

Laboratory Tests in the Diagnosis and Follow-Up of Pediatric Rheumatic Diseases: An Update

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Objectives: We reviewed the literature to evaluate the role of common laboratory tests and to examine the recent progress in the laboratory diagnosis of pediatric rheumatic diseases.

Methods: We used the PubMed database (1950-2008) to search for the keywords “laboratory,” “erythrocyte sedimentation rate” (ESR), “C-reactive protein” (CRP), “blood cytology,” “procalcitonin” (PCT), “complement system,” “ferritin,” “antistreptolysin O titer” (ASO), “autoantibodies,” “genetic studies,” in conjunction with “rheumatic disease in children” and “pediatric autoimmune diseases.” All relevant original and review articles in English were reviewed as well as textbooks of pediatric rheumatology.

Results: Laboratory tests (ESR, CRP, blood cytology, complement system, ferritin, ASO titer) play an important role in confirming a diagnosis and in the follow-up of rheumatic diseases in the pediatric age group. The ESR is probably the most widely measured index of the acute phase response. Measurement of CRP is very useful in the rapid diagnosis of infection as a progressive increase can be shown in the first 48 hours. Also, the subsequent fall in serum CRP concentration on resolution of inflammation is useful for monitoring the efficacy of treatment. In chronic diseases, a combination of CRP and ESR may provide the most useful information. Cytopenia and different forms of anemia can be encountered in many rheumatic diseases: they can be related to disease activity or to therapeutic side effects. Determination of complement levels (C3 and/or C4) is useful in the follow-up of systemic lupus erythematosus (SLE) and membranoproliferative glomerulonephritis. Ferritin is a laboratory hallmark of primary and secondary hemophagocytic lymphohistiocytosis. ASO titer should be obtained to confirm a diagnosis of acute rheumatic fever; other important antibody markers of streptococcal infection include antihyaluronidase, antideoxyribonuclease B, and antistreptokinase antibodies. We also found that, in the pediatric age, the main indication for synovial fluid analysis is suspected joint infection. Antinuclear antibodies, anti-Smith antigen, and anti-double-stranded DNA antibodies are important in the diagnosis of SLE, are useful prognostic markers, and facilitate clinical and treatment follow-up. Anti-SSA/Ro and anti-SSB/La antibodies are associated with Sjögren’s syndrome and congenital heart block, while the anti-U1 small nuclear ribonucleoprotein antibodies show high specificity for mixed connective tissue disease. Repetitive spontaneous abortions, thrombocytopenia, and many types of venous or arterial thrombosis are associated with antiphospholipid antibodies. The presence of cytoplasmic antineutrophil antibodies is essential in the diagnosis of Wegener granulomatosis. The discovery of underlying single causative gene defects led to the identification of several autoinflammatory diseases, a group of genetic disorders characterized by recurrent attacks of inflammation (hereditary periodic fever syndromes). These include familial Mediterranean fever due to mutations in the Mediterranean fever (MEFV) gene, hyperimmunoglobulinemia D syndrome due to mutations in the MK gene for mevalonate kinase, cryopyrinopathies such as Muckle-Wells syndrome or neonatal-onset multisystemic inflammatory disease (neonatal-onset multisystemic inflammatory disease or chronic infantile neurological cutaneous and articular (CINCA)) associated with cold-induced autoinflammatory syndrome 1 gene mutations, and tumor necrosis factor receptor-associated periodic syndrome due to mutation of TNF receptor I gene.

Conclusions: Laboratory investigations play an important role in the diagnosis and follow-up of inflammatory rheumatic diseases in children. A good history and a complete physical examination are the best screening

tests. Routine laboratory tests are useful to confirm a suspected diagnosis, to assess disease activity, and to measure the response and toxicity to treatment. Only a few tests represent diagnostic criteria such as antinuclear antibodies and anti-double-stranded DNA in SLE or cytoplasmic antineutrophil cytoplasmic autoantibodies in Wegener's granulomatosis. Recent advances in molecular genetics have impacted diagnosis, pathogenesis, and treatment in genetic fever syndromes.

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The diagnosis of most rheumatic diseases in children is based on clinical history and physical examination. Laboratory investigations also play a major role in the evaluation and care of children with a suspected or confirmed rheumatologic illness. Laboratory tests are useful as screening tools to provide evidence of inflammation, to assist in diagnosis, to assess disease activity, to measure the response to treatment, and, finally, to better understand the pathogenesis of the disease.

For appropriate use of laboratory tests, physicians should order and interpret tests within the context of the patient's clinical situation to increase the specificity of the results. Many laboratory tests should be performed selectively, only when there is a strong suspicion of rheumatic disease. To simplify and give general guidelines, we divided this review into 3 sections: in section A (first-line diagnostics), we discuss the utility of common tests which are of simple execution and commonly available in most laboratories; in section B (second-line diagnostics), we review tests that are required in patients with symptoms of autoimmune disorders; and in section C (third-line diagnostics), we analyze the advances in molecular genetics for the diagnosis of many inflammatory syndromes.

METHODS

We used the MEDLINE databases to June 2008 (no earlier date limit) to search the keywords: "laboratory," "erythrocyte sedimentation rate" (ESR), "C-reactive protein" (CRP), "procalcitonin" (PCT), "C3," "C4," "ferritin," "serum amyloid A" (SAA), "blood cytology," "autoantibodies," "antinuclear antibodies" (ANA), "rheumatoid factor" (RF), "anticyclic citrullinated peptide antibodies" (anti-CCP antibodies), "antiphospholipid antibodies" (aPL), "antineutrophil cytoplasmic autoantibodies" (ANCA), "genetic studies," "rheumatic disease in children," and "pediatric autoimmune diseases." All relevant articles were then retrieved and reviewed.

We only considered prospective, matched case-control studies, randomized, double-blind studies, and relevant reviews; we reviewed the majority of papers about rheumatic diseases in pediatric age group but also included some studies on adults because of the paucity of pediatric data. We found more than 400 publications and included 195 in our analysis.

The reference lists of pertinent review articles were also reviewed to check for the articles that were not captured by the MEDLINE search. This systematic literature anal-

ysis was restricted to English, French, Italian, and Spanish language articles.

RESULTS

Section A: First-Line Diagnostics

Acute Phase Reactants

The initial routine laboratory assessment should consist of a complete blood cell count, including a white blood cell and differential count, and the determination of acute phase indicators such as ESR and CRP.

Acute phase reactants are plasmatic proteins that increase during acute phase of inflammation (Table 1). They are produced by the liver under regulation of circulating cytokines such as interleukin-6 (IL-6), interleukin-1 β (IL-1 β), and tumor necrosis factor- α (TNF- α), released by monocytes and macrophages. The major inflammatory indicators are CRP and ESR (1,2).

ESR is 1 of the major monitoring tests for acute phase inflammation because it correlates with increased levels of acute phase reactants, in particular, fibrinogen. It is the measure of the height of the layer of red blood cells that settle in a tube of anticoagulated blood in 1 hour.

The ESR is higher in women than in men and in older persons. The upper limit of normal for persons aged 50 years and younger is 15 mm/h in men and 20 mm/h in women. Shearn and Kang, in a population study, showed that mean normal values of ESR were 9.6 mm/h for men and 10.4 mm/h for women younger than 20 years (3). About 60 to 70% of ESR depends on fibrinogen because of its neutralizing effect on erythrocyte sialic acid, which inhibits red blood cell aggregation (4-6). Fibrinogen is 1 of the acute phase reactants produced by the liver in response to IL-6, IL-1, and TNF. The ESR is affected by many factors including the patient's age, sex, red blood cell morphology, hemoglobin concentration, and serum levels of immunoglobulins (Table 2); it increases if the tube is not vertical or if it is subject to vibration. Consequently, the ESR cannot be used as a diagnostic test in rheumatology but can be helpful to monitor disease activity and treatment response. In rheumatoid arthritis (RA), the ESR correlates well with disease activity (1,5). In juvenile idiopathic arthritis (JIA), the ESR represents 1 of the 6 criteria adopted by pediatric rheumatologists for evaluating improvement in response to treatment in clinical trials (7). In oligoarticular juvenile arthritis, a higher

ESR at onset has been associated with a more severe course of the disease (8).

