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Laboratory Testing in Rheumatologic Disease

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Laboratory Testing in Rheumatologic Disease

INTRODUCTION

Laboratory tests and their correct interpretation are important components in evaluating patients both for and with rheumatologic diseases. However, it is important to remember that laboratory test results must be interpreted in the context of the patient’s history and physical examination. Unless the appropriate disease manifestations are present, laboratory abnormalities may only mean that the values have fallen outside 2 standard deviations of the mean. The more tests that are ordered, the more likely some clinically irrelevant laboratory abnormalities may be found. In the appropriate clinical context, laboratory tests help diagnose or support the diagnosis of disease, help predict the patient’s prognosis, aid in monitoring disease activity, and warn of toxicity of the treatment. This manual addresses the role and significance of laboratory testing in rheumatologic disease.

RHEUMATOID ARTHRITIS

CASE PRESENTATION

A 35-year-old white woman with a 3-year history of bilateral metacarpophalangeal (MCP), proximal interphalangeal (PIP), wrist, knee, ankle, and metatarsophalangeal (MTP) swelling presents to a rheumatologist. The patient reports that she experiences joint stiffness lasting 6 hours after arising from bed (“morning stiffness”), difficulty performing her activities of daily living, and significant fatigue.

On physical examination, she has significant bilateral synovitis of the MCP, PIP, wrist, knee, ankle, and MTP joints and a pea-size nodule on the extensor surface of her right forearm.

• What laboratory tests should be requested at the initial visit?

DISCUSSION

This patient has a chronic symmetric polyarthritis with constitutional symptoms. The differential diagnosis includes rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), chronic hepatitis, sarcoidosis, and psoriatic arthritis. Laboratory testing should consist of specific tests that will help determine a diagnosis, assess the degree of systemic disease activity (the extent of the inflammation), and determine which organs may be involved. In addition, testing should provide information regarding the patient’s general medical condition, thus allowing the physician to begin a reasonable treatment regimen.

Complete Blood Count

In autoimmune and chronic inflammatory diseases, the hemoglobin or hematocrit, platelet count, and leukocyte count are important initial laboratory measurements because they can reflect systemic inflammation or disease activity and its chronicity. They are also important tests with which to follow the response to treatment and side effects of the medications.

Patients with chronic inflammatory diseases can become anemic for several reasons. The most common is the anemia of chronic disease (AODC). Although its etiology is multifactorial, AODC is most likely due to the effects of circulating inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF-α) on bone marrow. In this anemia, the hematocrit is approximately 28% to 35%, hemoglobin is between 9 and 11 g/dL, the red blood cells are borderline normocytic with a mean corpuscular volume (MCV) of approximately 76 to 83 µm³, and the reticulocyte count is inappropriately low. Iron supply studies will reveal a low serum iron level, a low to normal total iron-binding capacity (TIBC), and a normal or elevated ferritin level. Furthermore, the patient will have normal bone marrow iron stores. The chronic systemic inflammation possibly impairs the ability of erythropoietic precursors to transport and utilize iron from the marrow iron stores, resulting in the anemia. As the inflammation abates, the hematocrit should increase toward normal. The most common rheumatologic cause of AODC is RA, but this anemia can be seen in any chronic inflammatory process (although infrequently in SLE).

Other types of anemia seen in autoimmune disease are hemolytic anemia (both intravascular and extravascular) and, more rarely, pure red cell aplasia. If the hematocrit is lower than 28% or the MCV is either less than 75 µm³ or greater than 83 µm³ in patients with anemia, other causes must be considered; these include gastrointestinal blood loss (causing microcytosis), folate/B12 deficiency, or reticulocytosis (which can...
Inflammatory disease can cause a benign secondary thrombocytosis. As inflammation is adequately treated, the platelet count decreases toward normal. The leukocyte count in autoimmune disease can be elevated without a left shift (no immature neutrophils or bands) as the result of demargination, reflecting systemic inflammation. Felty’s syndrome is characterized by neutropenia. Neutropenia also occurs in SLE but with less frequency than lymphopenia, which is caused by antibodies to lymphocytes. An absolute lymphopenia can be observed in scleroderma.

Acute Phase Reactants and Erythrocyte Sedimentation Rate

Acute phase reactants are proteins produced in increased quantities in response to physical stress, usually acutely, and their level remains elevated until the stress abates. Among the stresses that induce production of acute phase reactants are infection, vasculitis, inflammation, and tissue necrosis. Proteins in this group include fibrinogen, alpha globulin, gamma globulin, ferritin, C-reactive protein, complement proteins (C3, C4, CH50), soluble IL-2 receptor, haptoglobin, ceruloplasmin, and amyloid A precursor.

