A subgroup of juvenile idiopathic arthritis patients who respond well to methotrexate are identified by the serum biomarker MRP8/14 protein

Halima Moncrieffe¹, Simona Ursu¹, Dirk Holzinger²³⁴, Fiona Patrick¹, Laura Kassoumeri¹, Angie Wade⁵, Johannes Roth² and Lucy R. Wedderburn¹

Abstract

Objectives. In JIA there is an unmet need for biomarkers with which to identify patients who will respond well to MTX. The aim of this study was to define the prognostic value of baseline serum proteins and clinical variables in response to MTX to help inform the clinician at time of diagnosis whether the patient is likely to respond well to MTX.

Methods. JIA patients were recruited into the Childhood Arthritis Response to Medication Study (CHARMS). Clinical data and venous blood were collected before administration of MTX and at follow-up. MRP8/14 and inflammatory cytokines were measured by ELISA and multiplex immunoassay, respectively. CRP and ESR were measured as part of routine clinical assessment. To explore which baseline factors might predict successful treatment, binary logistic regression models were fitted for outcome.

Results. High disease activity (high serum MRP8/14, active joint count or physician’s score) pre-MTX was observed in a subgroup of patients with a better response to therapy. In a multivariable analysis, after accounting for MRP8/14 at baseline, no other factors were independently significantly associated with outcome. Patients with baseline MRP8/14 >3000 ng/ml were more likely to respond to MTX at ACR50 or better: odds ratio 16.07 (95% CI 2.00, 129.3).

Conclusion. We have demonstrated that high levels of baseline serum MRP8/14 have prognostic value in predicting a subgroup of patients whose arthritis will improve on MTX. Routine collection of serum prior to the start of medication would be a valuable step in collaborative validation of such biomarkers.

Key words: juvenile idiopathic arthritis, methotrexate, response to treatment, biomarker, paediatric, rheumatology, disease outcome, CHARM study.

Introduction

JIA carries a significant burden of morbidity into adult life, especially if joint inflammation is not fully controlled [1–3]. MTX remains the first-line treatment for JIA when simple joint injections fail. However, clinical care pathways have been radically altered by the increasing availability of biologic agents coupled with data indicating their efficacy [4–7]. This has allowed clinicians to adopt a zero-tolerance approach to inflammation, aiming for full early remission in every child [8–10].

Despite these developments, clinicians and families have little evidence upon which to base drug choices [11], since currently there are no reliable biomarkers with which to identify children who will respond, or fail to respond, to either MTX or biologic agents. Therefore the more expensive biologics are offered only once MTX has failed or severe intolerance makes its administration impractical. This sequential therapeutic approach exposes children to drugs that may be ineffective yet have side effects. During this time children accrue disability and...
impaired quality of life. A more tailored approach to drug choice, based upon the use of validated biomarkers, could facilitate early remission induction for more children, reducing suffering and the burden of JIA to society. The lack of such biomarkers is a key knowledge gap that currently impedes good care for JIA.

Considerable evidence suggests that there may be an early window of opportunity when successful treatment leads to better outcomes [12]. Furthermore, an early favourable response to MTX is correlated with good long-term outcome in JIA [13]. Therefore the development of reliable biomarkers for use in clinical protocols to identify children who will respond well to MTX would be a considerable step forward for children with arthritis.

To address this need for reliable predictive biomarkers, we have established the SPARKS-Childhood Arthritis Response to Medication Study (CHARMS) [14]. JIA patients are recruited to CHARMS when they are about to start disease-modifying drug treatment for arthritis. Clinical and laboratory data are collected in parallel with biological material, prior to drug commencement and at follow-up. We have previously reported on gene expression and genetic and psychological aspects of response to MTX in JIA using the CHARMS cohort [14–16]. We have now tested the hypothesis that measurement of serum inflammatory proteins, either alone or in combination with clinical variables, before starting MTX may be valuable as biomarkers to separate children with a high chance of good response from non-responders. We measured serum cytokines and the myeloid-related protein heterodimer MRP8/14, since these are implicated in disease pathogenesis.

