Laboratory Evaluation in Pediatric Autoimmune Diseases

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Educational Gaps

1. Pediatricians should recognize the complex assessment of pediatric autoimmune disease but feel comfortable with the initial evaluation of these diseases in children.
2. Clinicians should have an increased awareness of antibody testing in pediatric autoimmune disease and an understanding of the implications of antibody positivity.

Objectives

After completing the article, the reader should be able to:

1. Understand the diagnostic evaluation of pediatric autoimmune diseases.
2. Order initial laboratory evaluations for children with suspected autoimmune disease.
3. Explain what positive antinuclear antibody testing can indicate and what steps should be taken for further investigation when results are positive.

INTRODUCTION

The evaluation of pediatric autoimmune disease can be intimidating for the general pediatrician, especially when faced with the laboratory examination that is generally requested after consultation with a pediatric rheumatologist. In this article, we explain the reasons behind such comprehensive evaluations in an attempt to deepen understanding of why they are important.

Among the multiple autoimmune diseases (AIDs) in pediatric rheumatology are juvenile idiopathic arthritis (JIA), systemic lupus erythematosus (SLE), childhood scleroderma or pediatric systemic sclerosis (PSS), juvenile dermatomyositis (JDM), Sjögren syndrome (SS), antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides, and seronegative spondyloarthropathy.

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GENERAL EVALUATION

The general evaluation of AID in the pediatric population begins with a complete blood cell count, urinalysis, comprehensive metabolic panel as well as measurement of the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and muscle enzymes creatine phosphokinase (CPK), aldolase, and lactate dehydrogenase (LDH).

Complete Blood Cell Count

In children with AID, low hemoglobin and hematocrit values may be a sign of: 1) chronic anemia (anemia of chronic inflammatory disease) in the case of JIA, SLE, or any of the previously mentioned diseases; 2) acute anemia, which is most likely to occur in systemic-onset JIA; or 3) hemolytic anemia, especially in the case of SLE where Coombs test results would be expected to be positive for immunoglobulin (Ig)G and C3. The acute inflammatory cytokines, tumor necrosis factor-alpha and interleukin-1 (IL-1), cause delays in hemoglobin formation in the bone marrow, which may explain both the acute and chronic anemias found in JIA, SLE, and other AIDs. (1)(2)

When leukocytosis occurs, it may be due to the inflammatory response in JIA, corticosteroid therapy, or infection. Leukopenia may be a result of anti-white blood cell antibodies seen in patients with SLE and SS, especially those who have antibodies to SS-A and SS-B. Thrombocytosis may be seen in systemic-onset JIA due to acute inflammation and as an acute-phase reactant. Furthermore, thrombocytopenia may be seen in patients with SLE and SS who have antiplatelet antibodies. (1)(2)

Urine Testing

Urinalysis is an easy and effective method of screening for renal involvement in AID. Proteinuria can be an indicator of glomerular damage, likely associated with SLE or other AIDs. White and red blood cells on microscopy as well as red cell casts are also indicators of renal pathology seen in SLE. Renal tubular acidosis can be an initial sign of SS in childhood-onset disease. (3) Further evaluation based on urinalysis abnormalities consists of a 24-hour urine collection for creatinine clearance and total protein as well as calculation of the protein-to-creatinine ratio. (Spot urine collection for protein-to-creatinine ratios has also been shown to be useful and is much easier for patients.) Renal biopsies stained for hematoxylin and eosin (H&E) and immunofluorescence may be indicated, especially in evaluation of SLE and ANCA-associated vasculitides, to determine the underlying pathology and provide a guide for further medical therapy.

Inflammatory Markers

The normal ESR (by modified Westergren) in children should be less than 5 mm/hr. The normal CRP should be less than 0.3 mg/dL (28.6 nmol/L) (reported by some laboratories as 3 mg/L [28.6 nmol/L]). If the value is higher, some inflammatory component is present. Of course, neither test is specific to any disease process, but both are useful in initial evaluation and follow-up testing to monitor response to therapy, disease activity, or disease progression. Many laboratory reference ranges are too high for children. (4)

Comprehensive Metabolic Panel

As a general screen, increased creatinine (> 1.5 mg/dL [132.6 μmol/L]) may indicate renal damage due to SLE or medication toxicity. However, readings higher than 0.8 mg/dL (70.7 μmol/L) in children should be monitored, especially if they rise. Increased aspartate aminotransferase and alanine aminotransferase may be due to hepatitis, which could be a primary disease, drug-related, or an adverse effect of therapy. These values may also be elevated due to muscle disease. The globulin concentration must also be monitored because elevation and reversal of the albumin/globulin ratio may be a sign of early connective tissue disease (CTD). If the albumin/globulin ratio approaches 1 (should be approximately 1.5), an inflammatory process may be present.