The erythrocyte sedimentation rate (ESR) measures the rate at which red blood cells fall in plasma. This rate is a reflection of the repulsion of red blood cells and indirectly reflects the production of acute phase reactant proteins that interfere with this repulsion (e.g., fibrinogen, gamma globulin, and alpha globulin) (Figure 1). Inflammation (and the resulting production of acute phase proteins) increases the ESR by neutralizing red blood cell repulsion and by producing heavier red blood cells.

There are 2 methods for measuring the ESR: the Wintrobe method and the Westergren method (which is the most commonly available). In the Westergren procedure, 2 mL of anticoagulated blood (ethylenediaminetetraacetic acid or NaCitrate) is placed into an upright calibrated capillary tube. After an hour, the distance the red blood cells have fallen is reported as sedimentation rate in mm/h (Figure 2). Because the ESR test can be performed easily and has a quick turnaround time and low cost, it is the most widely used inflammatory measurement.

The ESR test must be performed within 4 to 6 hours of the blood draw to obtain accurate results; therefore, results of tests performed in reference laboratories may be artificially low due to a delay in processing. Although the ESR is very sensitive when performed within the specified time frame, it is influenced by many conditions (Table 1). There are several arguments against routinely measuring the ESR: It is impossible to correct for the red blood cell size and shape, plasma viscosity, or any of the conditions in Table 1. Additionally, it may take up to 10 days for the ESR to increase in inflammation. Nonetheless, the cost, ease, and turnaround time of the ESR make it a very attractive test.

C-Reactive Protein

The C-reactive protein (CRP) is an ancient protein initially isolated from the horseshoe crab, which predates the dinosaur. Its ancient origin is demonstrated by the lack of polymorphism across species. CRP is produced in the liver, probably by hepatic macrophages. Although its exact role is unknown, the protein activates the classic complement pathway, activates neutrophils, enhances phagocytosis, and may interfere with platelet activation by inhibiting platelet-activating factor.

The CRP level rises within a few hours of inflammation onset or tissue injury and peaks within 2 to 3 days of the acute stimulus. It remains elevated in chronic inflammatory states until adequate therapy is provided, whereupon it falls rapidly; thus, the CRP level parallels inflammation more closely than the ESR. In 80% to 85% of situations in which the CRP is greater than 10 mg/dL, a bacterial infection is present. Similar to the ESR, all rheumatologic conditions can cause an elevated CRP, but nonrheumatologic conditions can increase the CRP as well (Table 2).
A new CRP test called the highly sensitive CRP (hs-CRP) or ultrasensitive CRP has received significant attention over the past 2 years because it correlates with risk for ensuing cardiovascular ischemic events. This is a different test than the conventional CRP, and for many hospitals it is considered a reference (or referral/send out) test. To date, there are no studies evaluating the levels of the hs-CRP in systemic inflammatory diseases.

**Rheumatoid Factor**

Rheumatoid factor (RF) is an autoantibody directed against the Fc portion of IgG. Rheumatoid factors of immunoglobulin classes IgG, IgM, IgA, and IgE have been described, although IgM is the most easily detected and thus the most commonly measured RF. The test to detect RF can be performed either as an agglutination test or by nephelometry. The agglutination test uses beads (frequently latex) coated with human immunoglobulin. Patient serum is added to the beads in a 96 well plate. If the serum sample contains the pentavalent IgM RF, the beads will clump and a pellet will form at the bottom of the well, indicating a positive RF test. Because IgG, IgA, and IgE antibodies are divalent, a non-IgM isotype RF will not cause the beads to clump sufficiently for a visible pellet to form, resulting in a negative test. The nephelometer detection method uses a laser and computer to measure RF of all isotypes based on the light-scattering characteristics produced by the sample.

Based on data obtained using the agglutination method of detection, classically 75% to 80% of patients with RA are RF-positive (seropositive). Although the sensitivity for RA is approximately 75% to 80%, the specificity is much lower. Patients with many rheumatologic/autoimmune diseases, infectious diseases, and noninfectious nonrheumatologic diseases can have RF (Table 3). Therefore, RF activity is neither sensitive nor specific, and patients who are RF-positive can only be diagnosed with RA when they have the A (arthritis), not arthralgia. A high RF titer in RA patients may indicate more extensive joint involvement and extraarticular manifestations, and thus a worse prognosis.

**Liver Function Tests**

Measurement of liver enzymes, liver function, and creatinine are important initial tests to obtain in order to evaluate for diseases other than RA and because medications such as methotrexate, leflunomide, and nonsteroidal anti-inflammatory drugs can cause organ toxicity.