CRP is an acute-phase protein discovered and named because of its reactivity with the C polysaccharide of *Streptococcus pneumoniae*, resulting in calcium-dependent precipitation (9). CRP recognizes pathogens and medi-

| Abbreviations | |
|---------------|---|
| ACA | Anti-centromere antibodies |
| aCL | Anticardiolipin antibodies |
| ADB | Antideoxyribonuclease B |
| AID | Anemia of inflammatory disease |
| ANA | Antinuclear antibodies |
| ANCA | Antineutrophil cytoplasmic autoantibodies |
| anti-CCP | Anti-cyclic citrullinated peptide |
| anti-dsDNA | Anti-double-stranded DNA |
| anti-NCS | Antinucleosome |
| anti-Sm | Anti-Smith |
| aPL | Antiphospholipid |
| APS | Antiphospholipid syndrome |
| aPTT | Activated partial thromboplastin time |
| ASO | Antistreptolysin O titer |
| β_2 GPI | β_2 -glycoprotein I |
| c-ANCA | Cytoplasmic antineutrophil cytoplasmic autoantibodies |
| CH50 | Total hemolytic complement |
| CHB | Congenital heart block |
| CRP | C-reactive protein |
| ELISA | Enzyme-linked immunosorbent assay |
| EPO | Erythropoietin |
| ESR | Erythrocyte sedimentation rate |
| FMF | Familial Mediterranean fever |
| HLA | Human leukocyte antigens |
| HLH | Hemophagocytic lymphohistiocytosis |
| HPFS | Hereditary periodic fever syndromes |
| IDA | Iron deficiency anemia |
| IIF | Indirect immunofluorescence |
| IL-1 β | Interleukin-1 β |
| IL-6 | Interleukin-6 |
| JIA | Juvenile idiopathic arthritis |
| LA | Lupus anticoagulant |
| MCTD | Mixed connective tissue disease |
| p-ANCA | Perinuclear antineutrophil cytoplasmic autoantibodies |
| PCT | Procalcitonin |
| PPV | Positive-predictive value |
| PT | Prothrombin time |
| RA | Rheumatoid arthritis |
| RF | Rheumatoid factor |
| SAA | Serum amyloid A |
| sACE | Serum angiotensin-converting enzyme |
| SF | Serum ferritin |
| SLE | Systemic lupus erythematosus |
| SpA | Spondyloarthritis |
| TNF- α | Tumor necrosis factor- α |
| TRAPS | TNF receptor-associated periodic syndrome |
| WG | Wegener's granulomatosis |

Table 1 Acute Phase Reactants

Proteins that increase in response to inflammation
 C-reactive protein
 Fibrinogen
 Plasminogen
 Ferritin
 Ceruloplasmin
 Complement factors
 Haptoglobin
 Hemopexin
 Granulocyte colony-stimulating factor (G-CSF)
 Serum amyloid A
 Alpha-1 antitrypsin
 Interleukin-1 receptor antagonist

(Adapted from *Pediatr Ann* 2002;31:362-71.)

ates the complement system and phagocytic cells; it activates neutrophils, monocytes, and platelets, upregulates adhesion molecules, and appears to play a role in the clearance of apoptotic and necrotic host cells (10-13). These latter properties of CRP have been suspected to contribute to inflammation and autoimmune diseases (14).

CRP is a member of the pentraxin family of proteins, made in the liver under the control of cytokines such as IL-6, IL-1, and TNF- α . The human CRP gene maps to the chromosome 1 (q23-24), which has been linked with systemic lupus erythematosus (SLE) in many populations (15,16). In healthy humans, CRP concentration is less than 1 μ g/dL, but it can increase 1000-fold following tissue injury or inflammation. An advantage over the ESR is that plasma CRP levels change more quickly in response to inflammation and fall quickly with appropriate treatment. After appropriate stimuli, the CRP increases within 4 hours, reaches the maximum level after 24 to 72 hours, and then decreases to normal values when inflammation resolves. The magnitude of inflammation is related to the magnitude of the CRP concentration (6). Another advantage is that CRP level is not affected, as the ESR, by the number and morphology of red blood cells and by serum immunoglobulin concentration. Also, CRP can be analyzed at a later time; sera can be frozen and only small amounts of blood are required for its determination (2,6).

In febrile children, the CRP concentration has been shown to be superior to total white blood cells and absolute neutrophil count in predicting severe bacterial infections (17). In RA, CRP levels correlate with inflammation and disease activity better than ESR, while ESR may measure general severity of disease better than CRP (6,18). In JIA, the CRP seems to reflect severe disease more closely than ESR and to predict amyloidosis (19). A recent work has shown that high CRP levels at the time of diagnosis correlate with poor therapeutic response in JIA patients (20). In patients with SLE, CRP is usually normal and its increase may suggest a concomitant infection (21). Recently, CRP has been implicated in the pathogenesis of

Table 2 Factors That Influence the Erythrocyte Sedimentation Rate (ESR)

| |
|--|
| <p>Increase in ESR</p> <ul style="list-style-type: none"> • Old age • Female sex • Pregnancy • Anemia • Hypercholesterolemia • Macrocytosis • High room temperature • Technical factors: tilted ESR tube • High fibrinogen level: infection, inflammation, malignancy <p>Decrease in ESR</p> <ul style="list-style-type: none"> • Extreme leukocytosis • Polycythemia • Sickle cell disease • Anisocytosis • Spherocytosis • Acanthocytosis • Microcytosis • Bile salts • Protein abnormalities: hypofibrinogenemia, hypogammaglobulinemia, dysproteinemia (cachexia) • High doses of adrenal steroids • Congestive heart failure • Technical factors: clotting of blood sample, short tube, vibration during testing, low room temperature, >2-hour delay in running the test |
|--|

(Adapted from Ann Intern Med 1986;104:515-23.)

atherogenesis and in the tissue damage of myocardial infarction (10,22-24).

PCT, a 116-amino-acid prohormone of calcitonin, is a newly discovered acute-phase reactant produced in the C-cells of the thyroid gland (25-27). In healthy subjects, PCT levels are less than 0.10 ng/mL but rapidly increase up to 200-fold after bacterial infections (26). Several studies have shown that PCT is mainly produced during severe bacterial infections, whereas its level remains low during viral infections, autoimmune illnesses, and graft versus host disease (28-30). In septic children, PCT proved to be more specific than CRP and best correlated with severity of acute inflammation (29). For these reasons, PCT is considered an excellent test in detecting invasive infections in intensive care units and in emergency departments (31). Unfortunately this test is not available in all laboratories. In patients with autoimmune disease such as SLE, or immunodeficiency with high risk of severe infections, PCT is useful to distinguish between a bacterial infection and a disease flare (32). PCT is a useful aid for an early diagnosis in children with suspected osteomyelitis (33). In a recent study, the serum concentration of PCT was increased in patients with Kawasaki disease and was associated with the later development of coronary aneurysms (34). Other studies did not confirm these findings (35).

Peripheral Blood Cytology

Peripheral blood cytology is useful in the differential diagnosis between inflammatory and noninflammatory diseases. Different forms of anemia can be encountered in rheumatic diseases. A normocytic or microcytic anemia is associated with RA and is the prototype of the anemia of chronic disease or the anemia of inflammatory disease (AID). Although the underlying mechanisms of AID are not completely understood, inflammatory cytokines, such as TNF- α , IL-1 β , IL-6, and IFN γ , appear to play a major role in this syndrome (36,37). Evidence suggests that TNF- α inhibits bone marrow erythropoiesis by a direct negative effect on erythroid progenitor cell growth, by stimulating the production of inhibitory cytokines by bone marrow accessory cells, and by increasing apoptosis of erythroid progenitor cells (38-40). It is believed that IL-6 inhibits erythroid progenitor proliferation, blunts erythropoietin (EPO) production, or impairs iron supply for erythropoiesis (37,41). Inflammatory cytokines also interfere with the ability of erythroid progenitor cells to respond to EPO (42,43). Furthermore, some studies have shown that resistance to EPO action in systemic autoimmune disease can be due to anti-EPO autoantibodies (44,45).

In inflammatory diseases, anemia may also be related to iron deficiency (iron deficiency anemia, IDA) resulting from gastrointestinal blood loss associated with the use of nonsteroidal anti-inflammatory drugs. AID is correlated to some extent with the parameters of disease activity and may be indistinguishable from IDA (41). The presence of low serum ferritin (<50 g/L) in combination with high transferrin levels and decreased mean corpuscular volume of erythrocytes results in 100% sensitivity and specificity for the detection of iron deficiency (46). In the last few years, increase in serum transferrin receptor level has been proposed as a sensitive parameter for detection of iron deficiency in both RA and JIA (41,47).

Anemia is found in about 50% of patients with SLE with AID being the most common form; autoimmune hemolytic anemia, IDA, drug-induced myelotoxicity, and anemia of chronic renal failure can also be encountered in these patients (48,49).

Leukopenia may be present in SLE and is related to neutropenia and/or lymphopenia (50). There are several mechanisms of lymphopenia in SLE: antilymphocyte antibodies, including anti-DNA and antiribosomal P antibodies, were frequently found in patients with SLE; besides, lymphocyte apoptosis is increased in active SLE and lymphocytes may be sequestered at sites of inflammation or lymphoid tissues (51). Lymphopenia in patients with SLE flares has been recently associated with disease activity and neuropsychiatric manifestations in a pediatric population (51). Thrombocytopenia is also common in SLE and has been associated with a decreased survival. Antiplatelet antibodies have been found in 60 to 87% of lupus patients, being mostly (60%) of the IgG type (52).

The Complement System

The complement proteins are a cascade of proteins that can be activated by a variety of agents including immune or antigen-antibody complexes. The most frequently analyzed components are C3 and C4. Increased levels of complement components and hemolytic activity are frequently found in inflammatory disorders. Depressed complement levels (C3 and/or C4) are commonly present in SLE, acute postinfectious glomerulonephritis, membranoproliferative glomerulonephritis, liver disease, and congenital deficiency of complement components (53).

