FEATURE ARTICLE

Anti-CarP & Other Biomarkers for Diagnosis & Prognosis of Rheumatoid Arthritis

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For patients with rheumatoid arthritis (RA), treating the disease early and appropriately is essential for the best chance of remission or comfortable management. 1 Not all cases of RA are identical, however. Clinical features and prognosis of each patient's case help inform the best course of treatment for the individual. There are a few biological markers, measured by a blood test, which indicate a poor prognosis and the necessity for more robust treatment, such as biological therapy. [1,2]

The main biomarkers for RA are autoantibodies, which are indicative of an immune system imbalance. Which autoantibodies test positive can allow a doctor to diagnose and generate a prognosis for RA.[3,4] Here are the current commonly-used biomarkers and what they can mean for RA prognosis and treatment.

What are autoantibodies?

RA is an autoimmune disorder, meaning that the immune system reacts to parts of its own body. The proteins that cells produce sometimes become modified slightly, either due to inflammation or by other means. In RA and other autoimmune diseases, white blood cells incorrectly identify these modified proteins as foreign antigens, which typically come from pathogens like viruses or bacteria. In response, the immune system starts attacking self-cells as it would invading microbes. [3,4]

Antibodies produced by immune cells normally recognize and bind to pathogen proteins. However, some are made that will specifically recognize modified self-proteins. These are known as autoantibodies. The presence of autoantibodies in a person's blood are indicators that they have an autoimmune disorder, often long before the onset of any symptoms. A few autoantibodies have been identified in RA patients and are used to diagnose the disease. Which types are present, and their relative abundance, can also serve as prognostic factors. [3,4]

Rheumatoid factor (RF)

Rheumatoid factor (RF) is an autoantibody that reacts to and attacks healthy tissue in the body. Though RF is present in small amounts in some healthy individuals and people with conditions other than RA, it is found in 53-80% of RA patients. RF can be detected in the bloodstream up to ten years prior to the onset of symptoms in RA patients; however, not everybody who tests positive for RF ends up developing RA in the future.[4,5]

RF as a prognostic factor RF was the first autoantibody identified as an RA marker, and RF assays have an accuracy that has been eclipsed by more recent biomarkers. The presence of RF is associated with markers of inflammation in the joints. Similarly, patients who are positive for RF have a greater chance of developing joint erosion damage, and an even higher risk if present in conjunction with other RA biomarkers.[5,6]

Anti-citrullinated protein antibodies (ACPA)

During inflammation, some amino acids in proteins get converted into citrulline molecules. Sometimes white blood cells will produce antibodies that recognize and bind to proteins that have been citrullinated. These are called anti-citrullinated protein antibodies (ACPA). Like RF, ACPA are present in about 50-78% of RA patients and can be detected as early as ten years before symptoms develop. However, not all people who test positive for ACPA end up with RA later in their lives.[7,8]

ACPA as a prognostic factor

ACPA assays are currently the most sensitive and accurate tests for diagnosing RA, both before and after symptoms develop. Like RF, ACPA positivity is associated with higher inflammation, as well as higher disease activity, and very high levels have been shown to correlate with more severe disease. The presence of ACPA along with RF are included in the American College of Rheumatology 2010 diagnostic criteria for RA.[6]

Anti-CarP antibodies

Distinct from citrullinated proteins, some proteins undergo carbamylation. When this process is out of balance the body can develop antibodies which recognize and bind to carbamylated proteins. These are called anti-CarP antibodies, and have recently been identified as useful biomarkers for the diagnosis and prognosis of RA. While not as specific to RA as ACPA, 34-53% of RA patients have anti-CarP antibodies in their blood. They can also be detected years before the onset of symptoms and have been found in RA patients who do not test positive for either RF or ACPA. Therefore, Anti-CarP has recently become recognized as a useful marker for determining the status of RA in patients where the traditional biomarkers otherwise could not.[9-11]

