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Laboratory tests in rheumatology: A rational approach

ABSTRACT

Laboratory tests are useful in diagnosing rheumatic diseases, but clinicians should be aware of the limitations of these tests. This article uses case vignettes to provide practical and evidence-based guidance on requesting and interpreting selected tests, including rheumatoid factor, anticitrullinated peptide antibody, antinuclear antibody, antiphospholipid antibodies, antineutrophil cytoplasmic antibody, and human leukocyte antigen-B27.

KEY POINTS

If a test was requested without a clear indication and the result is positive, it is important to bear in mind the potential pitfalls associated with that test; immunologic tests have limited specificity.

A positive rheumatoid factor or anticitrullinated peptide antibody test can help diagnose rheumatoid arthritis in a patient with early polyarthritis.

A positive *HLA-B27* test can help diagnose ankylosing spondylitis in patients with inflammatory back pain and normal imaging.

Positive antinuclear cytoplasmic antibody (ANCA) can help diagnose ANCA-associated vasculitis in a patient with glomerulonephritis.

A negative antinuclear antibody test reduces the likelihood of lupus in a patient with joint pain.

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Laboratory tests are often ordered inappropriately for patients in whom a rheumatologic illness is suspected; this occurs in both primary and secondary care.[1] Some tests are available both singly and as part of a battery of tests screening healthy people without symptoms.

The problem: negative test results are by no means always reassuring, and false-positive results raise the risks of unnecessary anxiety for patients and clinicians, needless referrals, and potential morbidity due to further unnecessary testing and exposure to wrong treatments.[2] Clinicians should be aware of the pitfalls of these tests in order to choose them wisely and interpret the results correctly.

This article provides practical guidance on requesting and interpreting some common tests in rheumatology, with the aid of case vignettes.

RHEUMATOID FACTOR AND ANTICITRULLINATED PEPTIDE ANTIBODY

A 41-year-old woman, previously in good health, presents to her primary care practitioner with a 6-week history of pain and swelling in her hands and early morning stiffness lasting about 2 hours. She denies having any extraarticular symptoms. Physical examination reveals synovitis across her right metacarpophalangeal joints, proximal interphalangeal joint of the left middle finger, and left wrist. The primary care physician is concerned that her symptoms might be due to rheumatoid arthritis.

Would testing for rheumatoid factor and anticitrullinated peptide antibody be useful in this patient?

Rheumatoid factor is an antibody (immunoglobulin M, IgG, or IgA) targeted against the Fc fragment of IgG.[3] It was so named because it was originally detected in patients with rheumatoid arthritis, but it is neither sensitive nor specific for this condition. A meta-analysis of more than 5,000 patients with rheumatoid arthritis reported that rheumatoid factor testing had a sensitivity of 69% and specificity of 85%.[4]

Numerous other conditions can be associated with a positive test for rheumatoid factor (**Table 1**). Hence, a diagnosis of rheumatoid arthritis cannot be confirmed with a positive result alone, nor can it be excluded with a negative result.

Anticitrullinated peptide antibody, on the other hand, is much more specific for rheumatoid arthritis (95%), as it is seldom seen in other conditions, but its sensitivity is similar to that of rheumatoid factor (68%).[4–6] A positive result would thus lend strength to the diagnosis of rheumatoid arthritis, but a negative result would not exclude it.

TABLE 1

Conditions associated with rheumatoid factor

Condition	Frequency
Rheumatoid arthritis	70%
Other autoimmune rheumatic conditions	
Primary Sjögren syndrome	75%–95%
Systemic lupus erythematosus	15%–35%
Systemic sclerosis	20%–35%
Systemic vasculitis	5%–20%
Infections[a]	
Infective endocarditis	40%
Syphilis	8%–37%
Hepatitis B	25%
Hepatitis C	76%
Human immunodeficiency virus infection	10%–20%
Tuberculosis	15%
Other diseases	
Liver cirrhosis	25%
Mixed cryoglobulinemia	100%
Primary biliary cirrhosis	45%–70%
Healthy people	5%–25%[b]

[a]The rheumatoid factor in infectious diseases is produced by B cells, possibly to clear the immune complexes. They are usually transient and harmless.
[b]The frequency rises with age (5% at age 50, rising to 10% to 25% at age 70.

Data from reference 3.

Approach to early arthritis

When faced with a patient with early arthritis, some key questions to ask include[7,8]:

Is this an inflammatory or a mechanical problem? Inflammatory arthritis is suggested by joint swelling that is not due to trauma or bony hypertrophy, early morning stiffness lasting longer than 30 minutes, and elevated inflammatory markers (erythrocyte sedimentation rate or C-reactive protein). Involvement of the small joints of the hands and feet may be suggested by pain on compression of the metacarpophalangeal and metatarsophalangeal joints, respectively.

