchers, developed revised criteria for diagnosing SLE.(7) These criteria include more laboratory tests than the earlier ACR criteria, including elements of the complement system. Patients are classified as having SLE if they satisfy 4 or more of the 18 criteria below, including at least 1 clinical criterion and 1 immunologic criterion, or they have biopsy-confirmed nephritis compatible with SLE and with ANA or antidsDNA antibodies. In a sample of 690 patients, the SLICC criteria had a sensitivity of 97% and a specificity of 84% for diagnosing SLE, whereas the ACR criteria applied to the same sample had a sensitivity of 83% and a specificity of 96%. It is not clear how well-accepted the SLICC recommendations are in the practice setting. The SLICC criteria are outlined in Table 1.

Table 1. Clinical and Immunologic Criteria

Clinical Criteria
- Acute cutaneous lupus (including but not limited to lupus malar rash)
- Chronic cutaneous lupus (including but not limited to discoid rash)
- Oral ulcers
- Nonscarring alopecia in the absence of other causes
- Synovitis involving ≥2 joints, characterized by swelling or effusion or and ≥30 min of morning stiffness
- Serositis
- Renal: excessive protein in the urine or cellular casts in the urine
- Neurologic disorder: seizures, psychosis, mononeuritis complex, or peripheral, or cranial neuropathy
• Seizures
• Hemolytic anemia
• Leukopenia or lymphopenia
• Thrombocytopenia

**Immunologic Criteria**

- Antinuclear antibody above laboratory reference range
- Antibodies to double-stranded DNA above laboratory reference range
- Antibodies to Smith nuclear antigen
- Antiphospholipid antibody
- Low complement (low C3, low C4, or low CH150)
- Direct Coombs tests in the absence of hemolytic anemia

As previously noted, the SLICC classification system includes a wider range of laboratory tests than the ACR criteria. To date, the most common laboratory tests performed in the diagnosis of SLE are serum ANA, and, if positive, tests for anti-dsDNA and anti-Sm. ANA tests are highly sensitive (ie, with a high negative predictive value) but have low specificity and relatively low positive predictive value, particularly when the ANA is positive at a low level. Specificity of testing can be increased by testing for specific antibodies against individual nuclear antigens (extractable nuclear antigens) to examine the “pattern” of ANA positivity. These include antigens against single- and dsDNA, histones, Sm, Ro, La, and RNP antibodies. The presence of anti-dsDNA or anti-Sm is highly specific for SLE because few patients without SLE test positive; however, neither test has high sensitivity.(8) The presence of other antibody patterns may indicate the likelihood of other diagnoses; eg, the presence of Ro and La antibodies suggests Sjögren syndrome, while the presence of anti-histone antibodies suggests drug-induced lupus.

Better diagnostic tests for SLE and other CTDs would be useful in clinical practice. A variety of biomarkers, including markers associated with the complement system, are being explored to aid in the diagnosis of lupus. The complement system is part of the immune system and consists of 20 to 30 protein molecules that circulate in the blood in an inactive form until activated by a trigger (eg, an infection)—and when the protein molecules are activated, a sequence of events known as the complement cascade is initiated. This cascade involves the proteolysis of a complement protein into a smaller protein and a peptide. The smaller protein is able to bind to the complex one at the surface of the invading microorganism, and the peptide diffuses away. For example, in the first step, complement protein C3 is cleaved into C3b and C3a. C3b binds to the surface of the microorganism and activates the next step in the cascade, the proteolysis of C5, and the small peptide, C3a diffuses away. The precursors C3 and C4 and the complement activation products (eg, C3a, C5a, C4d) have been considered as SLE biomarkers. More recently, cell-bound complement activation products, which live longer than circulating complement activation products, have been investigated as biomarkers of SLE.

In addition to exploration of individual biomarkers with higher accuracy than accepted markers (eg, ANA, anti-dsDNA), there is interest in identifying a panel of tests with high sensitivity and specificity for SLE diagnosis. At least 1
multibiomarker test to aid diagnosis of SLE and other CTDs is commercially available. This panel, Avise CTD (Exagen Diagnostics), contains 22 different tests. It combines 2 smaller panels, a 10-marker panel that includes common SLE tests, as well as cell-bound complement activation products (known as Avise Lupus) and a 12-marker panel that focuses on CTDs other than SLE (known as Avise CTD). Avise CTD includes nuclear antigen antibodies markers to help distinguish CTD, a rheumatoid arthritis panel to rule-in or rule-out rheumatoid arthritis, an antiphospholipid syndrome panel to assess risk for thrombosis and cardiovascular events, and a thyroid panel to help rule-in or rule-out Graves disease and Hashimoto disease. Specific biomarkers in the panel are listed in Table 2.

