Methotrexate Withdrawal at 6 vs 12 Months in Juvenile Idiopathic Arthritis in Remission
A Randomized Clinical Trial

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Context Novel therapies have improved the remission rate in chronic inflammatory disorders including juvenile idiopathic arthritis (JIA). Therefore, strategies of tapering therapy and reliable parameters for detecting subclinical inflammation have now become challenging questions.

Objectives To analyze whether longer methotrexate treatment during remission of JIA prevents flares after withdrawal of medication and whether specific biomarkers identify patients at risk for flares.

Design, Setting, and Patients Prospective, open, multicenter, medication-withdrawal randomized clinical trial including 364 patients (median age, 11.0 years) with JIA recruited in 61 centers from 29 countries between February 2005 and June 2006. Patients were included at first confirmation of clinical remission while continuing medication. At the time of therapy withdrawal, levels of the phagocyte activation marker myeloid-related proteins 8 and 14 heterocomplex (MRP8/14) were determined.

Intervention Patients were randomly assigned to continue with methotrexate therapy for either 6 months (group 1 [n=183]) or 12 months (group 2 [n=181]) after induction of disease remission.

Main Outcome Measures Primary outcome was relapse rate in the 2 treatment groups; secondary outcome was time to relapse. In a prespecified cohort analysis, the prognostic accuracy of MRP8/14 concentrations for the risk of flares was assessed.

Results Intention-to-treat analysis of the primary outcome revealed relapse within 24 months after the inclusion into the study in 98 of 183 patients (relapse rate, 56.7%) in group 1 and 94 of 181 (55.6%) in group 2. The odds ratio for group 1 vs group 2 was 1.02 (95% CI, 0.82-1.27; $P=.86$). The median relapse-free interval after inclusion was 21.0 months in group 1 and 23.0 months in group 2. The hazard ratio for group 1 vs group 2 was 1.07 (95% CI, 0.82-1.41; $P=.61$). Median follow-up duration after inclusion was 34.2 and 34.3 months in groups 1 and 2, respectively. Levels of MRP8/14 during remission were significantly higher in patients who subsequently developed flares (median, 715 [IQR, 320-1110] ng/mL) compared with patients maintaining stable remission (400 [IQR, 220-800] ng/mL; $P=.003$). Low MRP8/14 levels indicated a low risk of flares within the next 3 months following the biomarker test (area under the receiver operating characteristic curve, 0.76; 95% CI, 0.62-0.90).

Conclusions In patients with JIA in remission, a 12-month vs 6-month withdrawal of methotrexate did not reduce the relapse rate. Higher MRP8/14 concentrations were associated with risk of relapse after discontinuing methotrexate.

See also Patient Page.

CHRONIC INFLAMMATORY DISeases are among the most relevant medical challenges. While these diseases have increased in prevalence, many, including rheumatoid arthritis, inflammatory bowel disease, and juvenile idiopathic arthritis (JIA), are now treatable.[1-3] However, JIA often takes a remitting disease course, and the long-term outcome is not easy to predict.[4-8] Therefore, physicians have to balance the risk of doing too little (eg, withdrawing medication and provoking flares) vs the risk of doing too much (eg, continuing medication despite a stable

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remission and thereby accepting the risk of adverse effects). While evidence-based advice for starting therapies in active disease is available, no controlled data exist to suggest the need for treatment continuation after remission is achieved.[9]

In the case of JIA, methotrexate is the most widely used disease-modifying drug.[1,3,10,11] Remission can be induced in most patients continuing medication (also referred to as "remission on medication"[5]) using combined anti-inflammatory treatment, and up to 50% of such patients reach a continuous status of remission after discontinuing medication (also referred to as "remission off medication"[5]).[7,12] However, about half of the patients have flares after withdrawing or tapering methotrexate—an intriguing ratio for statistical analysis of follow-up studies. Continuation of methotrexate for at least 12 months after induction of remission has been proposed,[11,13] although no controlled prospective studies have investigated the effect of early discontinuation of methotrexate treatment on the rate of flares.

