Laboratory Testing in Pediatric Rheumatology

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KEYWORDS

• Pediatric rheumatology • Diagnosis • Laboratory testing
• Biologic markers • Autoantibodies • Prognosis

Although pediatric rheumatic disorders are primarily diagnosed through history and physical examination, laboratory studies are valuable adjuncts to the care of patients with such disorders. Laboratory evaluations can assist in the screening of patients for inflammatory disorders, confirm diagnoses, allow for monitoring of disease activity and response to therapy, and suggest prognoses and risk of morbidities associated with rheumatic diseases.

Which laboratory tests are ordered should be dictated by a thorough history and physical examination, rather than by the indiscriminate use of a rheumatology screen. Although commonly ordered tests such as the complete blood count (CBC), antinuclear antigen (ANA), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) are useful in the appropriate clinical setting, they lack specificity and abnormal values may be found in the well child. This review provides an overview of the usefulness and interpretation of both the commonly ordered tests ordered by the general pediatrician as well as those frequently used in the pediatric rheumatology clinic for diagnosis and disease monitoring.

CBC

The CBC provides information about the 3 major cellular components of whole blood. Rheumatic diseases may be associated with alterations in each of them.

Red Blood Cell Count

Anemia is seen in many of the rheumatic diseases and can be multifactorial. An anemia of chronic disease is usually normocytic, but may be microcytic, and can be associated with any inflammatory disease. The cause likely relates to cytokine-mediated shortened red blood cell (RBC) survival and impaired bone marrow response to erythropoietin.[1] Another major contribution to the anemia of chronic

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disease is the inflammation-induced activation of the interleukin 6 (IL-6)-hepcidin axis, which leads to decreased intestinal iron absorption and decreased iron release from stores.[2] This condition must be distinguished from iron-deficiency anemia, which can also be seen in any of the rheumatic diseases. **Table 1** contrasts these 2 conditions based on commonly used serum parameters. Complicating factors include the frequent coexistence of these 2 conditions and the fact that ferritin is an acute phase reactant so it may be normal, or even increased, in iron deficiency. Measurement of the soluble transferrin receptor, which is gaining acceptance, may help to more reliably distinguish anemia of chronic disease from iron-deficiency anemia.[3]

Autoimmune hemolytic anemia may be seen in systemic lupus erythematosus (SLE) and related conditions (ie, Sjögren syndrome and mixed connective tissue disease [MCTD]). This diagnosis is made through a positive direct Coombs test and evidence of hemolysis (decreased hemoglobin or hematocrit, increased reticulocyte count, increased serum lactate dehydrogenase (LDH), increased unconjugated bilirubin, decreased haptoglobin, and hemoglobinuria). Nonimmune-mediated hemolysis is seen in the macrophage activation syndrome (MAS) (see later discussion) and thrombotic thrombocytopenic purpura.

White Blood Cell Count

SLE and related conditions frequently cause leukopenia, specifically lymphopenia and neutropenia. These abnormalities may confer an increased risk of infection in patients with SLE.[4–6] Increased white blood cell (WBC) counts can be seen in other inflammatory diseases, and particularly increased counts may be seen in systemic juvenile idiopathic arthritis (sJIA; often >30,000 cells/mm^3). Malignancies can cause either increased or depressed WBC counts, and they must be considered in any patient who presents with WBC abnormalities.

Platelet Count

Because of their role as an acute phase reactant, platelets are frequently modestly increased in the rheumatic diseases. Significant increases (occasionally more than 1,000,000 cells/mm^3) may be seen in sJIA, Kawasaki disease, or Takayasu arteritis. SLE and related conditions often cause thrombocytopenia, and a depressed platelet count in the face of signs of systemic inflammation or arthritis should raise suspicion for these diseases or for malignancy. Thrombocytopenia may also be seen in the antiphospholipid antibody syndrome (APS) and in thrombotic thrombocytopenic purpura.

Table 1
Distinguishing features of anemia of chronic disease and iron-deficiency anemia

Parameter	ACD	IDA
MCV	Normal (can be ↓ in prolonged disease)	↓
Serum iron	↓	↓
Transferrin	↓	↑
Ferritin	Normal or ↑	↓ (can be normal if ACD+IDA)
STfR-ferritin index	<1.0	>2.0 (can also indicate ACD+IDA)
Transferrin saturation %	Normal (can be ↓)	↓

Abbreviations: ACD, anemia of chronic disease; IDA, iron-deficiency anemia; MCV, mean corpuscular volume; STfR, soluble transferrin receptor.

Special Considerations

MAS is a potentially life-threatening complication of any of the rheumatic diseases, and is particularly associated with sJIA. An overwhelming inflammatory reaction is seen and phagocytosis of all hematopoietic components ensues. Thus, any of WBC, RBC, and platelets may decrease. Therefore, normalization of previously increased WBC or platelets (and significantly increased ferritin level) in the face of a clinically worsening patient should alert the provider to the possibility of MAS.

ACUTE PHASE REACTANTS

The acute phase reactants are nonspecific markers of inflammation. Although they are frequently increased in inflammatory conditions, normal acute phase reactants should not be reassuring in a patient who presents with other signs of systemic inflammation (eg, fever, arthritis, failure to thrive). Conversely, in a child who is otherwise growing and developing normally, is afebrile, and has no persistent musculoskeletal complaints, checking acute phase reactants is of low yield and could lead to unnecessary further investigations.