**TEST RESULTS AND DIAGNOSIS**

Laboratory evaluation of the case patient is reported as follows:

- RF = positive with a titer of 1:32 dilutions
- Hematocrit = 31% (normal = 39% to 44%), with an MCV of 78 µm³
- Platelet count = 578,000 (normal = 150,000 to 270,000)
- ESR = 51 mm/h (normal = < 20 mm/h)
- CRP = 7 mg/dL (normal = < 1 mg/dL)
- Antinuclear antibody (ANA) = negative
Chemistries = normal
Hepatitis panel = negative

The physician makes a diagnosis of seropositive nodular RA. Because the presence of nodules and a high RF titer are associated with a worse prognosis, the patient is started on a regimen of leflunomide and sulfasalazine.

How should this patient be monitored?

DISCUSSION

Rheumatoid factor does not correlate with disease activity, so this test does not need to be repeated. Tests that do correlate with disease activity include the ESR, CRP, and complete blood count (CBC). Baseline laboratory tests allow the physician to monitor for subsequent drug toxicity. Current American College of Rheumatology guidelines for methotrexate use recommend performing CBC and measuring aspartate aminotransferase (or alanine aminotransferase), albumin, and creatinine on a monthly or bimonthly basis. In addition, because of methotrexate’s potential liver effects, all patients should be tested for hepatitis B and C prior to initiation of therapy to preclude the possibility of additional liver toxicity. These recommendations also apply to leflunomide.

PATIENT FOLLOW-UP

At a follow-up visit 1 month after initiation of therapy, the patient complains of shortness of breath, dyspnea on exertion, bilateral knee swelling (right significantly greater than the left), and a temperature of 99.8°F. Chest radiograph with decubitus views reveals a layering right pleural effusion.

What diagnostic entities should be considered?
What testing should be done?

DISCUSSION

Serosal Fluid Analysis

The differential diagnosis includes RA lung disease, infection, malignancy, or pulmonary embolus. In addition to the standard laboratory tests for disease activity, aspiration of the pleural and synovial fluid should be performed. Analysis of serosal fluid (pleural effusion and pericardial effusion) is important, especially in RA and SLE where pleural effusions and increased risk of infection are both present. Table 4 shows the pleural fluid analysis findings characteristic of RA and SLE that many rheumatologists extrapolate to be valid for pericardial fluid as well. A low glucose level is characteristic of RA and is a result of impaired glucose transport into the

| Table 3. Conditions Associated with Positive Rheumatoid Factor |
|-----------------------------|-----------------------------|-----------------------------|
| Rheumatologic              | Infectious                  | Noninfectious/Nonrheumatologic |
| Cryoglobulinemia            | Viral                       | Normal individuals: 5% to 10% of the normal population |
| Dermatomyositis             | Cytomegalovirus             | Aging: > 20% of individuals 90+ years old |
| Hashimoto’s thyroiditis     | Epstein-Barr virus          | Periodontal disease          |
| Polymyositis                | Influenza                   | Malignancy                   |
| Rheumatoid arthritis        | Rubella                     | Chronic liver disease        |
| Sarcoidosis                 | Hepatitis                   | Interstitial pulmonary fibrosis |
| Scleroderma                 | Bacterial                   | Waldenström’s macroglobulinemia |
| Sjögren’s syndrome          | Subacute bacterial endocarditis |
| Systemic lupus erythematosus | Leprosy                     |
| Wegener’s granulomatosis    | Syphilis                    |
|                            | Tuberculosis                |
|                            | Parasitic-filariasis        |
|                            | Malaria                     |
|                            | Schistosomiasis             |
|                            | Trypanosomiasis             |

<table>
<thead>
<tr>
<th>Table 4. Serosal Fluid Findings in Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>SLE</td>
</tr>
<tr>
<td>RA</td>
</tr>
</tbody>
</table>

LDH = lactate dehydrogenase.

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It is important to note that the pleural space. A low glucose level can also be seen in sepsis, particularly in tuberculosis. The leukocyte count is variable, ranging from 100 to 3500/mm³ (mostly mononuclear cells). Pleural fluid ANA and complement levels are not specific for SLE. They have been found in patients with empyema and malignancy but have not been extensively tested in other autoimmune diseases. As a result, measurement of ANA or C3/C4 in serosal fluid is not recommended. Pleural RF is found in tuberculosis, other infections, and malignancy and therefore is also unhelpful. Lupus erythematosus cell evaluation is not used much anymore because it is difficult to perform, is not sensitive, and may not be specific for SLE.

Joint Fluid Analysis

Joint fluids are characterized as normal, noninflammatory (as seen in osteoarthritis or sympathetic effusions), inflammatory, or septic based on their leukocyte count. Although the term “septic” implies infection, it really refers to the number of leukocytes present, typically neutrophils. The acute crystalline arthropathies can all produce a leukocyte count in the septic range.

