Salina Pathology News

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UP FRONT

The Role of Anti-CCP in the Laboratory Diagnosis of Rheumatoid Arthritis

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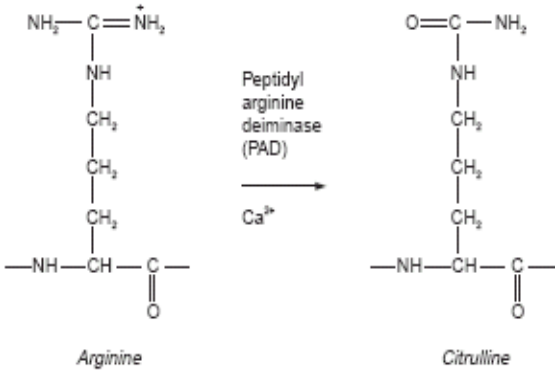
CAP Diagnostic Immunology Resource Committee

Rheumatoid Arthritis (RA) is one of the most common systemic autoimmune diseases, affecting approximately 0.5–1.0% of the world population. The American Rheumatism Association criteria for the classification of RA includes: 1) morning stiffness, 2) arthritis of 3 or more joint areas, 3) arthritis of hand joints, 4) symmetric arthritis, 5) rheumatoid nodules, 6) serum rheumatoid factor (RF), and 7) radiographic changes. A patient should have four of the seven criteria to be diagnosed with RA and the first four criteria should be present for at least six weeks.

Until recently, the only serological test routinely performed for the detection of RA was for the presence of IgM RF. RF is found in approximately 50%–90% of these patients, but it is also found in patients with infections, other autoimmune diseases, and some healthy individuals with increasing frequency in older age groups, thus limiting its specificity for RA.

Several studies have shown that anti-perinuclear autoantibodies, otherwise known as anti-keratin autoantibodies, are found in patients with RA. It has been discovered that these antibodies recognize an epitope that contains the deamidated form of arginine called citrulline. Enzyme-Linked Immunosorbent Assay (ELISA) testing for these autoantibodies directed against anti-cyclic citrullinated peptide (anti-CCP) is reasonably sensitive (68%) and highly specific (98%) in patients with RA.

The pathogenesis of anti-CCP antibodies in rheumatoid arthritis has been shown to be attributable to the body's humoral response to citrulline. Citrullination is the post-translational conversion of arginine to citrulline by an enzyme called peptidylarginine deiminase (PAD). See figure 1.



The enzymatic conversion of protein-contained arginine to citrulline.

Figure 1

PAD activation is assisted by calcium ions. PAD is normally present as inactive intracellular enzymes. During programmed cell death (apoptosis) in the synovial joints of patients with rheumatoid arthritis, PAD may leak out of the dying cells. Once activated, PAD will cause citrullination of extracellular arginine. In the synovium, the citrulline acts as an antigenic stimulant to induce anticitrullinated protein antibodies (ACPA) locally produced by plasma cells. The ELISA that detects these autoantibodies uses synthetic cyclical citrulline peptides.

The original ELISA for the anti-CCP sequence was not broadly marketed due to low sensitivity and technical complexity. However, the second generation anti-CCP test (often referred to in the literature as CCP-2/CCP2) shows superior performance compared to the original peptide. The vast majority of the laboratories

that offer this test utilize the second-generation CCP assay.

In 2005, a third generation of anti-cyclic citrullinated peptide (CCP3) was made available for the laboratory diagnosis of RA. These assays have been reported to recognize additional citrulline epitopes that are not identifiable with the second-generation CCP assays. The CCP3 assays have had reported results of up to 5% increased sensitivity compared to the CCP2 assays. To the contrary, however, several publications have shown similar diagnostic performance between the CCP3 and CCP2 assays.

Recently, Nishimura et al. performed a meta-analysis of published studies regarding the diagnostic accuracy of anti-CCP and RF for rheumatoid arthritis. Their results showed a positive likelihood ratio of 12.46 and a negative likelihood ratio of 0.36 for anti-CCP antibody in patients with RA. The same study showed a positive likelihood ratio of 4.86 and a negative likelihood ratio of 0.38 for RF. These results indicate that anti-CCP positivity alone is more specific than IgM RF for the diagnosis of RA.