ESR

The ESR evaluates the distance RBCs sediment in 1 hour. Its increase in inflammatory conditions primarily reflects increased fibrinogen production because fibrinogen is an acute phase reactant and causes RBCs to form rouleaux, which decrease at a faster rate than free RBCs. As an indirect measure of inflammation, the ESR can be influenced by several nonpathologic conditions. One of the most commonly encountered of these conditions in the pediatric outpatient setting is obesity, which can cause the ESR to be increased outside the normal age-specific range.[7] Other increasing factors include anemia, increasing age, and pregnancy.[8] For these reasons, mild increases in the ESR (ie, ESR <50 mm/h) in the absence of signs and symptoms of localized or systemic inflammation should not prompt further investigation or subspecialty referral. Conversely, an ESR of more than 100 mm/h is frequently associated with infection, malignancy, or inflammatory disease[9] and warrants further workup.

Although it is not specific for inflammatory diseases, the ESR is valuable as a tool to monitor disease activity and determine prognosis in patients with these conditions. Persistently increased ESR is predictive of a more aggressive course or worse outcome in sJIA,[10] enthesitis-related arthritis (ERA),[11] and oligoarticular JIA.[12,13] Increased ESR is associated with active synovitis in JIA[14] and a normal ESR is one of the provisional criteria for inactive disease in JIA.[15] The ESR is frequently normal in oligoarticular, and occasionally polyarticular, JIA, in contrast to malignancy (eg, leukemia or neuroblastoma), which may present with pain in 1 or a few joints but often has an extremely increased ESR. In SLE, an increased ESR has been shown to be associated with disease activity and damage accrual.[16] However, ESR increases are not always associated with disease activity. Likewise, a normal ESR does not invariably suggest inactive disease.

As previously mentioned, MAS is a severe complication of pediatric rheumatic diseases, most frequently seen with sJIA. Consequent to the intense systemic inflammation seen in MAS, hypofibrinogemia occurs, likely because of both liver dysfunction and fibrinogen consumption from coagulopathy.[17–19] This situation leads to a paradoxic decrease in the ESR. Therefore, a decreasing ESR in a patient who otherwise has active rheumatologic disease by clinical and other laboratory parameters should raise suspicion of MAS and indicate the need for emergent rheumatology consultation.

CRP

In contrast to the indirect association of ESR increase with inflammation, the CRP is produced by the liver as an acute phase reactant and plays a role in the innate host defense system.[20] It is a sensitive, but not specific, marker of inflammation. Plasma CRP more rapidly increases, in response to inflammation, and decreases, with its resolution, than does the ESR.

Similarly to the ESR, the CRP is predictive of disease course in JIA, although it may more closely reflect severe disease in polyarthritis.[21] Its normalization is a criterion for disease inactivity in JIA. One particular usefulness of the CRP in rheumatology is in the differentiation of flare from active infection in a patient with SLE. Given that either flare or infection can present with constitutional symptoms and that the immunosuppressive therapy used in SLE increases patients' risk for serious infection, this is often a difficult distinction to make clinically. Whereas the ESR can be increased in both disease flare and infection, the CRP tends not to increase with disease flare (with the exception of serositis) but does increase with active infection.[22] In a recent study of adult patients with SLE, a CRP greater than 5 mg/dL was 80% specific for infection.[23]

Ferritin

Ferritin is central to iron homeostasis and its synthesis and expression are regulated at multiple steps by iron, cytokines (IL-1, IL-6, and tumor necrosis factor α), hormones (thyroid hormones, insulin, and insulinlike growth factor 1), and oxidative stress.[24] During inflammation, serum ferritin level frequently increases in response to a decrease in serum iron. In sJIA, ferritin levels correlate with disease activity, with mild to moderate increases with active disease and normalization with quiescence.[25] Ferritin levels also correlate with disease activity in SLE,[26,27] but this is not a widely used method of monitoring patients, unlike in sJIA, in which it is part of routine surveillance and helps to guide treatment. In MAS, the ferritin level is often increased, and in hemophagocytic lymphohistiocytosis, which shares many similarities to MAS, the ferritin level is frequently more than 10,000 ng/mL.[28] Very low glycosylated ferritin percentage (<20%) has been seen in both adult-onset Still disease[29] and other hemophagocytic syndromes.[30] It remains to be established whether glycosylated ferritin measurement has a role in diagnosis of MAS associated with pediatric rheumatic diseases.