TESTING AND FURTHER COURSE

A thoracentesis is performed and the yellow-colored pleural fluid is sent for cell count, differential, cytology, Gram stain with culture, and measure of lactate dehydrogenase (LDH), protein, glucose, and pH. Analysis reveals 700 leukocytes/mm³ with 25% polymorphonuclear neutrophils, 35% lymphocytes, and 40% monocytes; test for malignancy is negative. Microbiology studies are negative. Protein is 4.5 g/dL, LDH is 850 U/L, glucose is 10 mg/dL, and the pH is 7.1.

Because the patient’s right knee is significantly more swollen, indicating the possible presence of infection, the knee is aspirated and 52 mL of turbid fluid is removed. The fluid is analyzed for crystals and sent for cell count and differential, Gram stain, and culture with sensitivity.

The cell count is 40,000/mm³ with 95% neutrophils; the other studies are negative with sterile cultures after 5 days. The physician concludes that the pleural effusion is due to RA lung disease and the knee swelling is due to the RA joint disease. He injects both knees with 40 mg of methylprednisolone and adds hydroxychloroquine sulfate to the patient’s regimen of leflunomide and sulfasalazine. After 8 months, the patient has 30 minutes of morning stiffness, no shortness of breath or dyspnea on exertion, minimal synovitis, and no recurrence of her pleural effusion. Hematocrit is 38%, platelet count is 280,000/mm³, ESR is 25 mm/h, and CRP is 2 mg/dL.

SYSTEMIC LUPUS ERYTHEMATOSUS

CASE PRESENTATION

A 35-year-old black woman presents to a rheumatologist for evaluation of joint pain. Past medical history is significant for 2 miscarriages, a 1-year history of intermittent MCP and PIP joint pain and swelling, alopecia, fatigue, facial rash, dyspnea after climbing 1 flight of stairs, and difficulty lifting her 3-year-old child. Physical examination shows thinned hair, a facial blush that spares the nasolabial folds, purplish discoloration of her eyelids, slight PIP synovitis, 4/5 strength on bilateral shoulder abduction, right thigh flexion of 4/5, and left thigh flexion of 4–5. Distal muscle strength is 5/5.

- What disease entities can explain these symptoms?
- What laboratory tests should be ordered at the initial visit?

DISCUSSION

This patient has a chronic symmetric polyarthritis, malar rash with heliotrope features, dyspnea on exertion, proximal muscle weakness, alopecia, and miscarriages. The differential diagnosis includes SLE, dermatomyositis, a viral syndrome (eg, chronic hepatitis C), sarcoidosis, or syphilis. In addition to the tests previously discussed in the Rheumatoid Arthritis section, tests particularly helpful in the initial evaluation for SLE and dermatomyositis should be ordered.

Antinuclear Antibody

Like other autoantibodies, the ANA is a normal immunoglobulin that can be present in many people but is increased to a more easily detectable level in the context of either temporary (as in the case of liver inflammation) or constant (as in autoimmune disease) activation of the immune system. There are 4 common ANA
immunofluorescent patterns in SLE: homogeneous (diffuse), peripheral (rim), speckled, and nucleolar (Figure 3, see page 11). The patterns are associated with particular autoantibodies. The homogeneous pattern is the least specific and can be seen in patients with SLE or other diseases and even in some normal individuals. The peripheral pattern correlates with antibodies to double-stranded DNA (dsDNA) and is specific for SLE. The speckled pattern is seen in SLE but also in scleroderma, Sjögren’s syndrome, and mixed connective tissue disease; it may correlate with antibodies to Smith (Sm) antigen, topoisomerase I (Scl-70), and Ro (Sjögren’s syndrome A [SS-A]) or La (Sjögren’s syndrome B [SS-B]) antigen. A subset of the speckled pattern is the centromere pattern, which is seen in limited scleroderma (formerly known as the CREST variant) and correlates with anticientromere antibodies. The nucleolar pattern is seen most commonly in scleroderma and correlates with antibodies to ribonucleoprotein (RNP). The use of ANA patterns has decreased because the specific autoantibodies are now easily measured.

The 2 methods for determining the presence of ANA are based on the 2 different tissue substrates with which patient sera is incubated. The substrates are either rodent (mouse or rat) liver or the human epithelial-2 cell line (HEp-2), which yield different background ANA detection. The rodent tissue produces a positive test in 5% to 10% of normal individuals at the screening serum dilution of 1/20, but only 1% have a positive test at a dilution of 1/160. Rodent liver was the original substrate for the ANA test, but it is now used infrequently. The HEp-2 cell line is more sensitive and is almost universally used. Between 10% and 15% of normal individuals will have a positive test at a 1/40 dilution, but only 1% will have a positive test at 1/320 dilution. ANAs occur in multiple diseases, including all rheumatic diseases, but appear most frequently and with the highest titer in SLE (Table 6).

