Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis

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Abstract

Objective

An unsolved problem in juvenile idiopathic arthritis (JIA) is to identify patients at special risk for relapse. It is important to adjust anti-inflammatory and immunosuppressive therapy to the children's actual disease activity especially in times of remission. Our aim was to analyze if the serum levels of MRP8/MRP14 are reliable predictive markers for the risk of relapse in clinically inactive juvenile idiopathic arthritis.

Methods

Serum concentrations of MRP8/MRP14 were determined by ELISA and correlated with laboratory and clinical parameters for disease activity in patients with JIA. 29 patients with changing disease activity were followed up for a mean time of 2.9 years. Two groups of patients – one before relapse (mean 3.7 months) but without clinical signs of disease reactivation, and one in remission for 12 further months – were compared.

Results

MRP8/MRP14 serum levels in patients before relapses were significantly higher than the levels in patients in stable remission for one year (662 ng/ml versus 395 ng/ml; $p < 0.05$). Using a cut-off for MRP8/MRP14 of 450 ng/ml the likelihood ratio for relapse was 3.7 (positive predictive value 80%), while no differences were found for C-reactive protein and erythrocyte sedimentation rate between the two groups.

Conclusion

MRP8/MRP14 correlate with individual disease activity in patients with JIA. Our data suggest that local disease activity may be present even months before flares become clinically apparent. Serum levels of MRP8/MRP14 can give a hint as to clinically occult disease activity, in this way helping to adjust therapy in times of low disease activity.

Key words

Juvenile idiopathic arthritis, remission, relapse, flare, myeloid related proteins, MRP.

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Introduction

JIAis a chronic relapsing inflammatory childhood disease characterized by arthritis with varying systemic inflammation. Patients can be subdivided into at least three major subgroups – oligoarticular, polyarticular and systemic JIA. The clinical course is characterized by changes in the degree of inflammation. The outcome is substantially variable, even within disease onset subtypes; some patients recover completely in adolescence, whereas others experience lifelong symptoms.

Remission is achieved in up to two-thirds of JIA patients (1). Thus, it is reasonable to strive for discontinuation of treatment once disease activity has been controlled, particularly for children in view of the potential acute toxicity and chronic effects of long-term immunosuppression. Unfortunately about 50% of children experience disease flares or relapses after discontinuation of immunosuppressive therapy, e.g. with methotrexate (MTX) (2). An unsolved problem in JIA is to assess which patient is at risk for relapses and to adjust anti-inflammatory and immunosuppressive therapy to the children's actual disease activity especially in times of remission. To date, commonly used parameters such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are markers for systemic inflammation that are of limited predictive value for the further course of the disease.

MRP8 (myeloid related protein 8; S100-A8) and MRP14 (S100A9) are calcium binding proteins expressed during myeloid differentiation. MRP8/MRP14 form a heterodimeric complex in a calcium-dependent manner and are predominantly found in granulocytes and monocytes (3, 4). In patients with a number of different inflammatory diseases, elevated serum concentrations of MRP8/MRP14 were found and previous studies have shown that it is also a useful marker for various rheumatic diseases including systemic lupus erythematosus (5), reactive arthritis (6), and rheumatoid arthritis (7). A significant correlation between MRP8/MRP14, CRP, ESR and several clinical parameters of disease activity was found in JIA(8, 9).

In the present study we demonstrate that serum levels of MRP8/MRP14 can indicate unapparent disease activity even in the absence of clinical signs or symptoms and thus predict disease flares.

Patients and methods

Patients

In order to determine the correlation of inflammatory parameters with changing disease activity in JIA we performed a prospective follow-up analysis of 29 consecutive patients (20 girls and 9 boys) over a mean period of 2.9 years (range 1.2 – 7.0). Prior to inclusion in this study, patients had documented disease activity of varying degrees and an indication for long-term follow-up in our out-patient clinic. JIA was defined according to the proposed ILAR criteria (10).

Ten patients (8 girls, 2 boys) showed a polyarticular and 19 patients (12 girls, 7 boys) an oligoarticular onset. The mean age of the patients at the end of the study was 12.6 years (range 3.10 – 20.2 years) and the mean disease duration was 7.4 years (2.3 – 14.1 years). The age at disease onset was on average 5.1 years (0.8 – 15.8 years). The patients were examined during visits at intervals of approximately 3 months. We analyzed 3 – 15 samples per patient, for a total of 216 serum samples. Patients were treated either with the disease-modifying drug MTX or with non-steroidal anti-rheumatic drugs or a combination of both.