In addition to diagnostic value, several studies have shown that anti-CCP may also add prognostic significance in the determination of development of erosive disease in RA. Kroot, EJ et al showed that anti-CCP positive patients developed significantly more severe radiologic damage than those patients who were anti-CCP negative.

Although the presence of anti-CCP is not currently required for the diagnosis of RA, future classification criteria will most likely incorporate its use as an adjunct to IgM RF as a laboratory diagnostic tool. Additionally, RA patients with positive CCP status may benefit from its prognostic value by receiving earlier customized treatment regimens that could potentially delay the development of erosive disease.

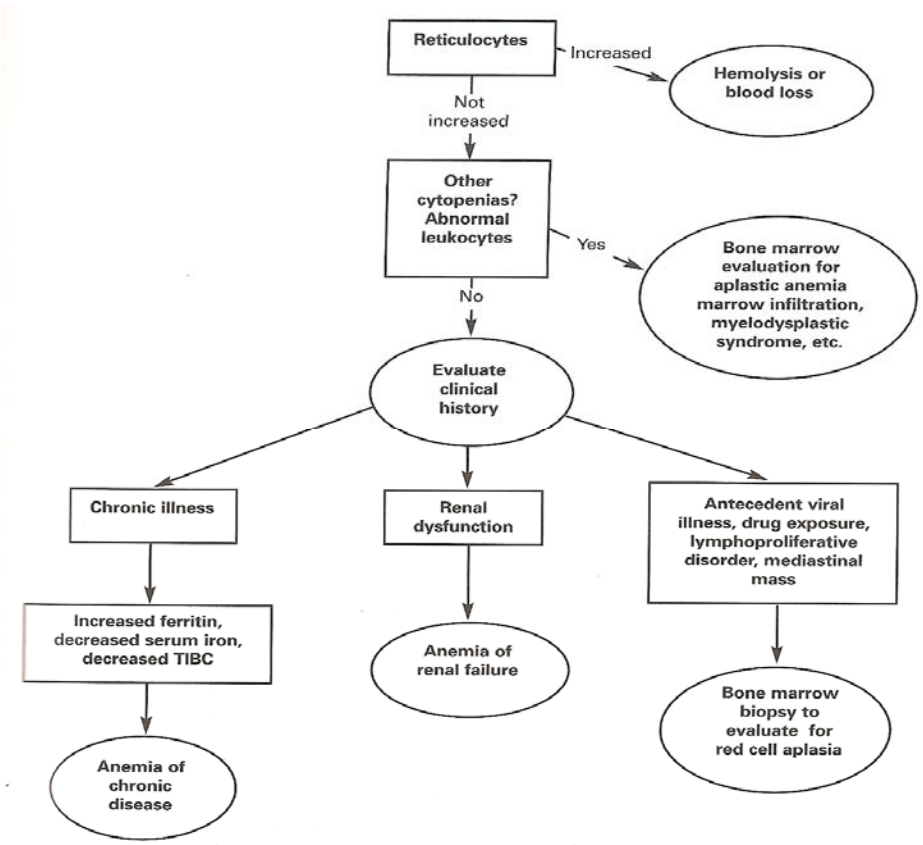
Laboratory Evaluation of Normocytic Anemia

Alexandra Harrington, MD
CAP Hematology and Clinical Microscopy Resource Committee

Anemias can be classified according to the mean corpuscular volume (MCV) into microcytic, normocytic and macrocytic. In normocytic anemias, the MCV is within the reference range, generally between 80-100 fL. Though anemia of chronic disease (ACD) is the most common cause of such anemias, the differential diagnosis is extensive, including blood loss, hemolytic anemias, anemia of renal disease, nutritional anemias and primary bone marrow disorders. Multiple diagnostic algorithms are available; a concise and clinically useful example is shown in the figure below.