Table 2. Avise Systemic Lupus Erythematosus Tests

<table>
<thead>
<tr>
<th>Systemic Lupus Erythematosus Tests</th>
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<tbody>
<tr>
<td>10-marker Avise Lupus test</td>
</tr>
<tr>
<td>Auto-antibodies: ANA, anti-dsDNA, antimitated citrullinated vimentin, C4d erythrocyte-bound complement fragment, C4d lymphocyte-bound complement, anti-Sm, Jo-1, Sci-70, CENP, SS-B/La</td>
</tr>
<tr>
<td><strong>Avise CTD test</strong></td>
</tr>
<tr>
<td>Avise Lupus test plus the following:</td>
</tr>
<tr>
<td>Auto-antibodies: U1RNP, RNP70, SS-A/Ro</td>
</tr>
<tr>
<td>Rheumatoid arthritis auto-antibodies: rheumatoid factor IgM, rheumatoid factor IgA, anticyclic citrullinated peptide IgG</td>
</tr>
<tr>
<td>Anti-phospholipid syndrome auto-antibodies: cardiolipin IgM, cardiolipin IgG, β2-glycoprotein 1 IgG, β2-glycoprotein 1 IgM</td>
</tr>
<tr>
<td>Thyroid auto-antibodies: thyroglobulin IgG, thyroid, thyroid peroxidase</td>
</tr>
</tbody>
</table>

ANA: antinuclear antibody; anti-dsDNA: antibodies to double-stranded DNA; anti-Sm: antibodies to Smith nuclear antigen; Ig: immunoglobulin.

All 22 markers are assessed when the Avise CTD test is ordered. Avise CTD uses a 3-step process.(9) The 10-marker panel is done in 2 tiers, and the add-on 12-marker panel is done in a third step to further assist with the differential diagnosis of CTD. In addition, ANA testing is done by enzyme-linked immunosorbent assay and by indirect immunofluorescence. The 2-tiered testing approach to the 10-marker panel is described next.

Tier 1: Tests for anti-Sm, EC4d, BC4d, and anti-dsDNA. If any tests are positive, the result is considered suggestive of SLE and no further testing is done. Cutoffs for positivity are greater than 10 U/mL for anti-Sm, greater than 75 U/mL for EC4d, greater than 200 U/mL for BC4d, and greater than 301 U/mL for anti-dsDNA. Positive findings for anti-dsDNA are confirmed with a *Crithidia luciliae* assay.

Tier 2: If the tier 1 tests are negative, an index score is created, consisting of results of tests for ANA, EC4d and BC4d, antimitated citrullinated vimentin, anti-Jo-1, anti-Sci-70, anti-CENP, and anti-Ss-B/La. In other words, there are 6 additional markers and the ratio of EC4d to BC4d, both of which were measured in tier 1.)
The index score, calculated using a proprietary algorithm, rates how suggestive test results are of SLE. Although there is information on cutoffs used to indicate positivity for individual markers, information is not available on how precisely the index score is calculated. The score can range from -5 (highly nonsuggestive of SLE) to 5 (highly suggestive of SLE), and a score of -0.1 to 0.1 is considered indeterminate.

Exagen also offers the Avise Lupus Prognostic test, a 10-marker panel that can be ordered with the Avise Lupus/Avise CTD panels. The prognostic test focuses on patients’ risk of lupus nephritis, neuropsychiatric SLE, thrombosis, and cardiovascular events. The test includes anti-C1q, anti-ribosomal P, antiphosphatidylserine/prothrombin immunoglobulin (Ig) M and IgG, anti-cardiolipin IgM, IgG, and IgA and anti-β2-glycoprotein 1 IgM, IgG, and IgA. Four of the 10 markers are included in both panel tests.

**Treatment**

Treatments for SLE can ameliorate symptoms, reduce disease activity, and slow progression of organ damage; however, there is no cure for SLE. Muscle and joint pain, fatigue, and rashes are generally treated initially with nonsteroidal anti-inflammatory drugs. Antimalarial drugs such as hydroxychloroquine can relieve some symptoms of SLE including fatigue, rashes, and joint pain. Patients with more severe symptoms (eg, heart, lung, or kidney involvement) can be treated with corticosteroids or immune suppressants. There are also biologic treatments (eg, rituximab) approved by the U.S. Food and Drug Administration for treatment of rheumatoid arthritis and are being evaluated for SLE.

