ORIGINAL RESEARCH

Serum calprotectin (S100A8/A9): a promising biomarker in diagnosis and follow-up in different subgroups of juvenile idiopathic arthritis

Céline La,1,2,3 Phu Quoc Lê,2 Alina Ferster,2 Laurence Goffin,2 Delphine Spruyt,4 Bernard Lauwerys,5 Patrick Durez,5,6 Cécile Boulanger,5 Tatiana Sokolova,6 Joanne Rasschaert,4 Valérie Badot1

ABSTRACT

Introduction In the management of juvenile idiopathic arthritis (JIA), there is a lack of diagnostic and prognostic biomarkers. This study assesses the use of serum calprotectin (sCal) as a marker to monitor disease activity, and as a classification and prognosis tool of response to treatment or risk of flares in patients with JIA.

Methods Eighty-one patients with JIA from the CAP48 multicentric cohort were included in this study, as well as 11 non-paediatric healthy controls. An ELISA method was used to quantify sCal with a commercial kit.

Results Patients with an active disease compared with healthy controls and with patients with inactive disease showed an eightfold and a twofold increased level of sCal, respectively. sCal was found to be correlated with the C-reactive protein (CRP) and even more strongly with the erythrocyte sedimentation rate. Evolution of DAS28 scores correlated well with evolution of sCal, as opposed to evolution of CRP. With regard to CRP, sCal could differentiate forms with active oligoarthritis from polyarthritides and systemic forms. However, sCal brought an added value compared with active oligoarthritis from polyarthritides and systemic forms. Indeed, patients with active disease and reaching minimal disease activity (according to Juvenile Arthritis Disease Activity Score) at 6 months following the test had higher sCal levels, while patients with inactive disease had higher sCal levels if a flare was observed up to 3–9 months following the test.

Conclusions This study confirms the potential uses of sCal as a biomarker in the diagnosis and follow-up of JIA.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) represents a very heterogeneous disease and is divided in seven subgroups according to the International League of Associations for Rheumatology (ILAR) classification: oligoarthritis, rheumatoid factor (RF)-positive and RF-negative polyarthritis, enthesitis-related arthritis (ERA), psoriatic arthritis, systemic arthritis and undifferentiated arthritis.1 JIA can be associated with significant morbidity and mortality and represents an important cause of short-term and long-term disability.2,3 Immune pathogenesis of JIA is still incompletely understood. Disease probably results, as with most autoimmune diseases, from a combination of genetic susceptibility (Human Leukocyte Antigen HLA) and non-HLA-related genes, environmental factors and over-activation of innate and adaptive (T-cell or B-cell) immunity.3 The course of the disease is characterised by alternating periods of flares and remission. Some predictors of poor outcome have been identified (presence of RF, early radiographic changes, symmetrical disease, extension of...
arthritis at onset, positivity of antinuclear antibodies), but a lack of precise and reliable prognosis factors remains a major problem in JIA.\textsuperscript{2,3}

Serum calprotectin (sCal), also known as myeloid-related protein 8/14, is part of the S100 proteins family (with proinflammatory effects) and is formed by a stable heterodimer of S100A8 and S100A9 subunits.\textsuperscript{4} It has been described in many studies involving numerous inflammatory diseases (Crohn’s disease, cystic fibrosis, sepsis, etc.)\textsuperscript{5-7} including rheumatological diseases (rheumatoid arthritis, systemic lupus erythematosus, etc)\textsuperscript{8-12} and, more recently, in severe forms of COVID-19.\textsuperscript{13}

In JIA, sCal was shown to be elevated in patients with active disease, particularly in the systemic form, helping to distinguish them from patients with infections or malignant disease (which can present as a hard differential diagnosis at first).\textsuperscript{14-16} sCal has also shown promising results as a biomarker by predicting disease relapse after stopping nonsteroidal anti-inflammatory drugs (NSAIDs),\textsuperscript{17} methotrexate (MTX)\textsuperscript{18,19} or etanercept\textsuperscript{20} treatment.