The inflammatory protein MRP8/14 (S100A8/9) has been shown to be a powerful biomarker with which to detect ongoing subclinical disease activity in children with JIA who have reached clinical remission on MTX [17, 18]. MRP8/14 is a calcium-binding protein and a proinflammatory ligand of Toll-like receptor 4 [19]. Our data suggest that a subset of children with a high chance of good response to MTX can be defined before starting treatment by high inflammatory activity at baseline, indicated by serum MRP concentration. In parallel, other measures of disease activity, including active joint count, physician’s assessment and inflammatory cytokines, show a similar pattern. In combination, these measurements are valuable in aiding clinicians to achieve a personalized medicine approach to JIA.

Patients and methods

Patients and samples

The CHARM study recruits children with all categories of JIA who fulfil ILAR criteria [20] and are about to start new disease-modifying medication for active arthritis [12]. The study has full ethical committee approval (Institute of Child Health/Great Ormond Street NHS Trust Ethics Committee) and is fully compliant with the Declaration of Helsinki. Subjects were recruited with fully informed parental consent, and child assent where appropriate, at Great Ormond Street Hospital, London, UK. Clinical data, including demographics, disease features, duration and activity, were collected prior to starting MTX (up to 4 weeks before commencing) and 6 months later (range 4–8 months). MTX was given at 10–15 mg/m²/week (median dose 13.2 mg/m²/week) by the oral (n = 70, 80.4%) or s.c. route (n = 14, 16.1%), according to physician’s choice (MTX route was unavailable for three patients). Data on IA joint injections and steroid use were collected. At each time point, venous blood was drawn. CRP and ESR were measured as part of routine clinical assessment and serum was stored at ~80°C for measurement of cytokines and other proteins.

Clinical assessment of response to treatment

To assess clinical response to medication, core set variables and Definition of Improvement (DOI) for JIA were used [21], comparing data at 0 and 6 months. These variables are physician’s global assessment of disease activity [visual analogue scale (VAS) of 0–10 cm: 0 = inactive, 10 = most severe], parent/patient global assessment of overall well-being (VAS of 0–10 cm: 0 = very well, 10 = very unwell), functional ability [Childhood Health Assessment Questionnaire (CHAQ): 0 = best, 3 = worst] [22], number of joints with active arthritis, number of joints with restricted range of movement and ESR (mm/h). The DOI of ACR30 requires at least 30% improvement from baseline in three of six variables, with no more than one remaining variable worsening by >30%. ACR50, ACR70 and ACR90 require improvements of 50%, 70% and 90% in at least three variables, with no more than one variable worsening by >30%. Non-responders (NRs) were defined as those who failed to reach even ACR30 improvement. In a secondary analysis, response was also measured using change in the Juvenile Arthritis Disease Activity Score (JADAS-10 [23]). JADAS-10 is a linear scale from 0 to 40 calculated from the parent VAS, physician VAS, ESR and number of active joints. Change in JADAS-10 score was calculated by subtracting the follow-up JADAS-10 score from the baseline score.

Patient classification

Using the DOI in JIA to assess response to treatment at 6 months [21], 69 (73.3%) patients reached an ACR30 response, 62 (71.3%) ACR50 and 49 (56.3%) ACR70. Note that all children who reach ACR70 automatically also reach ACR30 and ACR50, while those who achieve ACR50 also achieve ACR30. Subjects were categorized according to their highest response level and then divided into two groups depending on responder status at follow-up. Patients who showed no response or limited response to MTX (NR or ACR30) at follow-up were combined (designated N30), as were patients who achieved an outcome of ACR50 or above (designated R50).

Measurement of serological variables

Serum concentrations of MRP8/14 were determined by sandwich ELISA as previously described [24]. For comparison with earlier studies, internal control sera were used as a reference in all ELISA assays. The readers of
laboratory assays were blinded for diagnosis and inflammatory activity. CRP (mg/l) was measured as part of routine clinical assessment. Cytokines (IL-1β, IL-2, IL-6, IL-12, IL-17, IL-18, IL-22, IFN-γ and TNF-α) were measured using multiplex immunoassay as previously described [25].