In SLE, C3 concentration falls close to the time of a flare and returns to higher concentration after some weeks or months of appropriate treatment. Persistently low C3 is associated with SLE nephritis (54). In contrast, the C4 concentration may be low even during the inactive stage of SLE; thus, C4 alone is not a good predictor of disease activity (55). The low levels of C4 seen in inactive stages of SLE are usually due to partial C4 deficiencies, which occur quite often in SLE patients (56). Hereditary complement deficiencies of C1q, C4, and C2 have been associated with an increased risk of autoimmune disease, especially SLE (57). Heterozygous or homozygous deficiencies of C4A are present in 40 to 60% of SLE patients; the most common complement deficiencies in such patients are C4A, C4B, and C2 deficiencies (58). The total hemolytic complement (CH50) activity represents a screening test for inherited complement deficiencies. The CH50 assay assesses the total hemolytic complement activity, that is, the integrity of the complement system. In this method, complement components present in the serum of the patients are placed in solution with antibody-sensitized sheep erythrocytes: the titer at which 50% hemolysis occurs is proportional to the activity of the classical pathway in the serum (59). Boeckler and coworkers, in a retrospective series, found that about 50% of SLE patients had normal or elevated C3, C4, and CH50 levels. CH50 levels were decreased only in 50% of patients with complete C4B deficiency. The authors suggest that a combination of tests (CH50, C3, C4, and C2 levels, C4 polymorphisms, and human leukocyte antigens (HLA) class I and II typing and other component levels such as C1q) is necessary for the diagnosis of C4 and/or C2 complement deficiency in patients with SLE (58).

Serum Ferritin

Serum ferritin (SF) is an iron storage protein consisting of a spherical protein coat (apoferritin) and a core of hydrous ferric oxide [Fe (III)O₂·OH]. Apoferritin is composed of 24 identical subunits of 2 species, the heavy subunit (H subunit) and the light subunit (L subunit). A single functional H gene and L gene have been identified on chromosome 11q23 and 19q13.3, respectively (60,61). Ferritin is widely distributed in nature and is found in most cell types of humans and other vertebrates and in invertebrates, fungi, and bacteria. The principal function of this

Table 3 Pediatric Reference Intervals (2.5-97.5 percentiles) for Ferritin Concentrations in Women and Men Age 0 to 18 Years

| Age | Male Intervals ng/mL (μg/L) | Female Intervals ng/mL (μg/L) |
|----------------------|--------------------------------|----------------------------------|
| 0 to 90 days | 40 to 775 | 79 to 501 |
| 91 days to 12 months | 25 to 790 | 25 to 560 |
| 13 months to 3 years | 12 to 501 | 10 to 500 |
| 4 to 10 years | 25 to 280 | 22 to 158 |
| 11 to 14 years | 25 to 112 | 15 to 112 |
| 15 to 18 years | 18 to 158 | 10 to 125 |

(Adapted from Clin Chim Acta 2004;342:111-7.)

protein is the storage of iron in a soluble form, but it has also been implicated in different immunological effects including inhibition of T-lymphocytes, suppression of antibody production by B-lymphocytes, and decreasing granulocyte phagocytosis. Ferritin synthesis is regulated by intracellular iron, cytokines (TNF α , IL-1 α , IL-1 β , IL-6), oxidative stress products, hormones, and growth factors, throughout heavy-chain transcription and translation (61).

Pediatric reference intervals of ferritin vary depending on age and sex (Table 3); at all ages, levels below 12 μ g/L identify iron deficiency (62). However, inflammation, even in the presence of iron deficiency, causes elevation of SF. The pathogenesis of this condition is related to the accumulation of iron in macrophages that leads to ferritin overproduction (61).

Elevated SF levels act as a nonspecific marker for a large number of disorders. In certain inflammatory diseases such as adult-onset Still's disease, this finding may be an important tool. In systemic-onset juvenile idiopathic arthritis, high SF levels seem to reflect disease activity (63).

In a prospective study evaluating SF levels in adults with active SLE, SF appeared to provide a useful marker of disease activity and to be positively correlated with Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores (64).

Highly elevated ferritin levels are a laboratory hallmark of hemophagocytic lymphohistiocytosis (HLH), a condition characterized by a pathologic pro-inflammatory activity of T-cells and macrophages (65). Genetic (primary) HLH is inherited in an autosomal-recessive or x-linked manner, which frequently develops early in life. Acquired (secondary) HLH occurs in all age groups and may be triggered by severe infection, malignancy, and autoimmune disease (66).

The Histiocyte Society HLH-2004 treatment protocol includes SF over 500 μ g/L as 1 of the diagnostic criteria. Moreover, SF over 10,000 μ g/L has a sensitivity of 90% and a specificity of 96% for HLH (65).

High SF levels have also been reported in both children and adults with severe sepsis and septic shock. In these patients, a worse outcome has been associated with ferritin >500 μ g/L (67).

Serum Amyloid A

The acute-phase SAA protein is a multifunctional apolipoprotein involved in cholesterol metabolism. SAA is produced in hepatocytes after induction by cytokines, although an extrahepatic expression has also been documented (68,69). The protein is coded on chromosome 11p in humans and chromosome 7 in mice (68). SAA function is not completely understood. Recent investigations have suggested that SAA plays a role in the inflammatory process through its cytokine-like property and by induction of cytokines (70). The median plasma concentration of SAA in healthy persons is 3 mg/L, but it can increase to more than 2000 mg during the acute phase response (71). SAA is the serum precursor of the amyloid A protein, which is the principal component of the amyloid deposits found in inflammation-associated reactive amyloidosis (69). Sustained overproduction of SAA is a prerequisite for the development of amyloidosis, although this complication occurs only in a small proportion of patients with chronic inflammatory disorders (72). Still, monitoring SAA concentrations in patients at risk for amyloidosis seems justified. Clinical amyloidosis occurs in up to 10% of patients with chronic infections and idiopathic inflammatory disorders such as RA, JIA, or Crohn's disease (73). The kidneys are most often affected but any organ may be involved. Long-term outcome studies of JIA reported that 9% of patients developed amyloidosis with systemic JIA patients being most commonly affected (74). Amyloidosis is the most severe complication of familial Mediterranean fever (FMF) with nephrotic syndrome and eventually uremia being the most common clinical manifestations (75). The development of renal amyloidosis in FMF has been mainly associated with the presence of the M694V mutation in the FMF gene (MEFV) in both the homozygous and the heterozygous state (76). Nevertheless, recent studies have shown that the patient's country should be considered in addition to MEFV genotype as a risk factor of renal amyloidosis in patients with FMF (75).

Other Laboratory Tests

Elevated alanine aminotransferase and aspartate aminotransferase can be associated with drug toxicity (sulfasalazine, methotrexate, cyclosporine A, nonsteroidal anti-inflammatory drugs), autoimmune hepatitis, systemic vasculitis, and SLE (77,78).

Proteinuria or hematuria can be associated with lupus nephritis, vasculitis, or drug toxicity (cyclosporine A) (78,79). Serum electrolyte concentrations should be monitored in high-dose systemic steroid treatment because of possible potassium depletion and sodium retention (78).

Hypercalcemia is observed in approximately 30% of patients with sarcoidosis but is rarely symptomatic, although some cases of nephrocalcinosis have been reported (80).

Serum angiotensin-converting enzyme (sACE) should be measured when a diagnosis of sarcoidosis is being considered. Elevation of this enzyme constitutes a strong diagnostic factor as it is elevated in 50 to 80% of patients

(81). sACE originates from the epithelioid cells of the sarcoid granulomas (81-83) and sACE levels are helpful to the clinician in following sarcoidosis activity and in evaluating treatment response (84).

An increase of muscle enzymes (creatine kinase, lactate dehydrogenase, aldolase, aspartate aminotransferase) is the laboratory hallmark of juvenile dermatomyositis. At diagnosis, more than 90% of patients have an increase of at least 1 of these enzymes (85). Other serological indicators of disease activity include neopterin and von Willebrand factor antigen, markers of macrophage activation, and endothelial cell damage, respectively (86). In patients with musculoskeletal complaints, persistent elevation of lactate dehydrogenase together with blood cell count abnormalities suggest a lymphoproliferative disease. It is well known that serum lactate dehydrogenase is increased in most patients with acute lymphoblastic leukemia and non-Hodgkin's lymphoma and that these pathologies may begin with musculoskeletal symptoms (87).

Serum immunoglobulin concentrations (IgG, IgA, and IgM) are markedly raised in many autoimmune diseases. The magnitude of increase of these proteins is not related to the severity of disease; repeated measurements are therefore useless and a waste of money (88).

Cryoglobulins are antibodies that precipitate at temperatures below 37°C and redissolve on rewarming. They are classified into 3 groups; only type II and III cryoglobulins can activate complement cascade and are likely to cause vasculitis. Type II and III cryoglobulins are associated with rheumatic diseases and hepatitis C, while type I cryoglobulins can be encountered in malignancy. Symptoms generally associated with the presence of cryoglobulins are purpura, ulcerations, Raynaud's phenomena, arthralgias, proteinuria, and renal failure (88,89). The presence of cryoglobulins in children is rare.

