Rheumatology Panel in Pediatric Practice

RAKESH KUMAR PILANIA AND SURJIT SINGH

From Allergy Immunology Unit, Department of Pediatrics, Advanced Pediatrics Centre, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India.

Correspondence to: Dr Surjit Singh, Head, Department of Pediatrics, and Chief, Allergy Immunology Unit, Advanced Pediatrics Centre, PGIMER, Chandigarh 160 012, India. surjitsinghpgi@rediffmail.com.

Common rheumatological disorders encountered in pediatric practice are juvenile idiopathic arthritis, systemic juvenile idiopathic arthritis, Kawasaki disease, Henoch-Schonlein purpura, systemic lupus erythematosus, chronic uveitis and juvenile dermatomyositis. Diagnosis of these disorders requires a critical appraisal of the clinical history, physical examination and relevant investigations. Laboratory tests are helpful for screening purposes as also for confirmation of diagnosis and monitoring of disease activity. These tests should, however, only be ordered after due deliberation and in the context of clinical findings in a given patient.

Keywords: *Autoimmune disorders, Diagnosis, Juvenile idiopathic arthritis, Systemic lupus erythematosus*.

Rheumatological diseases commonly seen in children include juvenile idiopathic arthritis (JIA), systemic JIA (sJIA), Kawasaki disease (KD), Henoch-Schonlein purpura (HSP), systemic lupus erythematosus (SLE), chronic uveitis, Takayasu arteritis (TA) and juvenile dermatomyositis (JDM). The initial presentation may overlap with each other, and even with non-rheumatological disorders such as infections. The diagnosis of these conditions is primarily clinical. Laboratory tests can facilitate screening, confirmation of diagnosis, and monitoring the disease activity and response to treatment. However, these tests need to be used judiciously and always in context of the clinical setting [1]. In this review, we describe and discuss various laboratory tests that are performed in clinical practice.

BASIC LABORATORY TESTS

Hemogram: Anemia is seen in most rheumatological disorders and is usually indicative of chronicity or disease activity. Anemia is generally normocytic and normochromic but can be microcytic. A normal hemoglobin level on follow-up in JIA is reassuring as it indicates reasonable disease control. Autoimmune disorders like SLE results in associated autoimmune hemolytic anemia and a positive direct Coombs test [2]. Total leucocyte count (TLC) is usually increased in inflammatory disorders like JIA. SLE causes leucopenia specifically, lymphopenia. A high platelet count is usually indicative of ongoing inflammation seen in KD, JIA and TA. Fresh onset thrombocytopenia may indicate macrophage activation syndrome (MAS). Thrombocytopenia in active lupus disease is commonly associated with antiphospholipid syndrome (APS) [3].

C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR): The CRP and ESR are acute phase reactants and provide useful information regarding disease activity and prognostication. Increased ESR may be seen in severe anemia. In inflammatory disorders it is a reflection of elevated fibrinogen which is an acute phase reactant. An ESR of more than 100 mm/hour is usually indicative of a serious underlying disorder like rheumatologic disease, malignancy or infection. Children with JDM may show a discordance between disease activity and ESR levels [4]. A rapid fall in ESR with underlying rheumatological disorder may have sinister connotation as it may herald the onset of MAS [3]. CRP is a sensitive marker for inflammation which is synthesized in the liver. It has low specificity and may be elevated in infections. It reflects changes in disease activity earlier than ESR. CRP is elevated in active lupus when associated with ongoing infection, serositis or arthritis [5]. CRP is elevated in MAS where ESR is usually normal or decreased (***Fig.* 1**) [5,6].

Urinalysis: Urinalysis is important for assessment of patients with several rheumatological disorders. Renal involvement can be a primary manifestation of HSP, SLE, antineutrophilic cytoplasmic antibodies (ANCA) associated vasculitis and secondary amyloidosis due to sJIA. KD is the commonest cause of sterile pyuria in children which can lead to an erroneous diagnosis of a urinary tract infection [7]. Microscopic hematuria, proteinuria and casts are suggestive of active disease in

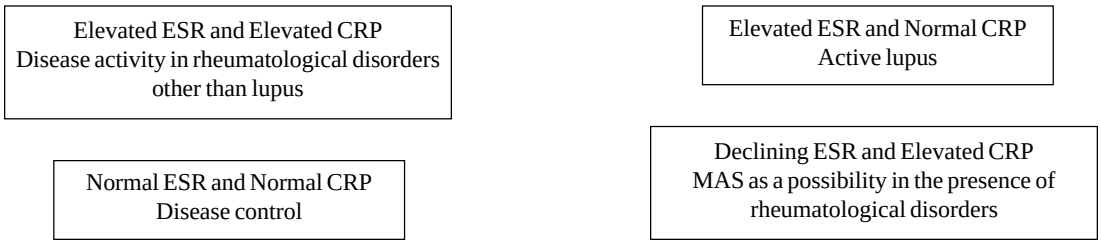


FIG. 1 *Role of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in rheumatological disorders.*

SLE nephritis. A 24-hour urinary protein quantification indicates severity of lupus nephritis. Frequent urine examination is recommended for early identification of amyloidosis in patients with sJIA and polyarticular JIA.In HSP, significant renal involvement is usually seen only in children above the age of 7 years. In some children with HSP, the renal involvement may not manifest at first presentation, and may become apparent only on follow-up [8]. Urine examination in HSP is recommended weekly for first 1 month and every 2-4 weeks thereafter till 6 months [9].