In this study, we intended to measure sCal in a Belgian population of JIA patients among the CAP48 cohort and to correlate its level with the different clinical subgroups of JIA, disease activity and outcome (to evaluate the response to treatment in patients with active disease or the risk of flares in inactive disease).

METHODS
Study design
The patients were enrolled from an observational multicentric study (CAP48 cohort) and followed for a duration of 2 years at the time of this study (from January 2014 to January 2016). Patients were included from five different hospitals in Brussels and Wallonia (including Hôpital Universitaire des Enfants Reine Fabiola, Cliniques Universitaires Saint-Luc, Hôpital Erasme, Cliniques Universitaires Mont-Godinne and Clinique Saint-Pierre Ottignies).

The CAP48 cohort is divided in two subgroups: patients naïve to any disease-modifying antirheumatic drug (DMARD) therapy at the time of inclusion (intraarticular corticosteroids were allowed) and patients with an established disease (already treated with a DMARD). The patients were followed every 3 months during first year of follow-up, and subsequently every 6 months. Details of this study are described elsewhere.

Eighty-one patients were included and classified according to their JIA category and disease activity. Forty-five patients were in a state of disease inactivity or remission (all with an established disease). Thirty-six patients had an active disease at baseline (of whom 35 started a new treatment at baseline), and include 16 naïve and 20 patients with established JIA.

It was decided not to rely on healthy paediatric controls, given the ethical and technical difficulties underlying this type of sampling. Healthy controls were thus young adults and included from the biobank of the Department of Rheumatology of Hôpital Erasme.

Definitions
The inclusion criterion was a diagnosis of JIA according to the ILAR criteria.\textsuperscript{1}

At the time of the creation of the CAP48 cohort, the Juvenile Arthritis Disease Activity Score (JADAS) was still recent and validated cut-off values had yet to be determined, so disease activity was followed by both DAS28-CRP and JADAS10-CRP scores.\textsuperscript{21-25} Disease was thus herein considered active with a DAS28-CRP $\geq 2.6$ or a JADAS10-CRP $>2$ for the oligoarticular and $>3.8$ for the polyarticular forms. The disease inactivity was defined according to the American College of Rheumatology criteria revised in 2011,\textsuperscript{26} while remission was defined as a persistent inactivity for 6 months under stable treatment.

Response to treatment was assessed by the patients’ ability to reach minimal disease activity state at 6 months, according to JADAS cut-offs.\textsuperscript{27} The cut-offs for classification of minimal disease activity are defined as 2 for oligoarticular forms of JIA and 3.8 for polyarticular forms of JIA.\textsuperscript{27} Finally, the definition of flare was based on the criteria developed in 2002, and characterised by worsening of 2 core outcome variables (COV) by $\geq 40\%$ without concomitant improvement of more than one of the remaining COV by $\geq 50\%$.\textsuperscript{28} The core set of outcome variables consists of the number of joints with active arthritis, the number of joints with limited range of motion, the physician’s global assessment of disease severity on a 10 cm Visual Analogue Scale (VAS), the parent’s or patient’s global assessment of overall well-being on a 10 cm VAS, one laboratory marker of inflammation (CRP or sCal) and, more recently, in severe forms of COVID-19.\textsuperscript{13}
Figure 2  Levels of sCal or CRP according to clinical subgroups. (A and B) Levels of sCal according to clinical subgroups, with all forms of oligoarthritis grouped (A) or separated according to their categories (B). (C) Levels of CRP according to clinical subgroups. Results are given as mean±SEM (A–C). Statistical significance (*p<0.05, **p<0.01, ****p<0.0001) was assessed by Mann-Whitney test. CRP, C-reactive protein; ERA, enthesitis-related arthritis; OA-E, extended form of oligoarthritis; OA-P, persistent form of oligoarthritis; PA, polyarthritis; sCal, serum calprotectin.