Is there a definite identifiable underlying cause for the inflammatory arthritis? The pattern of development of joint symptoms or the presence of extraarticular symptoms may suggest an underlying problem such as gout, psoriatic arthritis, systemic lupus erythematosus, or sarcoidosis.

If the arthritis is undifferentiated (ie, there is no definite identifiable cause), is it likely to remit or persist? This is perhaps the most important question to ask in order to prognosticate. Patients with risk factors for persistent disease, ie, for development of rheumatoid arthritis, should be referred to a rheumatologist early for timely institution of disease-modifying antirheumatic drug therapy.[9] Multiple studies have shown that patients in whom this therapy is started early have much better clinical, functional, and radiologic outcomes than those in whom it is delayed.[10–12]

The revised American College of Rheumatology and European League Against Rheumatism criteria[13] include the following factors as predictors of persistence:

- Number of involved joints (with greater weight given to involvement of small joints)
- Duration of symptoms 6 weeks or longer
- Elevated acute-phase response (erythrocyte sedimentation rate or C-reactive protein level)
- A positive serologic test (either rheumatoid factor or anticitrullinated peptide antibody).

If both rheumatoid factor and anticitrullinated peptide antibody are positive in a patient with early undifferentiated arthritis, the risk of progression to rheumatoid arthritis is

almost 100%, thus underscoring the importance of testing for these antibodies.[5,6] Referral to a rheumatologist should, however, not be delayed in patients with negative test results (more than one-third of patients with rheumatoid arthritis may be negative for both), and should be considered in those with inflammatory joint symptoms persisting longer than 6 weeks, especially with involvement of the small joints (sparing the distal interphalangeals) and elevated acute-phase response.

Rheumatoid factor in healthy people without symptoms

In some countries, testing for rheumatoid factor is offered as part of a battery of screening tests in healthy people who have no symptoms, a practice that should be strongly discouraged.

Multiple studies, both prospective and retrospective, have demonstrated that both rheumatoid factor and anticitrullinated peptide antibody may be present several years before the clinical diagnosis of rheumatoid arthritis.[6,14–16] But the risk of developing rheumatoid arthritis for asymptomatic individuals who are rheumatoid factor-positive depends on the rheumatoid factor titer, positive family history of rheumatoid arthritis in first-degree relatives, and copresence of anticitrullinated peptide antibody. The absolute risk, nevertheless, is still very small. In some, there might be an alternative explanation such as undiagnosed Sjögren syndrome or hepatitis C.

A positive antinuclear antibody result does not always mean lupus

In any event, no strategy is currently available that is proven to prevent the development of rheumatoid arthritis, and there is no role for disease-modifying therapy during the preclinical phase.[16]

Back to our patient

Blood testing in our patient reveals normal complete blood cell counts, aminotransferase levels, and serum creatinine concentration; findings on urinalysis are normal. Her erythrocyte sedimentation rate is 56 mm/hour (reference range 0–15), and her C-reactive protein level is 26 mg/dL (normal < 3). Testing is negative for rheumatoid factor and anticitrullinated peptide antibody.

Although her rheumatoid factor and anticitrullinated peptide antibody tests are negative, she is referred to a rheumatologist because she has predictors of persistent disease, ie, symptom duration of 6 weeks, involvement of the small joints of the hands, and elevated erythrocyte sedimentation rate and C-reactive protein. The rheumatologist checks her parvovirus serology, which is negative.

The patient is given parenteral depot corticosteroid therapy, to which she responds briefly. Because her symptoms persist and continue to worsen, methotrexate treatment is started after an additional 6 weeks.

ANTINUCLEAR ANTIBODY

A 37-year-old woman presents to her primary care physician with the complaint of tiredness. She has a family history of systemic lupus erythematosus in her sister and maternal aunt. She is understandably worried about lupus because of the family history and is asking to be tested for it.

Would testing for antinuclear antibody be reasonable?

Antinuclear antibody is not a single antibody but rather a family of autoantibodies that are directed against nuclear constituents such as single- or double-stranded deoxyribonucleic acid (dsDNA), histones, centromeres, proteins complexed with ribonucleic acid (RNA), and enzymes such as topoisomerase.[17,18]

Protein antigens complexed with RNA and some enzymes in the nucleus are also known as extractable nuclear antigens (ENAs). They include Ro, La, Sm, Jo-1, RNP, and ScL-70 and are named after the patient in whom they were first discovered (Robert, Lavine, Smith, and John), the antigen that is targeted (ribonucleoprotein or RNP), and the disease with which they are associated (anti-ScL-70 or antitopoisomerase in diffuse cutaneous scleroderma).