Anti-CarP antibodies as prognostic factor

% of Patients with Erosive Arthritis After 96 Months [13]

p=0.009

Data from Truchetet et al.
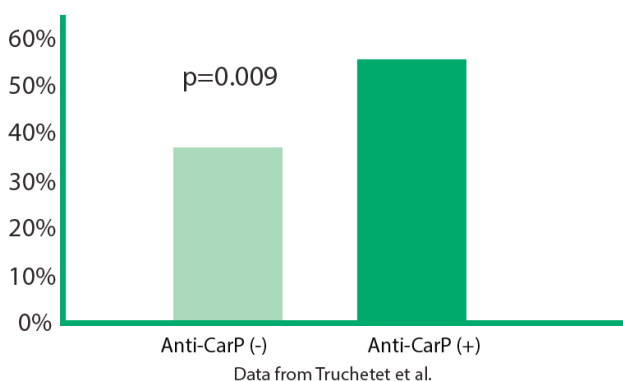
The presence of anti-CarP antibodies is associated with a more active disease status, both at the time of testing and later over time. RA patients who are positive for anti-CarP are nearly twice as likely to develop joint degradation as those who do not have anti-CarP antibodies in their bloodstream. When anti-CarP antibodies are present along with ACPA, the chance of joint erosion increases even more. [12,13] Disability due to inflammatory arthritis is more common in patients who are anti-CarP positive, even in those who are negative for both ACPA and RF.[14]

Testing for prognostic markers

The presence of autoantibodies in the bloodstream can be detected by performing a simple blood draw and running various tests. ACPA and RF are standard biomarkers for RA diagnosis, so there are several different tests that can be done for these. Often, a laboratory will use cyclic citrullinated peptides (CCP) to check for a reaction with ACPA.[6]

As it is a newer biomarker, fewer tests have been developed and validated for anti-CarP antibodies. Exagen, a life science company focused in rheumatology has recently developed an assay called AVISE® Anti-CarP that can be performed with a routine blood draw, the test is also included in the AVISE CTD test.

Treatment options

Since RA is so variable, there is no one standard of treatment based on specific combinations of RA markers. Ultimately, the course of therapy is left to doctor discretion. If a patient tests positive for anti-CarP antibodies, or more than one autoantibody, they may be considered to have a poor prognosis and require a more stringent treatment, such as biologic agents or combination therapy. Fortunately, poor prognosis patients respond to treatment as well as normal prognosis patients if treatment starts in the early stage of RA.[15]

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New AVISE SLE Monitor Test Incorporates A Powerful Combination of Biomarkers to Provide Actionable Results

PRACTICAL APPLICATIONS

CASE STUDY

A 57-year-old African American female with SLE was under the care of Dr. Macalester and started complaining of worsening symptoms. The patient had a medical history of fatigue and idiopathic thrombocytopenia, which had been successfully treated using intravenous immune globulin (IVIG).

The patient also had a medical history of:
- Multiple miscarriages
- Pleural and pericardial effusions
- Thoracentesis revealed increased white blood count, 26% neutrophils but gram stain and culture were negative
- An axillary lymph node biopsy, which was complicated by a staph infection, showed reactive changes
- Bone marrow biopsy showed hypercellularity consistent with an atypical myeloproliferative disorder, which was treated using prednisone;platelet count came up to 148,000
- Volume loss in both lung bases, prednisone dose was increased to 20 mg
- No history of blood clots
- Musculoskeletal pain in the large muscle groups, as well as pain in her palms and distalinterphalangeal joints

Previous Labs

Previous labs revealed border-line positivity for IgG anticardiolipin and moderate positive for IgM anticardiolipin. Additional labs also revealed, the patient had ANA positivity at 1:1280 and anti-dsDNA positivity. Lab results were also positive for: anti-RNP, anti-SSA, anti-SCL-70 and anti-SMA.

However, the patient had consistently normal levels of soluble complement at every visit. The patient had been treated with a combination of 200 mg hydroxychloroquine (HCQ) and tapered doses of prednisone. She was later placed on azathioprine, with remarkable clinical improvement. Quinacrine was briefly added for treatment of a skin rash.

Since being treated by Dr. Macalester, the patient had been stable for over 4 years. To assess serological evidence of disease activity, Dr. Macalester ordered the AVISE SLE Monitor test and the AVISE HCQ test to help assess adherence to 200 mg of HCQ.