Table 1  Clinical and biological parameters among patients achieving minimal disease activity and patients with an active disease after 6 months

<table>
<thead>
<tr>
<th></th>
<th>Minimal disease activity (n=24)</th>
<th>Active disease (n=11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at disease onset (years), median (range)</td>
<td>8.5 (1.1–15.8)</td>
<td>12.6 (2.8–16.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Disease duration (years), median (range)</td>
<td>2.5 (0.2–15.1)</td>
<td>3.3 (0.1–8.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>12 (50.0)</td>
<td>5 (45.5)</td>
<td>ns</td>
</tr>
<tr>
<td>Categories of JIA, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic</td>
<td>3 (12.5)</td>
<td>1 (9.1)</td>
<td>ns</td>
</tr>
<tr>
<td>Oligoarthritis</td>
<td>13 (54.2)</td>
<td>4 (36.4)</td>
<td></td>
</tr>
<tr>
<td>Persistent</td>
<td>9 (37.5)</td>
<td>3 (27.3)</td>
<td></td>
</tr>
<tr>
<td>Extended</td>
<td>4 (16.7)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>PA-RF+</td>
<td>1 (4.2)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>PA-RF−</td>
<td>2 (8.3)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ERA</td>
<td>5 (20.8)</td>
<td>4 (36.4)</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ANA+, n (%)</td>
<td>7 (29.2)</td>
<td>1 (9.1)</td>
<td>ns</td>
</tr>
<tr>
<td>Number of DMARDs used, mean (SEM)</td>
<td>1.3 (0.2)</td>
<td>2.1 (0.3)</td>
<td>ns</td>
</tr>
<tr>
<td>TJC, mean (SEM)</td>
<td>1.7 (0.5)</td>
<td>1.4 (0.5)</td>
<td>ns</td>
</tr>
<tr>
<td>SJC, mean (SEM)</td>
<td>1.9 (0.6)</td>
<td>3.1 (1.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Physician VAS, mean (SEM)</td>
<td>19.1 (3.8)</td>
<td>27.8 (6.1)</td>
<td>ns</td>
</tr>
<tr>
<td>Patient/parent VAS, mean (SEM)</td>
<td>18.9 (6.9)</td>
<td>25.4 (8.2)</td>
<td>ns</td>
</tr>
<tr>
<td>CHAQ, mean (SEM)</td>
<td>0.3 (0.1)</td>
<td>0.4 (0.2)</td>
<td>ns</td>
</tr>
<tr>
<td>CRP (mg/L), mean (SEM)</td>
<td>10.9 (3.5)</td>
<td>11.5 (3.4)</td>
<td>ns</td>
</tr>
<tr>
<td>ESR (mm/hour), mean (SEM)</td>
<td>21.5 (9.3)</td>
<td>36.5 (8.7)</td>
<td>ns</td>
</tr>
<tr>
<td>DAS-28CRP, mean (SEM)</td>
<td>2.5 (0.3)</td>
<td>2.7 (0.4)</td>
<td>ns</td>
</tr>
<tr>
<td>JADAS10-CRP, mean (SEM)</td>
<td>6.2 (1.3)</td>
<td>8.6 (2.2)</td>
<td>ns</td>
</tr>
<tr>
<td>sCal (ng/mL), mean (SEM)</td>
<td>12 138 (2133)</td>
<td>5165 (1255)</td>
<td>* (0.03)</td>
</tr>
</tbody>
</table>

Results are given as numbers, percentages, median±range or mean±SEM. Statistical significance (*p<0.05, ns: not significant) was assessed by Mann-Whitney test. ANA, antinuclear antibody; CHAQ, Childhood Health Assessment Questionnaire; CRP, C-reactive protein; ERA, enthesitis-related arthritis; ESR, erythrocyte sedimentation rate; JIA, juvenile idiopathic arthritis; PA, polyarthritis; RF, rheumatoid factor; sCal, serum calprotectin; SJC, swollen joint count; TJC, tender joint count; VAS, disease evaluation on a Visual Analogue Scale.
inflammation (erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP)), and a functional assessment tool based on the Childhood Health Assessment Questionnaire (CHAQ).\textsuperscript{28-29}

sCal measurements

sCal was measured at baseline in all 81 patients (with an active or inactive disease). The serum samples were frozen and stored at −80°C.

