Review Article

Role of Biomarkers in Juvenile Idiopathic Arthritis

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The aim of this review was to identify the utility of biomarkers used in the diagnosis and the monitoring and treatment of Juvenile idiopathic arthritis (JIA). JIA is a heterogeneous group of diseases characterized by arthritis of unknown etiology that lasts for at least 6 weeks, with onset before the age of 16 years. Unlike adult rheumatoid arthritis, JIA is a heterogeneous arthritis with different subtypes. The diagnosis of JIA is based on clinical evaluation and often involves ruling out other causes of arthritis. Many biomarkers have been studied or are under development to define the classifications of JIA, assess disease activity, predict disease course, treatment response, or the risk of relapse. The diagnostic and prognostic use of biomarkers in JIA could be applied differently depending on diverse subtypes or individuals. To obtain the best clinical outcomes in JIA, it is important to understand the pathophysiology of each JIA subtype and to select and use appropriate biomarkers. **(J Rheum Dis 2020;27:233-240)**

Key Words. Juvenile idiopathic arthritis, Biomarkers, Outcome measures

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in childhood, and may result in significant morbidity [1]. JIA refers to the onset of arthritis with no defined cause before 16 years of age and includes symptoms that persist for at least 6 weeks [2]. The diagnosis of JIA can be difficult because JIA is a heterogeneous group of conditions that manifests inflammatory arthritis without a definite test to confirm the diagnosis. Furthermore, there are many diseases that can mimic JIA [3]. The current classification categories created for JIA are primarily for research purposes and are not intended for use as diagnostic criteria in the clinical setting [4]. Therefore, JIA is diagnosed clinically and is based on a combination of presenting signs and symptoms, blood tests, and if necessary, medical imaging. A number of biomarkers have been used or are under development to assist with the diagnosis of JIA. These biomarkers not only help to predict the course of the disease but are also useful to predict the patient's response to treatment and relapse.

This review therefore sought to identify the utility of biomarkers used in the diagnosis and treatment monitoring of JIA.

MAIN SUBJECTS

The utility of biomarkers used in JIA include classifying the JIA subtypes, evaluating disease activity, predicting the disease course, treatment response, or the risk of relapse. Table 1 summarizes the biomarkers (existing and promising) that can be used in JIA.

Consideration for new classification of JIA

Several classification systems for chronic arthritis in childhood have been proposed over the past few decades [5]. The older terms "juvenile rheumatoid arthritis," commonly used by the American College of Rheumatology (ACR), and "juvenile chronic arthritis" by the European League Against Rheumatism, were replaced by the term "juvenile idiopathic arthritis" (JIA) at the Pediatric

Table 1. Biomarkers that can be used in juvenile idiopathic arthritis

Utility	Biomarkers
Biomarkers that define JIA subtypes	RF
	HLA-B27
	ANA
	Anti-CCP
	Biomarkers increased in systemic JIA (S100 proteins, IL-18)
Biomarkers that measure disease activity	CRP
	ESR
	S100 proteins
	MMP-3
Biomarkers that predict disease course	RF
	Anti-CCP
	Biomarkers predicting MAS in systemic JIA (ferritin, platelet count, AST, triglycerides, fibrinogen, soluble CD25, soluble CD163 etc.)
Biomarkers that predict response to therapy and relapse	S100 proteins
	IL-18

JIA: juvenile idiopathic arthritis, RF: rheumatoid factor, HLA: human leukocyte antigen, ANA: antinuclear antibody, CCP: cyclic citrullinatedpeptide, IL: interleukin, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, MMP-3: matrix metalloproteinase-3, AST; aspartate aminotransferase, MAS: macrophage activation syndrome.

Standing Committee of the International League of Associations for Rheumatology (ILAR) in 1993 [6,7]. In the current classification system established by ILAR, JIA is further categorized into 7 distinct subtypes, based on their presentation within the first 6 months (Table 2). Most pediatric rheumatologists agree that the ILAR criteria are require revision for several reasons: the criteria have been established for more than 20 years, and the number of affected joints or the presence of psoriasis does not reflect a homogeneous immune pathogenesis [8,9]. Although there is controversy regarding the method employed to change the criteria, a consensus conference led by the Pediatric Rheumatology International Trials Organization (PRINTO) recently proposed a new classification system that is in the process of validation (Table 3) [10].