Antiphospholipid Syndrome Testing

Antiphospholipid syndrome (APS) was first identified in patients with SLE. Further investigation has confirmed its association with SLE, but it can also be seen in other immune disorders (eg, RA) and as a primary syndrome. The hallmarks of APS as caused by the antiphospholipid antibody are recurrent vascular thrombosis (it is associated with the IgM isotype, and deep venous thrombosis is most common presentation); recurrent fetal loss (associated with IgM isotype); livedo reticularis (Figure 4, see page 11); thrombocytopenia; Libman-Sacks endocarditis of SLE; false-positive VDRL (Venereal Disease Research Laboratory) syphilis test or rapid plasmin reagent test (with nonreactive microhemagglutination-Treponema pallidium); and falsely prolonged partial thromboplastin time (PTT, the lupus anticoagulant).

The clinical manifestations of APS are caused by antibodies against phospholipids, notably cardiolipin and phosphatidylserine. As a result, several antiphospholipid antibodies (particularly the anticardiolipin and antiphosphatidylserine antibodies) have been identified in patients with APS. Lupus anticoagulants are antiphospholipid antibodies that bind and sequester the phospholipid reagent of the PTT assay, causing an artificially prolonged PTT; thus, the misnomer “anticoagulant” is derived. The lupus anticoagulant may be detected by performing mixing studies that help determine why the PTT is prolonged. The prolonged PTT is either due to a clotting factor deficiency (such as in patients on anticoagulants) or the presence of an inhibitor of the clotting assay (antiphospholipid antibody). A prolonged PTT that corrects (normalizes) with the addition of normal plasma indicates that an individual is deficient of a clotting factor that was replenished by the normal plasma. However, the PTT that does not correct with the addition of normal plasma contains an inhibitor, such as an antiphospholipid antibody, especially if it corrects with the addition of phospholipid or a source of phospholipid (freeze-thawed) platelets. The best test for a lupus anticoagulant is the dilute Russell’s viper venom time, although other tests such as the Kaolin clot time are also frequently used.

Unfortunately, not all clinically relevant antiphospholipid antibodies are revealed solely by the lupus

Table 6. Diseases Associated with a Positive Antinuclear Antibody Test

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency of Positive Test, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic lupus erythematosus</td>
<td>98</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>95</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>90</td>
</tr>
<tr>
<td>Primary Sjögren’s syndrome</td>
<td>75</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>50–75</td>
</tr>
<tr>
<td>Chronic juvenile arthritis</td>
<td>?</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>?</td>
</tr>
<tr>
<td>Infectious mononucleosis</td>
<td>?</td>
</tr>
<tr>
<td>Lepromatous leprosy</td>
<td>?</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>?</td>
</tr>
<tr>
<td>Subacute bacterial endocarditis</td>
<td>?</td>
</tr>
</tbody>
</table>

*Tests used human epithelial-2 cells as substrate.

Adapted with permission from Wallace DJ, Hahn BH, Quismorio FP, Klinenberg JR, editors. Dubois’ lupus erythematosus. 5th ed. Baltimore: Williams & Wilkins; 1997:398.
anticoagulants tests; therefore, complementary tests such as the anticardiolipin and antiphosphatidylserine antibody detection tests and the false-positive rapid plasmin reagin (RPR) test should be used to identify antiphospholipid antibodies. The presence of any of these abnormalities in sufficient titers is satisfactory to conclude that the patient has the antiphospholipid antibody. Diagnosis of APS and initiation of treatment, however, require the appropriate clinical scenario.

Complement (C3/C4/CH50)

Acute inflammation, infection, and tissue injury cause increased hepatic complement production. Thus, the complement proteins are considered acute phase reactants and are elevated in inflammatory conditions. However, in some diseases, including SLE, complement is consumed in the setting of increased immune complex formation, resulting in depressed levels of C3, C4, and total hemolytic complement (CH50 and CH100) (Table 7). Complement levels can be followed to evaluate whether a patient’s symptoms might be due to active lupus and to predict whether a patient with decreasing levels is at risk for a clinical flare. Measurement of complement split (or degradation) products can be used to distinguish between a lupus exacerbation and pre-eclampsia (or eclampsia) in a pregnant lupus patient with low C3 and C4. Lupus will consume complement and the split products will increase. In preeclampsia (or eclampsia), hepatic complement production will decrease and the split products will be either normal or decreased.