The levels of sCal were measured by ELISA method with a commercial kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland), chosen after a review of literature, that would theoretically deliver more consistent results. The intra-assay coefficient of variability was 5%.

Statistical analysis

The statistical analyses were made using SPSS V.23.0 (IBM) and GraphPad Prism V.6 (GraphPad Software, La Jolla, California, USA).

Categorical data are described as numbers (and in percentages), while continuous data are described according to their mean (and standard error of the mean (SEM)) or their median (and range).

Comparisons were done with non-parametric Mann-Whitney tests. Correlations between measures were assessed with Spearman’s rank correlation coefficient. Receiver operating characteristic (ROC) curves were also generated to appraise sensitivity and specificity of measuring sCal as a prognostic tool.

RESULTS

Demographic data at baseline

The patients had a median age of 12.6 years, with a predominance of female (F:M sex ratio of 1.9:1). The median disease durations were 0.8 and 3.9 years in the naïve and established cohort, respectively. Most of the patients were treated with MTX and approximately one-fifth of patients were treated with a biological DMARD. More detailed information about their baseline parameters is available in online supplemental additional file 1. Patients with active or inactive disease did not differ in age, gender or JIA subtypes.

The control population had a median age of 26.2 years, with a predominance of female (F:M sex ratio of 1.7:1).

Levels of sCal according to disease activity and clinical and biological parameters

The levels of sCal were significantly higher in patients with an active disease than in patients with an inactive disease, illustrated by a twofold increased level of sCal (11403 ng/mL compared with 6555 ng/mL) (figure 1). sCal was also significantly higher than in healthy controls, measured at 1737 ng/mL.

Furthermore, the sCal also correlates with some clinical (tender joint counts (TJC) and CHAQ) and biological (ESR and CRP) markers of disease activity. There is an even greater correlation with ESR (r=0.79, p<0.001) than with CRP (r=0.45, p<0.05), and sCal correlates to a lesser extent with JADAS10-CRP score (r=0.2, p<0.05). However, the evolution of sCal correlated well with the evolution of JADAS10-CRP over time (r=0.8, p<0.05). Moreover, the evolution of DAS28 score correlated strongly with the evolution of sCal over the same period of time (r=1, p<0.05), but not with the CRP.

Levels of sCal according to clinical subgroups

Systemic arthritis differs significantly from polyarthritis, ERA and oligoarthritis with regard to levels of sCal and CRP (figure 2). When all forms of oligoarthritis (ERA, persistent and extended) are grouped and considered as a whole, their levels of sCal (7515 ng/mL) also differ significantly from polyarthritis (14714 ng/mL) and systemic arthritis (26976 ng/mL). ERA was associated with the lowest levels of sCal and CRP. All subgroups included patients with both active and inactive disease, on or off treatment, and did not differ from each other according to these conditions.
Levels of sCal according to the response to treatment
Among the 35 patients with active disease at baseline and starting a new treatment, 68.6% will reach a state of minimal disease activity according to their JADAS score at 6 months (or 9 months if lack of follow-up data at 6 months). These patients did not differ from those with an active disease at 6 months with regard to any clinical or laboratory parameters at baseline, except for higher levels of sCal, whereas CRP did not allow similar discrimination (table 1, figure 3A).

With a threshold fixed at 9095 ng/mL, the ROC curves obtained a sensitivity of 91% and a specificity of 46%, with a likelihood ratio of 1.68 and an area under the curve (AUC) of 0.73 (figure 3B).

Levels of sCal according to the risk of flares
Within the 45 patients in remission at baseline, 8.9% will experience a flare in the following 3–9 months. They had significantly higher levels of sCal at baseline than patients who remained in prolonged remission under stable treatment (table 2, figure 4A). No difference in CRP levels was seen between the groups.

The analysis of ROC curves identified a threshold fixed at 10285 ng/mL associated with a sensitivity of 100%, a specificity of 78% and a likelihood ratio of 4.56 (figure 4B). The AUC was in this case of 0.95.