Determination of disease activity

Disease activity was determined on the basis of the core set criteria for JIA(11, 12). Documentation of the patient data included the medical history and physical examination, in particular the number of active joints (joint swelling or limitation of movement, with either pain on movement or tenderness), the physician's global assessment of disease activity, the patient's/parental assessment of overall well-being (visual analogue scale as part of the Child Health Assessment Questionnaire CHAQ), functional ability (disability as measured by the

CHAQ), and the number of joints with limited range of motion. Patients were categorized as having active or inactive disease. Active disease was defined by the presence of any active joint (joint swelling or limitation of movement, with either pain on movement or tenderness, or morning stiffness). Inactive disease was defined according to the ACR criteria for remission (13). Relapse was defined according to the preliminary criteria for disease flares of JIA (14). Assessors of disease activity were blinded to the MRP results.

Subgroup analysis for patients in remission

In order to investigate the predictive value of MRP8/MRP14 for disease flares we divided the samples of those patients considered to be inactive into two groups with either of the subsequent events "relapse" or "non-relapse". Samples from 29 patients may be found in both groups according to the disease activity of patients over the entire follow-up period, which reached a maximum of 7 years.

Patients without apparent disease activity for at least one subsequent year were characterized as "non-relapsers". Eight patients remained in stable remission throughout the follow-up period. Thirteen patients with changing disease activity had periods of stable remission of at least one year, in addition to periods with disease flares. Taken together, we had 21 cases with the event "non-relapse". In total, 46 samples within the non-relapse periods obtained more than one year before a disease flare were used for this analysis.

Patients who relapsed within one year were characterized as "relapsers". The last sample before relapse was included into the latter group (mean interval to relapse 3.7 months; SEM ± 0.4; range 1 – 8 months). Thirteen patients had one disease flare (with 13 samples before relapse), and 5 patients had two disease flares over the follow-up period (giving 10 samples before relapse). Taken together, we had 23 "relapse" events in 18 patients, and 23 samples before these relapses. The data for patients are summarised in Table I.

Table I. Data for the patients in subgroups.

	Relapsers	Non-relapsers
No. of patients/samples	18/23	21/46
Sex, no. male/no. female	5/13	5/16
No. of patients with oligoarthritis/polyarthritis	11/7	14/7
Age at end of study, mean years	12.2	12.6
Disease duration, mean years	7.2	7.4

Healthy controls

Thirty healthy children (16 girls, 14 boys; mean age 14.1 years; range 3.6 – 17.8 years) who underwent blood sampling for other reasons, e.g. exclusion of growth hormone deficit, and who had no history of inflammatory disorders or infections served as controls.