Laboratory markers currently in use cannot detect residual inflammation that influences the risk of flares when stopping treatment; hence, clinicians would benefit from improved molecular biomarkers of inflammation. Myeloid-related protein (MRP) 8 (S100A8) and MRP 14 (S100A9) are secreted by activated phagocytes and form MRPs 8 and 14 heterocomplex (MRP8/14 [calprotectin]).[14] In JIA, MRP8/14 has been shown to be a marker of subclinical disease activity not detectable by clinical investigation or laboratory tests.[9,15] In this study we analyzed whether the duration of methotrexate therapy during clinical remission of JIA influences the rate of flares after withdrawal and hypothesized that patients at risk for a flare may be identified by MRP8/14 analyses.

METHODS
Patients
Patients with any subtype of JIA[16] successfully treated with methotrexate were eligible for this study. The patients were included at first confirmation of clinical remission while continuing medication, ie, after clinically documented inactive disease for at least 3 months.[5,7,12] According to the criteria for inactive disease, the patients had no joints with active arthritis; no fever, rash, serositis, splenomegaly, or generalized lymphadenopathy attributable to arthritis; no active uveitis; no elevation in erythrocyte sedimentation rate, C-reactive protein level, or both attributable to arthritis; and no disease activity by physician's global assessment on a visual analog scale ranging from 0 to 10 cm. At inclusion only 1 nonsteroidal anti-inflammatory drug and methotrexate (maximum dose, 15 mg/m^2 per week) were permitted. Steroids had to be withdrawn at least 1 month prior to inclusion. Patients were excluded if they had received intra-articular corticosteroids, biologics, or other disease-modifying drugs up to 3 months prior to inclusion. Data on race/ethnicity were collected and racial/ethnic groups recategorized in the phase of the analysis as white, Hispanic, and other. Race/ethnicity had no relevance for subsequent analyses.

The study protocol was approved by independent ethics committees at each study site. Patients or their parents provided written informed consent. The study complied with the Consolidated Standards of Reporting Trials statement and the Standards for Reporting of Diagnostic Accuracy guidelines.[17,18]

Study Design
The study was designed as a prospective, open, multicenter, medication-withdrawal randomized clinical trial. Patients were included during clinical remission while continuing medication at participating pediatric rheumatology centers and were randomly assigned to 1 of 2 groups by the Pediatric Rheumatology International Trials Organization (PRINTO) using a computer-generated randomization schedule. In group 1, patients discontinued methotrexate (and nonsteroidal anti-inflammatory drugs, if applicable) 6 months after inclusion if remission was confirmed at that time. In group 2, patients discontinued therapy 12 months after inclusion if remission was confirmed at that time. Clinical remission after discontinuation of medication was evaluated in both groups in further follow-up examinations every 3 months over at least another year. Stable remission was defined as the absence of any flare, ie, continuous remission after discontinuation of medication.

Documentation of Clinical Parameters
Patients were evaluated using the preliminary criteria for inactive disease and clinical remission of JIA.[5] In addition, data were collected on subtype, disease onset and duration, time taking methotrexate, dosage, other medications, morning stiffness, or complications. Disease flare was defined as occurrence of any sign of active arthritis and/or active systemic symptoms, ie, when any of the criteria for inactive disease was no longer met. The clinical assessments were made by experienced pediatric rheumatologists at each visit.

Laboratory Examinations
Serum concentrations of MRP8/14 were determined by an in-house sandwich enzyme-linked immunosorbent assay as previously reported.[19] Interassay and intra-assay reliability were 9.7% and 8.2%, respectively. Serum MRP8/14 concentrations were analyzed once during clinical remission at the time treatment with methotrexate was stopped. The analyzing laboratory in Muenster was blinded for patients' characteristics. Erythrocyte sedimentation rate (by Westergren method) and C-reactive protein level (by nephelometry) were standardized based on the normal values provided by each local laboratory as previously described.[20]

Statistical Analysis
The primary outcome of the study was the relapse rate. The difference between flare rates in both treatment groups was determined for both the intention-to-treat population and the per-

protocol cohort. Intention-to-treat analyses were performed, including all randomized patients and evaluating the flare rate within 2 years after the patients' inclusion into the study. In per-protocol analyses, the flare rate within 1 year after methotrexate discontinuation was evaluated, including patients who discontinued methotrexate treatment according to the protocol.