TESTS SPECIFIC TO RHEUMATOLOGIC DISEASES

ANA

Since the first description more than 50 years ago by Friou[31] of the binding to nuclear antigens by serum of patients with SLE, the ANA has been used as a serologic marker to aid in the diagnosis of SLE as well as other rheumatic diseases. The ANA also has special significance in pediatric rheumatology because of the well-recognized association of a positive ANA with an increased risk of chronic uveitis in patients with JIA.[32,33] However, up to 20% of children who are either healthy or have benign musculoskeletal complaints have a positive ANA,[34–36] and, therefore, results of ANA testing must be interpreted in combination with clinical findings. Although this positivity may persist,[35] most patients who present to a rheumatology clinic with a positive ANA but no autoimmune condition do not go on to develop any autoimmune disease.[36] For these reasons, the ANA should not be used as a screening tool without concerning joint symptoms (morning stiffness, persistent swelling) or signs of SLE (**Box 1**). Conversely, a negative ANA has a strong (0.96–1) negative predictive value for SLE, MCTD, and overlap syndromes.[37]

Box 1
Common signs of lupus

1. Persistent malar rash
2. Discoid rash
3. Photosensitivity: rash in reaction to sunlight
4. Oral or nasopharyngeal ulcers: usually painless
5. Arthritis: tenderness, swelling or effusion
6. Serositis: by history, examination, electrocardiography, or imaging
7. Proteinuria: >0.5 g/d or 3+ protein on dipstick
8. Urinary cellular casts
9. Seizures or psychosis in absence of other causes
10. Cytopenia
11. Alopecia: rapid loss of large amount of scalp hair
12. Raynaud phenomenon (RP)

The method used to perform the ANA testing influences interpretation of the results. The gold standard method[38] is immunofluorescence (IF) against human epithelial (HEp-2) cells, which have large nuclei and express a large number (>100) of nuclear antigens. From the IF, information about the pattern and intensity of staining is provided. The pattern of ANA staining on IF reflects the specific nuclear antigens to which the ANA is binding (**Fig. 1**). However, using the ANA pattern to diagnose specific autoimmune disorders has low sensitivity and specificity, and is being replaced by specific antinuclear antibody tests (see later discussion).

Some laboratories have moved to more automated commercial enzyme-linked immunosorbent assays (ELISAs), which allow for higher throughput of samples. The ELISA tests for serum absorbance to specified nuclear antigens. A disadvantage of this method arises when a patient has an ANA that binds to a nuclear antigen not present among the ones tested, which may lead to a false-negative test. This finding is of particular concern in patients with JIA, because the antigen specificity in ANA-positive patients is not known and is likely heterogenous.[39] Exclusive use of the ELISA in these patients could lead to misclassification of chronic uveitis risk.[40] The practice at our institution is to order both IF and ELISA on all patients with JIA.

In addition to SLE and JIA, other rheumatologic conditions are associated with a positive ANA (**Box 2**). In children with RP, the ANA predicts the risk of an underlying connective tissue disease, such as lupus or systemic sclerosis. In a retrospective study, 85% of children with secondary RP (RP associated with an underlying connective tissue disease) had a positive ANA, whereas only 25% of those with RP alone (primary RP) were ANA positive.[41]

As mentioned earlier, a positive ANA correlates with an increased risk of chronic, insidious uveitis in patients with JIA. ANA positivity more than triples the risk of uveitis in JIA,[42] and more than 80% of patients with JIA who develop uveitis are ANA positive.[42,43] Therefore, patients with JIA and a positive ANA are screened more frequently for chronic uveitis,[44] which is generally asymptomatic and detected only through slit-lamp examination by an ophthalmologist.

Indications for ordering an ANA are listed in **Box 3**.

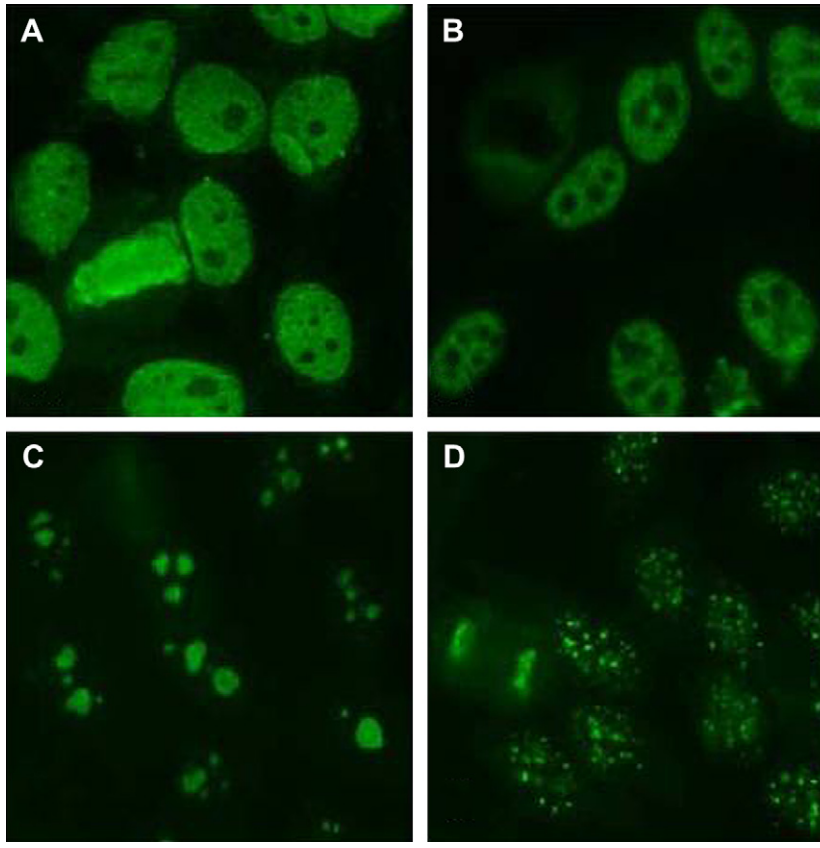

Fig. 1. ANA patterns on Immunofluorescence. (*A*) Homogenous; (*B*) Speckled; (*C*) Nucleolar; (*D*) Centromere. (*From* Damoiseaux JG, Tervaert JW. From ANA to ENA: how to proceed? Autoimmun Rev 2006;5:10–7; with permission.)