Muscle Enzymes

Increased CPK, aldolase, and LDH values are indicators of myositis. All three must be evaluated in children because only one or two may be elevated, even with severe disease. Elevated values may be due to polymyositis, dermatomyositis, or an underlying CTD. LDH is a cytoplasmic enzyme that is nonspecific and can be seen in myositis, hepatitis, and red blood cell destruction. Further evaluation that includes neopterin and von Willebrand factor antigen may also be necessary. These are macrophage functions, but changes may indicate possible underlying myositis, and both may reflect immune system activation.

DEFINITIVE EVALUATION

The definitive evaluation of AID includes rheumatoid factor (RF), anticyclic citrullinated peptide antibodies (anti-CCP), and antinuclear antibodies (ANAs). If the ANA is positive, an ANA profile should be obtained. The ANA profile usually includes anti-Sm, anti-double-stranded DNA, anti-Scl-70, anti-RNP, anti-chromatin, anti-SS-A, and anti-SS-B antibodies. Furthermore, complement levels (C3, C4, and CH50), antiphospholipid antibodies (anticardiolipin antibodies, anti-β2 glycoprotein antibodies, and lupus anticoagulant)

as well as ANCA and genetic studies are key components of the comprehensive evaluation.

Rheumatoid Factor

RF is a nonspecific antibody response to an exogenous antigen, meaning that it is a nonspecific immune response to various antigens from outside the body (ie, microbes). The classic molecule described is the 19S IgM molecule, but RF is found in all the immunoglobulin classes (IgG, IgA, IgD, and IgE). Evaluation of RF may not be that helpful in many cases of JIA. RF assessed by enzyme-linked immunosorbent assay (ELISA) is positive in only 10% to 30% of those with JIA (mostly polyarticular disease), 80% to 90% of those with rheumatoid arthritis (RA), 20% to 30% of those with SLE, 25% to 35% of those with pediatric systemic sclerosis, 30% to 40% of those with mixed CTD (MCTD), 20% to 30% of those with polymyositis/dermatomyositis, and 70% to 80% of those with SS. RF can also be positive in viral infections such as those caused by Epstein-Barr virus (EBV), cytomegalovirus, parvovirus B-19, and hepatitis B and C as well as subacute bacterial endocarditis. RF can be identified in serum and in synovial, pericardial, and pleural fluid. Its presence in JIA and RA indicate more severe disease, while its presence in other CTDs simply indicates a diffuse immune response. (5)

Anticyclic Citrullinated Peptide Antibodies

Anti-CCP antibodies react with synthetic peptides containing the amino acid citrulline, the posttranslationally modified arginine residue. They are found in approximately 75% of adult patients with RA with high specificity. Present commercial testing is only for IgG antibodies. Anti-CCP antibodies are also found in a percentage of patients with JIA, in all onset types but particularly in the polyarticular subtype. Anti-CCP antibodies are associated with early erosive disease and more aggressive disease in JIA. They may be the first response of the innate immune system to an exogenous antigen. They are found in all immunoglobulin classes, with the presence of IgA, IgM, and IgG antibodies at disease onset being associated with more severe and aggressive disease in JIA. (5)

Antinuclear Antibodies

ANAs are a diverse collection of antibodies directed against numerous macromolecules that are normal constituents of the cell nucleus. The presence of ANA is a hallmark of CTD but is *not* diagnostic. Low-titer ANA (≤ 1:320) can be found in approximately 15% to 20% of the healthy childhood population. Viral infections (eg, EBV, cytomegalovirus, and parvovirus) can cause a transiently positive ANA. Autoimmune thyroiditis is also a frequent cause of a positive ANA. In the case of a positive ANA but a negative ANA profile, thyroid antibodies (anti-thyroglobulin and anti-thyroperoxidase) should be assessed. (6)

The significance of ANA is that its assessment can screen patients for CTD. Specific antibodies can define a specific CTD, give evidence of possible organ system involvement, or predict possible disease course. However, rising or falling titers are not associated with disease activity or response to therapy except in specific cases (anti-dsDNA and anti-RNP antibodies).

The most widely used technique for the detection of ANA is indirect immunofluorescence using human epithelial cells (Hep-2) as substrate. The standard test may enable early detection of antibodies that are present in abundance (ds & ss DNA, histones, Sm, RNP, Scl-70, SS-B). However, those present in lower concentrations (SS-A) or cytoplasmic antigens (ribosomal P, antisynthetase) may require specific ELISAs or special substrates. Of note, some laboratories offer an "ANA screen," which is a direct method but provides only qualitative results.