Prolongation of activated partial thromboplastin time (aPTT) and/or prothrombin time (PT) and lack of correction of this phenomenon with normal plasma suggest the antiphospholipid syndrome (APS). APS is characterized by the presence of antibodies to phospholipids and a number of clinical manifestations such as venous thrombosis, recurrent fetal loss, and spontaneous bleeding (90). Abnormal coagulation tests (hypofibrinogenemia, low PT and APTT, increased fibrin products) are commonly present in the macrophage activation syndrome, a rare complication of systemic-onset juvenile idiopathic arthritis and juvenile SLE (91).

A diagnosis of acute rheumatic fever is supported by evidence of preceding group A streptococcal infection by the positive rapid streptococcal antigen test, positive throat culture, or rising antistreptolysin O titer (ASO) (92). For the diagnosis of streptococcus pyogenes pharyngitis, the American Academy of Pediatrics recommends that for any rapid antigen test that is negative, a follow-up culture should be done (93). A rise in ASO can be shown within 1 week of infection and a maximum titer will be reached after 3 to 6 weeks. ASO titer may be negative in

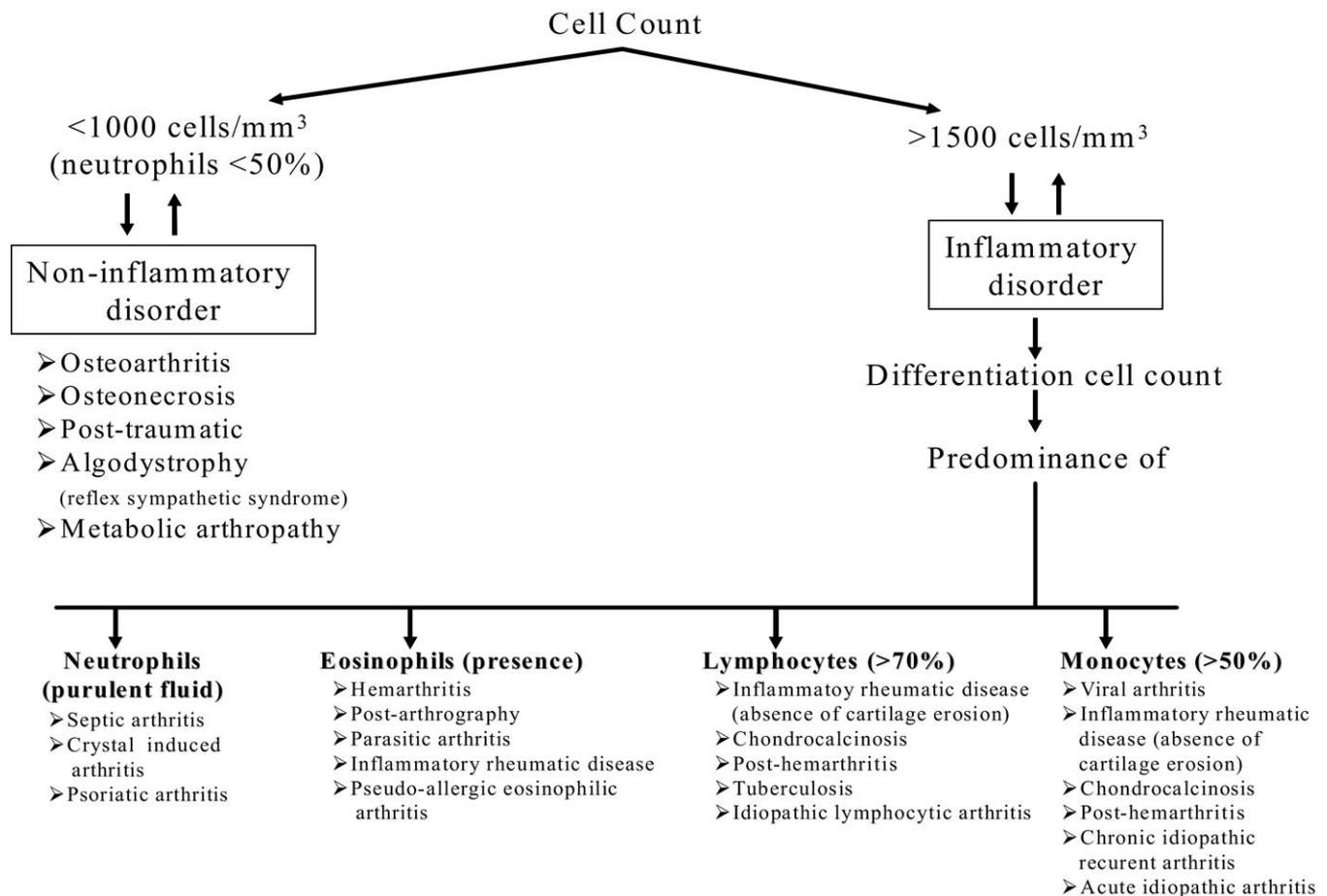


Figure 1 Flow diagram of differential diagnosis based on synovial fluid white blood cell analysis (From Dougados M. Synovial fluid cell analysis. Baillieres Clin Rheumatol 1996;10:519-34).

up to 20% of patients who develop acute rheumatic fever (94). Other important and more specific markers of streptococcal infection include antihyaluronidase, antideoxyribonuclease B (ADB), and antistreptokinase antibodies (92). The ADB titer can take up to 6 to 8 weeks to reach a maximum and starts to decline by 3 months. In the absence of reinfection, the ASO titer usually reaches normal values after 6 to 12 months, whereas the ADB titer tends to remain high for longer periods (95). In comparison with streptococcal pharyngitis, higher ASO titers are usually found in rheumatic fever, but no correlation exists with the severity of clinical manifestations (96).

Synovial Fluid Examination

Synovial fluid analysis is rarely necessary in the pediatric age group. Normal synovial fluid is clear, highly viscous, similar in consistency to egg white, and contains less than 100 cells/mm³. The majority of cells are small lymphocytes; the remainder are neutrophils and mononuclear phagocytes (97). In children, the main indication for arthrocentesis and synovial fluid examination is suspected septic arthritis, although this analysis may be useful in distinguishing between inflammatory and noninflamma-

tory articular effusions (98). Fluid samples should be examined for volume, viscosity, color, cells count, protein content, and glucose levels; culture and a Gram stain should be obtained. The total nucleated cell count is usually measured using a hematological counting chamber. Synovial cell counts higher than 100,000/mm³ usually suggests septic arthritis. However, lower cell counts do not exclude infective arthritis (Fig. 1). Septic synovial fluid also contains high levels of protein and low values of glucose (<25% of serum glucose concentration). A sterile synovial fluid does not exclude osteomyelitis especially in older children. Indeed, in the neonate, spread of infection from bone to joint is a relatively common sequel to osteomyelitis as the cartilaginous epiphyses receive their blood supply directly from metaphyseal blood vessels. In older children, the epiphyses and metaphyses have a separate blood supply, and spread of infection from bone to joint (or vice versa) is far less common (99). Synovial fluid from patients with inflammatory arthropathies usually contains more than 1500 cells/mm³ with a prevalence of polymorphonuclear cells (97).

Red blood cells may be present in synovial fluid due to traumatic joint aspiration or to hemarthrosis. If the fluid

aspirate immediately appears diffusely red, brown, or pink, hemarthrosis should be suspected. Hemarthrosis in children may also be related to hemophilia, von Willebrand's disease, Glanzmann thrombasthenia, pigmented villonodular synovitis, and synovial tumor (97).

Section B: Second-Line Diagnostics

Antinuclear Antibodies

ANA are autoantibodies directed against nuclear, nucleolar, or perinuclear antigens and are characteristic of autoimmune diseases. Although their pathogenetic role is not clear, ANA are suggestive of autoimmunity when they are persistently present in high titers together with the clinical features of the disease. Positive ANA testing is associated with several inflammatory rheumatic diseases and has been included in the classification criteria for SLE, mixed connective tissue disease (MCTD), and Sjögren syndrome. ANA can also be helpful for the diagnosis of several nonrheumatic autoimmune diseases such as autoimmune hepatitis, autoimmune thyroiditis, and drug-induced autoimmune syndromes (88). Screening tests for ANA are commonly performed by indirect immunofluorescence (IIF) using either frozen sections of animal tissue or, more appropriately, cultured cell lines (eg, human epithelial Hep-2 cells). Different immunofluorescent patterns can be recognized: the homogeneous pattern and the speckled pattern are more common (2). The immunofluorescence pattern can suggest target antigens and indicate the need for enzyme-linked immunosorbent assay (ELISA) to detect specific antibodies (ANA profile). ELISA technology dominates routine laboratory practice but tends to produce more false-positive and true weak-positive results. Where necessary, laboratories should use more reliable assays such as the immunoprecipitation (Farr) technique to confirm dubious ELISA results. The Farr is superior to the ELISA in detecting anti-double-stranded DNA (anti-dsDNA) antibodies in patients with SLE; indeed Farr correlates with global disease activity as well as renal and vascular involvement (100).