Specific Antinuclear Antibodies

Antibodies to specific nuclear antigens can be associated with specific diseases (**Table 2**). In addition, testing for anti-dsDNA antibodies has special value in the management of SLE, because increasing levels are predictive of flares of glomerulonephritis in patients with a history of renal disease.[45] For nonrenal manifestations of SLE, the relationship between persistently high or increasing anti-dsDNA levels and flare is less certain.[46–48]

Other than antihistone antibodies, tests for specific antinuclear antibodies are not usually positive in JIA. Therefore, the presence of specific antinuclear antibodies in a patient with chronic arthritis should suggest an alternative diagnosis than JIA.

Rheumatoid Factor

Rheumatoid factor (RF) is an antibody (typically IgM) directed against the F_c portion of IgG. The primary usefulness of RF measurement in the pediatric rheumatology clinic is differentiating between the 2 subtypes of polyarticular JIA: RF positive and RF negative. Unlike adult rheumatoid arthritis, most children with JIA do not have RF. Of patients with polyarthritis, approximately 85% are RF negative.[49,50] Testing for RF is generally not helpful in establishing or ruling out a diagnosis of JIA,[51] and it is indicated

Box 2
Conditions associated with a positive ANA

1. SLE
2. Drug-induced lupus
3. JIA
4. MCTD
5. Sjögren syndrome
6. Systemic sclerosis
7. RP
8. Juvenile dermatomyositis (JDM)
9. Malignancy
10. Autoimmune thyroiditis
11. Graves disease
12. Autoimmune hepatitis
13. Primary biliary cirrhosis
14. Autoimmune cholangitis
15. Chronic infections
16. Idiopathic pulmonary hypertension

only in patients who have objective signs of polyarthritis ($\geq$4 affected joints). In those patients with polyarthritis who are RF positive, the course tends to be more aggressive, with more long-term disability[52] and the lowest remission rates among juvenile arthritis subtypes.[53] Other rheumatologic conditions are associated with a positive RF (**Box 4**). The clinical significance of RF in these conditions is unknown.

Anticyclic Citrullinated Peptide Antibodies

In rheumatoid arthritis, inflamed synovium contains citrullinated peptides, and patients with RA produce antibodies that bind specifically to substrates that contain citrulline.[54] In adults, anticyclic citrullinated peptide (anti-CCP) antibodies are highly specific for RA and may be present before the onset of symptoms.[55] These antibodies are found primarily in children with polyarticular, and rarely other subsets of, JIA.[56–58] Anti-CCP antibodies have been associated with more aggressive disease,[58] even in RF-negative polyarticular patients, and may indicate the need for more aggressive therapy. The precise role of anti-CCP testing in JIA has not been established.

Antineutrophil Cytoplasmic Antibodies

Antineutrophil cytoplasmic antibodies (ANCA) were discovered to have an association with granulomatosis with polyangiitis (GPA; formerly known as Wegener granulomatosis)

Box 3
Indications for ordering an ANA

1. A patient with signs or symptoms suggestive of SLE (see **Box 1**)
2. Assessment of the risk of uveitis in a patient with JIA
3. Assessment of the risk of an underlying connective tissue disease in a patient with RP

Table 2
Specific antinuclear antibody disease associations

Antibody Specificity	Disease Association
SS-A (Ro)	Sjögren syndrome. SLE. NLE
SS-B (La)	Sjögren syndrome. SLE. NLE
Smith	SLE
Double-stranded DNA	SLE
Centromere	Limited cutaneous systemic sclerosis
Topoisomerase 1 (Scl-70)	Diffuse cutaneous systemic sclerosis
Pm-Scl	Myositis-scleroderma overlap
Ribonucleoprotein	MCTD
Histone	SLE. Drug-induced SLE

Abbreviation: NLE, neonatal lupus erythematosus.

in 1985.[59,60] Since then, it has been found to be closely associated with 2 other systemic vasculitides: microscopic polyangiitis (MPA) and Churg-Strauss syndrome. In these conditions, the target of ANCA-binding is usually either myeloperoxidase (MPO) or serine protease 3 (PR3), which are found in granules of neutrophils and macrophages. The binding of ANCA to its target likely plays a pathogenic role in the pathophysiology of ANCA-associated vasculitis (AAV).[61] This topic is discussed in more detail in the article on vasculitis by Weiss elsewhere in this issue.