Serum ferritin: Ferritin is an acute phase reactant and correlates with disease activity. It can differentiate sJIA and other inflammatory disorders like KD where serum ferritin levels are significantly elevated in sJIA with normal or slightly raised levels in KD [10]. Serum ferritin levels are helpful in early identification of MAS [11].

Rheumatoid factor (RF): RF is an IgM antibody (but may be IgG, IgA or IgE as well) directed against the Fc portion of IgG. It is a nonspecific inflammatory marker that can be elevated in infections like tuberculosis, infective endocarditis, hepatitis C infection, osteomyelitis and in connective tissue disorders. The usual methods for estimation of RF are nephelometry and enzyme-linked immunosorbent assays (ELISA). It is useful in classification of JIA and prognostication of polyarticular JIA. A positive RF may suggest onset of erosive disease. According to International League of Associations for Rheumatology (ILAR), RF should be positive on two separate occasions for the diagnosis of RF positive JIA (polyarthritis) as transient elevation of RF is seen in infectious illnesses. A positive RF does not indicate rheumatoid arthritis .

Anti-Citrullinated peptide antibodies (ACPA): These antibodies were originally identified in rheumatoid arthritis and are a marker of severe disease course . While the role of these antibodies in diagnosis and prognostication of adult rheumatoid arthritis is well established their role in JIA is still not clear. Approximately 20% children with JIA can have raised ACPA titres. ACPA titres are high in RF positive polyarticular JIA. ACPA positivity indicates more aggressive form of JIA and earlier onset of RF negative JIA [12,13].

Synovial fluid analysis: Synovial fluid is present in small quantity in normal joints. Synovial fluid examination is primarily used for ruling out infections or crystal arthropathy. The latter is extremely rare in children [14]. The aspirated fluid should be subjected to total and differential leucocyte counts, protein and glucose estimation, Gram stain and culture. Leucocyte count is helpful in differentiating between non-inflammatory arthritis (<2000/mm^3) and inflammatory arthritis (≥2000/mm^3). Leucocyte count in synovial fluid in children with active JIA may be as high as 50,000-100,000/mm^3 with neutophilic predominance, decreased glucose, increase protein and low complements which can mimic septic arthritis [14]. Several studies have shown that there are differences in cytokine and proteomic profiles in synovial fluids of different JIA subtypes [15,16]. Synovial fluid CD4+:CD8+ T cell ratio reversal and increased levels of CCL5 chemokine predicts development of extended oligoarthritis. Increased IL-18 levels in synovial fluid predicts activity in systemic arthritis. Rarely synovial fluid can be hemorrhagic in hemophilia, pigmented villonodular synovitis or hemagioma. Synovial biopsy is helpful in diagnosis of tuberculous arthritis.

Muscle enzymes: Muscle enzymes can be elevated in inflammatory myopathies like JDM and include creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and aldolase. All muscle enzymes may not be elevated in a patient at the same time. Serum levels of these enzymes usually decrease 3-4 weeks prior to clinical improvement in muscle strength. Similarly, an increase in muscle enzymes can predict disease relapse 5-6 weeks prior to clinical manifestations. CK is the first to rise and first to fall. A combination of LDH and AST is the best predictor for disease activity in children with JDM [17].

Complement system: The complement system represents a cascade of more than 30 proteins and has 3 distinct pathways for activation viz *(a)* classical pathway, (*b*) mannose-binding lectin (MBL) pathway and (*c*) alternative pathway. All three pathways result in membrane attack complex formation and cell lysis. There are two broad methods for screening of complement system: (*a*) measurement of individual complement level (*b*) functional assay to assess functional ability of whole complement pathway (CH_{50}) or alternative pathway (AH_{50}). CH_{50} testing effectively screens for diseases of whole complement system except MBL and factor B or D of alternative pathway. In classical pathway activation both C3 and C4 will be low while low C3 and a normal C4 indicate alternative pathway activation [15]. Low C3, C4 is an important clue to diagnosis of SLE. Assay of complements also serves to evaluate disease activity and treatment response in patients with lupus as decreased C3 or C4 levels correlate with disease flare. Complement levels normalize with disease improvement Complement deficiency (inherited) can also be associated with early onset lupus [16]. Lupus due to complement deficiency has early onset of disease (usually <7 years of age) and has a predilection for the central nervous system [16].

AUTOANTIBODIES

The presence of autoantibodies provide important clues to diagnosis of rheumatological disorders. Tests that are commonly used in clinical practice include ANA, anti-dsDNA, Immunoblot assay, Anti-neutrophil cytoplasmic antibody *(*ANCA) and anti-phospholipid antibodies (APLs).