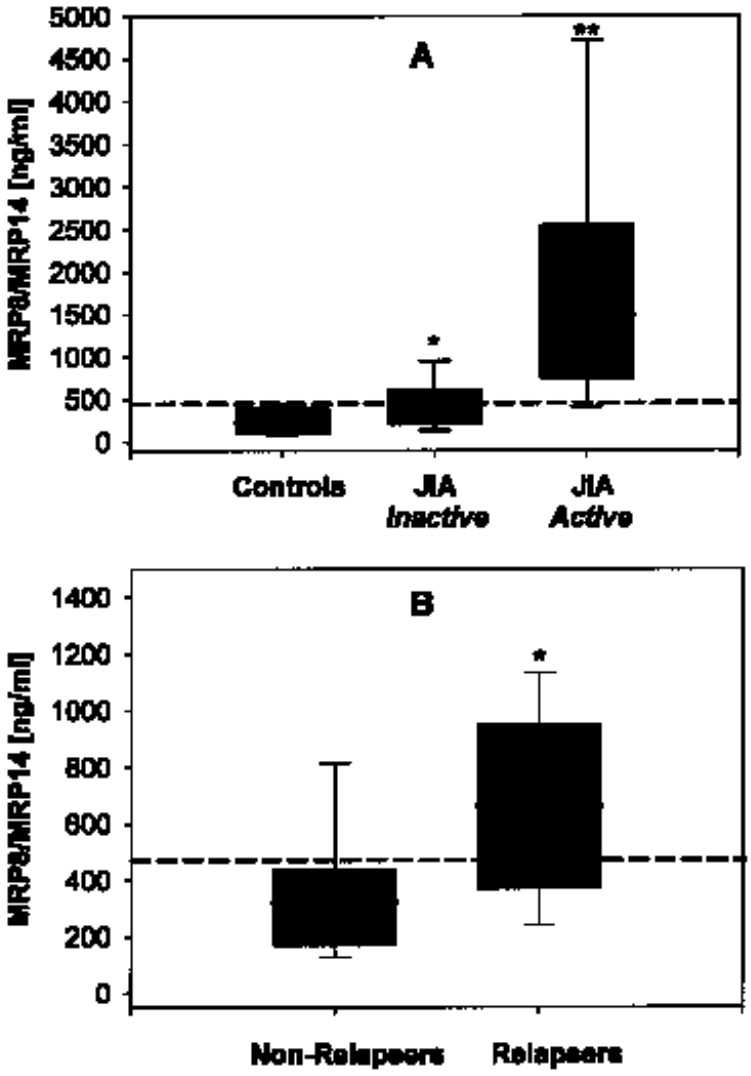

Fig. 1. Serum levels of MRP8/MRP14. Box plots show the median (thin line), mean (bold line), and 25th and 75thpercentiles. Error bars indicate the 5th and 95th percentiles, respectively. (**A**) Data on 29 patients with active disease (100 samples from 29 patients) or during inactive disease (116 samples from 28 patients) and on 30 healthy controls. (**B**) MRP8/MRP14 serum concentrations in patients with inactive disease, but a relapse within the next months were significantly higher than the MRP8/MRP14 of patients who continued in stable remission (*$p < 0.05$; **$p < 0.01$). The dotted horizontal lines indicate the upper limit of normal MRP8/MRP14 serum concentrations (e.g., 450 ng/ml).

Laboratory examinations

The blood samples were centrifuged within 2 hours and the serum was stored at –80°C until analyzed for MRP8/ MRP14. The concentrations of MRP8/ MRP14 were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) following the system established in our laboratory (4, 15). Serum concentrations of MRP8/MRP-14 are given as the mean ± standard error of the mean (SEM) if not mentioned otherwise. CRP in the serum was analyzed by nephelometry (mg/l) and ESR by the Westergren method (mm/h).

Statistical analyses

Correlations were calculated using Pearson's correlation. Student's t-test as well as the Mann-Whitney U test were used to analyze the differences in the means. Statistical analyses were performed using SPSS for Windows, version 11.0. Receiver-operating curves (ROC) were employed to analyse the diagnostic value of the MRP8/MRP14 serum levels. This method has been proposed to analyze the diagnostic value and accuracy of immunological tests in rheumatic disorders (16). It allows calculation of the sensitivity, specificity, and likelihood ratio over a broad range for tested parameters.

Results

Correlation between MRP8/MRP14 and disease activity

MRP8/MRP14 serum concentrations in patients with active disease were significantly higher compared to patients with inactive disease (1,997 ± 217 ng/ ml versus 640 ± 61 ng/ml). Both groups showed elevated serum levels compared to healthy controls (360 ± 50 ng/ ml) (Fig.1A). The mean MRP8/ MRP-14 serum concentration was 2,000 ± 225 ng/ml in patients with active oligoarticular JIA and 1,930 ± 187 ng/ml in active polyarticular JIA. In inactive disease, MRP8/MRP14 serum levels were 720 ± 79 ng/ml in oligoarticular JIAand 450 ± 59 ng/ml in polyarticular JIA, respectively. MRP8/MRP14 serum levels correlated well with disease activity as measured by the physician's global assessment (r= 0.43) and the number of active joints (r = 0.39) in individual patients. In oligoarticular JIA, MRP correlated better with the active joint count (r = 0.49) than with the physician's global assessment (r= 0.40). In polyarticular JIA, MRP correlated better with the physician's global assessment (r= 0.52) than with the active joint count (r = 0.35). Coefficients were statistically significant at the 0.01 level (2-tailed).

Predictive value of MRP8/MRP14 for the risk of relapse

The subgroup of relapsers contained 23 samples from 18 patients (5 boys, 13 girls), while the subgroup of non-relapsers contained 46 samples from 21 patients (5 boys, 16 girls). Eleven patients among the relapsers and 14 patients among the non-relapsers showed a polyarticular, and 7 patients from each subgroup an oligoarticular onset. The mean age of the patients at the end of the study was 12.2 years for the relapsers and 12.6 years for the non-relapsers. The mean disease duration was 7.2 years among the relapsers compared to 7.4 years among the non-relapsers.

The mean serum level of MRP8/MRP-14 among the non-relapsers was within the normal range (395 ± 60 ng/ml). The relapsers showed levels that were significantly higher than those of the non-relapsers (660 ± 70 ng/ml; $p < 0.05$) (Fig. 1B). No statistically significant differences between relapsers and non-relapsers were found for CRP (0.2 mg/ dl versus 0.1 mg/dl) or ESR (10.8 mm/ hour versus 8.6 mm/hour).