Antinuclear antibody testing is commonly requested to exclude connective tissue diseases such as lupus, but the clinician needs to be aware of the following points:

Antinuclear antibody may be encountered in conditions other than lupus

These include[19]:

- Other autoimmune diseases such as rheumatoid arthritis, primary Sjögren syndrome, systemic sclerosis, autoimmune thyroid disease, and myasthenia gravis

TABLE 2

Clinical and laboratory manifestations of systemic lupus erythematosus

Pathogenesis	Examples of disease manifestations
Nonspecific inflammatory response	Fever, fatigue, arthralgia, weight loss, anemia of chronic disease, elevated erythrocyte sedimentation rate
Immune complex deposition (commonly in the synovium, skin, serosa, kidneys, and lungs)	Inflammatory arthritis, photosensitive skin rashes, pleurisy, pericarditis, glomerulonephritis, pneumonitis, or interstitial lung disease
Direct antibody-mediated attack	Hemolytic anemia (red cell antibodies), thrombocytopenia (antiplatelet antibodies), lymphopenia (lymphocytotoxic antibodies), neuropsychiatric manifestations such as depression and psychosis (antiribosomal P antibodies)
Associated features	Recurrent thrombosis and miscarriages (antiphospholipid syndrome), dry eyes and mouth (Sjögren syndrome)
Laboratory clues	Hemolytic anemia, leukopenia, lymphopenia, thrombocytopenia, elevated erythrocyte sedimentation rate, normal C-reactive protein, low complement (due to immune complex formation), and abnormal urinalysis (proteinuria, hematuria, red cell casts, or dysmorphic red cells)

- Infection with organisms that share the epitope with self-antigens (molecular mimicry)
- Cancers
- Drugs such as hydralazine, procainamide, and minocycline.

Antinuclear antibody might also be produced by the healthy immune system from time to time to clear the nuclear debris that is extruded from aging cells.

A study in healthy individuals[20] reported a prevalence of positive antinuclear antibody of 32% at a titer of 1/40, 15% at a titer of 1/80, 7% at a titer of 1/160, and 3% at a titer of 1/320. Importantly, a positive result was more common among family members of patients with autoimmune connective tissue diseases.[21] Hence, a positive antinuclear antibody result does not always mean lupus.

Antinuclear antibody testing is highly sensitive for lupus

With current laboratory methods, antinuclear antibody testing has a sensitivity close to 100%. Hence, a negative result virtually rules out lupus.

Two methods are commonly used to test for antinuclear antibody: indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA).[22] While human epithelial (Hep2) cells are used as the source of antigen in immunofluorescence, purified nuclear antigens coated on multiple-well plates are used in ELISA.

Although ELISA is simpler to perform, immunofluorescence has a slightly better sensitivity (because the Hep2 cells express a wide range of antigens) and is still considered the gold standard. As expected, the higher sensitivity occurs at the cost of reduced specificity (about 60%), so antinuclear antibody will also be detected in all the other conditions listed above.[23]

To improve the specificity of antinuclear antibody testing, laboratories report titers (the highest dilution of the test serum that tested positive); a cutoff of greater than 1/80 is generally considered significant.

Test for antinuclear antibody only in patients with involvement of multiple organ systems

Do not order antinuclear antibody testing indiscriminately

If the antinuclear antibody test is requested indiscriminately, the positive predictive value for the diagnosis of lupus is only 11%.[24] The test should be requested only when the pretest probability of lupus or other connective tissue disease is high. The positive predictive value is much higher in patients presenting with clinical or laboratory manifestations involving 2 or more organ systems (**Table 2**).[18,25]

TABLE 3

Disease associations of specific antigen targets

Antigen target	Disease association
Double-stranded DNA and Sm	Systemic lupus erythematosus
Ro and La	Sjögren syndrome; Ro also associated with subacute cutaneous lupus
U1-RNP	Mixed connective tissue disease
Jo-1	Polymyositis (higher risk of interstitial lung disease)
ScL-70	Diffuse cutaneous scleroderma
Anticentromere	Limited cutaneous scleroderma

Categorization of the specific antigen target improves disease specificity. The antinuclear antibody in patients with lupus may be targeted against single- or double-stranded DNA, histones, or 1 or more of the ENAs. Among these, the presence of anti-dsDNA or anti-Sm is highly specific for a diagnosis of lupus (close to 100%). Neither is sensitive for lupus, however, with anti-dsDNA present in only 60% of patients with lupus and anti-Sm in about 30%.[17] Hence, patients with a positive antinuclear antibody and negative anti-dsDNA and anti-Sm may continue to pose a diagnostic challenge. Other examples of specific disease associations are listed in **Table 3**.

Antiphospholipid antibodies may be present in 1% to 5% of apparently health people

To sum up, the antinuclear antibody test should be requested only in patients with involvement of multiple organ systems. Although a negative result would make it extremely unlikely that the clinical presentation is due to lupus, a positive result is insufficient on its own to make a diagnosis of lupus.