**REGULATORY STATUS**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). The Avise® tests (Exagen Diagnostics, Vista, CA) are available under the auspices of CLIA. CLIA must license laboratories that offer laboratory-developed tests for highcomplexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

**Rationale**

This evidence review was created in August 2014 and has been updated regularly with searches of the MEDLINE database. The most recent literature update was performed through April 25, 2017.

Assessment of a diagnostic technology typically focuses on 3 categories of evidence: (1) technical performance (test-retest reliability or interrater reliability); (2) diagnostic accuracy (sensitivity, specificity, and positive and negative predictive value) in relevant populations of patients; and (3) clinical utility (demonstration that the diagnostic information can be used to improve patient outcomes).
SERUM BIOMARKER PANEL TESTING FOR SYSTEMIC LUPUS ERYTHEMATOSUS AND OTHER CONNECTIVE TISSUE DISEASES

Clinical Context and Test Purpose
The purpose of a serum biomarker panel is to inform the differential diagnosis of connective tissue diseases that share similar symptoms. This diagnosis may allow for earlier appropriate treatment and reduce organ damage.

The question addressed in this evidence review is: Does a serum biomarker panel for systemic lupus erythematosus (SLE) and other connective tissue diseases (CTDs) improve diagnosis compared with established clinical criteria and laboratory tests?

The following PICOTS were used to select literature to inform this review.

Patients
The relevant population(s) of interest are patients who have signs and/or symptoms of SLE or other CTDs but have not been diagnosed. Most of the initial clinical features of SLE are nonspecific and include fatigue, joint and muscle pain, rash, and headaches; initial laboratory features may also be nonspecific.

Interventions
This evidence review focuses on a commercially available multibiomarker test to aid in the differential diagnosis of SLE and other CTDs. This panel, Avise CTD, contains 22 different tests. This panel combines 2 smaller panels, a 10-marker panel that includes common SLE tests, as well as cell-bound complement activation products (CB-CAPs; known as Avise Lupus) and a 12-marker panel that focuses on CTDs other than SLE (known as Avise CTD).

Comparators
Diagnosis is based on a combination of clinical symptoms and laboratory results from the 1997 American College of Rheumatology (ACR) criteria (see Background section).

Outcomes
Beneficial outcomes include a differential diagnosis of SLE from other CTDs and appropriate treatment, leading to a reduction in joint and organ damage. Specifically, diagnostic accuracy is the outcome of primary interest because the intent of the Avise Lupus and Avise CTD is to rule out fibromyalgia and facilitate the differential diagnosis of SLE from other CTDs including rheumatoid arthritis, Graves disease, Hashimoto disease, Sjögren syndrome, antiphospholipid syndrome, and polymyositis and dermatomyositis.

Harmful outcomes include misdiagnosis. A false-positive test results can lead to adverse drug-related treatment effects. A false-negative test results can lead to a lack of appropriate treatment.
**Timing**
Follow-up for several years may be needed to assess the accuracy of the diagnosis.

**Setting**
These tests may be ordered by a specialist in autoimmune disorders and processed in a central laboratory.

**Technical Accuracy**
Some individual biomarkers (eg, antinuclear antibodies [ANA] and antibodies to double-stranded DNA [anti-dsDNA]) are considered standard of care in the diagnosis of CTDs, and, presumably, the technical accuracy of these tests has been established. In 2017, Dervieux et al reported the technical accuracy of the Avise Lupus biomarker panel.(10) Complement split product complement 4d (C4d) bound to erythrocytes (EC4d) and B lymphocytes (BC4d) were stable for 2 days at ambient temperature and for 4 days at 4°C. The median intraday coefficient of variation ranged from 2.9% to 7.8% (n=30) and the interday coefficient of variation ranged from 7.3% to 12.4% (n=66). The 2-tiered index score was reproducible over 4 consecutive days when blood was stored at 4°C.

**Diagnostic Accuracy**

**Novel Panel Components: CB-CAPs**
As previously discussed, CB-CAPs are key components of a commercially available biomarker panel test for lupus diagnosis. CB-CAPs include C4d levels on erythrocytes, platelets, and B cells. Preliminary investigations of each biomarker have been done by a U.S. research team.