Normocytic anemias can be initially categorized based on the reticulocyte count, into those characterized by effective erythropoiesis (elevated reticulocytes) or ineffective erythropoiesis (low to normal reticulocytes). Hemolytic anemias and anemias secondary to blood loss will have elevated reticulocyte counts in the days following episodes, as red cell production is not impaired. In contrast, nutritional anemias, ACD, anemia of renal disease and the anemias secondary to primary bone marrow disorders are examples of disorders of diminished red cell production and, therefore, have low to inappropriately normal reticulocyte counts.

Examination of the peripheral blood smear is as essential as reticulocyte enumeration in the initial work-up of a normocytic anemia. Attention must be paid to all three cell lineages, as abnormalities in each may provide diagnostic clues. For example, many of the hemolytic anemias have characteristic peripheral blood features, such as sickle cells in sickle cell disease, spherocytes in hereditary spherocytosis (HS) and auto-



NEWS AND NOTES

immune hemolytic anemia (AIHA), schistocytes in microangiopathic hemolytic anemia (MAHA) and bite cells in glucose-6-phosphate dehydrogenase deficiency. Cytopenias, circulating immature precursors, including a leukoerythroblastic reaction, and dysplastic features suggest underlying bone marrow disorders and require bone marrow examination.

If hemolysis is suspected and supported by decreased haptoglobin with elevated indirect bilirubin and lactate dehydrogenase levels, further evaluation is needed. Because the hemolytic anemias encompass a wide variety of disorders—including AIHAs, MAHAs, hereditary and acquired red cell membrane defects, such as HS and paroxysmal nocturnal hemoglobinuria and hemoglobinopathies—additional work-up is tailored based on clinical suspicion and peripheral blood findings. For example, a direct antiglobulin test (Coombs test), which assesses immunoglobulin and/or complement bound to red cells, is requested to rule out an AIHA in a patient with spherocytes.

For normocytic anemias with decreased reticulocyte counts and fairly unremarkable blood smears, the differential diagnosis includes ACD, anemia of renal disease, nutritional anemias and red cell aplasia. In these cases, the clinical history may guide further laboratory evaluation, if needed. For example, in patients with chronic illness, iron studies are obtained to evaluate for ACD, with elevated serum ferritin levels, decreased serum iron levels and decreased total iron-binding capacity (TIBC) confirming the diagnosis. Likewise, in patients with poor diets, it may be appropriate to assess iron status and/or vitamin B_{12} and folate levels, as deficiencies of these nutrients may rarely present as normocytic anemias. Finally, bone marrow examination is required for evaluating the etiology of red cell aplasia.

In summary, the differential diagnosis of normocytic anemia is vast. Evaluation begins with reticulocyte enumeration and blood smear examination, with further work-up based on these findings and clinical history.

CMS Centers for Medicare & Medicaid Services

MEDICARE TIP OF THE WEEK

Are you unsure if a Local Coverage Determination policy exists for a procedure you bill?

CMS offers a search tool that can locate and identify if an LCD exists. All you need to do is enter a keyword and the geographic area for the policy. A keyword can be a CPT code, a narrative descriptor, a Dx code or other information you may be seeking.

To access the LCD search on the CMS website, refer to the following address, choose "Local Coverage Documents," enter the keyword and geographic area, and click, "Search Now," to search only the title of LCDs. To search LCD documents in their entirety, you will need to click on "Advanced Search."

www.cms.hhs.gov/mcd/search.asp?from2=search.asp&

OUR JOURNEY TOWARD GREEN

We have recycled our alcohol, xylene and phone books for several years. Recently, we began recycling the plastic, aluminum, paper and cardboard we generate. Five feet of available floor space was

dedicated to our "recycling area." Lab couriers deliver the collected materials to the local recycling center as the containers fill.

In addition, we are vigilant about the paper we generate.

Did you know:

- Office paper is highly recyclable
- The average office worker uses **10,000 sheets** of copy paper each year — totaled with other office workers, it is enough to build a 12' wall from New York to San Francisco
- It takes more than 1½ cups of water to make one sheet of paper (picture a typical soda can).
- Reducing paper use reduces greenhouse gases: comparable to 3/4 acre of pine forest absorbing carbon for every office worker.
- The costs of using paper in the office can run 13 to 31 times the cost of purchasing the paper in the first place!