Diagnosing lupus is straightforward when patients present with a specific manifestation such as inflammatory arthritis, photosensitive skin rash, hemolytic anemia, thrombocytopenia, or nephritis, or with specific antibodies such as those against dsDNA or Sm. Patients who present with nonspecific symptoms such as arthralgia or tiredness with a positive antinuclear antibody and negative anti-dsDNA and anti-Sm may present difficulties even for the specialist.[25–27]

Back to our patient

Our patient denies arthralgia. She has no extraarticular symptoms such as skin rashes, oral ulcers, sicca symptoms, muscle weakness, Raynaud phenomenon, pleuritic chest pain, or breathlessness. Findings on physical examination and urinalysis are unremarkable.

Her primary care physician decides to check her complete blood cell count, erythrocyte sedimentation rate, and thyroid-stimulating hormone level. Although she is reassured that her tiredness is not due to lupus, she insists on getting an antinuclear antibody test.

Her complete blood cell counts are normal. Her erythrocyte sedimentation rate is 6 mm/hour. However, her thyroid-stimulating hormone level is elevated, and subsequent testing shows low free thyroxine and positive thyroid peroxidase antibodies. The antinuclear antibody is positive in a titer of 1/80 and negative for anti-dsDNA and anti-ENA.

We explain to her that the positive antinuclear antibody is most likely related to her autoimmune thyroid disease. She is referred to an endocrinologist.

ANTIPHOSPHOLIPID ANTIBODIES

A 24-year-old woman presents to the emergency department with acute unprovoked deep vein thrombosis in her right leg, confirmed by ultrasonography. She has no history of previous thrombosis, and the relevant family history is unremarkable. She has never been pregnant. Her platelet count is 84 × 10^9/L (reference range 150–400), and her baseline activated partial thromboplastin time is prolonged at 62 seconds (reference range 23.0–32.4). The rest of her blood counts and her prothrombin time, liver enzyme levels, and serum creatinine level are normal.

Should this patient be tested for antiphospholipid antibodies?

Antiphospholipid antibodies are important because of their association with thrombotic risk (both venous and arterial) and pregnancy morbidity. The name is a misnomer, as these antibodies are targeted against some proteins that are bound to phospholipids and not only to the phospholipids themselves.

According to the modified Sapporo criteria for the classification of antiphospho-

lipid syndrome,[28] antiphospholipid antibodies should remain persistently positive on at least 2 separate occasions at least 12 weeks apart for the result to be considered significant because some infections and drugs may be associated with the transient presence of antiphospholipid antibodies.

Screening for antiphospholipid antibodies should include testing for IgM and IgG anticardiolipin antibodies, lupus anticoagulant, and IgM and IgG beta-2 glycoprotein I antibodies.[29,30]

Anticardiolipin antibodies

Anticardiolipin (aCL) antibodies may be targeted either against beta-2 glycoprotein I (beta-2GPI) that is bound to cardiolipin (a phospholipid) or against cardiolipin alone; the former is more specific. Antibodies directed against cardiolipin alone are usually transient and are associated with infections and drugs. The result is considered significant only when anticardiolipin antibodies are present in a medium to high titer (> 40 IgG phospholipid units or IgM phospholipid units, or > 99th percentile).

Lupus anticoagulant

The antibody with "lupus anticoagulant activity" is targeted against prothrombin plus phospholipid or beta-2GPI plus phospholipid. The test for it is a functional assay involving 3 steps:

Demonstrating the prolongation of a phospholipid-dependent coagulation assay like the activated partial thromboplastin time (aPTT). (This may explain the prolongation of aPTT in the patient described in the vignette.) Although the presence of lupus anticoagulant is associated with thrombosis, it is called an "anticoagulant" because of this in vitro prolongation of phospholipid-dependent coagulation assays.

Mixing study. The phospholipid-dependent coagulation assay could be prolonged because of either the deficiency of a coagulation factor or the presence of the antiphospholipid antibodies. This can be differentiated by mixing the patient's plasma with normal plasma (which will have all the clotting factors) in a 1:1 ratio. If the coagulation assay remains prolonged after the addition of normal plasma, clotting factor deficiency can be excluded.

Addition of a phospholipid. If the prolongation of the coagulation assay is due to the presence of an antiphospholipid antibody, addition of extra phospholipid will correct this.

Beta-2 glycoprotein I antibody (anti-beta-2GPI)

The beta-2GPI that is not bound to the cardiolipin can be detected by separately testing for beta-2GPI (the anticardiolipin test only detects the beta-2GPI that is bound to the cardiolipin). The result is considered significant if beta-2GPI is present in a medium to high titer (> 99th percentile).

Studies have shown that antiphospholipid antibodies may be present in 1% to 5% of apparently healthy people in the general population.[31] These are usually low-titer anticardiolipin or anti-beta-GPI IgM antibodies that are not associated with thrombosis or adverse pregnancy outcomes. Hence, the term *antiphospholipid syndrome* should be reserved for those who have had at least 1 episode of thrombosis or pregnancy morbidity and persistent antiphospholipid antibodies, and not those who have asymptomatic or transient antiphospholipid antibodies.