Two methods are used to measure the ANCA: indirect IF and ELISA. Each method has clinical usefulness and both are usually ordered in patients in whom systemic vasculitis is suspected. IF is more sensitive, whereas ELISA is more specific. On IF, one of 2 staining patterns (**Fig. 2**) may be seen: cytoplasmic (c-ANCA) or perinuclear (p-ANCA). ELISA measures antibodies against MPO and PR3. c-ANCA staining on IF usually correlates with a positive ELISA for PR3, and this pattern is primarily seen in GPA. p-ANCA staining usually correlates with a positive MPO ELISA, and this pattern is primarily seen in MPA. However, the associations among IF staining, ELISA, and disease are not rigid and some crossover may be seen (**Table 3**). There are other conditions in which a positive ANCA by IF is found (**Box 5**). Other than drug exposure, these conditions are generally not associated with positive ELISA for anti-PR3 or anti-MPO antibodies.

Box 4
Rheumatologic conditions associated with a positive RF

1. Polyarticular JIA
2. Rheumatoid arthritis
3. SLE
4. Systemic sclerosis
5. MCTD
6. Sjögren syndrome
7. Sarcoidosis

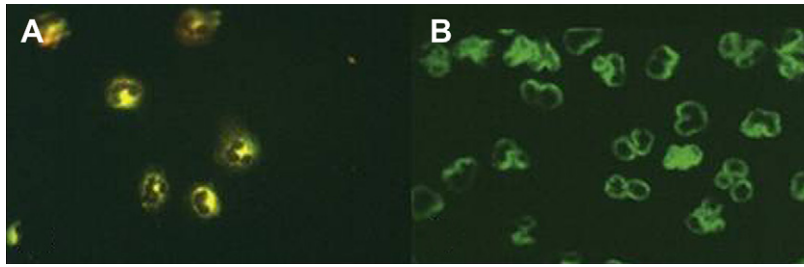

Fig. 2. ANCA patterns on immunofluorescence. (*A*) Cytoplasmic (c-ANCA); (*B*) Perinuclear (p-ANCA). (*From* Calabrese LH, Molloy ES, Duna G. Antineutrophil cytoplasmic antibody–associated vasculitis. In: Firestein GS, Budd RC, Harris ED, et al, editors. Kelley's Textbook of Rheumatology, 8th edition. Philadelphia: WB Saunders, 2008; with permission.)

Given the protean presentation of systemic vasculitis, the question of when to order ANCA testing arises. Situations in which systemic vasculitis should be suspected are listed in **Box 6**.

Antiphospholipid Antibodies

Antiphospholipid antibodies (APLs) are a group of antibodies that, through effects on platelets, endothelial cells, neutrophils, and monocytes, lead to an increased risk of arterial and venous thromboembolic events. These antibodies may be transiently found in healthy children, and, therefore, diagnosis of the APS requires at least 2 positive measurements (for the same positive antibody) 12 weeks apart along with clinical criteria (**Table 4**). Persistently positive APLs or the APS may be found in patients with no other findings or in patients with an underlying rheumatologic disorder (most commonly SLE).

There are 3 commonly used tests to assay for APLs: (1) lupus anticoagulant (LA) tests; (2) ELISA for anticardiolipin (ACL) IgG and IgM; (3) ELISA for anti-β_2-glycoprotein-I (anti-β_2GPI) IgG and IgM. LA is tested for through either the activated partial thromboplastin time (aPTT) or the dilute Russell viper venom time (DRVVT). In patients with APLs, the aPTT is paradoxically prolonged, despite the prothrombotic state that APLs confer. However, only half of patients with LA have a prolonged aPTT and the DRVVT is a more sensitive test for LA. The DRVVT test uses the ability of the venom of the Russell viper to activate factor X and set off the intrinsic clotting cascade. However, as with the aPTT test, APLs interfere with the ability of phospholipids to promote in vitro thrombosis and thus prolong the DRVVT. The prolongation of both

Table 3
ANCA by IF, ELISA, and disease association in AAV

Disease	IF (%)	ELISA (%)
Granulomatosis with polyangiitis (Wegener)[a]	66 c-ANCA 21 p-AWCA	68 PR3 14 MPO
Microscopic polyangiitis[b]	23 c-ANCA 58 p-ANCA	25 PR3 58 MPO

[a] *Data from* Cabral DA, Uribe AG, Benseler SM, et al. Classification, presentation and initial treatment of Wegener's granulomatosis in childhood. Arthritis Rheum 2009;60:3413–24.
[b] *Data from* Hagen EC, Daha MR, Hermans J, et al. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. Kidney Int 1998;53:743–53.

Box 5
Conditions associated with a positive ANCA

1. Inflammatory bowel disease (IBD)
2. Rheumatoid arthritis
3. SLE
4. Myositis
5. Cystic fibrosis
6. Sclerosing cholangitis
7. Autoimmune hepatitis
8. Infections
   a. Endocarditis
   b. Human immunodeficiency virus
9. Drugs
   a. Hydralazine
   b. Propylthiouracil
   c. *D*-penicillamine
   d. Minocycline

the aPTT and DRVVT is not reversed when patient's plasma is mixed with normal plasma. In children, a persistently positive LA test is only somewhat sensitive (72%)[62] for the APS, but confers a strong risk for thrombotic events,[63] especially in patients with underlying SLE.[64–66]