Statistical analysis
Spearman’s ranked correlation was performed to assess the correlation between MRP8/14 and clinical variables. Differences in subgroups were compared using Mann–Whitney U tests; Wilcoxon matched-paired signed-rank test was used to compare variables pre- and post-MTX. To explore which baseline factors might predict successful treatment, binary logistic regression models were fitted for ACR outcome and linear regression was fitted for change in JADAS-10. Good response to MTX is indicated by a negative value for the change in JADAS-10. The potential predictor variables assessed were serum MRP8/14, CRP, the cytokines listed above, disease duration, age of onset and the core set variables: active joint count, physician’s global assessment, ESR, restricted joint count, CHAQ and parent/patient assessment. A multivariable model was used to investigate combined associations. Missing data were imputed using multiple imputation by PASW Statistics (v18, IBM). Variables were entered into the model in a stepwise fashion in order of significance post-adjustment for variables already in the model. Unadjusted odds ratios (ORs) are presented with 95% CIs. The ability of baseline clinical and serological variables to predict responder status at follow-up was assessed using receiver operating characteristic (ROC) curves in univariable and multivariable models. Fisher’s exact test was used to test for differences in frequency of steroid usage between patient groups. Analyses were performed using PASW Statistics and Prism (v5, GraphPad).

Results
Greater disease activity in JIA patients who will respond to MTX
Within the CHARM study, children with all categories of JIA are recruited prior to starting MTX for treatment of arthritis [14]. Patients with systemic JIA (sJIA) have recently been reported separately [26]. Eighty-seven patients with serological and clinical data were analysed (Table 1). The two largest groups starting MTX were children with extended oligoarticular and polyarticular RF-negative JIA (Table 1).

Fig. 1 shows baseline data for clinical and serum parameters that differed between patients who achieved response to MTX of ACR50 or higher at follow-up (hereafter referred to as R50) compared with those who failed to respond or achieved only ACR30 status (hereafter referred to as N30). There was no significant difference in patient age, disease duration, gender, MTX dose or route between groups. Comparing the core set variables at baseline we found that the number of active joints prior to starting MTX was significantly higher in the group who would achieve a better response to MTX (R50) than in N30 patients; a similar trend for higher pre-treatment values in R50 patients was observed for physician’s VAS (Fig. 1A).

Since these data suggested that children with high inflammatory activity prior to taking MTX had a greater chance of achieving a good response, we next compared inflammatory markers, both those routinely measured in the clinic (ESR and CRP) and also levels of the pro-inflammatory serum protein MRP8/14, which we have previously shown to correlate with disease activity in JIA [27]. Serum MRP8/14 and CRP levels prior to MTX treatment were significantly higher in R50 patients than in N30 patients (Fig 1B); however, baseline levels of ESR were not significantly different between the groups: 22.5 mm/h (IQR 8.5–38.8) vs 25 mm/h (IQR 11–65) (N30 and R50, respectively, P = 0.31). Since these parameters all measure aspects of disease activity, we tested for correlations between them. There was a positive correlation between serum levels of CRP and MRP8/14 (Spearman’s r = 0.6), but only weak correlation between MRP8/14 and the number of active joints or physician’s VAS (r = 0.3 each) (Fig. 1C).

We next asked whether serum levels of inflammatory cytokines were different between the two patient groups at baseline. Serum cytokine analysis showed that, in parallel with our findings for MRP and CRP, baseline levels of several proinflammatory cytokines (IL-2, IL-6, IL-12, IL-18 and TNF-α) were significantly elevated (P < 0.05) in patients who would achieve ACR50 status or greater at follow-up (Fig. 2). IL-17 levels were below