Triple positivity (positive anticardiolipin, lupus anticoagulant, and anti-beta-2GPI) seems to be associated with the highest risk of thrombosis, with a 10-year cumulative incidence of 37.1% (95% confidence interval [CI] 19.9–54.3) for a first thrombotic event,[32] and 44.2% (95% CI 38.6–49.8) for recurrent thrombosis.[33]

A positive lupus anticoagulant test constitutes a high-risk profile

The association with thrombosis is stronger for lupus anticoagulant than with the other 2 antibodies, with different studies[34] finding an odds ratio ranging from 5 to 16. A positive lupus anticoagulant test with or without a moderate to high titer of anticardiolipin or anti-beta-2GPI IgM or IgG constitutes a high-risk profile, while a moderate to high titer of anticardiolipin or anti-beta-2GPI IgM or IgG constitutes a moderate-risk profile. A low titer of anticardiolipin or anti-beta-2GPI IgM or IgG constitutes a low-risk profile that may not be associated with thrombosis.[35]

Antiphospholipid syndrome is important to recognize because of the need for long-term anticoagulation to prevent recurrence.[36] It may be primary, when it occurs on its own, or secondary, when it occurs in association with another autoimmune disease such as lupus.

TABLE 4

Some indications to test for antiphospholipid antibodies[a]

Unprovoked deep vein thrombosis or pulmonary embolism (Antiphospholipid antibody testing is not recommended in patients with provoked venous thrombosis, as there is insufficient evidence to recommend long-term anticoagulation.)

Ischemic stroke (including transient ischemic attack) in patients under age 50

Patients with both arterial and venous events

Recurrent thrombosis

Thrombosis in an unusual site

Pregnancy morbidity (1 or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation; 1 or more premature births of a morphologically normal neonate before the 34th week of gestation because of preeclampsia, eclampsia, or placental insufficiency; or 3 or more unexplained consecutive spontaneous abortions before the 10th week of gestation)

All patients with systemic lupus erythematosus

[a]Knowledge of the antiphospholipid antibody status helps to decide if low-dose aspirin should be recommended for primary prevention of thrombosis.[32] Lupus anticoagulant is likely to be falsely positive in those with acute thrombosis and those receiving anticoagulant therapy. Hence, anticoagulant therapy should be interrupted for at least 7 days before testing for lupus anticoagulant. However, anticardiolipin and anti-beta-2 glycoprotein I can be tested at any time, as they are not affected by thrombosis or anticoagulant therapy.

Venous events in antiphospholipid syndrome most commonly manifest as lower-limb deep vein thrombosis or pulmonary embolism, while arterial events most commonly manifest as stroke or transient ischemic attack.[37] Obstetric manifestations may include not only miscarriage and stillbirth, but also preterm delivery, intrauterine growth retardation, and preeclampsia, all occurring due to placental insufficiency.

The frequency of antiphospholipid antibodies has been estimated as 13.5% in patients with stroke, 11% with myocardial infarction, 9.5% with deep vein thrombosis, and 6% for those with pregnancy morbidity.[38]

Some noncriteria manifestations have also been recognized in antiphospholipid syndrome, such as thrombocytopenia, cardiac vegetations (Libman-Sachs endocarditis), livedo reticularis, and nephropathy.

The indications for antiphospholipid antibody testing are listed in **Table 4**.[29] For the patient described in the vignette, it would be appropriate to test for antiphospholipid antibodies because of her unprovoked thrombosis, thrombocytopenia, and prolonged aPTT. Anticoagulant treatment is known to be associated with false-positive lupus anticoagulant, so any blood samples should be drawn before such treatment is commenced.

Back to our patient

Our patient's anticardiolipin IgG test is negative, while her lupus anticoagulant and beta-2GPI IgG are positive. She has no clinical or laboratory features suggesting lupus.

She is started on warfarin. After 3 months, the warfarin is interrupted for several days, and she is retested for all 3 antiphospholipid antibodies. Her beta-2GPI I IgG and lupus anticoagulant tests are again positive. Because of the persistent antiphospholipid antibody positivity and clinical history of deep vein thrombosis, her condition is diagnosed as primary antiphospholipid syndrome. She is advised to continue anticoagulant therapy indefinitely.

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY

A 34-year-old man who is an injecting drug user presents with a 2-week history of fever, malaise, and generalized arthralgia. There are no localizing symptoms of infection. Notable findings on examination include a temperature of 38.0°C (100.4°F), needle track marks in his arms, nonblanching vasculitic rash in his legs, and a systolic murmur over the precordium.