WEBSITE TRAINING RESOURCES

Like most of your facilities, our website has grown and developed since its inception five years ago.

- Visitors "hit" our website nearly 60,000 times last year, with 18,000 page visits
- We have seen particular interest in the area of archived training resources
- Nearly 80% of our visitors connect through a bookmark
- 42% of visitors add us to their favorites
- There are 113 total pages
- Among the most popular training resources recently are the Lab Safety audio-conference series, CLSI antimicrobial susceptibility standards handouts, and "Phlebotomy Made Simple"

To access the training opportunities page, go to:

www.petersonlab.com/about/inservice.html

IN THE SPOTLIGHT: HISTOLOGY DEPARTMENT

SPL Histology Dept Staff, from left to right: Lola Reichert, Transcriptionist; Rhonda Burt, HT, Histotechnician; Debbie James, HT(ASCP), Histotechnician/Laboratory Supervisor

HISTOLOGY DEPARTMENT

We are proud to introduce the team who processes and creates more than 5,500 surgical slides each year.

Debbie James, HT(ASCP), Laboratory Supervisor: Debbie loves to travel. Could it be because she lived in Texas, Georgia, Louisiana, Germany and Kansas—all before the age of 12? Following her graduation from Salina South, Debbie attended KU. She returned to Salina to work for United Way and the Human Resources, Nursing Administration and Laboratory Departments of Salina Regional Health Center. You're always welcome to drop by the lab. If you do, you'll often find Debbie doing what she loves best—embedding and cutting tissue.

Besides traveling for entertainment, Debbie enjoys fishing and reading "everything and anything." Debbie's two daughters (and five-year-old grandson) are within a 90-minute drive, though she yearns to travel even more. Her bucket list includes the Grand Canyon, Niagara Falls, Sequoia National Forest, an Alaskan cruise and more!

Rhonda Burt, HT: The Fisher Quintuplets aren't Aberdeen, South Dakota's only claim to fame—Rhonda was also born in that fair city. She has lived in the area since she was a preschooler, graduating from Central High. She brings a rich work experience to Salina Pathology, previously working as a CNA, P.T., Med Aide and 12 years as a histotechnician at Salina Regional Health Center. Her favorite work responsibilities? "Cutting tissue. I love it! I enjoy the challenge it takes to turn out perfect slides, no matter how many re-cuts are involved."

She and her husband enjoy visiting their daughter in Tonganoxie to see the grandchildren and their daughter in Keystone, Colorado to ski! Rhonda is a runner, recycler, cruciverbalist, shopper, Big Brothers/Big Sisters volunteer and movie go-er. "Want to go to a movie?"

Lola Richert, Transcriptionist: Though she was born and raised in Chicago, Lola also lived in Florida, Connecticut, and New York City prior to locating in the Salina/Wells area in 1990. Lola's first career found her traveling the western half of the U.S. as an executive training assistant for Advanced Systems, Inc. You may have met Lola as owner of "Lola's Bridal and Parties," which she operated from 1992 to 2003 — all while she worked full-time in the Medical Records Dept of Salina Regional Health Center until her retirement in 2005. In addition to her work for SPL, Lola performs transcription work for another health facility in the evenings. "I love everything about my job — the camaraderie, the typing, our team."

Lola is very proud of her daughter, Broadway singer/dancer/actress Wanda Richert. She has a 21-year old grand daughter and 11 year-old grandson. "Three beautiful cats have adopted me recently. I love my life!"

"I appreciate their promptness—its not uncommon to do surgery in the morning and have the result on my desk when I come back to the office later that afternoon. I also appreciate the (report) pictures, as do the patients and their families."

David Smith, MD,
Surgeon
Mowery Clinic

Tomorrow's Technology Today"

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- **Client representative**
- **Quarterly newsletter**
- **Collection and transport supplies**
- **Courier service**

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