A study on lymphocyte-bound complement activation products was published by Liu et al in 2009.(11) This cross-sectional study included 224 patients with SLE (according to ACR classification criteria), 179 patients with other autoimmune or inflammatory diseases, and 114 healthy controls. Levels of lymphocyte-bound complement activation products, T-cell bound C4d (TC4d) and C3d (TC3d), and B-cell bound C4d (BC4d), and C3d (BC3d) were measured in all participants. The diagnostic accuracy of these markers was assessed using receiver operating characteristic analysis. The area under the curve was 0.727 for TC4d and 0.770 for BC4d. TC4d was estimated to be 56% sensitive and 80% specific for differentiating SLE from other diseases. BC4d had 56% sensitivity and 80% specificity.

In addition, the authors compared the CB-CAPs with other, conventionally used, SLE markers. The markers were evaluated as a confirmatory test in patients who tested positive for ANA. This analysis only included the SLE patients, 223 (99.6%) of 224 of whom were positive for ANA. Of the 223 ANA-positive patients, 141 (63%) patients had elevated levels of TC4d and/or BC4d. In contrast, 59 (28%) of the 209 ANA-positive patients tested positive for anti-dsDNA. Moreover, when the more commonly used complement activation products, serum C3 and serum C4,
were evaluated, 67 (30%) of 221 of ANA positive patients tested positive for C3 and 82 (37%) of 221 patients tested positive for C4.

Previously, a 2006 cross-sectional study of platelet C4d was published by Navratil et al.(12) They assessed 105 patients with SLE (according to ACR criteria), 115 patients with other autoimmune or inflammatory diseases, and 100 healthy controls. Abnormal levels of platelet C4d were detected in 18% of SLE patients. False-negative rates and sensitivity rates were not reported. The authors reported that the marker was 100% specific for a diagnosis of SLE compared with healthy controls and 98% specific compared with patients who had other diseases.

**Serum Biomarker Panel Tests**

In 2014, Putterman et al published data from a large cross-sectional, industry-sponsored study evaluating serum biomarkers for the diagnosis of SLE.(13) This study analyzed the 10 markers in the Avise Lupus (plus ANA) using a 2-tier testing logic similar to that employed in the commercially available panel (see the Background section). The study evaluated 2 cohorts (total N=794 patients); 593 participants were enrolled between April and August 2010, and 201 participants enrolled between June 2011 and September 2013. Together, the 2 cohorts consisted of 304 patients who met ACR classification criteria for SLE, 161 patients diagnosed with other rheumatic diseases, and 205 healthy volunteers. Results of serum testing were available for 764 (96%) of 794 participants.

The diagnostic accuracy of the CB-CAP EC4d and BC4d were compared with reduced complement (C3, C4) and anti-dsDNA. The area under the receiver operating characteristic curve was significantly higher for EC4d (0.82) and BC4d (0.84) than for C3 (0.73) and C4 (0.72) (p<0.001). The area under the receiver operating characteristic curve was significantly higher for BC4d than for anti-dsDNA (0.79; p=0.009), but the difference was not a statistically significant between EC4d and anti-dsDNA. A total of 140 (46%) patients with SLE, 9 (3%) patients with other diseases, and 1 healthy volunteer tested positive for at least 1 of the 4 tier 1 markers. Patients testing negative for tier 1 tests underwent tier 2 testing and an index score was calculated. A total of 102 (62%) of 164 patients with SLE analyzed in tier 2 had an index score greater than 0 (ie, suggestive of SLE). Moreover, 245 of 276 patients with other rheumatic diseases had an index score less than 0 (ie, not suggestive of SLE). When results of tier 1 and 2 testing were combined, the overall sensitivity for SLE was 80% (242/304) and the overall specificity for distinguishing SLE from other diseases was 86% (245/285). The specificity for distinguishing between SLE and health volunteers was 98% (201/205).

As shown in Table 3, the specificity and area under the curve were higher for models including CB-CAPs than without these markers; sensitivity was slightly lower.