### Table 1 Baseline demographics of patients (n = 87) at time of starting MTX and enrolment to the study

<table>
<thead>
<tr>
<th>Patients (n = 87)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at JIA onset, years</td>
<td>5.17 (2.2–9.8)</td>
</tr>
<tr>
<td>Disease duration at MTX start, years</td>
<td>1.3 (0.4–4.9)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>62 (71.2)</td>
</tr>
<tr>
<td>JIA subtype at MTX start, n (%)</td>
<td></td>
</tr>
<tr>
<td>Oligoarticular persistent</td>
<td>15/87 (17.2)</td>
</tr>
<tr>
<td>Oligoarticular extended</td>
<td>18/87 (20.7)</td>
</tr>
<tr>
<td>Polyarticular RF−</td>
<td>35/87 (40.2)</td>
</tr>
<tr>
<td>Polyarticular RF+</td>
<td>8/87 (9.2)</td>
</tr>
<tr>
<td>Enthesitis-related arthritis</td>
<td>6/87 (6.9)</td>
</tr>
<tr>
<td>Psoriatic</td>
<td>5/87 (5.7)</td>
</tr>
<tr>
<td>Clinical variables at MTX start</td>
<td></td>
</tr>
<tr>
<td>Physician’s VAS</td>
<td>4.0 (2.1–6.0)</td>
</tr>
<tr>
<td>Active joints</td>
<td>5 (2–8)*</td>
</tr>
<tr>
<td>Restricted joints</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td>Parent VAS</td>
<td>3.3 (1.4–6.2)</td>
</tr>
<tr>
<td>CHAQ</td>
<td>0.8 (0.3–1.8)</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>25 (10–55)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>5 (3–13)</td>
</tr>
<tr>
<td>MTX dose (mg/m²)</td>
<td>13.2</td>
</tr>
</tbody>
</table>

Data are either median (IQR) or n (%) where indicated. Systemic patients were excluded from this study. *Significant (P < 0.05) difference between N30 and R50 groups.
Higher disease activity before starting MTX in patients who will have a better outcome at 6-month follow-up.

Patients were divided into two groups depending on ACR status at follow-up after MTX treatment: NR and ACR30 were combined (N30), and ACR50 and higher response were combined (R50). Each symbol represents the value of a given parameter for an individual patient prior to starting MTX treatment. (A) Active joint count and physician’s VAS prior to MTX therapy and (B) serum levels of MRP8/14 and CRP prior to MTX therapy. Horizontal bars represent median values and bars represent IQR. Statistical comparisons were performed using the Mann-Whitney test. (C) Scatter plots show levels of baseline serum MRP8/14 that had the highest $P$-value using DOI for MTX response in logistic regression analysis against baseline active joint count (left), physician’s VAS (right) and CRP (lower). Original (unimputed) data are shown. Each symbol represents the value of a given parameter for an individual patient prior to starting MTX treatment. Open circles represent patients who will have N30 outcome; black triangles represents patients who will have an outcome of R50.
the limit of detection (<2 pg/ml) in all samples (data not shown).

Since this was not a clinical trial, concomitant therapy was permitted as per physician’s choice. No patients received a biological agent in this study and the majority (77%) received NSAIDs. There was no significant difference in the percentage of patients who received IA joint injections between the N30 and R50 groups (44% vs 40.3%, respectively). However, two patients (8%) in the N30 group compared with 26 R50 patients (41.9%, \( P < 0.01 \)) had steroid treatment. Of patients who received concomitant prednisolone, the majority (81%) had a short course and had stopped after a median of 10 weeks, with only five patients still on prednisolone at the second time point of the study. Since the use of steroids at the start of MTX therapy may impact on treatment effectiveness, we next investigated baseline clinical and serological characteristics in only patients who were confirmed as not receiving any steroid treatment (n = 58). One patient did not have full steroid data available and was not included in this subanalysis. Among those not receiving steroids, again patients had significantly higher baseline MRP and CRP in the group who would respond to MTX. Median MRP was 2300 ng/ml (IQR 965–3460) in the R50 group vs 1495 ng/ml (IQR 923–1890) in N30 patients \( (P = 0.02) \) and CRP was 6 mg/l (IQR 4–12.5) vs 3 mg/l (IQR 3–5). In this subanalysis (children who had no steroids), there were no significant differences in baseline physician’s VAS or active joints between the two groups.