Anti-nuclear antibody (ANA): Most commonly used methods for detection of ANA are indirect immunofluorescence (IIF) and ELISA based assays. Gold standard for ANA detection is IIF based assay which is performed by incubation of the test serum with a substrate that allows antibody binding. Anti-human IgG antisera labelled with a flurochrome is added (***Fig. 2***). The preferred substrate is human epithelial (HEp-2) cells, although in the past this test was carried out on rat liver cells. The IIF test also shows the pattern and staining intensity of ANA positivity. The ANA pattern is dependent on the specific nuclear antigens that provide the substrate (***Fig.* 3**) [18]. ELISA is not the preferred method for detection of ANA and can be negative in at least a third of patients who test positive by IIF [18,19]. It allows high throughput of samples. The results are dependent upon specific nuclear antigens in the test system and can be false negative when a patient has an ANA that is not present in the commercial kit being used. A positive ANA is not synonymous with a diagnosis of lupus. Studies have shown that 9-15% healthy children can have positive ANA [20]. Results of ANA, therefore, must be interpreted in context of clinical findings [18]. ANA has very good negative predictive value but carries low specificity. Patients with other rheumatological disorders (*e.g.* scleroderma, mixed connective tissue disorders and overlap syndrome) can be ANA positive. In patients with oligoarticular JIA, positive ANA predicts risk of uveitis

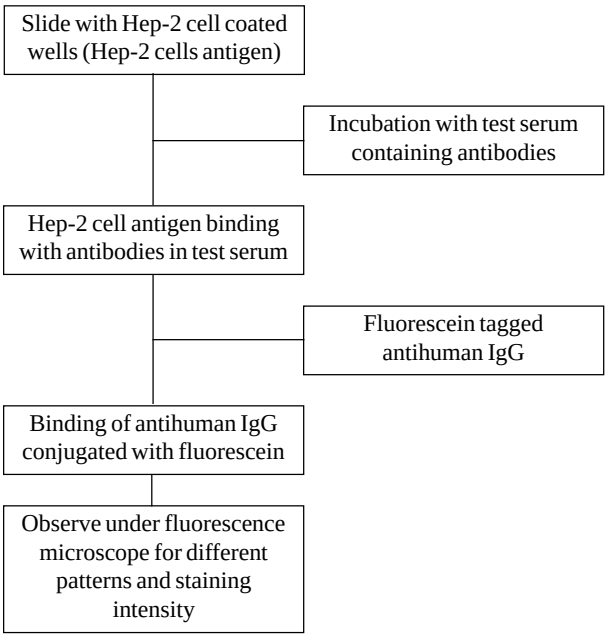
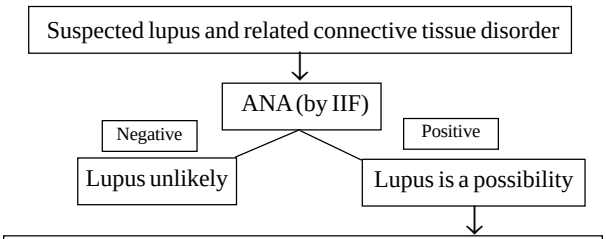


FIG. 2 *Indirect immunofluorescence method for measuring Anti-nuclear antibody (ANA).*



- *Diffuse ANA pattern:* usually seen in patients with lupus, especially lupus nephritis; also seen in drug induced lupus .
- *Speckled ANA pattern:* may be seen in lupus but is also associated with several other rheumatological disorders .
- *Nucleolar ANA pattern:* usually seen in patients with scleroderma.
- *Cytoplasmic ANA pattern:* usually seen in patients with overlap syndrome syndrome and lupus.

ANA: Anti-nuclear antibody

FIG. 3 *Different ANA patterns and association with disorders.*

[21]. ANA positivity can also predict the risk of development of underlying connective tissue diseases in children presenting with Raynaud phenomenon [22].

Anti-double-stranded DNA (Anti dsDNA) antibodies: These antibodies are highly specific and confirmatory for SLE. Measurement of these antibodies can be performed by various methods which include IIF and ELISA. Most specific method for estimation of anti-dsDNA is Crithidia lucilae based IF. It is highly specific for SLE but has poor negative predictive value. Elevated anti-dsDNA titers correlate well with active lupus nephritis and can be used to monitor disease activity [18,23,24].

Immunoblot assay: Immunoblotting is based on the principle of Western blotting where protein antigens (including nuclear and cytoplasmic antigens) are separated by using polyacrylamide gel electrophoresis. These antigens are then transferred to a nitrocellulose strip that is incubated with test serum. If antibody is present in the serum, it binds to a specific antigen over the membrane strip and that can be recognized by comparison with control results. The test will miss an antigen if not present in the commercial kit unlike IIF [18].