DISCUSSION
The levels of sCal were significantly higher in JIA patients than in healthy controls, being further increased with disease activity, as previously found by other teams. sCal correlates with TJC and may also be a reflection of disease severity (CHAQ), as previously reported by some authors. We confirm its correlation with inflammatory markers (CRP and ESR) but discover a large variability of sCal for normal CRP values. Moreover, unlike CRP, sCal is strongly correlated with the evolution of disease activity according to JADAS10 and DAS28. All these data are consistent with the results obtained in other inflammatory diseases, which underline the interest of sCal as a marker of disease activity and therefore of disease severity. In particular, a recent study showed that high plasma levels of calprotectin could be a robust biomarker for severe forms of COVID-19.

Our data show that sCal has the same ability as CRP to differentiate between oligoarthritis, polyarthritis and systemic forms, thus confirming the results described in systemic forms by Frosch et al. ERA forms also tend to have lower levels of sCal compared with other forms, and polyarthritis forms tend to have intermediate levels of sCal ranging between systemic and oligoarthritis forms. This highlights the potentially crucial role of sCal in aiding the diagnosis of JIA. Although interpretation of these results may be limited by the number of patients, it appears that the patients with ERA in our cohort predominantly had a combination of arthritis and enthesitis, or isolated enthesitis. Specifically, 8 of these 11 patients had arthritis (6 with oligoarticular and 2 with polyarticular forms), including 4 with associated enthesitis. Two patients had isolated enthesitis.

Table 2 sCal and CRP levels according to risk of flares among patients in remission

<table>
<thead>
<tr>
<th>Flare within 3–9 months (n=4)</th>
<th>No flare within 3–9 months (n=41)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCal (ng/mL), mean (SEM)</td>
<td>26073 (5295)</td>
<td>7066 (1126)</td>
</tr>
<tr>
<td>CRP (mg/L), mean (SEM)</td>
<td>0.6 (0.5)</td>
<td>1.2 (0.2)</td>
</tr>
</tbody>
</table>

Results are given as mean±SEM. Statistical significance (**p<0.001, ns: not significant) was assessed by Mann Whitney test.

CRP, C-reactive protein; sCal, serum calprotectin.

Figure 4 Levels of sCal according to the risk of flares. (A) Levels of sCal according to the risk of flare among patients in remission. (B) Receiver operating characteristic curves of risk of flares according to sCal or CRP. Results are given as mean±SEM (A). Statistical significance (**p<0.001) was assessed by Mann-Whitney test (A). CRP, C-reactive protein; sCal, serum calprotectin.
Moreover, 4 out of the 11 patients had sacroiliitis, including 2 isolated forms.

A major take-away of this study is the confirmation of sCal as a predictive marker of good response to treatment (all types of treatment combined), as also shown in two recent cohort studies with patients treated with methotrexate or Tumor Necrosis Factor (TNF)-inhibitors. It is important to note that the cut-off obtained from the ROC curve does not provide a perfect prediction, so the treatment decision cannot be based solely on sCal levels and must take into account other clinical factors. In addition, current medical practice suggests an increasingly personalised medicine. Thereby, more and more studies, such as ours, are investigating molecules of interest that can guide the therapeutic process, often derived from promising studies in the broad field of rheumatoid arthritis (sCal levels measured at baseline in patients with clinically inactive disease on anti-TNF therapy and followed up for a period of 6 months, and then at the time of long-term treatment discontinuation). In contrast, our study evaluated the use of sCal to predict the risk of flare-ups under treatment maintained during the 9 months of follow-up. The results are therefore not comparable and the duration of follow-up on stable therapy is not identical. Our study, therefore, highlights a group of patients at risk of relapse who may benefit from more frequent follow-up for a longer period, even if they are in an apparently reassuring state of remission. Another recent study could not find a relationship between sCal and prediction of treatment response and flare; this study excluded systemic forms (which may be more inflammatory and aggressive, and thus perhaps more related to sCal variations) and included two very different cohorts. Overall, this underlines the fact that JIA is a very heterogeneous disease with few comparable studies.