Together, these data suggest that a subset of patients with JIA who will respond favourably to MTX have higher baseline disease activity, indicated by higher MRP8/14, CRP or inflammatory cytokines, or greater numbers of

**Fig. 2** Serum cytokine levels are higher in patients who will respond better at follow-up.

Patients were divided into two groups depending on ACR status at 6-month follow-up after MTX treatment: NR and ACR30 were combined (N30), and ACR50 and higher response were combined (R50). Each symbol represents the value of a given cytokine for an individual patient prior to starting MTX treatment. Levels of IL-1β, IL-2 and IL-6 (top row), IL-12, IL-18 and IL-22 (middle row), and TNF-α and IFN-γ (bottom row) were measured in serum. Original (unimputed) data are shown.
active joints before starting MTX than those who fail to respond to MTX.

Modelling association of baseline clinical and serum variables with outcome

In order to calculate the association between baseline clinical and serum parameters and responder status at follow-up, logistic regression analysis and the area under the ROC curve were calculated. For the 87 JIA patients included in this study and used for the regression analysis, the dataset was 93.1% complete. Numbers (%)

Patients with missing data for each of the core set criteria were physician’s VAS ($n=3$, 3.4%), parent VAS ($n=1$, 1.1%), CHAQ ($n=1$, 1.1%), active joints ($n=0$, 0%), restricted joints ($n=0$, 0%) and ESR ($n=4$, 4.6%). Due to technical issues, some serum data were unavailable for the following patient numbers: cytokine measurements ($n=9$, 10.3%), MRP ($n=14$, 16.1%) and CRP ($n=16$, 18.4%). Using a listwise deletion method for handling missing data would result in 55 (63.2%) cases available for regression analysis if all variables were included in the model. Multiple imputation, a standard method for such datasets, was therefore performed. For univariable analysis, listwise deleted data gave similar results to imputed data (not shown), and the latter dataset was used to enable all children to contribute to both univariable and multivariable analyses.

Univariable logistic regression analysis showed that both clinical and serological parameters prior to starting MTX treatment had a significant association with outcome: high MRP/14, active joint count, physician’s VAS and IL-2 were all associated with a higher chance of achieving ACR50 or greater status at follow-up (Table 2). For each variable, the odds ratio of responding at ACR50 or higher was calculated per unit change (e.g. for CRP, per 1 mg/l), except for MRP/14, which has a wide range of values (170-18680 ng/ml), therefore 500 ng/ml was selected, and for MTX route, which is dichotomous (oral/s.c.). On average, each 500 ng/ml increase in MRP/14 serum concentration resulted in increased odds of achieving a response at ACR50 or higher at follow-up by 1.31-fold (95% CI 1.05, 1.63). In the multivariable analysis, after accounting for MRP/14 at baseline, no other factors were independently significantly associated with outcome. It was not possible to distinguish between ACR50 and better responders (supplementary Fig. S1, available at Rheumatology Online).

In order to determine the prognostic potential for MRP/14 and ascertain if this was greater than that of routine clinical measurement of CRP, ORs, sensitivity and specificity were calculated. Table 3 shows that patients with baseline MRP/14 >3000 ng/ml were more likely to be R50 patients: OR 16.07 (95% CI 2.00, 129.3). For CRP, serum concentration >25 mg/l provided an OR of 10.11 for likelihood of good response, although the CI was wide (0.56, 183.5) (supplementary Table S1, available at Rheumatology Online). Both MRP/14 and CRP had excellent positive predictive values, since high levels of these proteins were strongly associated with a high likelihood of good response.

**Table 2** Logistic regression analysis of MTX response in CHARMS JIA patients using baseline clinical factors, MTX route and serum MRP/14, CRP and cytokine levels