**Table 7. Interpreting the Results of Complement Testing**

<table>
<thead>
<tr>
<th>C3</th>
<th>C4</th>
<th>CH50</th>
<th>Condition/Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Acute inflammation; acute infection</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>Active SLE; active serum sickness; poststreptococcal glomerulonephritis</td>
</tr>
<tr>
<td>↓ Normal</td>
<td>↓</td>
<td>↓</td>
<td>Chronic SLE; C3 deficiency; membranoproliferative glomerulonephritis</td>
</tr>
<tr>
<td>Normal</td>
<td>↓</td>
<td>↓</td>
<td>Acute SLE; C4 deficiency</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>↓↓</td>
<td>C2 deficiency; membrane attack complex deficiency (C5–C9)*</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>C1 esterase inhibitor deficiency (hereditary angioedema)</td>
</tr>
</tbody>
</table>

*SLE = systemic lupus erythematosus.

This deficiency results in recurrent *Neisseria* infections or *N. meningitidis* meningitis without neurologic sequelae.


**TEST RESULTS**

Laboratory evaluation in the case patient reveals the following:

- ANA = positive with a titer of 1:1280 dilutions
- Hematocrit = 31%, with an MCV of 78 µm³ and reticulocytosis

- Leukocyte count = 3200/mm³ (4000 to 8600/mm³)
- Platelet count = 110,000/mm³
- Creatinine = 0.9 mg/dL
- Urinalysis = within normal limits
- C3 = 32 mg/dL (normal = 80 to 150 mg/dL)
- C4 = 5 mg/dL (normal = 24 to 60 mg/dL)
- Creatine kinase (CK) = 25 U/L (normal = 30 to 135 U/L)

**DISCUSSION**

**Specific ANA Tests**

This patient has evidence for SLE with a normal CK (thus myositis is less likely). Further testing in ANA-positive patients with a compatible history, physical examination, and initial laboratory testing will help define the nature of the positive ANA. Most panels of specific tests include tests for antibodies against ds-DNA, Sm, RNP, Ro, La, topoisomerase, and centromere (or some combination of these). However, because this patient has a rash and history consistent with dermatomyositis with lung involvement, antisynthetase antibodies would help define the antisynthetase antibody syndrome. Table 8 provides an overview of autoantibodies and their disease associations.

**Anti–Double-Stranded DNA (Native DNA) Antibody**

The anti-dsDNA antibody occurs in 50% to 60% of SLE patients and is highly specific for SLE. It is associated with the potential for significant SLE renal disease. The titers may parallel lupus activity, especially renal disease.

**Anti-Smith and Anti-Ribonuclear Protein Antibodies**

The antigens for Sm and RNP are extractable nuclear antigens, meaning they are found in the nuclear fraction
of cell lysate. Anti-Sm antibody is found in only 30% to 35% of SLE patients and is very specific for SLE. Any titer is significant. The antibody against RNP is seen in SLE, mixed connective tissue disease (at very high titers), RA, and scleroderma.

**Anti-Ro/La Antibodies**

The Sjögren’s antibodies, anti-Ro/La (SS-A/SS-B) antibodies, are made against antigens found in both the cell’s cytoplasm and nucleus. Anti-Ro and anti-La antibodies are present in Sjögren’s syndrome, SLE, RA, and diffuse scleroderma. Anti-Ro is also present in the few patients with ANA-negative lupus, manifested predominantly by photosensitive rash, Raynaud’s phenomenon, and serositis. Anti-Ro antibody also helps define the subset of lupus patients with subacute cutaneous LE (SCLE). SCLE is characterized by a nonscarring, exquisitely photosensitive papulosquamous or annular rash that typically appears on sun-exposed skin; 63% of patients with SCLE have anti-Ro antibody.

The presence of anti-Ro or anti-La antibodies also identifies mothers who are at risk for having babies with neonatal heart block.

**Anticentromere Antibody**

The anticentromere antibody occurs in 80% to 90% of patients with the subset of scleroderma now called limited or localized scleroderma. Only 10% to 15% of patients with diffuse scleroderma have this antibody.

**Antitopoisoiserase I Antibody**

Antitopoisoiserase (formerly anti-Scl-70) antibody is directed against topoisomerase I, an enzyme that participates in the initial uncoiling of DNA prior to transcription. This antibody is found in 20% to 40% of patients with diffuse scleroderma. However, 60% to 70% of patients with antitopoisoiserase antibody have diffuse scleroderma, and only 20% of limited scleroderma patients have antitopoisoiserase antibody. Its presence predicts a worse prognosis.

**Antisynthetase Antibodies**

The antisynthetase antibodies are directed against aminoacyl-transfer-RNA (tRNA), synthetase enzymes important in synthesis of proteins from messenger RNA (translation). This set includes antibodies to the following synthetases (their common names are given in parentheses):

- Histidyl-tRNA synthetase (Jo-1)
- Threonyl-tRNA synthetase (PL-7)
- Alanyl-tRNA synthetase (PL-12)
- Glycyl-tRNA synthetase (EJ)
- Isoleucyl-tRNA synthetase (OJ)

These antibodies define a syndrome called the polymyositis/dermatomyositis antisynthetase antibody syndrome; it is manifested by myositis of relatively acute onset, interstitial lung disease, fever, arthritis, Raynaud’s phenomenon, and mechanic’s hands (darkened or
dirty-appearing cracking and fissuring of the lateral and palmar aspects of the fingers as seen on an auto mechanic).