Anti-Neutrophil cytoplasmic antibodies (ANCA): ANCA are auto-antibodies against different antigenic components of azurophilic granules in neutrophils. ANCA is performed by IIF and ELISA based assays [25]. IIF is a more sensitive while ELISA is more specific. There are different IIF patterns of ANCA- cytoplasmic ANCA (c-ANCA); perinuclear ANCA (p-ANCA); and atypical ANCA. c-ANCA positivity results for binding to proteinase 3 (PR3) while p-ANCA positivity results for binding to Myeloperoxidase (MPO) enzyme. ELISA assay is used to confirm presence of specific antibody to PR-3 and MPO [18,26]. c-ANCA positivity is associated with granulomatosis with polyangiitis (GPA) while p-ANCA is positive in microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA).

Anti-phospholipid antibodies (APLs): The term APLs refers to a group of autoantibodies directed against phospholipid or plasma binding proteins. The three most important APLs that are screened are- lupus anticoagulant (LA); anti-β2 glycoprotein-I antibodies (anti-β2GPI) IgG and/or IgM; anticardiolipin antibodies (aCL) IgG and/or IgM. APS is an autoimmune syndrome and commonest prothrombotic disorder in children. APS is characterized by vascular thrombosis or pregnancy morbidity with persistently positive APLs. APS may occur as de novo (primary APS) or secondary to an autoimmune disorder (*e.g.* lupus). The prognosis and management of APS is determined by the type, number, and titer of specific APLs. APL positivity is defined with at least one of the antibodies positive twice at least 12 weeks apart (***Box* I**). It is recommended that all children with lupus should be screened for APLs at baseline and then annually [27,28].

IMAGING

While there are no specific imaging findings that can suggest diagnosis of JIA, it is helpful in disease assessment and monitoring [29] (***Table* I**).

BIOMARKERS

KD: Diagnosis of KD is largely clinical and no laboratory gold standard is available. Studies have shown that levels

BOX I COMMON ANTI-PHOSPHOLIPID ANTIBODIES

Lupus anticoagulant

- Best correlation with thrombotic events
- Defer testing if a patient has been started on anticoagulants

Anticardiolipin antibodies (aCL) IgG and IgM

- High titer positivity of IgG or IgM aCL on two or more occasions, at least 12 weeks apart
- High sensitivity for the APS syndrome, but specificity is low
- High titre of IgG aCL is suggestive of APS, while an elevated isolated IgM aCL is frequently detected in infectious diseases
- Transient/mild elevation is not of clinical significance. Titers to be repeated after 12 weeks

Anti-β2 glycoprotein-I antibodies IgG and IgM

- High titer positivity of IgG or IgM anti-β2GPI on two or more occasions at least 12 weeks apart
- Highest sensitivity for predicting APS
- Domain-I antibodies are strongly associated with thrombus formation

Triple positivity

- Positive predictive value for APS is highest when all three APLs are positive

TABLE I IMAGING IN PEDIATRIC RHEUMATOLOGY

Imaging	*Role in Pediatric Rheumatology*
X-rays (in JIA)	• Help in rule out structural damage • Help in ruling out other possible causes of joint pain and swelling, such as trauma, skeletal abnormalities, and bone tumors *Limitations* • Soft tissue changes are not identified • Virtually no role in diagnosis of JIA
Ultrasound imaging of joints	• Non-invasive, no radiation exposure, and can be repeated frequently • Helpful in detection of tenosynovitis, enthesitis, cartilage and bone abnormalities • Can detect inflammation and early bone erosions • Facilitates intra-articular injections *Limitations*: • Highly operator dependent; requires skill and experience • Interpretation in growing skeleton needs comprehensive knowledge of anatomical details at different ages
MRI of musculoskeletal system	• Ideal imaging modality for assessment of pathology of soft tissues (muscles, tendons, ligaments, fasciae), bones, and joints (especially during early stage of arthritis). Muscle involvement assessed in JDMS. • Gold standard modality for identification of subclincal disease, monitoring and to see response to therapeutics in patients with JIA • Special value in assessment for certain joints including temporomandibular joint, axial involvement (cervical spine- atlantoaxial instability, JIA with cervical spine involvement, and spinal cord compression) and sacroiliac joint involvement in HLA-B27 related arthritis *Limitations* • Is an expensive modality; may not be readily accessible • Longer scan times mandates sedation in young children • Expertise in interpretation of musculoskeletal MRI may not be easily available
2D-echocardiography	*KD*: assessment of coronary artery involvement and other cardiac manifestations *Takaysu arteritis*: assessment of cardiac dysfunction
CT angiography	Non-invasive method to asses degree of vascular involvement in Takayasu arteritis/ polyarteritis nodosa
Dual source CT coronary angiography	Assessment of coronary artery abnormalities in KD
PET scan	For assessment of disease activity in large vessels in Takayasu arteritis.

JIA: Juvenile idiopathic arthritis, JDMS: Juvenile dermatomyosistis syndrome, KD: Kawasaki Disease.

of interleukin (IL)-6, IL-17, IL-20 and tumor necrosis factor-α (TNF-α) are elevated in acute phase of disease [30]. Cardiac biomarker N terminal pro-B-type natriuretic peptide (NT-proBNP) is elevated in children with KD during the acute phase and is of value when the clinical presentation is incomplete or atypical [30-32]. Procalcitonin is a good marker to differentiate between viral infections and KD. High serum procalcitonin values are associated with increased risk of IVIg resistance in KD [33].