His white blood cell count is 15.3 × 10^9/L (reference range 3.7–11.0), and his C-reactive protein level is 234 mg/dL (normal < 3). Otherwise, results of blood cell counts, liver enzyme tests, renal function tests, urinalysis, and chest radiography are normal.

Two sets of blood cultures are drawn. Transthoracic echocardiography and the antineutrophil cytoplasmic antibody (ANCA) test are requested, as are screening tests for human immunodeficiency virus, hepatitis B, and hepatitis C.

Was the ANCA test indicated in this patient?

ANCAs are autoantibodies against antigens

located in the cytoplasmic granules of neutrophils and monocytes. They are associated with small-vessel vasculitides such as granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA), and isolated pauciimmune crescentic glomerulonephritis, all collectively known as ANCA-associated vasculitis (AAV).[39]

Laboratory methods to detect ANCA include indirect immunofluorescence and antigen-specific enzyme immunoassays. Indirect immunofluorescence only tells us whether or not an antibody that is targeting a cytoplasmic antigen is present. Based on the indirect immunofluorescent pattern, ANCA can be classified as follows:

- Perinuclear or p-ANCA (if the targeted antigen is located just around the nucleus and extends into it)
- Cytoplasmic or c-ANCA (if the targeted antigen is located farther away from the nucleus)
- Atypical ANCA (if the indirect immunofluorescent pattern does not fit with either p-ANCA or c-ANCA).

Indirect immunofluorescence does not give information about the exact antigen that is targeted; this can only be obtained by performing 1 of the antigen-specific immunoassays. The target antigen for c-ANCA is usually proteinase-3 (PR3), while that for p-ANCA could be myeloperoxidase (MPO), cathepsin, lysozyme, lactoferrin, or bactericidal permeability inhibitor. Anti-PR3 is highly specific for GPA, while anti-MPO is usually associated with MPA and EGPA. Less commonly, anti-PR3 may be seen in patients with MPA and anti-MPO in those with GPA. Hence, there is an increasing trend toward classifying ANCA-associated vasculitis into PR3-associated or MPO-associated vasculitis rather than as GPA, MPA, EGPA, or renal-limited vasculitis.[40]

Several audits have shown that the ANCA test is widely misused and requested indiscriminately to rule out vasculitis. This results in a lower positive predictive value, possible harm to patients due to increased false-positive rates, and increased burden on the laboratory.[41–43] At least 2 separate groups have demonstrated that a gating policy that refuses ANCA testing in patients without clinical evidence of systemic vasculitis can reduce the number of inappropriate requests, improve the diagnostic yield, and make it more clinically relevant and cost-effective.[44,45]

TABLE 5

Clinical indications to test for antineutrophil cytoplasmic antibody

Glomerulonephritis
Pulmonary hemorrhage (especially with pulmonary-renal syndrome)
Multiple lung nodules
Mononeuritis multiplex or unexplained peripheral neuropathy
Cutaneous vasculitis, especially with systemic features
Scleritis
Retroorbital mass
Chronic destructive disease of the upper airways
Chronic sinusitis or otitis
Subglottic tracheal stenosis

The clinician should bear in mind that:

ANCA testing should be requested only if the pretest probability of ANCA-associated vasculitis is high. The indications proposed by the International Consensus Statement on ANCA testing[46] are listed in **Table 5**. These criteria have been clinically validated, with 1 study even demonstrating that no cases of ANCA-associated vasculitis would be missed if these guidelines are followed.[47]

Current guidelines recommend using one of the antigen-specific assays for PR3 and MPO as the primary screening method.[48] Until recently, indirect immunofluorescence was used to screen for ANCA-associated vasculitis, and positive results were confirmed by ELISA to detect ANCAs specific for PR3 and MPO,[49] but this is no longer recommended because of recent evidence suggesting a large variability between the different indirect immunofluorescent methods and improved diagnostic performance of the antigen-specific assays.

In a large multicenter study by Damoiseaux et al, the specificity with the different antigen-specific immunoassays was 98% to 99% for PR3-ANCA and 96% to 99% for MPO-ANCA.[50]

Many conditions are associated with ANCA; consider the result in the context of the clinical picture

TABLE 6

Conditions associated with antineutrophil cytoplasmic antibody (ANCA) other than ANCA-associated vasculitis

Gastrointestinal disorders
Inflammatory bowel disease
Primary sclerosing cholangitis
Primary biliary cirrhosis
Autoimmune hepatitis
Viral hepatitis

Infections
Infective endocarditis
Tuberculosis
Malaria

Drugs
Propylthiouracil
Minocycline
Hydralazine
Allopurinol
Levamisole

Autoimmune diseases
Rheumatoid arthritis
Systemic lupus erythematosus (SLE)[a]
Antiglomerular basement membrane disease

[a]Antinuclear antibody (ANA) and p-ANCA resemble each other closely and are difficult to differentiate. Thus, SLE sera may show positive p-ANCA staining due to presence of ANA.