ELISA for ACL is the most sensitive test for APS in children,[62] and its specificity increases with increasing titer.[67] Many APLs are directed against β_2-glycoprotein-I and the anti-β_2GPI ELISA tests for these antibodies. Anti-β_2GPI ELISA is the least sensitive of the APLs in children[62] but is more specific than the ACL for thrombosis, especially in patients with SLE.[68] It is unknown whether other frequently tested APLs, such as ACL IgA and anti-β_2GPI IgA antibodies, and antibodies to prothrombin, phosphatidylserine, and phosphatidylethanolamine confer an increased thrombosis risk.[69]

Recent work by Wahezi and colleagues[70] has suggested that the annexin A5 resistance assay, a newly developed test of annexin A5 anticoagulant activity, is strongly associated with persistent APLs. In addition, decreased annexin A5 resistance was seen in children with rheumatic diseases who had thrombotic events. At our institution, we obtain annexin A5 resistance testing on all lupus patients and on those with suspected APS.

Box 6
When to consider ANCA testing

1. Fever of unclear cause >2 weeks
2. Multiple organ disease (especially lung, kidney, or neurologic disease)
3. Petechial or purpuric rash
4. Unexplained signs of systemic inflammation (increased ESR of CRP, anemia of chronic disease, thrombocytosis

Table 4
Classification criteria for APS

Clinical Criteria		
1	Vascular thrombosis	≥1 clinical episode of arterial, venous, or small vessel thrombosis in any tissue or organ
2	Pregnancy morbidity	a. ≥1 unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation OR b. ≥1 premature births of a morphologically normal neonate before the 34th week of gestation because of: (1) eclampsia or severe preeclampsia or (2) placental insufficiency OR c. ≥3 unexplained consecutive spontaneous abortions before the 10th week of gestation
Laboratory Criteria (require 2 or more positive tests at least 12 weeks apart):		
1	Lupus anticoagulant present in plasma	
2	Anticardiolipin antibody of IgG or IgM isotype in serum or plasma, present in medium or high titer (ie, >40 GPL or MPL, or >99th percentile)	
3	Anti-β2 glycoprotein-I antibody of IgG or IgM isotype in serum or plasma (in titer >99th percentile)	

APS criteria met if ≥1 clinical criteria and ≥1 laboratory criteria.
Abbreviations: GPL, IgG phospholipid standardized units; MPL, IgM phospholipid standardized units.
Adapted from Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome. J Thromb Haemost 2006;4:295–306; with permission.

APS should be suspected and APLs testing obtained in patients with a history of any of the clinical criteria in **Table 4** or with the suggestive findings described in **Box 7**.

LABORATORY TESTS FREQUENTLY USED IN RHEUMATOLOGY

Muscle Enzymes

Serum levels of muscle enzymes are most commonly used in pediatric rheumatology for the diagnosis and management of JDM. Individual patients may have increases in any of the commonly tested enzymes throughout their disease course, and, therefore, serial measurement of creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), LDH, and aldolase is recommended. However, normal values for these enzymes do not rule out a diagnosis of JDM; up to 26% of children with JDM, in a retrospective study, had a normal value for at least 1 enzyme at presentation.[71] In a retrospective study in adults with dermatomyositis or polymyositis, increases in CK heralded a flare of disease up to a month before relapse and normalization of CK was present weeks before muscle strength improvement.[72] However, in children with JDM, it was reported that LDH and AST predicted flares, and neither CK nor ALT was associated with disease flare.[73] It has been suggested that LDH is the most useful muscle enzyme because it best predicts global disease activity in patients with long-standing disease.[74]

Each of these enzymes has sources other than muscle, and, therefore, other conditions may lead to their increase (**Table 5**).

Box 7
Nonthrombotic clinical findings suggestive of APS

1. Dermatologic
   a. Livedo reticularis
   b. RP
   c. Digital necrosis
   d. Splinter hemorrhages
   e. Skin ulcers
2. Hematologic
   a. Thrombocytopenia
   b. Autoimmune hemolytic anemia
3. Neurologic
   a. Migraine headaches
   b. Seizures
   c. Chorea
4. Cardiac
   a. Sterile endocarditis
   b. Cardiomyopathy

Serum Complement Levels

Complement C3 and C4 levels are frequently measured as an adjunct to the clinical diagnosis and monitoring of patients with SLE. The rationale for this situation is activation of the classic and alternative pathways with antibody-antigen binding in SLE. Multiple studies have shown that decreased C3 or C4 levels correlate with increased renal and extrarenal activity or disease flare.[75–79] In addition, normalization of complement levels has been associated with disease improvement.[75] However, flares are not always accompanied by decreases in C3 and C4,[80] and some patients have persistently low complement levels despite disease inactivity.[48,81] Therefore, complement levels need to be considered in the context of clinical findings and other markers of disease activity when making decisions about therapy.