Consequently, the intention-to-treat analysis of the primary outcome evaluated the relapse rate within 24 months after the patients' inclusion into the study. Missing values were imputed using multiple imputation. In a corresponding per-protocol analysis, the relapse rate over at least 1 year after methotrexate discontinuation was analyzed. Differences between treatment groups were evaluated by a 2-sided χ^2 test at an α level of .05. A flare rate of 50% within 1 year after withdrawal of therapy was assumed. Thus, power analysis revealed that a total of 193 evaluable patients were required to provide an 80% power of detecting treatment differences of at least 20%.

The secondary outcome was time to relapse. A prespecified observational cohort analysis assessed the prognostic accuracy of MRP8/14 concentration for risk of flares.

Demographic and baseline characteristics were summarized using descriptive statistics. Data are given as mean or median as well as range, interquartile range (IQR), or 95% confidence interval (CI). The 2-sided Mann-Whitney U test was applied to analyze differences of quantitative parameters between groups of patients. Qualitative parameters were evaluated with the χ^2 test.

Survival analyses were performed, comprising Kaplan-Meier plots and log-rank tests as well as hazard ratios and associated CIs based on a proportional hazards model. The proportional hazards assumption was verified using the Grambsch-Therneau residual-based test. Survival analyses were performed in patients who received an analysis of serum MRP8/14 level at the time treatment was discontinued during remission, to determine the difference between the flare rates in patients with high vs low MRP8/14 levels. Biased results due to differences between JIA subtypes were excluded by Cox models with disease subtypes as covariable. Classification and regression tree analysis were used to determine the optimal cutoff level for MRP8/14.[21] Receiver operating curves (ROCs) were used to deter-

**Table 1.** Baseline Demographic and Clinical Characteristics of Patients[a]

| | Median (Range) | | |
Characteristics	Group 1 (n = 183)	Group 2 (n = 181)	P Value
Female sex, No. (%)	119 (65)	123 (68)	.55
Age at inclusion, y	11.2 (2-18)	10.7 (2-18)	.81
Age at disease onset, y	5.4 (1-16)	6.1 (1-15)	.43
Disease duration, y	3.2 (0-13)	3.0 (0-16)	.62
Juvenile idiopathic arthritis subtypes, No. (%)			
Persistent oligoarthritis	54 (30)	42 (23)	.17
Extended oligoarthritis	34 (19)	22 (12)	.09
Polyarthritis, negative rheumatoid factor	54 (30)	81 (45)	.003
Polyarthritis, positive rheumatoid factor	9 (5)	8 (4)	.82
Systemic-onset juvenile idiopathic arthritis	14 (8)	21 (12)	.20
Enthesitis-related arthritis	7 (4)	3 (2)	.34
Psoriatic arthritis	11 (6)	4 (2)	.07
Treatment and laboratory values at inclusion			
Methotrexate dose, mg/m² per wk	10.0 (1-17)	11.0 (4-20)	.11
Time taking methotrexate, y	1.3 (0-12)	1.4 (0-10)	.50
Taking nonsteroidal anti-inflammatory drugs, No. (%)	76 (42)	60 (33)	.12
Erythrocyte sedimentation rate, mm/h	9 (0-20)	8 (0-19)	.48
C-reactive protein, mg/L	1.0 (0-2.5)	0.9 (0-2.2)	.62
Laboratory values at withdrawal			
Erythrocyte sedimentation rate, mm/h	9 (5-17)	8 (4-19)	.78
C-reactive protein, mg/L	0.3 (0-1.4)	0.2 (0-2.0)	.70
MRP8/14, ng/mL	510 (60-2640)	480 (110-3310)	.94

Abbreviation: MRP8/14, myeloid-related proteins 8 and 14 heterocomplex.
SI conversion factor: To convert C-reactive protein values to nmol/L, multiply by 9.524.
[a] Group 1 comprised patients discontinuing methotrexate after 6 months in remission; group 2, those discontinuing methotrexate after 12 months in remission.