Biomarkers used to define the JIA subtypes

Biomarkers that are useful to classify the subtypes of JIA include rheumatoid factor (RF), human leukocyte antigen (HLA)-B27, antinuclear antibody (ANA), and anti-cyclic citrullinated peptide (CCP) antibodies.

RFs are antibodies directed against the Fc fragment of immunoglobulin G (IgG). They are heterogeneous and usually composed of IgM; therefore, most assays detect only IgM [11]. RFs cannot be used to diagnose JIA because IgM RFs are rarely found in children younger than 7 years of age, and can be detected not only in children with other autoimmune diseases but also in healthy children. Nevertheless, RFs are an important component of both the ILAR classification criteria and the newly proposed PRINTO criteria (Tables 2 and 3). RFs are most commonly detected in children with a later age at JIA onset and in those with polyarticular involvement [12]. In the PRINTO classification, RF-positive JIA is considered to be the pediatric counterpart of RF-positive rheumatoid arthritis (RA) in adults [10].

HLA-B27 is a class I surface antigen encoded by the B locus in the major histocompatibility complex, and has an affinity for antigenic peptides and presents them to cytotoxic T-lymphocytes [13]. The presence of HLA-B27 is an important part of the inclusion criteria used in the definition of enthesitis-related arthritis (ERA) based on the ILRA criteria or according to the definition of the enthesitis/spondylitis-related JIA provided by PRINTO (Tables 2 and 3). Approximately 60%~80% of children with ERA represent positive for HLA-B27 [12]. Although HLA-B27 is frequently present in these children, the presence or absence of HAL-B27 cannot be relied upon to confirm or exclude the diagnosis of JIA. The allele is an indicator of risk rather than a diagnostic test. HLA-B27 also has a strong association with ankylosing spondylitis (AS), which is notable considering that ERA is thought to be the pediatric counterpart of adult AS.

ANAs are antibodies that can be directed against cellular antigens and are detected predominantly in the nucleus and in other cellular components. ANAs may be found in 5%~18% of healthy children who do not show evidence of arthritis [14], which limits their use for diagnostic testing. However, ANAs are most frequently found in 65%~85% of young girls with oligoarthritis and uveitis [15,16]. These findings suggest that ANA positivity may represent a unique condition in JIA. Therefore, the PRINTO proposed a new diagnostic criterion called early-onset ANA-positive JIA that was not addressed in the ILAR criteria (Table 3) [10]. This subtype exists only in JIA and does not have a matching form in adults.

Table 2. International League of Associations for Rheumatology (ILAR) classification for juvenile idiopathic arthritis

Categories	Criteria

1. Systemic arthritis	• Fever of at least 2 weeks' duration and arthritis in ≥1 joint
	• Plus one or more of the following:
	    Erythematous rash
	    Lymphadenopathy
	    Serositis
	    Hepatomegaly and/or splenomegaly
	• Exclusions: a, b, c, d

2. Oligoarthritis	• Arthritis affecting ≤4 joints during the first 6 months of disease
	    There are 2 subcategories:
	    Persistent: affect 4 or fewer joints throughout the disease course
	    Extended: affect more than 4 joints after the first 6 months of disease
	• Exclusions: a, b, c, d, e

3. Polyarthritis RF-negative	• Arthritis affecting ≥5 joints during the first 6 months of disease • Test for RF is negative • Exclusions: a, b, c, d, e
4. Polyarthritis RF-positive	• Arthritis affecting ≥5 joints during the first 6 months of disease • Two or more test for RF at least 3 month apart during the first 6 months of disease are positive • Exclusions: a, b, c, e

5. Psoriatic arthritis	• Arthritis and psoriasis, or arthritis and at least 2 of the following:
	    Dactylitis
	    Nail pitting or onycholysis
	    Psoriasis in a first-degree relative
	• Exclusions: b, c, d, e

6. Enthesitis-related arthritis	• Arthritis and enthesitis, or arthritis or enthesitis with at least 2 of following:
	    The presence of or a history of sacroiliac joint tenderness and/or inflammatory
	     lumbosacral pain
	    The presence of HLA-B27 antigen
	    Onset of arthritis in a male over 6 years of age
	    Acute (symptomatic) anterior uveitis
	    History of ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory
	     bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative
	• Exclusions: a, d, e