A prospective non-randomised clinical trial investigating stratified biomarker-based treatment approaches in patients with polyarticular course has just completed recruitment and results are expected in December 2021 (ISRCTN 69963079). In light of recent results, it might also be interesting to study sCal in oligoarticular and systemic forms to evaluate its efficacy as a prognostic marker in other specific subgroups of patients (the results from Hinze et al tend to show that dosage of sCal under stable treatment might be discriminating in extended oligoarthritis and seropositive polyarthritis, but not in seronegative polyarthritis). It may also be of interest to assess sCal levels in patients who taper, but do not completely discontinue, their treatment.

A limitation of our study is our control cohort consisting of young adults, instead of children comparable to our patients. However, sCal levels measured in previous studies did not differ significantly with regard to age or sex distribution in the control cohort. It should also be noted that patients under anti-TNF therapy could theoretically exhibit modified sCal secretion due to decreased TNF levels following treatment and thus potential down-regulation of the S100 proteins inflammatory pathway.

In our study, 18% of patients were on anti-TNF therapy, whereas the vast majority (75% of patients) was on methotrexate treatment (which would affect the S100 proteins pathway less). Another limitation is a potential selection bias regarding the patients included from specialised centres, potentially having more severe disease, and therefore requiring more treatment. The heterogeneity of the JIA spectrum also leads to a more strenuous analysis in subgroups. Finally, partial retrospective data collection may also misrepresent some results.

**CONCLUSIONS**

This study confirms the interest of sCal as a diagnostic tool and as a marker of activity. In particular, it could represent a predictive marker of response to treatment, or persistence of inactive disease, that could be used routinely. This protein is indeed relatively stable and easily measurable in serum.

**Author affiliations**

1. Department of Rheumatology, CHU Brugmann, Bruxelles, Belgium
2. Department of Pediatric Rheumatology, Hôpital Universitaire des Enfants Reine Fabiola, Bruxelles, Belgium
3. Department of Rheumatology, Hôpital Erasme, Bruxelles, Belgium
4. Laboratory of Bone and Metabolic Biochemistry, Université Libre de Bruxelles, Bruxelles, Belgium
5. Department of Rheumatology, Cliniques universitaires Saint-Luc, Bruxelles, Belgium
6. Institut de Recherche expérimentale et Clinique (IREC), Université catholique de Louvain Secteur des sciences de la santé, Bruxelles, Belgium
Collaborators  Julie Smet; Jean-Pierre Brasseur; Benoît Brasseur; David Tuermilckx.

Contributors  CL performed the ELISA tests, analysed and interpreted all data, and wrote the manuscript. All authors made substantial contributions to the acquisition of data. All authors read, revised and approved the final manuscript.

Funding  This work was supported by the Radio-Télévision belge de la Communauté française (RTBF) via the CAP48 programme. The funding body had no role in the design of the study, or in the collection, analysis and interpretation of data or in the writing of the manuscript.

Competing interests  None declared.

Patient consent for publication  Not required.

Ethics approval  This study complies with the Declaration of Helsinki. The involved Institutional Review Board (IRB) names and approval numbers are as follows: Commission d’Éthique Biomédicale Hospitalo-Facultaire de l’UCL (P1200_14) and Comité d’Éthique Erasme-ULB (PE2013_211 for the CAP48 cohort; PE2012_251 for the biobank of the Department of Rheumatology). Informed consent was obtained from each patient and their parents.

Provenance and peer review  Not commissioned; externally peer reviewed.

Data availability statement  All data generated or analysed during this study are included in this published article and its supplementary information files.

Open access  This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Céline La http://orcid.org/0000-0002-6065-2970
Patrice Durez http://orcid.org/0000-0002-7156-2356

REFERENCES
10. Tweehusyen L, den Broeder N, van Herwaarden N. Predictive value of serum calprotectin (S100A8/A9) for clinical response after starting or tapering anti-TNF treatment in patients with rheumatoid arthritis. RMD Open 2018;4:e000654.

35 Foell Det al. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remission: a randomized clinical trial. *JAMA* 2010;303:1266–73.