**Table 3. Diagnostic Accuracy of Various Combinations of Markers**

<table>
<thead>
<tr>
<th>Measures</th>
<th>dsDNA, Sm, and</th>
<th>dsDNA, Sm, ANA, Plus Antibody</th>
<th>Two-Tiered Testing Using All Markers,</th>
</tr>
</thead>
</table>


Previously, an industry-sponsored study published in 2012 by Kalunian et al reported on the first cohort of 593 individuals included in the Putterman analysis.(14) The sample consisted of 210 patients with SLE who met ACR classification criteria, 178 patients with other rheumatic diseases, and 205 healthy volunteers. Authors evaluated the performance of a 7-marker biomarker panel for the diagnosis of SLE; some markers are included in a commercially available panel test.(14) The biomarkers included ANA, anti-dsDNA, and antimutated citrullinated vimentin measured by enzyme-linked immunosorbent assay. In addition, the authors assessed the CB-CAPs (EC4d, PC4d, BC4d), determined by fluorescence-activated cell sorting.

In a multivariate logistic regression, SLE diagnosis was associated with a positive ANA test, a negative antimutated citrullinated vimentin test, and elevated EC4d and BC4d levels (area under the curve, 0.92; p<0.001). The weighted sum of these 4 markers correctly categorized 106 (71.6%) of 148 SLE patients who were anti-dsDNA-negative. (The investigators evaluated the 4-marker index score among individuals who tested negative for anti-dsDNA because of the low sensitivity of this test [29.5%], thus the high false negative rate.) The specificity of the 4-marker index was 98.0% (200/204 healthy volunteers with test results were correctly classified). When anti-dsDNA was added to the 4-marker panel, the test had 80% sensitivity for SLE (168/210 SLE patients were correctly classified). Moreover, this 5-marker test had 97.6% specificity among healthy individuals (200/205 were correctly classified as not having SLE). The 5-marker test also had 87% specificity in patients with other rheumatic diseases; the most false positives (n=9) were in patients with rheumatoid arthritis. The biomarkers in the 5-marker test are part of the 10-marker Avise 2.0 SLE test marketed by Exagen. It is not clear whether the index score reported along with the Avise 2.0 panel is the same as or different from the index score reported in the Kalunian study.

A limitation of the Putterman and Kalunian studies is that study sample populations included patients with SLE who met ACR classification criteria, but not patients with symptoms suggestive of SLE who failed to meet ACR criteria. It is not known how the diagnostic accuracy of the panel test compares with the ACR classification criteria or with concurrent clinician diagnosis (in the Putterman study, the mean time since SLE diagnosis was 11 years). Furthermore, although they are included in the Systemic Lupus International Collaborating Clinics classification criteria, the complement factors C3 and C4 are not widely used in clinical practice.

<table>
<thead>
<tr>
<th></th>
<th>ANA</th>
<th>Specificity Components But Not CB-CAPs</th>
<th>Including CB-CAPs EC4d and BC4d</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity, %</td>
<td>89</td>
<td>83</td>
<td>80</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>53</td>
<td>76</td>
<td>86</td>
</tr>
<tr>
<td>Area under the</td>
<td>0.78</td>
<td>0.80</td>
<td>0.91</td>
</tr>
</tbody>
</table>

ANA: antinuclear antibodies; CB-CAP: cell-bound complement activation product; dsDNA: double-stranded DNA; Sm: Smith nuclear antigen.
to diagnose lupus and, therefore, the clinical significance of higher diagnostic accuracy for EC4d and BC4d is unclear.

In 2016, Mossell et al reported on an industry-sponsored retrospective study of 23 patients who had a positive Avise Lupus test result and 23 patients who had a negative result.(15) All patients were ANA positive but negative for auto-antibodies specific for SLE, representing cases difficult to diagnosis. Each positive Avise test case was matched to a control (negative test) from the same clinic with the same ANA level. A chart review was performed by a nonblinded rheumatologist approximately 1 year after the test results were available. Of the cases with a positive Avise Lupus test, 20 (87%) were diagnosed with SLE during follow-up. This compared with 4 (17%) individuals who had a negative result on the Avise Lupus test, resulting in sensitivity of 83.3% and specificity of 86.4%. Interpretation of this study is limited due to the retrospective design, relatively short follow-up to monitor progression of disease, and the lack of an independent reference standard, because diagnosis was based in part on the results of that test. The authors noted that prospective studies would be performed.

Clinical Utility
No studies were identified that provide direct evidence on the impact of serum biomarker panel testing for SLE on patient outcomes. A more accurate and timelier diagnosis of SLE (ie, before multiorgan system involvement) and other CTDs could lead to better patient management (eg, more appropriate medical treatment). This, in turn, could improve health outcomes (eg, less joint or organ damage, improved survival).

