



Toward a Multibiomarker Panel to Optimize Outcome and Predict Response in Juvenile Idiopathic Arthritis

Juvenile idiopathic arthritis (JIA) is a complex disease with heterogeneous pathogenesis, autoinflammatory and autoimmune, involving both innate and adaptive immunity. All JIA subtypes display joint inflammation, but with distinct clinical phenotypes, disease courses, outcomes, and response to different treatment approaches¹. In the last decade, much attention was focused on discovery and potential use of different biomarkers that could provide support in diagnostic and prognostic evaluations. In the sense of diagnostics and personalized therapy decisions, biomarkers could play a major role to support initial diagnosis, allow disease monitoring, and possibly indicate the reoccurrence of inflammatory responses even before clinical manifestation. Such a candidate biomarker(s) should be validated and proven as highly sensitive, obtained by standardized methodology and evaluable in everyday clinical practice. Two reviews by Swart, *et al*² and Gohar, *et al*³ exhaustingly elaborated current knowledge and possible clinical usage of different biomarkers in JIA, pointing out applicability of S100 proteins.

The phagocyte-specific S100 proteins (calgranulins) S100A8 (calgranulin A, also referred to as myeloid-related protein, MRP8), S100A9 (calgranulin B, MRP14), and S100A12 (calgranulin C) are calcium-binding proteins and phagocyte activation markers acting as proinflammatory ligands of Toll-like receptor-4 (TLR-4), which are constitutively expressed predominantly in phagocytic myeloid cells (i.e., granulocytes and monocytes)⁴. S100 proteins (S100A8/A9 and S100A12) have been studied extensively in JIA and other inflammatory diseases and have been found to correlate with inflammatory indices, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)⁵. Synovial fluid concentrations of these proteins in the inflamed joints correlate and are about 10- to 20-fold higher compared to the serum levels⁶. MRP8/14 protein complex (also known as calprotectin) has been shown to be useful for diagnosing systemic-onset JIA. Serum MRP8/14 concentra-

tions in patients with active systemic JIA were found to be higher than in healthy controls, patients with systemic infections, and patients with autoinflammatory chronic infantile neurological cutaneous and auricular syndrome (44-, 6-, and 5-fold higher, respectively)⁷. MRP8/14 serum concentrations can also be used to detect subclinical inflammatory activity and predict relapse of the disease after therapy withdrawal in systemic JIA⁸.

The levels of circulating S100A8/A9 and S100A12 in patients with nonsystemic JIA are clearly not as high as those in patients with systemic JIA, but their possible role as immune biomarkers for (subclinical) disease activity is promising in both JIA patient groups. Both S100A8/A9 and S100A12 are validated predictors of relapse risk and disease activity in JIA^{9,10}. Interestingly, S100A12 concentration measured at the time of treatment withdrawal in patients with JIA predicted the development of flare better than MRP8/14, while the combination of S100A12 plus high-sensitivity (hs)CRP had the best prediction potential¹¹. Details about studies performed in nonsystemic JIA are presented in Table 1.

In this issue of *The Journal*, Gohar, *et al*¹² present results of the S100A12 concentrations (measured by commercial and in-house method) in patients with nonsystemic JIA from 3 prospective cohort studies [UK Childhood Arthritis Response to Medication Study for MTX treatment, and the Dutch Arthritis and Biologicals in Children Register and the German Registry for Biologics in Paediatric Rheumatology for anti-tumor necrosis factor (TNF) treatment]. In the Gohar, *et al* study, baseline serum S100A12 was found to be associated with response to both MTX and anti-TNF therapy in patients with JIA who have had a high baseline concentration that decreased significantly with either MTX or anti-TNF treatment¹². Patients with higher baseline S100A12 concentration had higher disease activity and ESR and were more likely to be treatment responders. Addition of S100A12 to multivariate model analysis improved the

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Table 1. Evidence of S100A8/A9 and S100A12 biomarker capabilities in nonsystemic JIA.