Figure 1. Study Flow
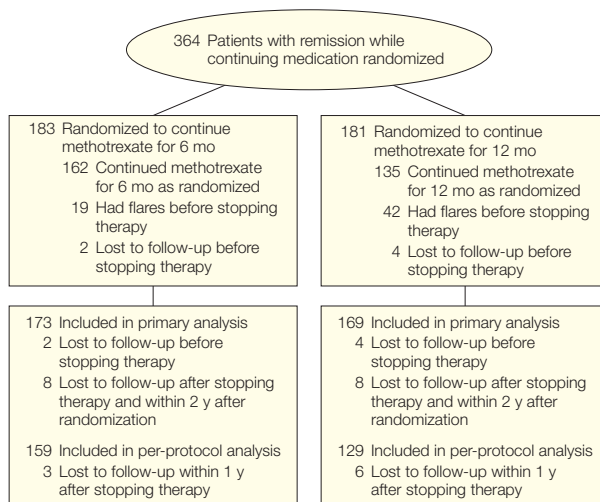
mine accuracy. As a summary statistic, the area under the ROC curve was calculated. This area is equal to the probability that the MRP8/14 marker will rank a randomly chosen patient who will experience a flare higher than a randomly chosen patient who will not.

Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, North Carolina). All reported *P* values are 2-sided and are considered significant at $P < .05$.

RESULTS
Baseline Patient Characteristics
A total of 364 patients in remission while continuing medication were enrolled in 61 PRINTO study centers from 29 countries between February 2005 and June 2006. The patients were randomized at enrollment to continue therapy for either 6 (group 1) or 12 (group 2) months. Differences in demographic characteristics and disease parameters at baseline between the 2 groups have been checked for statistical significance (**TABLE 1**). Only one JIA subtype, polyarthritis with negative rheumatoid factor, proved not equally distributed in both groups. Consequently, to prevent biased results, all subsequent analyses were additionally confirmed in multivariate model approaches adjusting for JIA subtype.

Randomization and Drug Withdrawal
At inclusion, 183 patients were randomized into group 1 and 181 into group 2. Six patients (1.7%) were lost to follow-up before stopping treatment; in 61 (16.8%), a flare occurred during the period of remission while continuing medication. The rate of flares during the period while continuing medication was comparable in both groups when correcting for time at risk, ie, 17.9 (95% CI, 11.4-28.1) and 21.9 (95% CI, 16.2-29.7) per 1000 patient-months in groups 1 and 2, respectively. In group 1, 162 patients withdrew methotrexate and entered a phase of remission after discontinuing medication. In group 2, 135 patients reached this point (**FIGURE 1**). At the time of

withdrawal, serum was collected whenever possible and stored frozen for MRP8/14 analysis.

Analysis of the Primary and Secondary Outcome
Intention-to-treat analyses of the primary outcome revealed relapse within 24 months after the inclusion into the study in 98 of 183 patients (relapse rate, 56.7%) in group 1 and 94 of 181 (55.6%) in group 2 (odds ratio, 1.02; 95% CI, 0.82-1.27; *P* = .86). This result was qualitatively confirmed in a multivariate model approach adjusting for JIA subtype. The median relapse-free interval after inclusion was 21.0 months in group 1 and 23.0 months in group 2. The hazard ratio for group 1 vs group 2 was 1.01 (95% CI, 0.82-1.41; *P* = .61). Median follow-up duration after inclusion was 34.2 and 34.3 months in groups 1 and 2, respectively.