<table>
<thead>
<tr>
<th>Variable pre-MTX</th>
<th>Unadjusted OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP8/14, per 500-unit ng/ml increase</td>
<td>1.31 (1.05, 1.63)</td>
<td>0.02</td>
</tr>
<tr>
<td>Active joints, per one joint increase</td>
<td>1.18 (1.02, 1.36)</td>
<td>0.03</td>
</tr>
<tr>
<td>Physician’s VAS disease activity, per 1 cm increase</td>
<td>1.28 (1.01, 1.64)</td>
<td>0.04</td>
</tr>
<tr>
<td>IL-2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.06 (1.00, 1.12)</td>
<td>0.04</td>
</tr>
<tr>
<td>CRP, per 1-unit mg/l increase</td>
<td>1.13 (0.99, 1.29)</td>
<td>0.08</td>
</tr>
<tr>
<td>MTX route (s.c. vs oral)</td>
<td>3.00 (0.62, 14.5)</td>
<td>0.17</td>
</tr>
<tr>
<td>Restricted joints, per one joint increase</td>
<td>1.08 (0.95, 1.22)</td>
<td>0.26</td>
</tr>
<tr>
<td>ESR, per 1-unit mm/h increase</td>
<td>1.01 (0.99, 1.02)</td>
<td>0.29</td>
</tr>
<tr>
<td>IL-10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.02 (0.98, 1.06)</td>
<td>0.30</td>
</tr>
<tr>
<td>IL-1&lt;sub&gt;1&lt;/sub&gt;&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.58 (0.62, 4.00)</td>
<td>0.33</td>
</tr>
<tr>
<td>Age of JIA onset, per year increase</td>
<td>1.05 (0.94, 1.18)</td>
<td>0.40</td>
</tr>
<tr>
<td>IL-6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 (0.96, 1.10)</td>
<td>0.42</td>
</tr>
<tr>
<td>IL-18&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.00 (0.99, 1.01)</td>
<td>0.49</td>
</tr>
<tr>
<td>IL-22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.01 (0.99, 1.03)</td>
<td>0.51</td>
</tr>
<tr>
<td>TNF-α&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.07 (0.85, 1.35)</td>
<td>0.54</td>
</tr>
<tr>
<td>IL-12&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.00 (1.00, 1.01)</td>
<td>0.57</td>
</tr>
<tr>
<td>Disease duration, per year increase</td>
<td>0.97 (0.86, 1.10)</td>
<td>0.62</td>
</tr>
<tr>
<td>CHAQ, per 1-point increase</td>
<td>1.12 (0.65, 1.94)</td>
<td>0.69</td>
</tr>
<tr>
<td>IFN-γ&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.00 (0.99, 1.02)</td>
<td>0.79</td>
</tr>
<tr>
<td>Parent VAS, per 1 cm increase</td>
<td>1.01 (0.86, 1.19)</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Odds ratios are per unit increase, apart from MRP8/14, which has a large scale, and MTX route. Outcome is ACR DOI. This table was generated using imputed data. <sup>a</sup>Cytokines are per 1-unit pg/ml increase. <sup>b</sup>Unadjusted OR IL-2 rounded from 1.06 (1.002-1.123), IL-18 rounded from 1.004 (0.993-1.014), IL-12 rounded from 1.002 (0.995-1.009) and IFN-γ rounded from 1.002 (0.985-1.02).
In addition to analysis using the core set variables for JIA, we undertook a secondary analysis using the recently proposed JADAS score [23], which is a linear scale and allows disease activity to be assigned on a numerical spectrum; a decrease in JADAS score indicates improvement of disease. Change in JADAS score was calculated and univariable linear regression analysis was performed. Again, high baseline MRP8/14, active joint count and physician’s VAS were significantly associated with better response to MTX therapy (data not shown).

ROC areas under the curve for the four significant variables associated with ACR outcome were IL-2, 0.70 (95% CI 0.58, 0.81); MRP8/14, 0.68 (95% CI 0.57, 0.79); active joint count, 0.65 (95% CI 0.53, 0.77) and physician’s VAS, 0.63 (95% CI 0.50, 0.76). In a multivariable analysis, the area under the ROC curve for these four parameters rises to 0.82 (95% CI 0.73, 0.91). A comparison of the analysis using imputed and listwise deleted data showed similar results (data not shown).

### TABLE 3 Diagnostic value of various cut-off values of MRP8/14 serum protein concentration for predicting R50 outcome

<table>
<thead>
<tr>
<th>MRP cut-off (ng/ml)</th>
<th>OR</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>5.71 (1.67, 19.61)</td>
<td>60 (46, 74)</td>
<td>79 (54, 94)</td>
</tr>
<tr>
<td>2500</td>
<td>5.97 (1.55, 22.95)</td>
<td>53 (39, 67)</td>
<td>84 (60, 97)</td>
</tr>
<tr>
<td>3000</td>
<td>16.07 (2.00, 129.3)</td>
<td>47 (33, 61)</td>
<td>95 (74, 100)</td>
</tr>
<tr>
<td>3500</td>
<td>18.70 (1.07, 328.2)</td>
<td>32 (20, 46)</td>
<td>100 (82, 100)</td>
</tr>
</tbody>
</table>

Values in brackets are 95% CIs.