7. Undifferentiated arthritis	• Arthritis that fulfills criteria in no category or in 2 or more of the above categories

RF: rheumatoid factor, HLA: human leukocyte antigen. One of the major aims of the ILAR classification is the mutual exclusivity of the subtypes. Therefore, the following list of possible exclusion for each category was defined; a) Psoriasis or a history ofpsoriasis in the patient or first-degree relative; b) Arthritis in an HLA-B27-positive male beginning after the sixth birthday; c) Ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease or acute anterior uveitis, or a history of one of these disorders in a first-degree relative; d) The presence of immunoglobulin M rheumatoid factor and at least two occasions at least 3 months a part; e) The presence of systemic juvenile idiopathic arthritis in the patient.

Anti-CCP antibodies are a group of autoantibodies that are directed against peptides and proteins that are citrullinated. Although the major clinical relevance of anti-CCP antibodies is their association with adult RA, they are also detected in children with JIA. In a study by Tebo et al. [17], anti-CCP antibodies were found in 73% of children with RF-positive polyarticular JIA, 19% with extended oligoarticular JIA, 13% with systemic JIA, 8% with RF-negative polyarticular JIA, 4% with ERA or persistent oligoarticular JIA, and in 2% of controls. Although anti-CCP antibodies have not yet been included in the ILAR classification criteria, they are included in the new PRINTO criteria, in which RF-positive JIA was defined as arthritis that persisted over 6 weeks, along with 2 positive tests for RF at least 3 months apart, or at least 1 positive test for anti-CCP antibodies (Table 3).

Among the JIA subtypes, systemic JIA is considered to be an autoinflammatory condition that is both clinically and immunologically distinct from the other categories of JIA and therefore requires different therapy. In addition,

Table 3. Pediatric Rheumatology International Trials Organization (PRINTO) classification for juvenile idiopathic arthritis

Categories	Criteria
1. Systemic JIA	• Fever of unknown origin that is documented to be daily for at least 3 consecutive days and reoccurring over a duration of at least 2 weeks and • Accompanied by 2 major criteria OR 1 major criterion and 2 minor criteria. Major criteria are (1) evanescent erythematous rash; and (2) arthritis. Minor criteria are (1) generalized lymph node enlargement and/or hepatomegaly and/or splenomegaly; (2) serositis; (3) arthralgia lasting 2 weeks or longer (in the absence of arthritis); and (4) leukocytosis ($\geq$ 15,000/mm^3) with neutrophilia
2. RF-positive JIA	• Arthritis for $\geq$ 6 weeks, and • Association with 2 positive tests for RF at least 3 months apart or at least 1 positive test for antibodies to cyclic citrullinated peptide
3. Enthesitis/spondylitis-related JIA	• Peripheral arthritis and enthesitis, or • Arthritis or enthesitis, plus $\geq$ 3 months of inflammatory back pain and sacroiliitis on imaging, or • Arthritis or enthesitisplus 2 of the following: (1) sacroiliac joint tenderness; (2) inflammatory back pain; (3) presence of HLA-B27 antigen; (4) acute (symptomatic) anterior uveitis; and (5) history of a spondyloarthritis in a first-degree relative
4. Early-onset ANA-positive JIA	• Arthritis for $\geq$ 6 weeks, and • Early-onset ($\leq$ 6 years), and • Presence of 2 positive ANA tests with a titer $\geq$ 1/160 (tested by immunofluorescence) at least 3 months apart Exclusions are systemic JIA, RF-positive arthritis, and enthesitis/spondylitis-related JIA
5. Other JIA	• Arthritis for $\geq$ 6 weeks • Does not fit criteria for disorders 1 to 4
6. Unclassified JIA	• Arthritis for $\geq$ 6 weeks • Fits > 1 disorder 1 ~ 4

JIA: juvenile idiopathic arthritis, RF: rheumatoid factor, ANA: antinuclear antibody, HLA: human leukocyte antigen.

children with this subtype are at risk of a severe, life-threatening complication called macrophage activation syndrome (MAS). In this unique type of JIA, the concentrations of S100 proteins such as S100A8–S100A9, S100A12, and interleukin (IL)-18 are increased in the plasma of children with systemic JIA [18]; therefore, these proteins may be supportive candidate biomarkers for the diagnosis of systemic JIA.