In the 297 patients who stopped therapy while in remission, 63 of 162 (39.6%) in group 1 and 51 of 135 (39.5%) in group 2 had a flare within 1 year. Thus, a per-protocol analysis of the primary outcome revealed an odds ratio of 1.00 (95% CI, 0.62-1.61; *P* = .99). Because the number of flares occurring later than 12 months after withdrawal differed between the groups (25 in group 1 vs 8 in group 2), there were 88 flares among 162 patients (54%) in group 1 and 59 flares among 135 patients (44%) in group 2 over the whole study period. These values are influenced by the fact that beyond 12 months of follow-up after withdrawal, patients in group 1 were censored later than those in group 2. Median follow-up thus differed considerably, constituting 28.2 (IQR, 24.5-34.2) months in group 1 and 22.6 (IQR, 16.8-27.3) months in group 2. However, the overall flare rates were not different, with 40.2 (95% CI, 31.4-51.5) and 40.3 (95% CI, 30.6-53.0) per 1000 person-months in groups 1 and 2, respectively (*P* = .44). The intention-to-treat sensitivity analysis of the relapse-free interval on the whole study population revealed no difference in rate of flares between the groups (**FIGURE 2**).

The flare rates at 12 months were considered for performing significance tests as well as the per-protocol analysis (**FIGURE 3**). The timing of discontinuation of methotrexate during remission had no effect on the likelihood of flares within 1 year after discontinuation of treatment.

### Assessment of Residual Disease Activity by MRP8/14 Level
Of the 297 patients who stopped taking methotrexate, serum was available

Figure 2. Analysis of Flare Rates
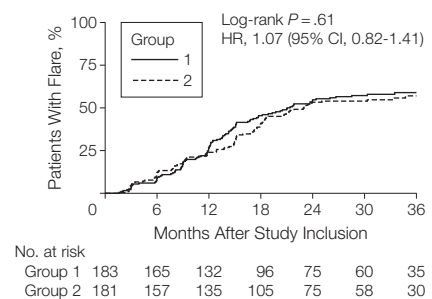
Group 1 comprised patients discontinuing methotrexate after 6 months in remission; group 2, those discontinuing methotrexate after 12 months in remission. Survival time starts at inclusion into the study and was performed without restricting follow-up. There was no difference in the rate of flares between the treatment groups. The y-axis shows the proportion of patients with flares after discontinuing medication. CI indicates confidence interval; HR, hazard ratio.

Figure 3. Per-Protocol Analysis of Flare Rates
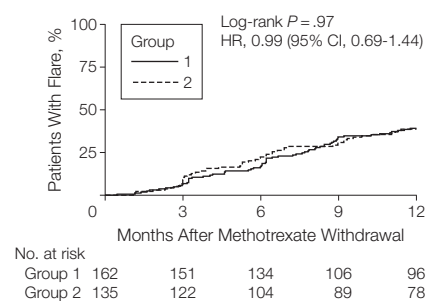
Group 1 comprised patients discontinuing methotrexate after 6 months in remission; group 2, those discontinuing methotrexate after 12 months in remission. Analysis was restricted to a follow-up of 12 months after withdrawal of therapy. There was no difference in the rate of flares between the treatment groups. The y-axis shows the proportion of patients with flares after discontinuing medication. CI indicates confidence interval; HR, hazard ratio.

for 188. These patients had a serum analysis for MRP8/14 level during remission (64% in group 1 and 63% in group 2). The patients were well representative of the total study population (**TABLE 2**). At this point, erythrocyte sedimentation rates and C-reactive protein levels were within normal ranges. The median MRP8/14 levels were higher than in healthy controls but comparable to patients with juvenile arthritis in clinical remission, as analyzed in previous studies.[15,22] Because MRP8/14 is a marker of phagocyte activation, this indicates ongoing innate immune reactions in at least some patients. Although all patients were in remission at this point according to the remission criteria on clinical grounds and considering routine laboratory parameters, MRP8/14 as molecular marker of innate immunity suggested that not all patients were in immunological remission.