AVISE SLE Monitor Results

Assays	Results	Interpretation
+ EC4d- Erythrocyte-Bound C4d	44 Net MFI	POSITIVE
Complement C3	100 mg/dL	Normal
! Complement C4	9 mg/dL	ABNORMAL- LOW
Anti-dsDNA IgG	10 IU/mL	Negative
Anti-C1q IgG	4 Units	Negative
+ PC4d-Platelet-bound C4d	321 Net MFI	POSITIVE
Hydroxychloroquine	<30 ng/mL	Underexposed

Conclusion

Testing revealed EC4d and PC4d positivity as well as under exposure to HCQ. As a result, Dr. Macalester increased HCQ to 400 mg and reinforced the importance of adherence. The patient's history of multiple miscarriages, positive anti-SCL-70, EC4d and PC4d suggested the patient may have SLE and APS overlap. This alerted Dr. Macalester to monitor the patient more closely for risk of thrombosis.

High Tech ANA Testing

LAB TECH HIGHLIGHT

Lab testing for antinuclear antibodies (ANA) is a relatively old test with its origins dating back to 1950. Even so, the ANA test has stood the test of time and remains regarded as the gold standard screening assay for systemic autoimmune conditions. Advances in computer aided instrumentation, specifically digital microscopy, have recently ushered in a new era for the old ANA test helping to address many longstanding shortcomings of that approach.

One challenge with ANA testing by immunofluorescence (IFA) is that it is a tedious and labor-intensive process. As requests for ANA testing increased, some larger general reference laboratories began to substitute their own version of the test using a method that could be automated. Rheumatologists quickly made their opinions known through a position statement from the American College of Rheumatology, that no substitutions were permissible without clear identification as the substituted versions included only a small fraction of the antigens of the original HEp-2 substrate used with the IFA method. This new digital microscopy approach is compliant with the position statement by using the preferred substrate and also automates the most labor-intensive darkroom slide review process.

Reproducibility of ANA testing results is another significant challenge that digital microscopes help to overcome. It is well documented that inter and intra observer variability can be a problem for ANA testing results, causing test values to fluctuate and in rare instances change from positive to negative and vice versa for the same patient. In contrast the computerized system does not get tired at the end of a long day or make errors in subjective determinations. Although these systems are not perfect and still require the review and approval by a clinical laboratory scientist for all results, the criteria for the image interpretation are consistent and not subjected to human error.
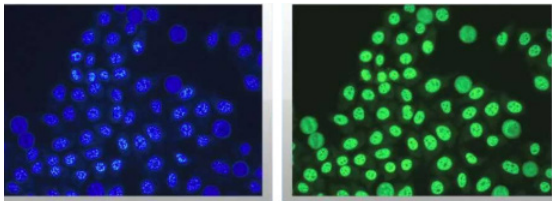
DAPI & FITC fluorescence staining used to locate cells and identify ANA patterns.

There is one additional luxury to using a digital microscope and that is the high-resolution image capture. Using the traditional IFA approach the visible fluorescent intensity for a given result begins to fade over-time and with each exposure. This means that getting multiple opinions or going back to try to re-interpret an IFA slide will result in a different visual observation. Newer automated methods have overcome this challenge, producing digital images that are archived and preserved in their original state, allowing for unlimited transferability of the image across any media device.

Not all laboratories have adopted innovative approaches to IFA testing and patients and providers should be mindful to ask about "how" their ANA test result was derived. Exagen Inc. was the first lab in the US to validate the leading-edge digital IFA system NOVA View®, which plays an important role as part of their AVISE® Lupus and AVISE CTD panels. Validation studies have shown an agreement between manual and this digital method: 96.5% / 96.2% for 1:40/1:80 dilution.[1] The use of this system untimely helps to provide physicians with confidence in their test results. Learn about an advanced autoimmune test incorporating this innovative testing technology, visit **AviseTest.com**.

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NOVA View® is a registered trademark of Inova Diagnostics, Inc.