ANCA-associated vasculitis should not be considered excluded if the PR3 and MPO-ANCA are negative. In the Damoiseaux study, about 11% to 15% of patients with GPA and 8% to 24% of patients with MPA tested negative for both PR3 and MPO-ANCA.[50]

If the ANCA result is negative and clinical suspicion for ANCA-associated vasculitis is high, the clinician may wish to consider requesting another immunoassay method or indirect immunofluorescence. Results of indirect immunofluorescent testing results may be positive in those with a negative immunoassay, and vice versa.

A positive ANCA result is not diagnostic of ANCA-associated vasculitis. Numerous other conditions are associated with ANCA, usually p-ANCA or atypical ANCA (**Table 6**). The antigens targeted by these ANCAs are usually cathepsin, lysozyme, lactoferrin, and bactericidal permeability inhibitor.

Thus, the ANCA result should always be interpreted in the context of the whole clinical picture.[51] Biopsy should still be considered the gold standard for the diagnosis of ANCA-associated vasculitis. The ANCA titer can help to improve clinical interpretation, because the likelihood of ANCA-associated vasculitis increases with higher levels of PR3 and MPO-ANCA.[52]

Back to our patient

Our patient's blood cultures grow methicillin-sensitive *Staphylococcus aureus* in both sets after 48 hours. Transthoracic echocardiography reveals vegetations around the tricuspid valve, with no evidence of valvular regurgitation. The diagnosis is right-sided infective endocarditis. He is started on appropriate antibiotics.

Tests for human immunodeficiency virus, hepatitis B, and hepatitis C are negative. The ANCA test is positive for MPO-ANCA at 28 IU/mL (normal < 10).

The positive ANCA is thought to be related to the infective endocarditis. His vasculitis is most likely secondary to infective endocarditis and not ANCA-associated vasculitis. The ANCA test need not have been requested in the first place.

HUMAN LEUKOCYTE ANTIGEN-B27

A 22-year-old man presents to his primary care physician with a 4-month history of gradually worsening low back pain associated with early morning stiffness lasting more than 2 hours. He has no peripheral joint symptoms.

In the last 2 years, he has had 2 separate episodes of uveitis. There is a family history of ankylosing spondylitis in his father. Examination reveals global restriction of lumbar movements but is otherwise unremarkable. Magnetic resonance imaging (MRI) of the lumbar spine and sacroiliac joints is normal.

Should this patient be tested for human leukocyte antigen-B27 (HLA-B27)?

The major histocompatibility complex (MHC) is a gene complex that is present in all animals. It encodes proteins that help with immunologic tolerance. HLA simply refers to the human version of the MHC.[53] The HLA gene complex, located on chromosome 6, is

categorized into class I, class II, and class III. *HLA-B* is one of the 3 class I genes. Thus, a positive *HLA-B27* result simply means that the particular gene is present in that person.

HLA-B27 is strongly associated with ankylosing spondylitis, also known as axial spondyloarthropathy.[54] Other genes also contribute to the pathogenesis of ankylosing spondylitis, but *HLA-B27* is present in more than 90% of patients with this disease and is by far considered the most important. The association is not as strong for peripheral spondyloarthropathy, with studies reporting a frequency of up to 75% for reactive arthritis and inflammatory bowel disease-associated arthritis, and up to 50% for psoriatic arthritis and uveitis.[55]

About 9% of healthy, asymptomatic individuals may have *HLA-B27*, so the mere presence of this gene is not evidence of disease.[56] There may be up to a 20-fold increased risk of ankylosing spondylitis among those who are *HLA-B27*-positive.[57]

Some HLA genes have many different alleles, each of which is given a number (explaining the number 27 that follows the B). Closely related alleles that differ from one another by only a few amino-acid substitutions are then categorized together, thus accounting for more than 100 subtypes of *HLA-B27* (designated from HLA-B*2701 to HLA-B*27106). These subtypes vary in frequency among different racial groups, and the population prevalence of ankylosing spondylitis parallels the frequency of *HLA-B27*.[58] The most common subtype seen in white people and American Indians is B*2705. *HLA-B27* is rare in blacks, explaining the rarity of ankylosing spondylitis in this population. Further examples include HLA-B*2704, which is seen in Asians, and HLA-B*2702, seen in Mediterranean populations. Not all subtypes of *HLA-B27* are associated with disease, and some, like HLA-B*2706, may also be protective.

When should the clinician consider testing for HLA-B27?