Predictive Validity of MRP8/14 Levels for Risk of Flare

The flare rate in the patients undergoing biomarker analysis was not different from that in the overall study population (**TABLE 3**). We tested the hypothesis that patients with subclinical disease activity, indicated by higher MRP8/14 serum concentrations during remission while continuing medication, were more likely to experience a flare during follow-up while in remission after discontinuing medication. Patients with stable remission had a median MRP8/14 level of 400 (IQR, 220-800) ng/mL, while patients with flares had a median MRP8/14 level of 715 (IQR, 320-1110) ng/mL (P=.003). The hazard ratio was highest when comparing levels of 690 (IQR, 270-895) ng/mL or greater vs less than 690 ng/mL (2.24; 95% CI, 1.39-3.62; P<.001). A log-rank analysis confirmed the difference of flare rates at this cutoff (**FIGURE 4**), while ROC curves revealed a sensitivity of 69% and a specificity of 66% for predicting flares. In multivariate Cox regression analyses, potential confounders beyond MRP8/14 concentrations were included (ie, treatment group, sex, age at disease onset, and JIA subtype). In the multivariate model, significant interactions of any regressor variables with MRP8/14 could be excluded.

The flare rates per 1000 patient-months were significantly higher in individuals with MRP8/14 levels of 690 ng/mL or greater vs less than 690 ng/mL when compared at 3, 6, 12, or 24 months (Table 3). More detailed analyses showed that higher MRP8/14 levels especially relate to the risk of early flares within the following 3 months (**FIGURE 5**). The group of patients with flares within 3 months had a median MRP8/14 level of 1260 (IQR, 850-1900) ng/mL. The accuracy of the biomarker was especially useful for the prediction of early relapses within this short time (**TABLE 4**). Considering flares within the next 3 months following the laboratory test, the MRP8/14 marker showed an area under the ROC curve of 0.76 (95% CI, 0.62-0.90).

COMMENT

Longer continuation of methotrexate therapy after induction of remission while continuing medication does not generally improve the stability of remission after discontinuing medication in JIA. The risk of relapsing disease remains similar after treating patients longer. A significant difference in follow-up times between the study groups beyond 1 year resulted in a disadvantage for the 6-month group and impeded a comparison of later flares. However, that the overall flare rates were not significantly different between the groups argues strongly against a late difference that would not have been detected within the 1 year follow-up period. Indeed, most of the

Table 2. Patients with MRP8/14 Analysis

Characteristics	No. (%) (n = 188)
Female sex	122 (65)
Age at inclusion, median (range), y	11.3 (2.3-18.0)
Age at disease onset, median (range), y	6.4 (1.1-16.3)
Disease duration, median (range), y	2.9 (0.4-13.9)
Juvenile idiopathic arthritis subtypes	
Persistent oligoarthritis	59 (31)
Extended oligoarthritis	27 (14)
Polyarthritis, negative rheumatoid factor	64 (34)
Polyarthritis, positive rheumatoid factor	10 (5)
Systemic-onset juvenile idiopathic arthritis	12 (6)
Enthesitis-related arthritis	8 (4)
Psoriasis arthritis	8 (4)

Abbreviation: MRP8/14, myeloid-related proteins 8 and 14 heterocomplex.

Table 3. Patients With MRP8/14 Analysis and Their Outcome After Withdrawal

Flare Rate Stratification	MRP8/14 <690 ng/mL	MRP8/14 ≥690 ng/mL	P Value
Flares per patient, No./total (%)			
Within 0-3 mo	2/113 (2)	13/75 (17)	<.001
Within 0-6 mo	13/113 (12)	22/75 (29)	.004
Within 0-12 mo	31/113 (27)	37/75 (49)	.004
Within 0-24 mo	39/113 (35)	45/75 (60)	.001
After 24 mo	6/50 (12)	2/14 (14)	>.99
Overall	45/113 (40)	47/75 (63)	.004
Flares per 1000 patient-months			
Within 0-3 mo	5.9	60.3	<.001
Within 0-6 mo	19.9	57.3	.001
Within 0-12 mo	26.0	57.0	.001
Within 0-24 mo	19.5	48.2	<.001
After 24 mo	11.2	39.8	.22
Overall	17.7	47.8	<.001

Abbreviation: MRP8/14, myeloid-related proteins 8 and 14 heterocomplex.

flares after withdrawal occurred within the first 6 months, with only a few occurring later than 12 months after stopping therapy. Interestingly, there was also a background risk of flares that was not prevented during the maintenance therapy, but the flare rate during remission while continuing medication was significantly lower than that during remission after discontinuing medication. Therefore, it cannot be recommended that methotrexate therapy be continued in all patients for longer than 6 months after remission is induced. The current definition of remission may be refined, adding "immunological remission" as a status that will be robust enough to last after discontinuing medication.