If the ANA is positive by Hep-2 cell substrate or there is high suspicion for AID, the ANA profile should be ordered, which yields more definitive information. Most ANA profiles contain antibodies to dsDNA, SSA, SSB, Sm, RNP, and chromatin. Not all profiles include antibodies to Scl-70, histone, Jo-1, RNA polymerase III, centromere, and other less frequent antibodies.

In addition to a titer, the pattern of the ANA seen on immunofluorescence (under microscopy) is also reported. Table 1 shows the pattern associations with antibodies. Table 2 reviews the association of specific antibodies with various AIDs.

Autoantibodies in SLE

More than 99% of patients with SLE have a positive ANA, and 75% of patients are anti-dsDNA antibody-positive at some time in the course of their disease. This autoantibody correlates with renal disease, central nervous system disease, vasculitis, and low complement levels and is an indicator of active disease. It correlates with the homogenous and rim patterns of ANA. It is evaluated by ELISA or Crithidia luciliae immunofluorescence. The Crithidia method is specific for the dsDNA antibody. High titers are very specific for SLE and can correlate with disease activity. The Farr and ELISA methods are more sensitive but can give positive results for ssDNA. The ELISA can produce false-positive results for cross-reactive antibodies, such as hepatitis B and C, and certain drug-induced syndromes.

TABLE 1. **Antinuclear Antibody (ANA) Pattern-Antibody Associations**

ANA PATTERN	ASSOCIATED ANTIBODIES
Homogenous	dsDNA, chromatin, histone
Speckled	Sm, U1RNP, SSA, SSB, Scl-70, RNA polymerase III
Discrete Speckled	Centromere
Rim	dsDNA, laminin
Cytoplasmic	ribosomal P
Nucleolar	Scl-70, RNA polymerase III, PM-Scl

Seventy-five percent of patients with SLE are also anti-chromatin (anti-nucleosome) antibody-positive. Performed by ELISA, this antibody correlates with the diagnosis of nephritis along with dsDNA. This can also be positive in drug-induced syndromes and correlates with the homogenous pattern of ANA.

Anti-histone antibodies are performed by ELISA, and 95% of patients with a drug-induced syndrome have a positive anti-histone antibody. Common causative drugs are blood pressure medications, oral contraceptives with high estrogen content, and propylthiouracil. Anti-histone antibodies correlate with the homogenous pattern of ANA.

Approximately 30% to 40% of patients have a positive anti-Sm antibody. This autoantibody tends to correlate with pleural and pericardial disease. It is associated with a speckled ANA pattern.

Anti-U1RNP is positive in 30% to 40% of patients with SLE. This correlates with more mild disease, Raynaud phenomenon, and restrictive lung disease. It is associated with a coarse speckled ANA pattern.

Anti-SSA (Ro) antibody occurs in 30% to 60% of patients with SLE. It correlates with subacute cutaneous lupus, neonatal lupus, sicca (dry eyes or mouth) complaints, and C2 and C4 deficiencies. It is associated with a fine speckled ANA pattern. (This antibody can be negative with some ANA substrates.)

Anti-SSB (La) antibody occurs in 20% to 30% of lupus patients and correlates with neonatal lupus. Like SSA, it is associated with a fine speckled ANA pattern.

Anti-ribosomal P is positive in only 10% to 20% of patients with SLE. This autoantibody correlates with central nervous system vasculitis (cerebritis) with psychosis. It occurs with a dense cytoplasmic ANA pattern. (7)

TABLE 2. **Specificity of Antinuclear Antibodies**

DISEASE	SPECIFIC ANTIBODIES
Systemic Lupus Erythematosus	anti-dsDNA, Sm, RNP, SSA, SSB
Mixed Connective Tissue Disease	anti-RNP
Scleroderma	anti-centromere, topoisomerase I (Scl-70), RNA polymerase III
Polymyositis	anti-Jo-1, PM-Scl, PL-7, PL-12, Mi-2
Sjögren Syndrome	anti-SSA, SSB

Autoantibodies in Mixed Connective Tissue Disease

More than 99% of patients with MCTD have a positive ANA. Anti-U1RNP is also positive in more than 99% of these patients. This correlates with Raynaud phenomenon, myositis, and restrictive lung disease and is associated with a coarse speckled ANA pattern. (7)