TEST RESULTS

Testing for specific ANA in the case patient reveals the following:

- Anti-dsDNA = positive with a titer of 1:320 dilutions (highly positive)
- Anti-Sm = positive with a titer of 1:160 dilutions (highly positive)
- Anti-Ro and La = negative

How should this patient be monitored?

DISCUSSION

Tests that can demonstrate disease activity prior to clinical manifestations should be used on a periodic basis to monitor the patient. Any change in the patient’s clinical status would mandate immediate testing. Standard testing includes CBC, urinalysis, renal function assessment, and measurement of complement levels and ESR and/or CRP. Anti-dsDNA levels may also vary in individual patients, reflecting their disease/nephritis activity. Several lupologists advocate following anti-dsDNA antibody levels to both monitor response to treatment and predict renal exacerbations. Antibodies to dsDNA may increase prior to or during a lupus nephritis flare. Although both complement levels and anti-dsDNA antibody levels may change, thus indicating a flare or the possibility for imminent flare, these trends need to be individualized among patients.

VASCULITIS MIMIC

CASE PRESENTATION

A 50-year-old white man with hypertension and a 3-month history of increased temperature (100.7°F), unintentional weight loss (10 lb), fatigue, achy joints and muscles, rash, and digital ulcerations presents to a rheumatologist.

On physical examination, the patient looks tired. Blood pressure is 155/93 mm Hg in the right arm and 143/90 mm Hg in the left arm. Heart rate is 92 bpm, and temperature is 99°F. The physician detects a 2/6 systolic ejection murmur loudest at the right upper sternal border without radiation to the carotids. The right long finger has an ulcer with necrosis, and the right ring and small fingers also have ischemic lesions (Figure 5).

- What disease entities can explain these symptoms?
- What laboratory tests should be ordered?

DISCUSSION

The patient has a systemic illness involving the vasculature with fever and constitutional symptoms. The differential diagnosis list includes vasculitis, dermatomyositis, malignancy, endocarditis, HIV, cryoglobulinemia, and atherosclerosis with cholesterol emboli. Diagnostic testing should investigate each of the possible diagnoses. Because vasculitis is among the possible diagnoses for this patient, testing for the antineutrophil cytoplasmic antibody (ANCA) should be considered.

Antineutrophil Cytoplasmic Antibody

ANCAs are directed against proteolytic enzymes in the alpha granule of the neutrophil. Their role in vasculitis is not clear: They either activate neutrophils, thus contributing to disease, or they are a marker of the disease. In some vasculitis patients, the ANCA titers may parallel disease activity; however, because this does not occur in all patients, the significance of the ANCA titer needs to be individualized.

ANCAs have 2 immunofluorescence staining patterns when the patient’s neutrophils are fixed with ethanol: perinuclear staining (pANCA) and diffuse cytoplasmic staining (cANCA). During the ethanol fixation step, the neutrophil granule membranes are disrupted and the positively charged granule proteins (such as myeloperoxidase) are attracted to the negatively charged nucleus, giving the pANCA staining pattern. The negatively charged proteins (such as proteinase-3) remain distributed within the cytoplasm, resulting in the
cANCA staining pattern. Thus, the perinuclear pattern is an artifact of the fixation procedure because when the cells are fixed with formalin, cells only have the cANCA staining pattern (Figure 6).

At least 70% of the pANCA staining is produced by antineutrophil antibodies directed against myeloperoxidase. Other pANCA antigens include cathepsin G, human leukocyte elastase, and lactoferrin. This antibody-staining pattern is associated with polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome. More than 90% of the cANCA is directed against proteinase-3; this antibody-staining pattern is associated with Wegener’s granulomatosis. However, the particular ANCA staining pattern or antigen specificity that is present is neither 100% sensitive nor 100% specific; thus, these patterns are not diagnostic but only support the diagnosis. Table 9 shows the prevalence of the ANCA’s in connective tissue diseases, the vasculitides, and other diseases. Other diseases reported with ANCA include bronchogenic carcinoma, colon carcinoma, endocarditis, glomerular basement membrane disease (Goodpasture’s syndrome), HIV, mucoviscidosis, sclerosing cholangitis (pANCA), and ulcerative colitis (pANCA).

TESTING AND DIAGNOSIS

To evaluate for the possible diagnoses, an ANCA and hepatitis panel (for hepatitis-associated polyarteritis nodosa or cryoglobulinemia), HIV titer, cryoglobulins, and 2 pairs of blood cultures (each pair containing an aerobic and anaerobic culture bottle) are obtained. Because of the heart murmur and fever, an echocardiogram is ordered to further evaluate for endocarditis.