HSP: Renal involvement in HSP is vital for long term outcome. A rapid decline of factor XIII in HSP with severe gastrointestinal involvement is associated with greater risk of renal involvement [34]. Elevated D-dimers, elevated urinary monocyte chemotactic protein-1/creatinine ratio, and HLA-B35 has been found to associated with higher chances of development of HSP nephritis [35-37].

JIA: Increased levels of S100 proteins and IL-18 are associated with disease activity and predict therapeutic response in sJIA. Increased levels of soluble CD163, soluble IL 2 receptor-α, follistatin-like protein-1 and IL-18 are helpful in detection of subclinical MAS in sJIA. Elevated levels of serum matrix metalloproteinase

(MMP) 3 correlate with disease activity in patients with enthesitis-related arthritis. and structural joint damage in patients with sJIA [38,39].

SLE: Several blood (e.g. C4d, C5a) and urinary (*e.g.* monocyte chemoattractant proteins, NGAL, TWEAK, FOXP3) markers predict lupus nephritis in children with SLE. As most of these biomarkers are not specific for lupus nephritis, clinical extrapolation of these findings remains conjectural.

JDM: Patients with JDM can be classified on the basis of myositis-specific autoantibodies (MSA) and myositis associated autoantibodies (MAA) which predict treatment response, clinical course and long term outcome in these patients (***Table* II)** [38,40].

TA: Assessment of disease activity in patients with TA is fraught with problems because disease activity does not correlate with inflammatory markers like ESR and CRP. Levels of IL-6, MMP-2, -3 and -9, CCL5 chemokine, vascular cell adhesion molecules (VCAM) and Pentraxin-3 correlate well with disease activity [41].

GENETIC MARKERS

KD: Several susceptibility genes including inositol 1,4,5-trisphosphate 3-kinase C (ITPKC), caspase-3 calcium release-activated calcium modulator 1 (ORAI1), Fcγ receptor 2A (FCGR2A), B-cell lymphoid kinase (BLK) and CD 40 have been linked to the development of KD [42]. Microarray based studies have developed transcript blood gene expression signature to distinguish KD from other febrile conditions

JIA: Several HLA association have been identified for subtypes of JIA. HLA-B27 has a high degree of genetic polymorphism and encompasses more than 100 known subtypes. Most common subtype include HLA-B*27:05 followed by HLA-B*27:04 [43]. HLAB27 is assayed in the laboratory by ELSIA, flow cytometry, microcytotoxity or by polymerase chain reaction. Flow cytometry is the most widely used method though less specific than PCR which is the gold standard [43]. There is well established relationship between HLAB27 and juvenile spondyloarthropathies and ankylosing spondylitis. HLAB27 is also present in 5-10% of normal individuals. HLAB27 can also be seen in reactive arthritis, psoriatic arthritis and inflammatory bowel disease associated arthritis in variable frequency [44]. Testing for HLAB27 is usually considered in older children who present with asymmetrical large joint arthritis, enthesitis, sacroiliitis, acute symptomatic uveitis, arthritis with family history of ankylosing spondylitis, psoriatic arthritis and inflammatory bowel disease. RF negative polyarthritis shows genetic similarity to oligoarticular JIA, while RF positive polyarthritis is similar to adult onset rheumatoid arthritis (***Table* III**). Other important gene associations with oligoarticular JIA and RF negative polyarticular JIA include protein tyrosine phosphatase nonreceptor 22 (PTPN22), and signal transducer and activator of transcription factor 4 (STAT4) loci. Single nucleotide polymorphism in the *IL-1 ligand* gene, and *TNFRSF1A*, *mevalonate kinase (MVK)*, and *MEFV* genes have been identified in patients with sJIA [45].

SLE: Monogenic forms of lupus are seen in approximately 1% of patients with SLE [16]. Strongest association has been seen with C1q deficiency, where the risk of developing SLE is 90%. Other complement deficiencies that have variable association with SLE include C4 deficiency (75%), C1s or C1r deficiency (50%), and homozygous C2 deficiency (10-30%). Lupus has been linked to several HLA haplotypes as well (***Table* III**) [46].

TABLE II IMPORTANT MYOSITIS SPECIFIC AUTOANTIBODIES IN ASSOCIATION WITH JUVENILE DERMATOMYOSITIS

Autoantibody	*Incidence*	*Remarks*
Anti-p155/140 autoantibodies (anti-TIF 1)	~25%	Associated with chronic disease, cutaneous predominance, and lipodystophy.
Anti-MJ (NXP-2), also known as anti-p140	~20%	Associated with predominate muscle manifestations, caclinosis, and gastrointestinal manifestations.
Anti-synthetase autoantibodies	Usually not seen in children	Most common is anti-Jo-1. These antibodies are associated with severe disease, interstitial lung disease and higher mortality.
Mi-2	~8%	Associated with typical cutaneous disease and amyopathic forms. Disease responds very well to conventional treatment and better long term prognosis.
Ant-SRP	Usually not seen in children	Associated with severe and refractory disease. There are higher chances of Raynaud phenomenon and cardiac disease.