Autoantibodies in Sjögren Syndrome

More than 90% of patients with SS are ANA-positive. Anti-SSA antibody is positive in 90% of patients with primary SS. It correlates with vasculitis, leukopenia, thrombocytopenia, positive RF, and increased IgG values. Anti-SSB antibody is positive in 75% of patients with primary SS. Anti-SSA/B antibody positivity is associated with increased risk of lymphoma, and immunoglobulin levels should be evaluated annually. (7)

Autoantibodies in Juvenile Dermatomyositis or Polymyositis

Antisynthetase autoantibodies are most frequently detected in polymyositis or juvenile dermatomyositis (JDM). Anti-Jo-1 is the most frequently found antibody in JDM. The synthetases are a distinct group of enzymes that catalyze the binding of specific amino acids to their cognate transfer RNA. Among patients who experience myositis and interstitial pneumonitis, up to 95% have positive anti-PL-12, KS, or OJ antibodies. In patients who experience arthritis, fever, Raynaud phenomenon, mechanic's hands, Gottron papules/rash, and heliotrope rash, the anti-PL-7 and especially the anti-Jo-1 antibodies are most likely positive. (8)

Anti-TIF1 antibody (anti-p155/140) is positive in up to 40% of children with JDM, generally those with more cutaneous involvement. The p155 target is a transcriptional intermediary factor, a nuclear protein involved in cellular differentiation. This antibody is associated with an increased risk of malignancy. (8)(9)

Anti-NXP2 antibody, anti-p140 (also known as anti-MJ), has been associated with JDM in patients who experience

calcinosis. The autoantigen is the nuclear matrix protein (NXP-2). It is positive in up to 40% of children with JDM.

Anti-CADM-140 antibody is seen in patients with skin lesions and mild muscle involvement. Interstitial pneumonia can be seen. This antibody primarily occurs in the Japanese population.

Complement

There are three complement pathways. The classical pathway can be induced by immune complexes, apoptotic cells, and CRP bound to ligand. The mannose-binding lectin pathway is induced by microbes with terminal mannose groups. The alternative pathway may be induced by many types of bacteria, fungi, viruses, and malignant cells. C3 is the link between the pathways and leads to activation of the membrane attack complex.

CH50 (total complement) measurement is a screen to rule out complement deficiencies. It measures the ability of a serum specimen to lyse 50% of a standard suspension of sheep red blood cells coated with rabbit anti-sheep red blood cell antibody. Baseline levels of C3 and C4 should be obtained at diagnosis. However, C4 is a more sensitive method to monitor episodes of complement activation of the classical pathway (ie, disease activity). Low concentrations of C3 are associated with more severe renal disease. (10)

Complement Deficiency and Systemic Lupus Erythematosus

Complement deficiencies lead to persistent immune complex formation, circulation, and deposition. A complete C4 deficiency is rare. A complete C4A deficiency occurs in 10% to 15% of those who have SLE, and complete C4B deficiency occurs in fewer than 5%. Heterozygosity for C4A occurs in 50%.

In a cohort of children who had SLE, levels of anti-C1q antibodies were higher in patients with active renal disease and showed significant correlation with proteinuria and urinary casts. A combination of antibodies to histone, chromatin, C1q, and dsDNA indicated more severe disease. (11)

Antiphospholipid Syndrome

The antiphospholipid antibodies consist of anticardiolipin antibodies, lupus anticoagulant, and anti-β-2 glycoprotein antibodies. The IgG antibody form is associated with more clotting abnormalities, migraine headaches, and miscarriages in the family. These antibodies may be present or disease-causing in several AIDs but are seen primarily in SLE. (12)

Antineutrophil Cytoplasmic Antibodies

Cytoplasmic ANCA correlates with antibodies to proteinase 3 (PR3), which are seen in 90% of patients with granulomatosis with polyangiitis, formerly known as Wegener granulomatosis. It is also seen in microscopic polyangiitis.

Perinuclear ANCA correlates with antibodies to myeloperoxidase, which are seen in patients with eosinophilic granulomatosis with polyangiitis (formerly known as Churg-Strauss), microscopic polyangiitis, inflammatory bowel disease, and drug-induced syndromes (minocycline, propylthiouracil). (13)

Human Leukocyte Antigens in Seronegative Spondyloarthropathy

Human leukocyte antigens are genetic markers found on chromosome 6 that are involved in regulation of the immune system in humans. Some disease-specific genetic markers may be useful in diagnosis of the diseases seen in Table 3. (14)