The homogeneous pattern of ANA is due to the presence of antihistone and/or anti-dsDNA antibodies that are highly specific of SLE; the presence of anti-dsDNA may also result in a peripheral or rim pattern. The speckled pattern is associated with anti-Sm (anti-Smith antigen), anti-SSA, and anti-SSB antibodies of Sjögren's syndrome and MCTD. The nucleolar pattern is related to anti-Scl70 antibodies in systemic sclerosis, and the centromere pattern is seen with antibodies to the kinetochore in the CREST (Calcinosis, Raynaud's phenomenon, Esophageal disease, Sclerodactyly, Telangiectasia) syndrome and in primary biliary cirrhosis. Cytoplasmic fluorescence reveals different patterns and is indicative of the distinct antigens involved: antimitochondrial autoantibodies display a granular pattern; autoantibodies to the Golgi organelle show granules arranged in clusters at 1 or both cell poles; the antikeratin antibody pattern is characterized by a fibrous network that spreads out from the

perinuclear region while autoantibodies to nonmuscle myosin decorate the stress fibers of the cytoskeleton (101).

The patterns of IIF are neither specific nor diagnostic of any autoantibody or disease entity. They are only indicative of the presence of an autoantibody, but its presence should be verified using more specific tests such as the double-immunodiffusion assays, the immunoblot test, and ELISA.

ANA are frequently found in SLE and drug-induced SLE, having a sensitivity nearly 100% and a specificity of about 90% (1). Low positive-predictive value (PPV) and high negative-predictive value have been reported both at low cutoff ANA titers and at high titers for juvenile SLE and MCTD (PPV between 0.06 and 0.6; negative-predictive value between 0.96 and 1.0) (102). As a consequence, a negative ANA test makes the diagnosis of SLE and MCTD extremely unlikely, while most people with a positive ANA test do not have SLE or MCTD (102). The diagnostic utility of ANA as a screening test is limited because of the large number of healthy children with low positivity. ANA in low titers (1:40, 1:80) may be present in patients with various nonautoimmune diseases (viral and bacterial infections, neoplasms) and in healthy subjects. Up to 30% of healthy individuals have an ANA-positive test at a titer of 1:40 and 5% at a titer of 1:160 (Table 4) (102-104). In addition, various studies have

Table 4 Selected Conditions Associated with ANA Positivity

| | |
|-----------------------------------|--|
| Healthy individuals | <ul style="list-style-type: none"> • Increase frequency with aging • Females more than males |
| Systemic autoimmune diseases | <ul style="list-style-type: none"> • Systemic lupus erythematosus • Mixed connective tissue disease • Scleroderma • Polymyositis • Dermatomyositis • Sjögren's syndrome • Rheumatoid arthritis • Juvenile idiopathic arthritis |
| Organ-specific autoimmune disease | <ul style="list-style-type: none"> • Autoimmune hepatitis • Primary autoimmune cholangitis • Autoimmune thyroid disease |
| Drugs | <ul style="list-style-type: none"> • Procainamide • Hydralazine • Isoniazid • Minocycline • Penicillamine • Quinidine • Anticonvulsivants • Diltiazem • Chlorpromazine • Methyldopa |
| Infections | <ul style="list-style-type: none"> • Epstein Barr virus • Tuberculosis • Subacute bacterial endocarditis • Malaria • Hepatitis C • Parvovirus B19 |

(Adapted from *Pediatr Ann* 2002;31:362-71.)

shown that the majority of ANA-positive children without signs of rheumatic conditions will not develop an autoimmune disease later in life (88,102,104-106).

In a retrospective chart review, Perilloux and coworkers showed that of 245 children with a positive ANA test, only 55% had specific autoimmune diseases, the most common being JIA (37%) followed by SLE (30%). Forty-five percent of patients with a positive ANA test did not have an autoimmune disease. One-third of these patients were found to have fibromyalgia, joint hypermobility, and nonspecific musculoskeletal complaints. Infections accounted for almost one-quarter of the positive tests. Patients with autoimmune disorders had a significantly higher ANA titer ($\geq 1:160$) than those with a nonautoimmune etiology. The authors concluded that patients with a high ANA titer $\geq 1:160$ should undergo further evaluation for autoimmune diseases. Moreover they suggested that an ANA profile should be performed for those children with an ANA titer $\geq 1:640$ and/or in those with a high clinical index of suspicion for SLE (104). Other authors found that ANA titers higher than 1:1280 are highly predictive for SLE (88,102,107).

In children with JIA, a positive ANA test identifies a subset of patients that shares the same characteristics: high risk for developing iridocyclitis, asymmetric arthritis, early disease onset, and female prevalence (108,109).

Whenever an ANA profile is indicated (Table 5), various techniques, including ELISA, immunoblotting, and FARR radioimmunoassay, are available (2).

Since their discovery in 1957, attention has focused on anti-dsDNA antibodies in an attempt to determinate their role in disease pathogenesis. Anti-dsDNA antibodies can be detected with different techniques: ELISA, FARR, and Crithidia luciliae. The Crithidia luciliae technique is based on the use of a protozoa whose kinetoplast at the base of the flagellum consists of dsDNA. A typical fluorescence of the kinetoplast is evident in the presence of anti-dsDNA antibodies. Positive anti-dsDNA are rarely encountered in healthy individuals and appear highly specific of SLE; they are observed in 84 to 95% of children and in about 50 to 70% of untreated adults with this disease (110-113). The titer of anti-dsDNA antibodies is also clinically important for monitoring disease activity. A rise in anti-dsDNA antibodies usually precedes SLE exacerbation by a few weeks (114). High titers have been identified in lupus nephritis and their levels tend to rise and fall with disease activity (115). Several lines of experimental evidence have demonstrated a direct link between anti-dsDNA antibodies and nephritis; some anti-dsDNA antibodies can induce immune deposits and nephritis in nonautoimmune mice (116).

Antihistones antibodies are often associated with drug-induced SLE (90%) but are not specific of this condition. Medications most commonly associated with drug-induced SLE are procainamide and isoniazid (1). The presence of antibodies to histones has been reported in about

50% of patients with SLE; for this reason this test has limited diagnostic utility (117).

The SSA antigen consists of 52- and 60-kDa proteins (called Ro52 and Ro60, respectively). Antibodies to the Ro52 antigen appear in association with antibodies to the Ro60 antigen and few techniques are able to distinguish between antibodies against the 2 different antigens. Antibodies (mainly of the IgG isotype) to Ro52 are more often seen in patients with primary Sjögren's syndrome, whereas antibodies to Ro60 are found more often in patients with SLE (88). The SSB protein is thought to participate in the termination of transcription of RNA polymerase III. Anti-SSA/Ro antibodies are associated with adult Sjögren's syndrome (75%) and SLE (40% of adults and 33% of children). They may even be present in ANA-negative patients, are strongly associated with neonatal SLE, and are involved in the pathogenesis of congenital heart block (CHB) (1,2,88,110,117).

Anti-SSB/La antibodies are present in SLE (20% of adults and 15% of children) and in adult Sjögren's syndrome (40%); they are also involved in neonatal SLE but less frequently than anti-SSA/Ro (1,2,110). In juvenile Sjögren's syndrome, about 60 to 70% of patients have autoantibodies to SSA or SSB (118,119). Accumulated data suggest that women with anti-SSA/Ro antibodies face a 2% risk of having a child with neonatal lupus CHB, most often third degree (120). Recent reports suggest that this risk increases to 5% in the presence of both anti-SSB/La and anti-SSA/Ro antibodies (121). Several lines of evidence support the pathogenic role of anti-SSA/Ro and anti-SSB/La maternal antibodies, which presumably cross the placenta and damage the conduction system of the developing fetus. Direct implication of Ro/La antibodies has been demonstrated in fatal CHB, and maternal IgG-bearing anti-La idiotypes were identified on the surface of fetal cardiac myocytes (122). In pediatric SLE, anti-SSB/La antibodies are also associated with class IV lupus nephritis and central nervous system disease (110).

The extractable nuclear antigens are a group of antigens that leach from the cell when extracted with saline. There are many antigens within the nucleus but only a small number have clinical utility (88). The interest in these antigens was stimulated by the description of MCTD, in which anti-ribonucleoprotein antibodies are the immunologic marker (123).

Anti-extractable nuclear antigens antibodies are detectable in SLE, MCTD, and juvenile systemic sclerosis.

The Sm antigen is a complex group of 4 proteins complexed with U1, U2, U4-U6, and U5 snRNAs. Anti-Sm antibodies are highly specific for SLE, although not sensitive (present in only 20% of patients with SLE), and are associated with renal involvement and a poor prognosis (88,117).