TABLE III IMPORTANT HLA ASSOCIATIONS IN PEDIATRIC RHEUMATOLOGY

Disease type	*HLA associations*
Oligoarticular JIA	A2, DRB1:01, DRB1:08, DRB1:11, DRB1:13, DPB1:02, DQA1:04, DQB1:04, DR5, DR8
Polyarticular RF- JIA	DRB1:08, DQAI*04 and DPB1:03
Polyarticular RF+JIA	DR4, DRB1*04, DQA1*03, and DQB1*03
Enthesiits related arthritis, psoriatic arthritis	B27
SLE	DRB1*15:03, DRB5*01:01, DRB1*03:01, DQA1*01:02, HLA, DQA1*05:01, DQB1*06:02, and DQB1*02:01.
Behcet's disease	HLAB*5101

JIA: Juvenile idiopathic arthritis; RF: Rheumatoid factor; SLE: Systemic lupus erythematosus.

Behcet disease: HLAB*5101 has the strongest genetic association with BD. However, the frequency of HLA-B51 varies from population to population. In patients where typical features of Behcet disease are not present, the presence of HLA-B51 helps in diagnosis [47].

MONITORING AND FOLLOW-UP OF DISEASE ACTIVITY

Treatment goals in rheumatological disorders include control of inflammation and maintenance of functional integrity. Monitoring is largely clinical and is supported by laboratory investigations. Several scoring systems have been developed for monitoring of disease activity and damage in these disorders [48,49] (***Web Table*** I).

CONCLUSIONS

The diagnosis of rheumatological disorders in pediatric practice is based on a detailed clinical assessment and a judicious use of relevant laboratory investigations. It is imprudent to order 'panel' of rheumatological investigations without keeping the clinical context in mind.

REFERENCES

1. Spencer CH, Patwardhan A. Pediatric rheumatology for the primary care clinicians-recognizing patterns of disease. Curr Probl Pediatr Adolesc Health Care. 2015;45:185-206.
2. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. J Allergy Clin Immunol. 2010;125:S238-47.
3. Ravelli A, Minoia F, Davì S, Horne A, Bovis F, Pistorio A, *et al*. 2016 classification criteria for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis: A European League Against Rheumatism/ American College of Rheumatology/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. Ann Rheum Dis. 2016;75:481-9.
4. Breda L, Nozzi M, De Sanctis S, Chiarelli F. Laboratory tests in the diagnosis and follow-up of pediatric rheumatic diseases: an update. Semin Arthritis Rheum. 2010;40:53-72.
5. Dima A, Opris D, Jurcut C, Baicus C. Is there still a place for erythrocyte sedimentation rate and C-reactive protein in systemic lupus erythematosus? Lupus. 2016;25:1173-9.
6. Ravelli A, Davì S, Minoia F, Martini A, Cron RQ. Macrophage Activation Syndrome. Hematol Oncol Clin North Am. 2015;29:927-41.
7. Pilania RK, Bhattarai D, Singh S. Controversies in diagnosis and management of Kawasaki disease. World J Clin Pediatr. 2018;7:27-35.
8. Singh S, Devidayal, Kumar L, Joshi K, Minz RW, Datta U. Severe henoch-schönlein nephritis: Resolution with azathioprine and steroids. Rheumatol Int. 2002;22:133-7.
9. Watson L, Richardson ARW, Holt RCL, Jones CA, Beresford MW. Henoch schonlein purpura-a 5-year review and proposed pathway. PloS One. 2012;7:e29512.
10. Mizuta M, Shimizu M, Inoue N, Kasai K, Nakagishi Y, Takahara T, *et al*. Serum ferritin levels as a useful diagnostic marker for the distinction of systemic juvenile idiopathic arthritis and Kawasaki disease. Mod Rheumatol. 2016;26:929-32.
11. Singh S, Chandrakasan S, Ahluwalia J, Suri D, Rawat A, Ahmed N, *et al*. Macrophage activation syndrome in children with systemic onset juvenile idiopathic arthritis: clinical experience from northwest India. Rheumatol Int. 2012;32:881-6.
12. Spârchez M, Miu N, Bolba C, Iancu M, Spârchez Z, Rednic S. Evaluation of anti-cyclic citrullinated peptide antibodies may be beneficial in RF-negative juvenile idiopathic arthritis patients. Clin Rheumatol. 2016;35:601-7.
13. Tebo AE, Jaskowski T, Davis KW, Whiting A, Clifford B, Zeft A, *et al*. Profiling anti-cyclic citrullinated peptide antibodies in patients with juvenile idiopathic arthritis. Pediatr Rheumatol Online J. 2012;10:29.
14. Akikusa J, Choo S. Laboratory investigations. In: Ross E. Petty, Ronald M. Laxer, Carol B. Lindsley, Lucy R. Wedderburn, editors. Textbook of Pediatric Rheumatology. Seventh Ed. 1600 John F. Kennedy Blvd. Ste 1800 Philadelphia, PA 19103-2899: Elsevier; *P*. 117-28.
15. Singh S, Bansal A. Twelve years experience of juvenile dermatomyositis in North India. Rheumatol Int. 2006;26:510-5.
16. Vignesh P, Rawat A, Sharma M, Singh S. Complement in autoimmune diseases. Clin Chim Acta Int J Clin Chem. 2017;465:123-30.
17. Bhattad S, Rawat A, Gupta A, Suri D, Garg R, de Boer M, *et al*. Early complement component deficiency in a single-centre cohort of pediatric onset lupus. J Clin Immunol. 2015;35:777-85.
18. Saikia B, Rawat A, Vignesh P. Autoantibodies and their Judicious Use in Pediatric Rheumatology Practice. Indian J Pediatr. 2016;83:53-62.
19. Meroni PL, Schur PH. ANA screening: An old test with new recommendations. Ann Rheum Dis. 2010;69:1420-2.