Not all patients with low back pain need an HLA-B27 test. First, it is important to look for clinical features of axial spondyloarthropathy (**Table 7**). The unifying feature of spondyloarthropathy is enthesitis (inflammation at the sites of insertion of tendons or ligaments on the skeleton). Inflammation of axial entheses causes spondylitis and sacroiliitis, manifesting as inflammatory back pain. Clinical clues to inflammatory back pain include insidious onset, aggravation with rest or inactivity, prolonged early morning stiffness, disturbed sleep during the second half of the night, relief with movement or activity, alternating gluteal pain (due to sacroiliitis), and good response to anti-inflammatory medication (although nonspecific).

TABLE 7

Features of spondyloarthritis

Inflammatory back pain
Arthritis
Enthesitis of the heel
Dactylitis
Uveitis
Psoriasis
Inflammatory bowel disease
Good response to nonsteroidal anti-inflammatory medication
Family history of spondyloarthritis
Positive *HLA-B27*
Elevated C-reactive protein

Not all patients with low back pain need an HLA-B27 test

Peripheral spondyloarthropathy may present with arthritis, enthesitis (eg, heel pain due to inflammation at the site of insertion of the Achilles tendon or plantar fascia), or dactylitis ("sausage" swelling of the whole finger or toe due to extension of inflammation beyond the margins of the joint). Other clues may include psoriasis, inflammatory bowel disease, history of preceding gastrointestinal or genitourinary infection, family history of similar conditions, and history of recurrent uveitis.

For the initial assessment of patients who have inflammatory back pain, plain radiography of the sacroiliac joints is considered the gold standard.[59] If plain radiography does not show evidence of sacroiliitis, MRI of the sacroiliac joints should be considered. While plain radiography can reveal only structural changes such as sclerosis, erosions, and ankylosis, MRI is useful to evaluate for early inflammatory changes such as bone marrow edema. Imaging the lumbar spine is not necessary, as the sac-

roiliac joints are almost invariably involved in axial spondyloarthropathy, and lesions seldom occur in the lumbar spine in isolation.[60]

The diagnosis of ankylosing spondylitis previously relied on confirmatory imaging features, but based on the new International Society classification criteria,[61–63] which can be applied to patients with more than 3 months of back pain and age of onset of symptoms before age 45, patients can be classified as having 1 of the following:

- Radiographic axial spondyloarthropathy, if they have evidence of sacroiliitis on imaging plus 1 other feature of spondyloarthropathy
- Nonradiographic axial spondyloarthropathy, if they have a positive *HLA-B27* plus 2 other features of spondyloarthropathy (**Table 7**).

These new criteria have a sensitivity of 82.9% and specificity of 84.4%.[62,63] The disease burden of radiographic and nonradiographic axial spondyloarthropathy has been shown to be similar, suggesting that they are part of the same disease spectrum. Thus, the HLA-B27 test is useful to make a diagnosis of axial spondyloarthropathy even in the absence of imaging features and could be requested in patients with 2 or more features of spondyloarthropathy. In the absence of imaging features and a negative HLA-B27 result, however, the patient cannot be classified as having axial spondyloarthropathy.

Back to our patient

The absence of radiographic evidence would not exclude axial spondyloarthropathy in our patient. The HLA-B27 test is requested because of the inflammatory back pain and the presence of 2 spondyloarthropathy features (uveitis and the family history) and is reported to be positive. His disease is classified as nonradiographic axial spondyloarthropathy.

He is started on regular naproxen and is referred to a physiotherapist. After 1 month, he reports significant symptomatic improvement. He asks if he can be retested for *HLA-B27* to see if it has become negative. We tell him that there is no point in repeating it, as it is a gene and will not disappear.

SUMMARY: CONSIDER THE CLINICAL PICTURE

When approaching a patient suspected of having a rheumatologic disease, a clinician should first consider the clinical presentation and the intended purpose of each test. The tests, in general, might serve several purposes. They might help to:

Increase the likelihood of the diagnosis in question. For example, a positive rheumatoid factor or anticitrullinated peptide antibody can help diagnose rheumatoid arthritis in a patient with early polyarthritis, a positive *HLA-B27* can help diagnose ankylosing spondylitis in patients with inflammatory back pain and normal imaging, and a positive ANCA can help diagnose ANCA-associated vasculitis in a patient with glomerulonephritis.

Reduce the likelihood of the diagnosis in question. For example, a negative antinuclear antibody test reduces the likelihood of lupus in a patient with joint pains.

Monitor the condition. For example DNA antibodies can be used to monitor the activity of lupus.

Plan the treatment strategy. For example, one might consider lifelong anticoagulation if antiphospholipid antibodies are persistently positive in a patient with thrombosis.

Prognosticate. For example, positive rheumatoid factor and anticitrullinated peptide antibody increase the risk of erosive rheumatoid arthritis.

If the test was requested in the absence of a clear indication and the result is positive, it is important to bear in mind the potential pitfalls associated with that test and not attach a diagnostic label prematurely. None of the tests can confirm or exclude a condition, so the results should always be interpreted in the context of the whole clinical picture.

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