The ribonucleoprotein antigen is formed by 3 proteins complexed with U1 snRNA. The anti-U1 small nuclear ribonucleoprotein antibodies show high specificity (about 100%) for MCTD. They are also present in 37% of chil-

| Autoantibody | Disease | Sensitivity (%) | Specificity and Associations |
|------------------------------------|-----------------------------|-----------------------|--|
| ANA screen | SLE | 90 to 95 | 90% |
| | Drug-induced SLE | 100 | |
| | Juvenile SLE | 100 | |
| | Juvenile systemic sclerosis | 80.7 | |
| | Juvenile Sjögren's syndrome | 92 | |
| Anti-dsDNA | SLE | 50 to 70 | 90 to 98% |
| | Juvenile SLE | 54 | 84 to 95% |
| | Juvenile systemic sclerosis | 5.7 | |
| Anti-histone | Drug-induced SLE | 90 | Nephritis and disease activity in SLE |
| | SLE | 50 | Low |
| Anti-Sm | SLE | 8 to 20 | 99% |
| | Juvenile SLE | 48 | Class III lupus nephritis in juvenile SLE |
| Anti-U1 snRNP | MCTD | 95 to 100 | 98% |
| | SLE | 30 to 40 | Disease activity in SLE |
| | Juvenile SLE | 37 | Class III lupus nephritis in juvenile SLE |
| Anti-Ro (SSA) | Sjögren's syndrome | 40 to 80 | Low |
| | Juvenile Sjögren's syndrome | 60 to 70 ^a | |
| | SLE | 30 to 50 | Low |
| | Juvenile SLE | 33 | Photosensitive skin rash, pulmonary disease, lymphopenia, and congenital cardiac block in SLE |
| Anti-La (SSB) | Sjögren's syndrome | 40 to 50 | Intermediate |
| | Juvenile Sjögren's syndrome | 60 to 70 ^a | |
| | SLE | 20 | Low |
| | Juvenile SLE | 15 | Secondary Sjögren's syndrome, late-onset SLE, neonatal SLE, class IV lupus nephritis, and central nervous system disease in juvenile SLE |
| Anti-ribosome | SLE | 10 to 20 | High |
| Anti-centromere (ACA) | Scleroderma | 22 to 36 | SLE psychosis |
| | Juvenile systemic sclerosis | 7 | Intermediate |
| Anti-topoisomerase I (anti-Scl-70) | Scleroderma | 20 to 25 | CREST syndrome and Raynaud's phenomenon in scleroderma |
| | Juvenile systemic sclerosis | 34 | 99% |
| Anti-Jo1 | Polymyositis | 20 to 40 | Lung fibrosis in scleroderma |
| | Dermatomyositis | 20 to 40 | 98% |
| | Juvenile myositis | 5 to 10 | |
| Anti-p155 | Dermatomyositis | 20 | Arthritis, fever, Raynaud's, lung fibrosis in dermatomyositis |
| | Juvenile myositis | ≤30 | Typical dermatomyositis patients with prominent rash including Gottron's papules, ulceration, and edema; might be associated with cancer in adults |
| Antiphospholipid (any) | Juvenile SLE | 45 | Risk of thrombosis |
| aCL | Juvenile SLE | 39 to 49 | Class V lupus nephritis, pericarditis, cerebrovascular disease, and chorea in juvenile SLE |
| | Juvenile systemic sclerosis | 15 | |

Table 5 Autoantibody Sensitivity, Specificity, and Associations with Different Autoimmune Systemic Rheumatic Diseases (continued)

| Autoantibody | Disease | Sensitivity (%) | Specificity and Associations |
|---------------------|--------------|-----------------|--|
| Anti- β_2 GPI | Juvenile SLE | 35 | Neuropsychiatric disease |
| LA | Juvenile SLE | 13 to 35 | Central nervous system disease in juvenile SLE |

(Adapted from Clin Exp Rheumatol 2004;22:349-55; Mayo Clin Proc 1996;71:391-6; J Pediatr 2008;152:550-6; Lupus 2006;15:496-500; Rheumatology (Oxford) 2008;47:183-7; Arthritis Rheum 2008;59:206-13; J Rheumatol 2005;32:2225-32; Eur J Pediatr 2003;162:661-5; Arthritis Rheum 2006;54:3971-8; Lancet 2008;371:2201-12; Rheumatology(Oxford) 2008;47:324-8.)
 †Include both anti-SSA and anti-SSB antibodies.

dren with SLE and are associated with lupus nephritis (110).

Anti Scl70 antibodies are directed against DNA topoisomerase I; they are seen in 20 to 25% of adults with systemic sclerosis but are unusual in pediatric patients and rarely observed in other autoimmune diseases. Patients with anti Scl-70 antibodies are particularly affected by lung fibrosis (88,124); children with these antibodies should be screened periodically with pulmonary function tests such as diffusing capacity for carbon monoxide.

Anti-centromere antibodies (ACA) were first described in 1980 and are directed against epitopes found in the kinetocore domain of the chromosome. ACA are found in systemic sclerosis in its limited cutaneous form and in the Calcinosis, Raynaud's phenomenon, Esophageal disease, Sclerodactyly, Teleangiectasia syndrome variant. These antibodies are also found in a subset of patients with primary biliary cirrhosis in whom liver disease may precede the manifestations of systemic sclerosis (88). In pediatric patients, ACA are closely associated with the presence of Raynaud's phenomenon and pulmonary hypertension, but the predictive and prognostic value of these antibodies are not completely understood (125). ACA-positive patients may have a rapid increase in pulmonary arterial systolic pressure and should be periodically screened with echocardiography (126).

Anti-Jo1 antibodies are a group of antibodies directed to a family of aminoacyl-tRNA synthetases that give a fine granular cytoplasmic staining on Hep2 IIF (88,127). They are present in 20 to 40% of adults with dermatomyositis and polymyositis, usually in association with interstitial lung disease, but are uncommon in childhood (5-10%) (1,2,88,117,128). A new myositis-associated autoantibody, anti-p155, has been recently identified in 30% of patients with juvenile dermatomyositis and in 20% of adults with dermatomyositis (128,129). This antibody has been associated with cutaneous involvement, including Gottron's papules, ulceration, and edema (130).

Antiribosomal P antibodies are highly specific for SLE, although they are present in only a few patients. These antibodies have been associated with lupus psychosis, but their predictive value is uncertain and controversial (115,131-133).

In recent years, antinucleosome antibodies (anti-NCS) have been demonstrated to correlate with disease severity

in both pediatric and adult patients with SLE (134-136). Nucleosomes are generated during cell apoptosis by cleaving the chromatin with endonucleases. In SLE, programmed cell death might be aggravated and result in the increased release of nucleosomes. Anti-NCS antibodies are superior to anti-dsDNA antibodies in terms of sensitivity and may thus be a better tool for diagnosing SLE. In 1 study anti-NCS antibodies had a sensitivity of 61% and a specificity of 98% in SLE (135).

It is recommended that positivity in 1 of the tests mentioned above should be confirmed by an alternative technique. The combination of ELISA and immunoblot appear to have high sensitivity (100%) and specificity for the detection of autoantibodies.

Many markers of autoimmunity are found in aging: although B- and T-lymphocytes decline with age, paradoxically there is an increase in autoantibodies. The development of autoimmune responses has been hypothesized to be secondary to thymus involution, with a decline in naive T-cells and the accumulation of memory cells with activation from "neoantigens" during the aging process. Autoantibodies like RF and ANA can be detected in a significant proportion of elderly people without necessarily causing pathology (137,138).

Rheumatoid Factor

RF was first described in 1940; these antibodies react with the Fc portion of the IgG molecules. RF is present in about 85% of adults with RA but only in a few children with JIA (about 5-10% of the polyarticular subset with an estimated PPV of 0.5-0.7). RF in both children and adults with arthritis correlates with a more aggressive joint disease course (2,139). Recently, in addition to IgM RF, IgA RF has been associated with disease severity in JIA, including erosive disease, joint damage, and functional disability. The detection of IgA and IgM RF by ELISA is more sensitive and specific than traditional methods, up to 50% of children with JIA being positive by IgA or IgM RF by ELISA (140). Children with RF-negative JIA rarely become RF-positive during the course of the disease, so retesting RF is unnecessary in these patients. RF is also frequently present in other rheumatic disorders such as SLE (10-30%), scleroderma (25-45%), mixed cryoglobulinemia (40-100%), and infections, in particular, subacute

Table 6 Conditions Associated with a Positive Rheumatoid Factor Test

| |
|---|
| <p>Rheumatic conditions associated with RF positivity (prevalence)</p> <p>Rheumatoid arthritis (50 to 90%)</p> <p>Juvenile idiopathic arthritis (5 to 10%)</p> <p>Systemic lupus erythematosus (15 to 35%)</p> <p>Juvenile SLE (11%)</p> <p>Sjögren's syndrome (75 to 95%)</p> <p>Juvenile Sjögren's syndrome (58%)</p> <p>Systemic sclerosis (20 to 30%)</p> <p>Juvenile systemic sclerosis (17%)</p> <p>Cryoglobulinemia (40 to 100%)</p> <p>Mixed connective tissue disease (50 to 60%)</p> <p>Nonrheumatic conditions associated with RF positivity</p> <p>Aging</p> <p>Infections: bacterial endocarditis, tuberculosis, syphilis, viral infections (mumps, rubella, influenza, parvovirus B19, hepatitis B and C)</p> <p>Sarcoidosis, silicosis, asbestosis</p> <p>Primary biliary cirrhosis</p> <p>Malignancies (leukemia, colon cancer)</p> |
|---|

(Adapted from Am J Med 1991;91:528-34; J Pediatr 2008;152:560-6; Arthritis Rheum 2006;54:3971-8.)

bacterial endocarditis and hepatitis B and C (Table 6) (1,2,88,117).