Receiver-operating curves (ROC) confirmed the diagnostic value of MRP8/ MRP14 serum levels (area under curve 0.78; $p < 0.01$). MRP8/MRP14 levels over 450 ng/ml had a diagnostic sensitivity for relapse of 65% while the specificity was 83% (likelihood ratio 3.7). The positive predictive value (i.e. the probability that an individual with a screening result above the cut-off threshold would develop a relapse) was 80%. Using a cut-off value of 350 ng/ml, the sensitivity was 78% and the specificity was 63%. The negative predictive value (i.e. the probability that an individual with a screening result below the threshold would stay in stable remission) was 85%. Table II summarizes our analyses of the accuracy of the tests for the prediction of relapses at these cut-off values.

Table II. MRP8/MRP14 test accuracy in predicting relapses.

	MRP8/MRP14	
Cut-off threshold	350 ng/ml	450 ng/ml
Sensitivity (%)	78	65
Specificity (%)	63	83
Positive likelihood ratio	2.1	3.7
Negative likelihood ratio	0.35	0.42
Positive predictive value (%)	68	80
Negative predictive value (%)	85	83

Discussion

MRP8/MRP14 are released under inflammatory conditions at the site of inflammation. A strong correlation between concentrations of MRP8/MRP14 in the synovial fluid and serum has been demonstrated, which is due to its expression in inflamed tissue (8). Thus, MRP8/MRP14 serum levels reflect local inflammation within the synovium. It is worth noting that MRP8/MRP14 was higher in the serum of patients with oligoarticular JIA than in patients with polyarticular JIA both in active and in inactive disease. There was also a closer correlation to the active joint counts in oligoarthritis. It seems conceivable that MRP8/MRP14 serum concentrations are linked to the volume of inflamed synovial tissue rather than simply to the number of joints. This could explain the higher levels found in oligoarticular JIA patients with the involvement of fewer, but larger joints compared to polyarticular JIA patients

who show the involvement of many smaller joints.

In general, we confirm that the serum levels of MRP8/MRP14 correlate well with disease activity in children with oligoarticular and polyarticular juvenile idiopathic arthritis, which has also been found in previous studies (8,9). In this study we also show that MRP8/MRP14 are reliable markers for clinically occult disease activity and may predict disease flares.

To our knowledge a predictive marker for relapse in juvenile idiopathic arthritis has not been found so far. Routine inflammatory markers such as CRPand ESR are not useful. Several adhesion molecules (17) as well as cytokines such as the interleukins (18, 19) and tumor necrosis factors (20) have been studied to discover if they could serve as markers of disease activity, but a predictive value has not yet been demonstrated.

In general, patients with inactive disease showed higher MRP8/MRP14 serum levels compared to healthy controls, while ESR, CRP and clinical assessment were in the normal range. These results indicate that even patients considered to be inactive differ in basal disease activity, detected only by MRP8/MRP14. As increased MRP8/MRP14 levels are found during bacterial infections (21) which thus could have influenced our results, all patients with obvious infections were excluded from this study. Possible latent infections, which could cause increases, can be considered as a risk factor for disease flares.

Comparing a subgroup of patients who relapsed with those who did not, we found significantly lower serum levels of MRP8/MRP14 for the latter group. We chose a cut-off at 1 year to distinguish these groups, which is arbitrary but considered to be a reasonable time to confirm the stability of disease-free remission. We found that patients with serum levels that had increased to over 450 ng/ml have a high risk of relapse (positive likelihood ratio 3.7). With respect to the high pre-test probability for disease flares in JIA, the likelihood of a relapse is significantly elevated in patients with high levels of MRP8/MRP14 despite clinically inactive disease. The positive predictive value for the risk of relapse is 80% in this group. As a consequence, in clinical practice the reduction of therapy would not be reasonable for this group of patients. In contrast, in patients with serum levels under 355 ng/ml the risk to relapse is low (negative likelihood ratio 0.35), and the negative predictive value (i.e. the probability of staying in stable remission in the presence of lower MRP8/MRP14 serum concentrations) is 85%. Thus, anti-inflammatory therapy can probably be adjusted without provoking an early reactivation in this group of patients.

Although our results have to be interpreted carefully since the number of patients analyzed in this study was relatively small, the data suggest that patients with inactive disease showing a higher level of MRP8/MRP14 run an increased risk of disease flares. This may indicate the need for careful follow-up in a subgroup of patients we consider to be inactive or in remission according to our clinical impression and routine diagnostic parameters. Withdrawal of medication might even be harmful for these patients. Our data indicate that local synovial inflammation may be present even months before a clinically apparent relapse is diagnosed.

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