While erythrocyte sedimentation rate and C-reactive protein level are not sensitive enough to assess residual inflammatory joint disease, MRP8/14 is a marker of local disease activity, and analysis of MRP8/14 levels detects subclinical inflammation that would exclude immunological remission.[14,23] MRP8/14 is a member of the family of "danger signals" and an endogenous activator of toll-like receptor 4, which is involved in innate immune mechanisms in inflammatory conditions such as arthritis and infections.[24,25] It is specifically secreted from activated phagocytes at local sites of inflammation and is a novel therapeutic target in autoimmunity.[26,27]

Flares after withdrawal of therapy are most likely related to the fact that the local disease process is not completely resolved, even though clinical impression and acute phase reactants suggest remission. Levels of MRP8/14 reflect subclinical inflammation that will influence the risk for flares, especially within the following 3 months. In the clinical setting it is clearly useful to use such a biomarker of synovial inflammation, because levels less than 690 ng/mL make it relatively unlikely that subclinical disease activity is present at the time the test is performed (negative predictive value, 98%). The likelihood of disease activity that could progress into a full-

blown disease flare over the following 3 months is small (negative likelihood ratio, 0.2), which helps with deciding to stop treatment.

Previous studies have revealed that the probability of stable remission is similar among subtypes of JIA. Patients with polyarticular disease and positive rheumatoid factor may have a slightly higher risk for flares, but this group was very small in our study. Because across all subtypes approximately 50% of patients relapse and 50% stay in remission after discontinuation of therapy,[5,7,12] JIA is an ideal statistical model to study the flare risk during remission after discontinuing medication. However, our data are of general relevance, because many chronic inflammatory diseases regularly take a relapsing course. Treatment over years is often necessary, even though patients may respond to the initial therapies. Sufficient advice exists for starting therapies once the diagnosis is established or for escalating treatment if the therapy is not sufficient to induce remission.[2] However, in clinical practice physicians are frequently faced with the question of what to do with patients who are clinically well after induction of remission. Physicians have to decide whether continuation of drug therapy is meaningful, because it may maintain inactive disease and induce a more stable remission that may last even after later withdrawal of treatment. Besides methotrexate therapy in JIA, expert opinions for the continuation of treatment during a state of remission while continuing medication exist for inflammatory bowel disease, for which continuation of azathioprine for more than 3 years while in remission is recommended.[28] In rheumatoid arthritis, for which remission after discontinuing medication is now the accepted goal of management, the duration of treatment during remission while continuing medication is a matter of debate.[29] In pediatric autoimmune hepatitis, therapy continued for at least 1 to 2 years while in complete remission has been proposed.[30] However, the duration of maintenance therapy in JIA dur-

Figure 4. Analysis of Flares Using MRP8/14 as a Molecular Marker of Relapse Risk
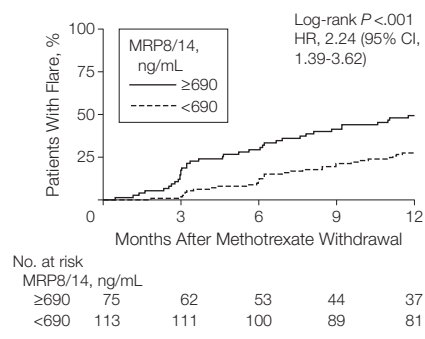
Survival analysis confirmed the significant difference of flare rates between patients with myeloid-related proteins 8 and 14 heterocomplex (MRP8/14) levels of 690 ng/mL or greater compared with those with lower levels. The analysis considered a follow-up of 12 months after withdrawal of therapy, which was the time when the biomarker analysis was performed. The y-axis shows the proportion of patients with flares after discontinuing medication. CI indicates confidence interval; HR, hazard ratio.