Anticyclic Citrullinated Peptide Antibodies

Anti-CCP antibodies are directed against citrulline residues formed in posttranslational modifications of arginine (88). It has been suggested that the inflamed synovial tissue is the site of anticitrullinated protein production in patients with RA (117). Anti-CCP are detectable with ELISA and are highly specific (89-98%) and sensitive (41-88%) for RA. The appearance of these antibodies may occur several years before the onset of RA and represents a marker of the future disease. To the contrary, many studies have shown that anti-CCP is a poor test in JIA because only children with RF-positive JIA have circulating antibodies against CCP (141). Therefore, routine determination of these antibodies is not recommended in JIA (141-144). However, the role of anti-CCP antibodies in JIA remains controversial since several studies have shown that they may play a role in the pathogenesis and perhaps in the development of joint damage. In addition, the presence of anti-CCP antibodies early in the course of JIA may be useful in identifying those patients requiring more aggressive treatment (140).

Antiphospholipid Antibodies

aPL are a heterogeneous group of autoantibodies directed against plasma proteins that bind to cell membrane phospholipids. Phospholipids are a characteristic of cellular membranes and mitochondria and are abundant in the brain, spinal cord, and plasma (145). aPL were detected

for the first time in sera of patients with syphilis in 1906 by Wassermann; the test was called Wassermann reagin and was introduced as a serologic test for syphilis (146). About 50 years later, a plasma coagulation inhibitor was found in patients with SLE and was denominated lupus anticoagulant (LA). In 1990, β_2 -glycoprotein I (β_2 GPI) was identified as 1 of the major target antigens for autoimmune aPL. The physiological role of this protein is uncertain but it has been suggested that β_2 GPI may exhibit anticoagulant properties (145,147,148). aPL have been associated with increased risk of thrombotic events (both venous and arterial), as hemolytic anemia, thrombocytopenia, and spontaneous abortion (146,148,149) (Table 5). aPL frequently occur in autoimmune diseases such as SLE and in primary APS (2,150-152).

aPL also occur in infectious disease but these antibodies are usually not associated with thromboembolic events (146,153,154). However, some observations suggest that in susceptible individuals infection-associated aPL may persist and induce autoimmunity (146).

As in adults, the APS can be seen in children with SLE (secondary APS) or in children without evidence of an underlying autoimmune disorder (primary APS). Primary APS in children is rare; the clinical features are similar to those in adults manifesting commonly with central nervous system thrombosis and uncommonly with skin, heart, and kidney disease (152,155). Evidence suggests a high incidence of transient aPL following viral infections in pediatric patients (153,156-159). In 1 study of 88 children, aPL were found positive in 30%. In a recent work, at least 1 antibody to phospholipids was demonstrated in 89% of 122 children during various infections. In these patients, aPL positivity should be confirmed at least twice (158). In some children these antibodies are transient and disappear in 2 to 3 months; in others, they persist and may trigger the development of autoimmune disease (146).

The standard methods for detecting aPL include coagulation assays for LA, aPTT, and dilute Russell's viper venom time, for detecting anticardiolipin antibodies (aCL) and anti- β_2 GPI by ELISA. In general there is a good correlation across assays for aPL detection (2,147), as follows:

LA: In 1952, Conley and Hartman described 2 patients with SLE whose plasma contained a unique coagulation inhibitor that prolonged whole-blood clotting time and PT; fresh plasma addition did not correct this phenomenon (160). About 50% of patients, with a positive test, do not have SLE. Nevertheless, a positive LA appears to identify a subset of SLE patients who are susceptible to arterial or venous thrombosis (147). LA activity is screened by standard methods including aPTT, kaolin clotting time, dilute Russell's viper venom time, and plasma clotting time. LA assay is generally accepted as the assay that correlates best with thrombosis (145), although in patients with a positive

LA and negative aCL and anti- β_2 GPI antibodies, a false-positive LA should be considered (161).

aCL: The aCL test was originally described in 1983 with a radioimmunoassay and was later modified (162). aCL antibodies of the IgG, IgM, and IgA isotypes are routinely tested by solid-phase ELISA. The aCL test has high sensitivity (80-90%) for the APS syndrome, but low specificity as infections and some drugs may induce formation of these antibodies. High levels of IgG are present in the APS, while elevated IgM levels are frequently detected in infectious diseases. The value of the IgA aCL test in the diagnosis of the APS is uncertain but appears to be similar to IgG aCL in terms of thrombogenicity (145,163).

Anti- β_2 GPI: These antibodies (IgM or IgG) are detected by ELISA and are thought to have the most clinical significance. The β_2 GPI is a single-chain plasma protein of 326 amino acids arranged into 5 short domains. Recently it has been demonstrated that while antibodies directed against each of the 5 domains exist, only those that react with a specific epitope on domain I have LA activity and are strongly associated with thrombotic manifestations (145). There are conflicting opinions about which antibodies should be tested to detect patients at risk of thrombosis. aPL detected with anticardiolipin and anti- β_2 GPI ELISA does not correlate significantly with thrombosis, with exclusion of antibodies against domain I of β_2 GPI. The LA assay is generally considered as the assay that best correlates with thrombosis (164). This is probably due to the lack of standardization of the ELISA tests, so that large differences are found when assay materials from different manufacturers are used. Nevertheless, the recently updated Sapporo criteria for APS diagnosis includes the presence of IgG and IgM antibodies against β_2 GPI as a third serological criterion for APS (Table 7) (165).

Antineutrophil Cytoplasmic Autoantibodies (ANCA)

ANCA constitute a family of antibodies that targets antigens present mostly in azurophilic granules of polymorphonuclear leukocytes. ANCA were first reported in 1982 by Davies and coworkers in patients with necrotizing glomerulonephritis (166). They can be detected by II on human granulocytes. A granular cytoplasmic fluorescence pattern is called cytoplasmic or classic ANCA (c-ANCA): the target of this antibody is the enzyme proteinase 3. A perinuclear fluorescence is observed in the presence of antibodies (p-ANCA) directed to different antigens such as cathepsin, elastase, lysozyme, and myeloperoxidase (167-169). The c-ANCA pattern shows granular cytoplasmic fluorescence with central interlobular accentuation in the ethanol-fixed human neutrophils. The p-ANCA pattern shows staining localized just around the nucleus. The formation of ANCA depends on both genetic predisposition factors and environmental triggers.

Table 7 Laboratory Requirements for Diagnosing a Patient with the Antiphospholipid Syndrome

| |
|--|
| 1. Lupus anticoagulant Lupus anticoagulant present in plasma, on 2 or more occasions at least 12 weeks apart, detected according to the guidelines of International Society on Thrombosis and Hemostasis |
| 2. Anticardiolipin ELISA Anticardiolipin antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (ie, >40 GPL or MPL [1 GPL or MPL is equivalent to 1 μ g/mL of an affinity-purified IgG or IgM sample, respectively], or >99th percentile), on 2 or more occasions, at least 12 weeks apart, measured by a standardized ELISA |
| 3. Anti- β_2 GPI ELISA Anti- β_2 GPI antibody of IgG and/or IgM isotype in serum or plasma (in titer >99th percentile), on 2 or more occasions, at least 12 weeks apart, according to recommended procedures |

(Adapted from J Thromb Haemost 2006;4:295-306.)

ANCA may stimulate oxidative burst and degranulation of neutrophils, leading to tissue damage and perpetuation of chronic inflammation (170). ANCA have good sensitivity and specificity for the primary systemic vasculitides, including microscopic polyarteritis and necrotizing crescentic glomerulonephritis. It has been demonstrated that the PPV of a positive ANCA result is much higher in patients with severe glomerular disease (Table 8) (171). c-ANCA are highly specific for Wegener's granulomatosis (WG), being positive in more than 80% of cases (2,171). Positive c-ANCA results may be helpful in suggesting the possibility of WG, while increasing titers appear to precede disease relapse (172). As ANCA are present in the early stages of WG, their testing increases the likelihood of early diagnosis.

Table 8 Estimated Positive- and Negative-Predictive Values (PPV and NPV, respectively) of ANCA Results for Pauci-Immune Crescentic Glomerulonephritis (PI-CGN) in Children (<18 years old) with Different Presentations

| Clinical Presentation of Patient | Prevalence of PI-CGN (%) | PPV for PI-CGN | NPV for PI-CGN |
|---|--------------------------|----------------|----------------|
| Rapidly progressive glomerulonephritis | 48 | 0.98 | 0.80 |
| Hematuria, proteinuria, and creatinine >3 mg/dL | 16 | 0.90 | 0.95 |
| Hematuria, proteinuria, and creatinine 1.5 to 3 mg/dL | 15 | 0.89 | 0.95 |
| Hematuria, proteinuria, and creatinine <1.5 mg/dL | 1 | 0.30 | 1.0 |

(Adapted from Kidney Int 1998;53:796-8.)

p-ANCA may be found in the Churg–Strauss syndrome, microscopic polyangiitis, inflammatory bowel diseases (ulcerative colitis), primary sclerosing cholangitis, and, rarely, polyarteritis nodosa and Kawasaki disease. They have been found in up to 70% of children with SLE (173). Therefore, the clinical usefulness of p-ANCA is limited (2,88,117).