20. Hilário MOE, Len CA, Roja SC, Terreri MT, Almeida G, Andrade LEC. Frequency of antinuclear antibodies in healthy children and adolescents. Clin Pediatr (Phila). 2004;43:637-42.
21. Solomon DH, Kavanaugh AJ, Schur PH, American College of Rheumatology Ad Hoc Committee on Immunologic Testing Guidelines. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. Arthritis Rheum. 2002;47:434-44.
22. Falcini F, Rigante D, Candelli M, Martini G, Corona F, Petaccia A, *et al*. Anti-nuclear antibodies as predictor of outcome in a multi-center cohort of Italian children and adolescents with Raynaud's phenomenon. Clin Rheumatol. 2015;34:167-9.
23. Singh S, Abujam B, Gupta A, Suri D, Rawat A, Saikia B, *et al*. Childhood lupus nephritis in a developing country-24 years' single-center experience from North India. Lupus. 2015;24:641-7.
24. Abujam B, Gupta A, Suri D, Rawat A, Singh S. Trends and predictors of mortality in childhood onset lupus in a single North-Indian centre over 23 years: A retrospective study. Clin Exp Rheumatol. 2016;34:554-9.
25. Csernok E, Holle JU. Twenty-eight years with antineutrophil cytoplasmic antibodies (ANCA): how to test for ANCA — evidence-based immunology? Auto-Immun Highlights. 2010;1:39-43.
26. Bossuyt X, Cohen Tervaert J-W, Arimura Y, Blockmans D, Flores-Suárez LF, Guillevin L, *et al*. Position paper: Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. Nat Rev Rheumatol. 2017;13:683-92.
27. Ahluwalia J, Singh S, Naseem S, Suri D, Rawat A, Gupta A, *et al*. Antiphospholipid antibodies in children with systemic lupus erythematosus: A long-term clinical and laboratory follow-up status study from Northwest India. Rheumatol Int. 2014;34:669-73.
28. Groot N, de Graeff N, Avcin T, Bader-Meunier B, Dolezalova P, Feldman B, *et al*. European evidence-based recommendations for diagnosis and treatment of paediatric antiphospholipid syndrome: The SHARE initiative. Ann Rheum Dis. 2017;76:1637-41.
29. Colebatch-Bourn AN, Edwards CJ, Collado P, D'Agostino M-A, Hemke R, Jousse-Joulin S, *et al*. EULAR-PReS points to consider for the use of imaging in the diagnosis and management of juvenile idiopathic arthritis in clinical practice. Ann Rheum Dis. 2015;74:1946-57.
30. Rawat A, Singh S. Biomarkers for diagnosis of Kawasaki disease. Indian Pediatr. 2015;52:473-4.
31. Reddy M, Singh S, Rawat A, Sharma A, Suri D, Rohit MK. Pro-brain natriuretic peptide (ProBNP) levels in North Indian children with Kawasaki disease. Rheumatol Int. 2016;36:551-9.
32. Dionne A, Meloche-Dumas L, Desjardins L, Turgeon J, Saint-Cyr C, Autmizguine J, *et al*. N-terminal pro-B-type natriuretic peptide diagnostic algorithm versus American Heart Association algorithm for Kawasaki disease. Pediatr Int Off J Jpn Pediatr Soc. 2017;59:265-70.
33. Dominguez SR, Martin B, Heizer H, Jone P-N, Tong S, Davidson J, *et al*. Procalcitonin (PCT) and Kawasaki disease: Does PCT correlate with ivig-resistant disease, admission to the intensive care unit, or development of coronary artery lesions? J Pediatr Infect Dis Soc. 2016;5:297-302.
34. Kawasaki Y, Ono A, Ohara S, Suzuki Y, Suyama K, Suzuki J, *et al*. Henoch-Schönlein purpura nephritis in childhood: Pathogenesis, prognostic factors and treatment. Fukushima J Med Sci. 2013;59:15-26.
35. Fuentes Y, Hernández AM, García-Roca P, Valverde S, Velásquez-Jones LF, Sosa G, *et al*. Urinary MCP-1/ creatinine in Henoch-Schönlein purpura and its relationship with nephritis. Pediatr Nephrol Berl Ger. 2014;29:1047-52.
36. Mahajan N, Kapoor D, Bisht D, Singh S, Minz RW, Dhawan V. Levels of interleukin-18 and endothelin-1 in children with Henoch-Schönlein purpura: A study from northern India. Pediatr Dermatol. 2013;30:695-9.
37. Mahajan V, Singh S, Khullar M, Minz RW. Serum and urine nitric oxide levels in children with Henoch-Schonlein purpura during activity and remission: A study from North India. Rheumatol Int. 2009;29:1069-72.
38. Consolaro A, Varnier GC, Martini A, Ravelli A. Advances in biomarkers for paediatric rheumatic diseases. Nat Rev Rheumatol. 2015;11:265-75.
39. Swart JF, de Roock S, Prakken BJ. Understanding inflammation in juvenile idiopathic arthritis: How immune biomarkers guide clinical strategies in the systemic onset subtype. Eur J Immunol. 2016;46:2068-77.
40. Hussain A, Rawat A, Jindal AK, Gupta A, Singh S. Autoantibodies in children with juvenile dermatomyositis: A single centre experience from North-West India. Rheumatol Int. 2017;37:807-12.
41. Alibaz-Öner F, Aydýn SZ, Direskeneli H. Recent advances in Takayasu's arteritis. Eur J Rheumatol. 2015;2:24-30.
42. Onouchi Y. The genetics of Kawasaki disease. Int J Rheum Dis. 2018;21:26-30.
43. Chheda P, Warghade S, Mathias J, Dama T, Matkar S, Shah N, *et al*. HLA-B27 testing: A journey from flow cytometry to molecular subtyping. J Clin Lab Anal. 2018;32:e22382.
44. Weiss PF. Update on enthesitis-related arthritis. Curr Opin Rheumatol. 2016;28:530-6.
45. Singh S, Bhattad S, Danda D. Genetics of juvenile idiopathic arthritis. Int J Rheum Dis. 2014;17:233-6.
46. Costa-Reis P, Sullivan KE. Monogenic lupus: It's all new! Curr Opin Immunol. 2017;49:87-95.
47. Ozen S, Eroglu FK. Pediatric-onset Behçet disease. Curr Opin Rheumatol. 2013;25:636-42.
48. Demirkaya E, Consolaro A, Sonmez HE, Giancane G, Simsek D, Ravelli A. Current research in outcome measures for pediatric rheumatic and autoinflammatory diseases. Curr Rheumatol Rep. 2016;18:8.
49. Brunner HI, Ravelli A. Developing outcome measures for paediatric rheumatic diseases. Best Pract Res Clin Rheumatol. 2009;23:609-24.