Figure 5. MRP8/14 as Molecular Marker of the Risk of Relapse
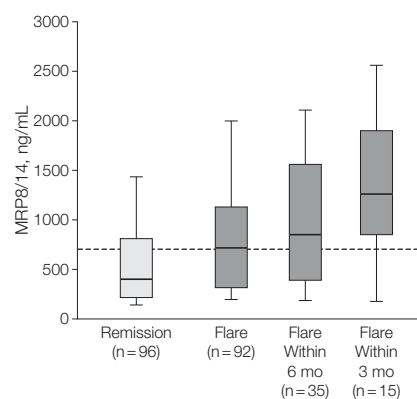
Patients with a flare over the whole study period had significantly higher levels of myeloid-related proteins 8 and 14 heterocomplex (MRP8/14) than patients who remained in stable remission (P=.003). The 92 patients with flares were further divided into subgroups of patients who had early flares within 6 months (n=35) or 3 months (n=15) after the biomarker analysis. When restricting to the 3-month follow-up, MRP8/14 levels of patients with relapse showed only little overlap with values from patients who remained in stable remission (P=.001 within 6 months; P<.001 within 3 months). The upper and lower bounds of each box indicate the 25th and 75th percentile, respectively; heavy lines within the box, the median; whiskers, the 10th and the 90th percentile. Dashed line indicates the cutoff at 690 ng/mL. P values report a comparison with patients without relapses while in remission after discontinuing medication, ie, in stable remission (Mann-Whitney U test).

Table 4. Performance of MRP8/14 at Cutoff of 690 ng/mL for Predicting Relapses

	Months		
Measure	0-3	0-6	0-24
Flares, No. (%)			
≥690 ng/mL (n = 75)	13 (17)	22 (29)	45 (60)
<690 ng/mL (n = 113)	2 (2)	13 (12)	39 (35)
Sensitivity %, (95% CI)	87 (60-98)	63 (45-79)	54 (42-65)
Specificity %, (95% CI)	64 (57-71)	65 (57-73)	71 (62-80)
Positive predictive value %, (95% CI)	17 (10-28)	29 (19-41)	60 (48-71)
Negative predictive value %, (95% CI)	98 (94-100)	89 (81-94)	66 (56-74)
Positive likelihood ratio	2.42	1.80	1.38
Negative likelihood ratio	0.20	0.57	0.79
Area under ROC curve (95% CI)	0.76 (0.62-0.90)	0.65 (0.54-0.76)	0.63 (0.55-0.71)

Abbreviations: CI, confidence interval; MRP8/14, myeloid-related proteins 8 and 14 heterocomplex; ROC, receiver operating characteristic.

ing remission while continuing medication was never before tested in a controlled trial.

Although data regarding the primary outcome of this study are clear, our study has some limitations. The real length of remission can be determined only in patients who experienced a relapse during the study. If patients did not show a flare during the study, then the total length of remission cannot be determined, because it may even last for the remaining years of life. Another concern is the variety of disease subtypes. As in previous studies, patients in our study with systemic JIA had higher MRP8/14 levels even while in stable remission, but the patients with systemic JIA had the lowest relapse rate. We thoroughly analyzed all data with regard to disease subtype as a confounder and could exclude that subgroups did influence the overall results. However, because of the small numbers, cutoff MRP8/14 levels for different subtypes cannot be provided from our study. Also, generalization from our data on the biomarker to all patients undergoing other therapies such as biologics cannot be made. Lastly, the secondary outcome of the biomarker findings are based on a cohort analysis, which weakens the validity strength, and the results will eventually need to be confirmed in larger cohorts. This limitation especially relates to the determination of the cutoff value of 690 ng/mL, which has to be confirmed in future trials.

In summary, a 12-month withdrawal of methotrexate compared with a 6-month withdrawal did not reduce the relapse rate in patients with JIA in remission. Higher MRP8/14 concentrations were associated with the risk of relapse after discontinuing methotrexate. These data indicate a need for the stratification of patients with chronic inflammatory diseases to ensure that the intensity of treatment is adjusted to the patients' individual needs.

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