Section C: Third-Line Diagnostics

Genetic Tests

Recent advances in genetic methodology have increased our understanding about the contribution of genetics to the susceptibility and pathogenesis of rheumatic diseases. Much of the genetic work undertaken in the past 3 decades has centered around the HLA genes.

Many antigens of the major histocompatibility complex, in particular, the HLA of class I and II, have been associated with rheumatic disorders. The class I gene HLA-B27 is present in about 90 to 95% of white patients affected with ankylosing spondylitis and only in 7 to 8% of general population (174). Thus, the HLA-B27 genotype is highly sensitive for ankylosing spondylitis but it has a PPV of only 3% in the general population (175). HLA-B27 is also present in a disproportionate number of patients with other forms of spondyloarthritis (SpA) including reactive arthritis, psoriatic arthritis, inflammatory bowel disease, and isolated acute anterior uveitis (174). For these reasons, HLA-B27 testing is useful only in patients with clinical manifestations that suggest a diagnosis of SpA. Although the role of HLA-B27 in the pathogenesis of SpA is not completely understood, there is increasing evidence that it plays a direct causative role (174).

The genetic basis of JIA is complex, first because it is not a single disease but a group of clinical syndromes and second because classification of childhood arthritis has long been problematic. A number of susceptibility genes for JIA have been identified including HLA genes, genes encoding cytokines, their receptors, and other molecules playing a role in the pathogenesis of JIA. Several studies of genetic polymorphisms confirmed the association between JIA and the HLA antigens (176–180). Pauciarticular JIA with late onset and a strong male preponderance is associated with HLA-B27 and represents the group of juvenile spondyloarthropathies related to adult SpA. About 76% of patients with enthesitis-related arthritis have been reported to be HLA-B27-positive compared with a population frequency of 10% (180).

Among the class II genes, HLA-DR1 and HLA-DR4 increase the risk for polyarticular JIA in many populations. DR4 is strongly associated with IgM RF-positive polyarticular JIA, the subset that resembles adult RA (178). Oligoarticular JIA has been associated with HLA-A2, HLA-DRB1*11 (a subtype of HLA-DR5), and HLA-DRB1*08. The combination of HLA-A2, DR5, and DRB1*0201 occurs in about 10% of affected children (178).

Table 9 Single Gene Defects in Hereditary Periodic Fever Syndrome (see text for abbreviations)

| Disease | Inheritance | Gene/Protein |
|---------------|-----------------------------|--------------------------|
| FMF | Autosomal recessive | MEFV/pyrin |
| TRAPS | Autosomal dominant | TNFRSA1A/TNFR1 (p55) |
| HIDS | Autosomal recessive | MVK/mevalonate kinase |
| Blau syndrome | Autosomal dominant | NOD2 |
| FCAS | Autosomal dominant | CIAS1/cryopyrin or NALP3 |
| MWS | Autosomal dominant | CIAS1/cryopyrin or NALP3 |
| NOMID | Sporadic/autosomal dominant | CIAS1/cryopyrin or NALP3 |

See text for abbreviations.
(Reproduced with permission from *Arthritis Rheum* 2004;50:345–49.)

Uveitis is the most common eye involvement in JIA and can lead to significant visual impairment, with a high prevalence in the oligoarthritis disease subgroup. In a recent matched case-control work, Zeggini and coworkers reported an association of HLA-DRB1*13 and uveitis in Caucasian JIA patients (181).

The systemic autoinflammatory syndromes, or hereditary periodic fever syndromes (HPFS), are a newly recognized group of immune disorders characterized by recurrent episodes of fever and serosal, mucosal, and/or cutaneous inflammation. These include FMF, TNF receptor-associated periodic syndrome (TRAPS), hyper-IgD syndrome, familial cold autoimmune syndrome, Muckle-Wells syndrome, and neonatal-onset multisystemic inflammatory disease (NOMID or CINCA) (182–184). More than 170 mutations have been identified in the 4 genes responsible of these 6 recurrent fever syndromes: identification of single genetic defects have impacted diagnosis, pathogenesis, and treatment (Table 9) (182). The mutated gene in FMF (MEFV gene) was identified in 1997 on the short arm of chromosome 16, consisting of 10 exons and 781 codons (185). The MEFV gene encodes a protein called marenostriin or pyrin, belonging to the family of proteins involved in the innate immune response (186). At present, >40 mutations have been detected with M694V and V726A being most prevalent (187). Homozygous M694V mutation is associated with more severe disease and a higher risk of developing amyloidosis, whereas E148Q homozygosity accounts for a mild phenotype (188,189).

Since 1999, hyper-IgD syndrome has been attributed to a recessive mutation in the mevalonate kinase gene, located on the long arm of chromosome 12, encoding an enzyme involved in cholesterol biosynthesis (190).

Mutations in the cold-induced autoinflammatory syndrome gene are associated with a spectrum of autoinflammatory diseases including familial cold autoimmune syndrome,

Muckle-Wells syndrome, and CINCA, the so-called cryopyrinopathies. Cold-induced autoinflammatory syndrome 1 encodes for cryopyrin (or NALP3), a protein involved in regulation of the proinflammatory cytokine IL-1 β and that participates in nuclear factor-kappa B activity. Both pyrin and cryopyrin bind to another common adaptor protein called ASC, which activates caspase 1, resulting in IL-1 release. The IL-1 proteins have a central role in the pathogenesis of fever and inflammatory diseases (191). TRAPS, also known as familial Hibernian fever, a rare autosomal-dominantly inherited fever, is caused by mutation in the p55 TNF receptor I gene on chromosome 12 (192). The reduction in plasma levels of the soluble receptor of TNF has been proposed as the pathogenic mechanism underlying these HPFS. It is supported by preliminary reports demonstrating that etanercept, a TNF α antagonist, is effective in reducing the severity, duration, and frequency of symptoms in TRAPS (193).

In patients with clinical manifestations suggesting in an autoinflammatory syndrome, the rate of detection of mutations is usually low. Because the clinical manifestations of these disorders overlap, it is difficult to decide which gene should be screened first. Recently, Gattorno and coworkers, in a population study, identified a set of clinical parameters that can predict the probability of carrying mutations in 1 of the genes associated with the most frequent HPFS (MEFV, mevalonate kinase, TNF receptor I genes). Young age at onset, positive family history of periodic fever, thoracic pain, abdominal pain, diarrhea, and oral aphthosis were highly associated with positive genetic test results. The authors proposed a flowchart based on the clinical profile of patients with suspected HPFS to identify patients that need to undergo genetic analysis. The aim of this diagnostic flowchart was to reduce the number of tests performed and the related costs of analyses (194).

In the last decade, the study of the human genome (genomics) led to the development of new sophisticated methodologies such as microarray and proteomic-based techniques (proteomics) that allowed much progress in the study of the rheumatic diseases. Proteomic techniques, including mass spectrometry and multidimensional high-pressure liquid chromatography, allow a global analysis of the genes that are expressed in cells and tissues of an individual under different conditions and during disease. Measurement of gene expression profile produced an increasing panel of molecules that may represent early biomarkers in various rheumatic diseases (195). These biomarkers can be used as diagnostic and prognostic measures in clinical practice and will help researchers find defective proteins associated with a particular disease and may lead to the development of new drugs. The ultimate goal is individualized therapy, with a much higher degree of success than is currently available.

DISCUSSION

Laboratory investigations play an important role in the diagnosis and follow-up of inflammatory rheumatic diseases of children. They can allow the confirmation of a suspected diagnosis, assess disease activity, and measure the response to treatment (1,2). Testing of the ESR, CRP, hemoglobin level, white blood cell count, protein electrophoresis, and urinalysis is helpful in many cases. Autoantibody determination and genetic studies are required in some patients. However, physicians must be cognizant of the fact that commonly used tests such as ANA and RF often give positive results even in patients who do not have a rheumatic disease (100), to avoid misdiagnosis and inappropriate treatment. Therefore, these tests should be ordered and interpreted in the context of clinical suspicion. Repeated measurements of autoantibodies in specific rheumatic diseases are suggested only if the utility of these tests for disease monitoring has been demonstrated (117).

In recent years, the identification of susceptibility genes for autoinflammatory diseases has broadened the clinical spectrum as well as the molecular basis of these syndromes. These advances offer new tools to help clinicians achieve an accurate diagnosis and to establish targeted treatment. Activation of the IL-1 β pathway is a common mechanism in the pathogenesis of autoinflammatory diseases. The pivotal roles of IL-1 β in cryopyrin-associated periodic syndromes and TNF in TRAPS have resulted in effective therapies targeting these proinflammatory cytokines and have uncovered other new potential targets for anti-inflammatory therapy (192).

In the future, proteomic technologies will probably revolutionize clinical care of children with rheumatic disease. This science promises to provide tools for the discovery of biomarkers for diagnosis and prediction of disease course, guiding therapeutic selection and monitoring response to therapy (195).

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