ORGAN EVALUATION

Certain organ testing methods may be key to proper identification of AID. Occasionally a reliable diagnosis hinges upon tissue biopsy, especially in cases of an unclear clinical picture or diseases with overlapping symptoms. These studies include: 1) skin biopsy with H&E stain as well as immunofluorescence, 2) 24-hour urine for creatinine clearance and total protein, 3) renal biopsy with H&E and immunofluorescence staining, 4) pulmonary function tests with diffusion capacity of the lung for carbon monoxide, 5) 6-minute walk time, 6) high-resolution chest computed tomography scan, 7) barium swallow with upper gastrointestinal tract evaluation, 8) esophageal manometry, and

TABLE 3. **Human Leukocyte Antigens (HLAs) Associated With Autoimmune Disease**

DISEASE	ASSOCIATION
Ankylosing spondylitis	> 90% (HLA-B27+)
Reactive arthritis	50%+ (HLA-B27+)
Inflammatory bowel disease	50%+ (HLA-B27+)
Psoriatic arthritis with spondylitis	50%+ (HLA-B27+)
Behçet	60% (HLA-B51+)

9) echocardiography with two-dimensional and M-mode to include measurement of right ventricular pressure.

CONCLUSION

The clinician who suspects AID in a patient should begin with a general evaluation (complete blood cell count, urinalysis, comprehensive metabolic panel, ESR, CRP). This is a good screen for organ involvement and inflammation. If indicated by muscle symptoms, the muscle enzymes (LDH, CPK, aldolase) should also be assessed. Completing the ANA and RF assessment may be helpful before referral to pediatric rheumatology. Depending on the symptoms, EBV and parvovirus titers can be very useful in explaining possible viral causes, especially in the setting of a positive ANA test result.

We hope that outlining the process of evaluation in pediatric rheumatology has demonstrated to general practitioners why such evaluations are necessary. Many of the diseases have overlapping signs and symptoms, prompting the need for laboratory testing in conjunction with history and physical examination to reach the correct diagnosis and determine appropriate treatment.

Summary

- Pediatric autoimmune diseases are chronic lifelong disorders associated with potential disability and increased morbidity and mortality if not properly recognized and treated. On the basis of largely expert opinion in addition to observational studies, children with suspected autoimmune disease should undergo general laboratory and autoantibody screening. (1)(2)(4)(6)(7)(11)
- There can be many causes of positive antinuclear antibody test results, including, but not limited to, autoimmune disease. On the basis of expert opinion and observational studies, a thorough history and physical examination as well as laboratory evaluation is recommended (often in consultation with a pediatric rheumatologist) to elucidate the cause for a positive test result. (4)(6)(11)

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1. When might a renal biopsy be helpful in the evaluation of a patient with an autoimmune disease to determine the underlying pathology and provide guidance for further medical therapy?
    A. If the erythrocyte sedimentation rate is elevated and proteinuria is present.
    B. In the evaluation of systemic lupus erythematosus and antineutrophil cytoplasmic antibody-associated vasculitides.
    C. When the albumin/globulin ratio is elevated in a patient with connective tissue disease.
    D. When a metabolic acidosis is found on a comprehensive metabolic panel.
    E. When there is thrombocytosis associated with juvenile idiopathic arthritis.
2. What is the most appropriate initial definitive evaluation of a patient suspected of having an autoimmune disease based on screening tests?
    A. Aldolase, erythrocyte sedimentation rate, and von Willebrand factor antigen.
    B. Antiphospholipid antibodies, C3, and C4.
    C. Anti-Sm antibodies, anti-dsDNA antibodies, and CH50.
    D. Chromosome analysis and antineutrophil cytoplasmic antibodies.
    E. Rheumatoid factor, anti-cyclic citrullinated peptide antibodies, and antinuclear antibodies.
3. In which patient population is rheumatoid factor most often positive?
    A. Juvenile idiopathic arthritis.
    B. Mixed connective tissue disease.
    C. Pediatric systemic sclerosis.
    D. Sjögren syndrome.
    E. Systemic lupus erythematosus.
4. What is the most widely used technique for detection of antinuclear antibodies (ANA)?
    A. ANA profile.
    B. ANA screen.
    C. Anti SS-A enzyme-linked immunosorbent assay.
    D. Direct immunofluorescence with human liver cell substrate.
    E. Indirect immunofluorescence with human epithelial cell substrate.
5. Measuring C4 in autoimmune diseases is a sensitive method for which of the following?
    A. To monitor for activation of the classical complement pathway.
    B. To monitor for immune complex formation.
    C. To monitor for progression of myositis.
    D. To monitor for progression of systemic lupus erythematosus.
    E. To monitor for renal disease progression.

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