### Discussion

In this study we demonstrate the potential of MRP8/14 serum protein to identify a subset of patients who respond well to MTX. This pattern of high disease activity prior to treatment correlating with good response was also observed for other parameters, including CRP, joint count and physician’s global assessment, as well as for proinflammatory cytokines. Of clinical relevance, MRP8/14 has a higher specificity for distinguishing patients who will be good responders than any other single variable. Using OR, sensitivity and specificity, MRP8/14 provides advantages over the routine clinical measure CRP.

Our findings corroborate a previous retrospective study in JIA that found higher baseline physician’s global assessment associated with good response to MTX, although in that study a different definition of

### Changes in clinical and serum parameters at follow-up

In order to assess changes in serum and clinical parameters over time in relation to clinical response to treatment, measurements were also made at 6-month follow-up. As expected, in patients with a higher level of response at follow-up the active joint count decreased from 5 (IQR 2–10) to 0 (IQR 0–1), and physician’s VAS also decreased (data not shown); similar observations were made for serum MRP8/14 and CRP (Fig.3).

Interestingly, six patients in the R50 group had increased MRP8/14 levels at follow-up from their individual baseline values, despite reaching ACR50 or higher. Follow-up data were available on five of these patients: three subsequently had a disease flare (mean follow-up 2 years). Future studies will be important to test if absolute MRP8/14 concentrations over time for an individual patient will be useful in predicting response to treatment.

### Fig. 3 Changes in serum MRP8/14 and CRP in JIA patients after treatment with MTX.

Patients were grouped by ACR status at follow-up: NR and ACR30 were combined (N30), ACR50 and higher responses were combined (R50). Line graphs for each patient are shown before (pre) and at follow-up (post) of MTX for serum MRP8/14 (left panel) and CRP (right panel). Original (unimputed) data are shown. Comparisons were made using the Wilcoxon matched pairs signed-rank test.
response was employed [12]. That study also found that
time to treatment was an important effect: we found no
difference in time to treatment between responders and
non-responders, perhaps because time to treatment was
short overall (1.3 years) in this cohort.

MRP8/14 is a relatively abundant stable protein that can
be measured in serum without the need for cold storage,
unlike cytokines, which are highly labile and require rapid
sample processing and measurement or −80 °C storage of
serum prior to measurement [28]. Although quantification
of MRP8/14 in serum is not yet a routine clinical measure
in JIA, the protein is measured by many centres in stool to
quantify disease activity in IBD [29]. Given that serum
samples could be sent by post for MRP8/14 quantifica-
tion, it might be practical for a reference laboratory to offer
this measurement for several centres.

Different thresholds of MRP8/14 concentration result in
different confidence levels in correctly classifying the
patient’s response on follow-up, with a higher threshold
resulting in a greater likelihood of correctly classifying
patients. All patients with a baseline MRP8/14 serum
concentration ≥3500 ng/ml achieved ACR50 or better
response (R50) in our cohort. Notably, patients with a
baseline serum concentration of MRP8/14 below this
threshold may be either N30 or R50 patients. Thus,
while a low MRP8/14 level cannot distinguish all good
responders from non-responders, a high serum MRP8/14
level will provide clinicians with a raised OR for good
response to MTX.

MRP8/14 is secreted into serum following phagocyte
activation and signifies an activated innate immune
system. MRP8/14 had only moderate correlation with
CRP and weak correlation with other variables of disease
activity and therefore is not simply another measure of
inflammation. Our data suggest that there are several
mechanisms by which MTX can act to control inflamma-
tion in JIA, and that in the subgroup of good responders
identified by having high serum MRP8/14 early in disease,
MTX acts via suppression of the innate immune system.
That high baseline MRP8/14 was observed in some, but
not all, responders presumably reflects the heterogeneity
of disease mechanisms in JIA.