WEB TABLE I CURRENTLY AVAILABLE SCORING SYSTEMS FOR MONITORING OF DISEASE ACTIVITY AND TREATMENT RESPONSE IN PEDIATRIC RHEUMATOLOGY

Disease	*Scoring system*	*Remarks*
JIA	• JADAS • Core set criteria	JADAS provides a numeric score. Hence it provides more objective assessment of disease activity
SLE	• SLEDAI • SELENA-SLEDAI • BILAG • SLAM • ECLAM	SLEDAI is one of the most commonly used scoring system for lupus
JDM	• CMAS • MMT-8 • DAS • MDAAT • CHAQ • CAT • MDI	Myositis activity is measured mainly by CMAS or MMT-8. DAS and MDAAT provide global assessment of disease activity. Accurate assessment of muscle disease and skin involvement helps the clinician in planning therapy; disease activity in muscle and skin may not always go hand in hand. Nail fold capillaroscopy is now an essential component for assessment of JDM in children.
TA	• DEI.-Tak • ITAS2010 • TADS	ITAS2010 and TADS are currently used for disease assessment in patients with TA; however, these have not been validated in children with TA.
Systemic sclerosis	• mRSS • J4S • Nail fold capillaroscopy • LoSCAT	mRSS is useful for skin scoring in systemic sclerosis. J4S is a multidimensional severity score and includes growth parameters, skin, vascular, osteoarticular, muscles and internal organ involvement (respiratory, renal, cardiac, gastrointestinal). Nail fold capillaroscopy is also important for monitoring patients with scleroderma. LoSCAT is useful for assessment for localized scleroderma

BILAG: British Isles Lupus Assessment Group; CAT: Cutaneous Assessment Tool; CMAS: Childhood Myositis Assessment Scale; CHAQ: Childhood Health Assessment Questionnaire; DAS: Disease Activity Scale; DEI.-Tak: Disease-extent index; ECLAM: European Consensus Lupus Activity Measurement; ITAS2010: Indian Takayasu Clinical Activity Score; JADAS: Juvenile Arthritis Disease Activity Score; J4S: Juvenile systemic scleroderma severity score; LoSCAT: Localized Scleroderma Assessment Tool; MDAAT: Myositis Disease Activity Assessment Tool; MDI: Myositis Damage Index; MMT: Manual Muscle Testing; mRSS: Modified Rodnan Skin Score; SLAM: Systemic Lupus Activity Measure, SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; TADS: Takayasu Arteritis Damage Score.