SUMMARY OF EVIDENCE
For individuals with signs and/or symptoms of systemic lupus erythematosus (SLE) or connective tissue disease (besides SLE) who receive serum biomarker panel testing, the evidence includes several diagnostic accuracy studies. Relevant outcomes are test accuracy, symptoms, and quality of life. One study evaluated a panel similar to a commercially available test; it found that the panel test had somewhat higher specificity and lower sensitivity than the most common currently used biomarkers. The clinical significance of this degree of difference in diagnostic accuracy is unclear. One case-control study found a high sensitivity and specificity for a commercially available test for diagnosing SLE, but this retrospective analysis has several limitations, and prospective studies are therefore needed. The evidence is insufficient to determine the effects of the technology on health outcomes.

SUPPLEMENTAL INFORMATION
PRACTICE GUIDELINES AND POSITION STATEMENTS
In 2014, an international group including participants from the European autoimmunity standardization initiative and the International Union of Immunologic Societies published recommendations on the assessment of auto-antibodies to cellular antigens.(16) The recommendations included the following statements relevant to the diagnosis of systemic lupus erythematosus (SLE):
“1. The diagnosis of SARD [systemic autoimmune rheumatic diseases] requires a panel of specific laboratory tests (ie, ANA [antinuclear antibodies], anti-dsDNA [double-stranded DNA], anti-ENA [extractable nuclear antigen] antibodies)

2. The detection of ANA is the first-level test for laboratory diagnosis of SARD....

14. If the ANA test is positive, testing for anti-dsDNA antibodies is advised when there is clinical suspicion of SLE

19. In case of a positive ANA test ... it is recommended to perform specific tests for anti-ENA antibodies....”

**U.S. PREVENTIVE SERVICES TASK FORCE RECOMMENDATIONS**

Not applicable.

**MEDICARE NATIONAL COVERAGE**

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

**ONGOING AND UNPUBLISHED CLINICAL TRIALS**

A search of ClinicalTrials.gov in June 2017 did not identify any ongoing or unpublished trials that would likely influence this review.

References:


**Billing Coding/Physician Documentation Information**

**83520** Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified

**86038** Antinuclear antibodies (ANA);
**86039** Antinuclear antibodies (ANA); titer

**86146** Beta 2 Glycoprotein I antibody, each
**86147** Cardiolipin (phospholipid) antibody, each Ig class

**86200** Cyclic citrullinated peptide (CCP), antibody
**86225** Deoxyribonucleic acid (DNA) antibody; native or double stranded
**86235** Extractable nuclear antigen, antibody to, any method (eg, nRNP, SS-A, SS-B, Sm, RNP, Sc170, J01), each antibody

**86376** Microsomal antibodies (eg, thyroid or liver-kidney), each
**86800** Thyroglobulin antibody

**88184** Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
**88185** Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker (List separately in addition to code for first marker)

**88187** Flow cytometry, interpretation; 2 to 8 markers

**84999** Unlisted chemistry procedure

**ICD-10 Codes**

**D89.82** Autoimmune lymphoproliferative syndrome [ALPS]
**D89.89** Other specified disorders involving the immune mechanism, not elsewhere classified

**D89.9** Disorder involving the immune mechanism, unspecified

**L40.50-** Arthropicathic psoriasis, code range

**L40.59**

**M06.4** Inflammatory polyarthritis

**M12.80-** Other specific arthropathies, not elsewhere classified, code range
M12.89  Arthropathy, unspecified
M12.9   Arthropathy, unspecified
M19.90- Osteoarthritis, unspecified site, code range
M19.93  Osteoarthritis, unspecified site, code range
M32.0- Systemic lupus erythematosus (SLE), code range
M32.9   Systemic lupus erythematosus (SLE), code range
M35.9   Systemic involvement of connective tissue, unspecified
M79.0   Rheumatism, unspecified
M79.1   Myalgia
R53.8   Other malaise and fatigue

Due to the reporting of an index score for the entire panel, the test would more accurately be reported with the unlisted multianalyte assay with algorithmic analysis (MAAA) CPT code – 81599.

**Additional Policy Key Words**

N/A

**Policy Implementation/Update Information**

8/1/15  New Policy. Considered Investigational.
8/1/16  No policy statement changes.
8/1/17  No policy statement changes.
8/1/18  The phrase “and other connective tissue diseases” added to policy statement and title.

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