Inherited and acquired deficiencies of certain complement components (C1, C2, C3, C4, mannose-binding lectin, and C1-inhibitor) are associated with SLE or SLE-like diseases.[82] The CH_{50} is a reliable screen for homozygous deficiencies in the classic pathway components, which have the strongest association with lupus and

Table 5
Nonmuscle sources of muscle enzymes

Enzyme	Sources
CK	Heart, brain
ALT	Liver
AST	Liver, heart, kidney, brain, erythrocytes
LDH	Liver, erythrocytes, heart, kidney, brain, lung
Aldolase	Liver, heart, erythrocytes, brain, kidney

lupuslike illnesses. This test quantifies complement activity by assaying the ability of patient's serum to lyse sheep erythrocytes coated with rabbit antisheep IgM. A homozygous deficiency in any of the classic pathway components gives an undetectable CH_{50} (except homozygous C9 deficiency, which may give a low CH_{50}). Indications for screening for complement deficiencies are listed in **Box 8**.

Urinalysis

The kidney can be affected as a primary manifestation of SLE (and related disorders) and the AAV, as well as a complication of amyloidosis in sJIA and the periodic fever syndromes. The American College of Rheumatology criteria for renal involvement in SLE require either persistent proteinuria of greater than 0.5 g/d (or 3+ albumin on dipstick) or cellular casts of any type.[83] For proteinuria measurements, urine spot protein/creatinine ratios have been increasingly accepted as a more convenient method for determining protein excretion. The rule of thumb that the ratio of spot urine protein to creatinine approximates the grams of urine protein excretion per day has been shown to hold true in lupus nephritis[84] and other glomerular diseases.[85] Thus, a ratio of greater than 0.2 is of concern and may warrant further monitoring and testing. Conversely, the urine dipstick for albumin lacks precision and correlation with the 24-hour total protein measurement and should not be used for diagnosis or monitoring of glomerular disease.[86]

Detection of cellular casts, which may be seen in SLE and AAV, requires prompt examination of urine as casts deteriorate over time. Community-based clinical laboratories often are not able to reliably detect pathologic casts,[87] and, therefore, urine from those patients for whom the clinician has a high suspicion of renal disease should be examined microscopically by an experienced hospital-based clinical pathology laboratory or by a nephrologist. Whereas proteinuria may be seen with any of the classes of lupus nephritis, the presence of RBC casts reflects a nephritic syndrome that is typically seen only with diffuse proliferative (class IV), advanced sclerotic (class VI), or a thrombotic microangiopathy.[88]

Angiotensin-converting Enzyme and Lysozyme

The angiotensin-converting enzyme (ACE) level is frequently increased in sarcoidosis, and the level of increase corresponds to clinical disease activity.[89] The source of increased ACE in sarcoidosis is alveolar macrophages[90] and granulomatous epithelioid cells.[91] A large, multicenter study of patients with sarcoidosis revealed the sensitivity of ACE for sarcoidosis to be 57%, specificity of 90%, positive predictive value of 90%, and negative predictive value of 60%.[92] Increases in the ACE level in an individual patient may reflect progressive, rather than stable, disease.[93] ACE gene polymorphisms contribute to interindividual variability in ACE levels between both

Box 8
Indications for suspecting a complement deficiency

1. Recurrent, unexplained pyogenic or *Neisseria* infections
2. Patients with SLE with a family history of SLE
3. Subacute cutaneous lupus
4. Very young (<7 years old) patient with SLE or SLE-like disease
5. Recurrent angioedema

patients with sarcoidosis[82] and normal controls,[54] and it has been suggested that ACE genotyping be used in the interpretation of ACE levels.[66]

Stimulated mononuclear phagocytes in sarcoidosis secrete lysozyme, and increased lysozyme levels are seen in patients with sarcoidosis.[94] The serum lysozyme level, like the ACE, can be followed as an indicator of disease activity.[95] Lysozyme is more sensitive than ACE for sarcoidosis (79% vs 59% in 1 study[96]). However, the specificity is only 60% and abnormal values can be seen in many other diseases, including tuberculosis. In patients for whom sarcoidosis is suspected, assaying both ACE and lysozyme is recommended.

Streptococcal Antibody Tests

Streptococcal antibody tests provide evidence for previous infection with group A streptococcus (GAS) and are used in pediatric rheumatology to aid in the diagnosis of acute rheumatic fever (ARF) and poststreptococcal reactive arthritis (PSRA). The most commonly used ones are the antistreptolysin O (ASO), anti-DNase B (ADB), and streptozyme. The streptozyme provides no useful information beyond that provided by the ASO and ADB and, furthermore, lacks reproducibility. It should, therefore, not be used as a test of GAS exposure.

Antibodies to GAS peak approximately 3 weeks after an acute infection.[70] Both ASO and ADB should be obtained in patients for whom either of these diseases are suspected because almost 20% of patients with ARF, in 1 study, had a negative result for at least 1 of the tests[29] but 92% were positive for at least 1 of these 2 tests. In contrast, a study of 25 patients with PSRA showed an increase in at least 1 antibody for all patients.[30] After treatment of ARF, ASO levels may begin to normalize after 2 months but can stay increased for 6 to 12 months[97] (longer if reinfection occurs). There is no correlation between level of ASO and clinical manifestations of ARF.[98] The height of ASO increase does not help in differentiating between ARF (mean 1011 IU) and PSRA (mean 889 IU).[99]