We previously demonstrated that MRP8/14 can serve
as a marker to predict risk of relapse after MTX withdrawal
in patients with clinical remission on MTX [17]. In that
study a level of MRP8/14 >690 ng/ml correlated with a
high risk of relapse after stopping MTX. Together these
studies indicate that the MRP8/14 protein may be useful in
predicting outcome in a variety of settings in childhood
arthritis (MTX withdrawal and predicting MTX response).
However, laboratories will require a test that is able to
quantify a wide range of concentrations of this protein to
apply this biomarker to clinical practice.

There are some limitations to this study. The CHARM
study was designed to collect data at baseline and after
6 months of MTX use (range of time at follow-up
4–8 months). With the increasing availability of biologics
and earlier switching of treatments after only 3–4 months
of MTX use, future studies may need to include data col-
collection and biological measurements at earlier time points.

Our study is relatively small and needs to be validated
in replication cohorts of patients. Future studies should
also be designed to allow analysis of inactive disease
and the outcome of clinical remission on medication [9],
since this is now the agreed clinical goal for JIA [30]. While
the proposed test alone will not be sufficient to drive
fully informed choices for use of MTX in JIA, this study
increases our evidence base for understanding and pre-
dicting response.

The prognostic power of serum MRP8/14 to predict
response to MTX that we have observed in our study
must now be tested in other patient cohorts and ultimately
in clinical trials. However, accurate prediction of response
to treatment for all children with JIA is likely to require
more than just one protein measurement. We and others
have demonstrated that both genetic and clinical factors,
such as time to treatment or pattern of joint involvement,
may be useful contributors to models for prediction
of response [12, 15, 31]. Similarly, in adult RA, clinico-
pharmacogenomic models have been proposed, and are
being tested, for prediction of response to both MTX [32,
33] and etanercept [34]. To achieve adequate statistical
power in such attempts, large cohorts will be required
to allow the combination of genetic, clinical and sero-
logical data and to validate such tools. With increasing
international collaboration and large inception cohorts
in JIA being studied [35, 36], we propose that routine stor-
age of a serum sample and a sample for DNA prior to
the start or switch of medication would be a valuable
step to enable validation of such biomarkers and so
to move towards effective, personalized medicine for
children with JIA.

**Rheumatology key messages**

- JIA patients with higher disease activity before MTX
  have a greater chance of achieving good clinical
  response to MTX.
- Serum MRP levels may predict a JIA patient
  subgroup likely to respond to MTX.

**Acknowledgements**

The authors thank all the patients and their families for
participating in this study, the ward and clinical staff for
help collecting samples and members of the CHARMS
and laboratory teams for sample handling and processing.
Childhood Arthritis Response to Medication Study (CHARMS)
study group: K. Burkle, A. Etheridge, P. Gilbert, A. Hinks, S.
Hirani, L. Kassoumeri, S. Lal, H. Moncrieffe, K. Mulligan, S.
Wedderburn and P. Woo. The authors thank Professor G.
Moore, Drs K. Nistala and D. Bending for feedback on this manu-
script and M. Saers and S. Schleifenbaum for excellent
technical assistance. The SPARKS-CHARMS study
was funded by SPARKS UK (grant reference 08ICH09)
and the Big Lottery Fund UK (grant reference RG/1/
010135231). The CHARM study is supported by the
UK Medicines for Children Research Network. L.W. is also supported by the Great Ormond Street Hospital Children’s Charity. D.H. and J.R. are funded by Bundesministerium für Bildung und Forschung (AID-NET, project 01GM08100), FP7 programme (Pharmachild; GA-No 260353) and the Interdisciplinary Centre of Clinical Research at the University of Muenster (IZKF CRA04). The funding sources had no involvement in study design, data analysis, report writing or decision to submit the article for publication.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data
Supplementary data are available at Rheumatology Online.

References
17 Foell D, Wulffraat N, Wedderburn LR et al. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remission: a randomized clinical trial. JAMA 2010;303:1266–73.