Biomarkers used to measure disease activity

The biomarkers most commonly used to assess disease activity in JIA are C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). CRP is an acute phase protein of hepatic origin the expression of which increases following IL-6 secretion by macrophages and T cells. CRP levels begin to increase within hours of the onset of inflammation, with levels peaking between 1 and 3 days [14]. The plasma half-life of CRP is 19 hours, making it a more sensitive and accurate reflection of the acute phase response than ESR [19]. In addition, CRP levels normalize more rapidly than ESR in response to therapy.

However, CRP does not have sufficient specificity to differentiate infectious from noninfectious inflammation in JIA. ESR is the rate at which red blood cells settle though plasma to form a sediment in whole blood in a standardized tube over a period of 1 hour. ESR is also a non-specific measure of inflammation that is included in both the Juvenile Arthritis Disease Activity Score (JADAS) and the core response variables identified by the ACR, which are the standardized and certified assessment tools of JIA disease activity. The JADAS includes 4 measures: physician global assessment of disease activity, parent/patient global assessment of well-being, active joint count, and ESR [20]. The ACR pediatric core criteria for JIA comprises 6 items: physician global assessment of disease activity, parent/patient global assessment of overall well-being, functional ability, number of joints with active arthritis, number of joints with limited range of motion, and ESR [21].

Other candidate biomarkers that are useful to measure disease activity are the S100 proteins and matrix metalloproteinase 3 (MMP3). The S100 proteins are a class of

pro-inflammatory calcium-binding proteins that are involved in the regulation of calcium homeostasis, enzyme activities, energy metabolism, cell growth and differentiation, and cytoskeletal dynamics [22]. In response to injurious or infectious stimuli, they also act as damage-associated molecular pattern molecules, with increased expression and release at sites of inflammation [23]. The most studied S100 proteins in JIA are S100A8 (also known as myeloid-related protein [MRP] 8 and calgranulin A), S100A9 (also known as MRP 14 and calgranulin B), and S100A12 (also known as MRP 6 and calgranulin C). S100A8 and S100A9 often exist as a complex of S100A8 and S100A9, (also known as calprotectin, and S100A8–S100A9), which is the most prevalent form under most physiological conditions. Increased levels of S100A8–S100A9 and S100A12 in the serum and synovial fluid were shown to correlate with disease activity in JIA, suggesting that these proteins may be promising markers for monitoring disease activity [24-26].

MMP-3 is an enzyme that in humans is involved in the breakdown of extracellular matrix, and may induce degradation of cartilage and bone in arthritis [27]. Concentrations of MMP-3 in the serum were higher in children with ERA than in healthy subjects and were correlated with clinical parameters of disease activity [28,29]. Therefore, MMP-3 is expected to serve as a useful biomarker for monitoring disease activity in JIA.

Biomarkers used to predict the disease course

RFs and anti-CCP antibodies are not only components of the classification criteria for JIA, but are also important predictors of disease prognosis. Older children with RF tend to have a longer disease duration, subcutaneous rheumatoid nodules or articular erosions, or a poorer functional class, which mimics adult RA [12,18]. In most studies, anti-CCP antibodies have been detected predominantly in patients with RF-positive polyarticular JIA [17]. The prognostic value of anti-CCP antibodies in JIA is yet to be studied, but in small studies they seem to be associated with a worse prognosis [30,31].

MAS is an aggressive and life-threatening complication of systemic inflammatory disorders. MAS occurs most frequently with systemic JIA [32]. The hallmark clinical features of MAS include high fever, hepatosplenomegaly, lymphadenopathy, hemorrhagic manifestations, and encephalopathy [33]. The characteristic laboratory features are pancytopenia, liver dysfunction, disseminated intravascular coagulation, hypofibrinogenemia, hypoalbuminemia, hyperferritinemia, hypertriglyceridemia, increased levels of lactate dehydrogenase, soluble CD25, soluble CD163, and decreased ESR levels [33]. With the recognition that MAS is clinically similar to hemophagocytic lymphohistiocytosis (HLH), some have applied the HLH-2004 diagnostic guidelines to the diagnosis of MAS (Table 4) [34]. However, the main shortcoming of using HLH-2004 guidelines is that some individual criteria may not be met