Frosch, <i>et al</i> ⁵	MRP8/14 concentrations in the synovial fluid of inflamed joints are about 10- to 20-fold higher compared to the serum levels with a correlation between these 2 levels. Serum concentrations of MRP8/14 correlated with the state of disease activity or remission in patients with oligoarticular JIA treated with IA steroids.
Foell, <i>et al</i> ⁶	Clear difference between S100A12 serum concentrations in oligoarticular or polyarticular JIA and those with systemic JIA. Remarkable elevation of S100A12 serum concentrations in samples obtained from patients with JIA experiencing a relapse of their arthritis during the following weeks.
Foell, <i>et al</i> ¹⁰	After MTX treatment discontinuation, MRP8/14 levels during remission were significantly higher in patients who subsequently developed flares compared with patients maintaining stable remission. Higher MRP8/14 concentrations were associated with risk of relapse after discontinuing MTX.
Moncrieffe, <i>et al</i> ¹⁶	High levels of baseline serum MRP8/14 have prognostic value in predicting a subgroup of patients whose arthritis will improve with MTX.
Anink, <i>et al</i> ¹⁷	High levels of baseline MRP8/14 are associated with good response to anti-TNF treatment, whereas elevated MRP8/14 levels at discontinuation of anti-TNF were associated with higher chance to flare in patients with JIA.
Kahn, <i>et al</i> ¹⁸	In nonsystemic JIA patients who fail MTX and need anti-TNF therapy, baseline MRP8/14 levels are found to be higher in responders compared to nonresponders, while after start of treatment the MRP8/14 levels decrease only in responders.
Gerss, <i>et al</i> ¹¹	S100A12 and MRP8/14 are especially useful in predicting early flares within 3 months after treatment withdrawal, and predictive performance of the marker S100A12 and MRP8/14 was improved by adding hsCRP.
Rothmund, <i>et al</i> ⁹	Biomarkers MRP8/14 and S100A12 assays that are available for routine practice are validated to be used for the prediction of JIA relapse after stopping medication.
Yamasaki, <i>et al</i> ¹⁹	Patients with JIA who stopped anti-TNF treatment flared within 6 months after treatment discontinuation if they have had significantly higher MRP8/14 levels compared to patients with stable remission.

JIA: juvenile idiopathic arthritis; MRP: myeloid-related protein; IA: intraarticular; MTX: methotrexate; TNF: tumor necrosis factor; hsCRP: high-sensitivity C-reactive protein.

prediction of response to treatment. In-house ELISA method assay was comparable with the commercial assay, but was found to be slightly more sensitive.

No single biomarker can be sufficiently sensitive or specific for predicting response¹³, especially in a heterogeneous disease such as JIA, which can follow an oligoarticular or polyarticular pattern emerging for different treatment strategies. The best proven candidates for multi-biomarker panel in nonsystemic JIA at the moment are S100A8/A9, S100A12, hsCRP, and antinuclear antibody. S100 proteins are involved in the amplification of the inflammatory process and are present in the crucial sites of joint destruction, in the synovial membrane and the cartilage-pannus junction¹⁴. So the extent of clinically or subclinically affected joints probably affects sensitivity of these biomarkers in nonsystemic JIA subtypes. That is why it would be rational to focus further efforts toward development and validation of separate multi-biomarker panels for the oligoarticular and polyarticular JIA course, with specific cutoffs. A recently published longitudinal study of patients with established rheumatoid arthritis in whom calprotectin (S100A8/A9) was shown to have the strongest association with ultrasound-detected synovitis and predicts response to biologic treatment¹⁵ provides the rationale for involvement of ultrasound in the clinically meaningful prediction panel for oligoarticular and polyarticular JIA courses. Therefore, longitudinal study of JIA patients with regular followup visits

using a multi-biomarker panel combined with imaging (i.e., ultrasound) is planned by the Outcome Measures in Rheumatology Clinical Trials (OMERACT) pediatric ultrasound group.

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