Laboratory evaluation reveals the following:

- Hematocrit = 31%, with an MCV of 78 µm³
- Leukocyte count = 12,000/mm³
- Platelet count = 450,000/mm³
- Creatinine = 0.9 mg/dL
- CK = within normal limits
- Urinalysis = 15 to 20 red blood cells/hpf and proteinuria
- ESR = 55 mm/h

The ANCA is reported positive with a pANCA pattern; the cryoglobulins and hepatitis and HIV serologies are negative. Angiograms of the right upper extremity
and kidneys reveal abrupt termination of several distal digital arteries with intraluminal filling defects and normal renal vessels. The transthoracic echocardiogram (although a poor study) shows a bicuspid aortic valve. Blood cultures grew methicillin-sensitive Staphylococcus aureus, and a transesophageal echocardiogram reveals an aortic valve vegetation. The physician makes a diagnosis of infectious endocarditis. The patient is treated with intravenous oxacillin for 6 weeks with resolution of his symptoms.

DISCUSSION

This patient was experiencing emboli to his finger, toe, and kidneys resulting in this vasculitis mimic. The important point to remember is that the positive ANCA test only supports a diagnosis—it is not specific enough to be diagnostic. Had this patient not had such an extensive evaluation, he might have received a diagnosis of vasculitis (based on the positive ANCA) and been treated with immunosuppressive therapy (steroids and cyclophosphamide), which would have caused his infected aortic valve to quickly worsen with potentially catastrophic consequences.

CONCLUSION

In conclusion, for the results of the laboratory tests discussed in this manual to be helpful to the diagnostician, they must be interpreted in the context of the patient’s history and physical examination. By themselves they do not diagnose any disease entity, but with the correct clinical scenario they can be very important in the correct diagnosis and treatment of a patient.

SUGGESTED READINGS


Table 9. Prevalence of Antineutrophil Cytoplasmic Antibodies in Connective Tissue Disease and Vasculitis

<table>
<thead>
<tr>
<th>Connective tissue disease</th>
<th>cANCA</th>
<th>pANCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>0/241</td>
<td>6/241</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td>0/32</td>
<td>5/32</td>
</tr>
<tr>
<td>SLE</td>
<td>0/109</td>
<td>4/109</td>
</tr>
<tr>
<td>Felty’s syndrome</td>
<td>0/14</td>
<td>3/14</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>0/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>0/50</td>
<td>0/50</td>
</tr>
<tr>
<td>MCTD</td>
<td>0/32</td>
<td>0/32</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>0/43</td>
<td>0/43</td>
</tr>
<tr>
<td>Reiter’s syndrome</td>
<td>0/29</td>
<td>0/29</td>
</tr>
<tr>
<td>SLE vasculitis</td>
<td>0/21</td>
<td>0/21</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vasculitis</th>
<th>cANCA</th>
<th>pANCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wegener’s granulomatosis</td>
<td>295/383</td>
<td>20/383</td>
</tr>
<tr>
<td>Polyarteritis nodosa</td>
<td>14/49</td>
<td>2/49</td>
</tr>
<tr>
<td>Unclassified vasculitis</td>
<td>8/110</td>
<td>9/110</td>
</tr>
<tr>
<td>Churg-Strauss syndrome</td>
<td>4/13</td>
<td>1/13</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
<td>0/62</td>
<td>5/62</td>
</tr>
<tr>
<td>Temporal arteritis</td>
<td>0/24</td>
<td>2/24</td>
</tr>
<tr>
<td>Behget’s syndrome</td>
<td>0/21</td>
<td>0/21</td>
</tr>
<tr>
<td>Henoch-Schönlein purpura</td>
<td>0/18</td>
<td>0/18</td>
</tr>
<tr>
<td>Takayasu’s arteritis</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Other*  
Bronchogenic carcinoma, colon carcinoma, endocarditis, glomerular basement membrane disease, HIV, mucoviscidosis, sclerosing cholangitis, ulcerative colitis

cANCA = cytoplasmic staining; MCTD = mixed connective tissue disease; pANCA = perinuclear staining; SLE = systemic lupus erythematosus.

*Data were compiled from case reports.


and kidneys reveal abrupt termination of several distal digital arteries with intraluminal filling defects and normal renal vessels. The transthoracic echocardiogram (although a poor study) shows a bicuspid aortic valve. Blood cultures grew methicillin-sensitive Staphylococcus aureus, and a transesophageal echocardiogram reveals an aortic valve vegetation. The physician makes a diagnosis of infectious endocarditis. The patient is treated with intravenous oxacillin for 6 weeks with resolution of his symptoms.