Table 4. Diagnostic criteria for macrophage activation syndrome

HLH-2004 diagnostic guidelines	Classification criteria for MAS complicating systemic JIA
A. Molecular diagnosis B. Diagnostic criteria • Fever • Splenomegaly • Cytopenia (at least 2 ~ 3 lineages: hemoglobin <90 gm/L, platelets $<100 \times 10^9$/L, neutrophils $<1.0 \times 10^9$/L) • Hypertriglyceridemia and/or hypofibrinogenemia (triglycerides ≥ 265 mg/dL, fibrinogen ≤ 1.5 g/L) • Hemophagocytosis BM, spleen, or lymph nodes • Low or absent NK cell activity • Ferritin ≥ 500 ng/mL • Soluble CD25 $\geq 2,400$	Ferritin >684 ng/mL and any 2 of the following: • Platelet count $\leq 181 \times 10^9$/L • AST >48 U/L • Triglycerides >156 mg/dL • Fibrinogen ≤ 360 mg/dL
The diagnosis of HLH can be established in presence of a molecular diagnosis consistent with HLH or by meeting 5 of 8 clinical and laboratory diagnostic criteria	A febrile patient with known or suspected systemic JIA is classified as having MAS if the above criteria are met

HLH: hemophagocytic lymphohistiocytosis, BM: bone marrow, MAS: macrophage activation syndrome, AST: aspartate aminotransferase, NK: natural killer cells.

for systemic JIA patients. Due to the remarkable inflammatory properties of systemic JIA, a relative decrease in leukocyte count, platelet count or fibrinogen level may be more useful for early diagnosis of systemic JIA than the absolute reduction required by the HLH-2004 standard. Therefore, the classification criteria of MAS complicating systemic JIA were developed in 2014, based on a combination of expert consensus, evidence from the medical literature, and analysis of patient data (Table 4) [34].

Biomarkers that predict response to therapy and risk for relapse

The S100 proteins are not only biomarkers for disease activity monitoring in JIA, but also serve as useful biomarkers to predict the response to treatment or disease flares in JIA. High serum levels of S100A8–S100A9 proteins before anti-rheumatic therapies were associated with good responses to methotrexate [35-37], and anti-tumor necrosis factor (TNF) agents [36]. In contrast, the higher levels of S100A8–S100A9 in the serum during remission were associated with a risk of relapse after discontinuation of methotrexate, which supported the hypothesis that the levels of these proteins in the serum could be useful parameters to inform the decision to discontinue treatment [37].

Similar to the findings for S100A8–S100A9, JIA patients with increased levels of serum S100A12 were also more likely to respond favorably to methotrexate or anti-TNF treatment. Increased levels of serum S100A12 during remission were associated with an increased risk of disease flare [38-40]. Similar to non-systemic JIA, increased serum levels of S100A8–S100A9 during clinical remission was also a useful biomarker to predict relapse after therapy withdrawal in systemic JIA [25,35]. IL-18 and S100A8–S100A9 serum levels may be candidate biomarkers for response to therapy in systemic JIA [41]. IL-18 serum levels increased in 4 out of 8 children who experienced failure in tapering of therapy and slightly increased in only 1 out of 7 patients who experienced successful tapering of therapy [42]. S100 proteins and IL-18 are considered for useful markers for predicting treatment response and relapse in systemic JIA and for the diagnosis of the JIA subtype.

CONCLUSION

Biomarkers serve as useful tools for the diagnosis of JIA subtypes, prediction of disease progression, and establishment of treatment strategies by assessing the response to therapy and relapse risk. The current classification of JIA remains a challenging area, especially given the heterogeneous nature of the disease. Therefore, to achieve the best outcome, it is important to carefully select appropriate biomarkers based on the disease subtype. As the genetic and pathophysiological understanding of JIA improves, new classification systems will define homogeneous groups of children, each with a biologically distinct disease. The identification of new biomarkers with more effective diagnostic/prognostic value could help to achieve the ultimate goal of facilitating the implementation of targeted therapies and tailored treatment interventions and improving remission rates while minimizing disease damage.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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