GENETIC TESTS USED IN RHEUMATOLOGY

HLA-B27

There is a well-established relationship between the HLA class I allele HLA-B27 and ankylosing spondylitis (AS) in both children (in whom it is known as ERA)[100] and adults.[101] ERA is one of a group of diseases known as the juvenile spondyloarthropathies (JSpA), which have as their defining characteristic inflammation of the axial skeleton and entheses (the sites of tendon, ligament, fascia, and capsule attachment to bone). JSpA is an umbrella term encompassing ERA, juvenile AS, reactive arthritis (ReA), arthritis associated with IBD, and juvenile psoriatic arthritis (PsA). Detailing the nuances in classification of JSpA is beyond the scope of this review, but each of these diseases is associated with HLA-B27. Testing for HLA-B27 is most useful in a male older than 8 years who presents with any of the symptoms listed in **Box 9**. The presence of a first-degree relative with similar symptoms increases the usefulness of testing.

Although the diagnosis of JSpA is made on clinical grounds, testing for HLA-B27 helps for classification purposes, because patients with JAS are more likely to be positive than those with other types of JSpA and the presence of HLA-B27 in patients with undefined JSpA predicts evolution to an identifiable disease. However, 5% to 10% of the normal population is positive for HLA-B27,[102,103] and only about 1% of all HLA-B27–positive individuals in the general population go on to develop AS.[103]

Box 9
Indications for testing HLA-B27

1. Arthritis in a male >8 years old
2. ReA
3. Inflammatory back pain[a]
4. Sacroiliac joint tenderness
5. Enthesitis
6. Arthritis in a patient with a family history of AS, ERA, PsA, ReA, acute uveitis, or IBD
7. Acute uveitis

[a] Inflammatory back pain is pain that is present in the morning (or the second half of the night), improves with exercise, has an insidious onset, and does not improve with rest.

Genetic Testing for Hereditary Periodic Fever Syndromes

Periodic fever syndromes (PFS) are defined as 3 or more episodes of fever, with no other defined medical illness to explain the fevers, in a 6-month period, with at least 7 days between episodes.[104] The hereditary PFS, which can be distinguished by their clinical characteristics and gene abnormalities, are listed in **Table 6**. Further details on the clinical presentation, genetics, and ethnic predilection for each of these PFS are found in the article on autoinflammatory diseases by Hashkes and Toker elsewhere in this issue. Commercial testing (GeneDx; Gaithersburg, MD) for mutations underlying familial Mediterranean fever (FMF); tumor necrosis factor receptor-associated periodic syndrome (TRAPS); hyper-IgD syndrome (HIDS); Muckle-Wells syndrome; familial cold autoinflammatory syndrome; neonatal-onset multisystem inflammatory disorder; cyclic hematopoiesis; pyogenic arthritis, pyoderma gangrenosum, and acne syndrome; and Majeed syndrome is available as a single panel. The sensitivity

Table 6
Gene abnormalities and clinical features of hereditary PFS

Syndrome	Gene	Distinctive Clinical Features
FMF	*MEFV*	Arthritis, peritonitis, erysipeloidlike rash
TRAPS	*TNFRS1A*	Macular migratory erythema, myalgia
HIDS	*MVK*	Erythematous macules, oral and vaginal ulcers
FCAS	*CIAS1*	Cold-induced symptoms, urticarial rash
MWS	*CIAS1*	Urticarial rash, sensorineural hearing loss
NOMID	*CIAS1*	Urticarial rash, meningitis, bone abnormalities
CH	*ELA2*	ANC <200/μL, recurrent oral infections
PAPA	*PSTPIP1/CD2BP1*	Recurrent arthritis, pyoderma gangrenosum, acne

Abbreviations: CH, cyclic hematopoiesis; FCAS, familial cold autoinflammatory syndrome; FMF, familial Mediterranean fever; HIDS, hyper-IgD syndrome; MWS, Muckle-Wells syndrome; NOMID, neonatal-onset multisystem inflammatory disorder; PAPA, pyogenic arthritis, pyoderma gangrenosum, and acne syndrome; TRAPS, tumor necrosis factor receptor-associated periodic syndrome.

and specificity of the individual tests varies widely, because only the most common mutations are tested and some mutations may be polymorphisms in certain ethnicities. Given this situation, only patients whose clinical symptoms, ethnicity, or family history lead to a strong clinical suspicion of a hereditary PFS should be tested. Gottorno and colleagues[105] used a set of clinical criteria including age, abdominal pain, aphthosis, thoracic pain, diarrhea, and family history to develop a decision tree for which patients should receive genetic testing for FMF, HIDS, and TRAPS. This algorithm had good sensitivity (82%) and moderate specificity (72%).

SUMMARY

Laboratory tests can suggest or confirm diagnoses, offer prognosis, and guide therapy in children with rheumatic diseases. They may also be a source of unnecessary consternation and cost for patients, families, and health care providers, especially given the varied sensitivity and specificity of many of these tests. Therefore, primary care and rheumatology providers are encouraged to let the patient's signs and symptoms guide the laboratory workup. The otherwise well child with occasional musculoskeletal complaints likely does not need an extensive laboratory evaluation; conversely, the child who presents with persistent complaints or signs of systemic illness is well served by a focused and sequential workup. When used in this way, these tests play an invaluable role in the care of our patients.

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