RHEUMATOLOGY

Rheumatology 2020;59:2392–2401
doi:10.1093/rheumatology/kez590
Advance Access publication 6 January 2020

Original article

MRP8/14 and neutrophil elastase for predicting treatment response and occurrence of flare in patients with juvenile idiopathic arthritis

Anouk M. Barendregt [1,2], Saskia R. Veldkamp[1], Petra C. E. Hissink Muller[3], Annemarie van de Geer[4], Cathelijn Aarts[4], E. Charlotte van Gulik[1,2], Marco W. Schilham[3], Christoph Kessel[5], Mischa P. Keizer[4], Robert Hemke[2], Amara Nassar-Sheikh Rashid[1], Koert M. Dolman[6,7], Dieneke Schonenberg-Meinema[1], Rebecca ten Cate[3], J. Merlijn van den Berg[1], Mario Maas[2] and Taco W. Kuijpers[1,4]

Abstract

Objective. To study two neutrophil activation markers, myeloid-related protein (MRP) 8/14 and neutrophil elastase (NE), for their ability to predict treatment response and flare in patients with JIA.

Methods. Using samples from two cohorts (I and II), we determined MRP8/14 and NE levels of 32 (I) and 81 (II) patients with new-onset, DMARD-naïve arthritis and compared patients who responded to treatment (defined as fulfilling $\geq$ adjusted ACRpedi50 response and/or inactive disease) with non-responders (defined as fulfilling $<$ adjusted ACRpedi50 response and/or active disease) at 6 and 12 months. Secondly, we compared biomarker levels of 54 (I) and 34 (II) patients with clinically inactive disease who did or did not suffer from a flare of arthritis after 6 or 12 months. Receiver operating characteristic analyses were carried out to study the predictive value of MRP8/14 and NE for treatment response and flare.

Results. For both cohorts, baseline MRP8/14 and NE levels for patients who did or did not respond to treatment were not different. Also, MRP8/14 and NE levels were not different in patients who did or did not flare. Receiver operating characteristic analysis of MRP8/14 and NE demonstrated areas under the curve <0.7 in both cohorts.

Conclusion. In our cohorts, MRP8/14 and NE could not predict treatment response. Also, when patients had inactive disease, neither marker could predict flares.

Key words: juvenile idiopathic arthritis, biomarkers, MRP8/14, S100A8/A9, calprotectin, neutrophil elastase, disease activity, flare, treatment response, prediction

CLINICAL SCIENCE

Rheumatology key messages

- Myeloid-related protein 8/14 and neutrophil elastase could not predict treatment response in children with active JIA.
- Myeloid-related protein 8/14 and neutrophil elastase could not predict flares in JIA patients with inactive disease.

[1]Department of Paediatric Immunology, Rheumatology and Infectious Disease, Emma Children's Hospital, Amsterdam UMC, University of Amsterdam, Amsterdam, [2]Department of Radiology and Nuclear Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, [3]Department of Paediatric Rheumatology, Leiden University Medical Center, Leiden, [4]Department of Blood Cell Research, Sanquin Research, Amsterdam, The Netherlands, [5]Department of Paediatric Rheumatology and Immunology, University Children's Hospital Muenster, Muenster, Germany, [6]Department of Paediatric Rheumatology, Reade, Amsterdam and [7]Department of Paediatrics, Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands

Submitted 9 July 2019; accepted 22 October 2019

Correspondence to: Anouk M. Barendregt, Department of Paediatric Immunology, Rheumatology and Infectious Disease, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, room G1-226, Amsterdam, 1105 AZ, The Netherlands and Department of Radiology and Nuclear Medicine, Meibergdreef 9, Room G1-226, Amsterdam 1105 AZ, The Netherlands.
E-mail: a.m.barendregt@amsterdamumc.nl

Introduction

JIA is the most common chronic rheumatic disease of childhood with an estimated annual incidence of 8.2/100 000 [1]. Following the introduction of biologicals two decades ago, the majority of patients with JIA reach clinical remission within a few years after disease onset. Nevertheless, individual patients' response to treatment is still difficult to predict. Next, due to the unstable nature of the disease, up to 55% of patients experience a disease flare after induction of clinical remission [2]. Thus far, clinical characteristics and genetic or immunological markers are not able to predict treatment response or flares [3, 4]. To facilitate individualized treatment aiming at prevention of irreversible joint damage, a strong need exists for validated biomarkers that enable a personalized treat-to-target approach.

Earlier research showed that the neutrophil and monocyte activation marker, myeloid-related protein (MRP) 8/14 heterocomplex (a protein from the S100-family also known as S100A8/A9 and calprotectin), was useful for predicting treatment response. Increased pre-treatment MRP8/14 levels in patients starting MTX or anti-TNF were indicative for better treatment response, with odds ratios for obtaining an ≥ACRpedi50 response or inactive disease from 1.31–1.55 [5–7]. In addition, increased MRP8/14 levels in patients with inactive disease predicted subsequent flares [6, 8, 9]. Notwithstanding these promising results, a recent article on 137 JIA patients with clinically inactive disease could not confirm that MRP8/14 levels before anti-TNF withdrawal had any value for the prediction of flares [10].

Since the majority of circulating MRP8/14 is derived from neutrophils [11], we decided to compare this biomarker with another leucocyte activation marker, neutrophil elastase (NE). This biomarker has not been studied in JIA with regard to treatment response and flare prediction. NE has been assessed in respiratory diseases [12] and auto-immune inflammatory bowel disorders [13], and generally serves as a marker of inflammation.

Using data from two Dutch cohorts of JIA patients, we tested the hypotheses that (i) patients with increased MRP8/14 and NE levels are better responders to therapy, and (ii) elevated biomarker levels during clinical remission are prognostic for subsequent flares.

Methods

Cohort I

In total, 206 consecutive patients with diagnosed or suspected non-systemic JIA were prospectively included in cohort I between May 2011 and March 2015. All patients (when ≥12 years) and parents gave written informed consent for participating in the study. The institutional review board of our medical centre approved the conduct of this study. Patients were recruited from the outpatient department of one of the following medical centers in Amsterdam: Amsterdam University Medical Center, Onze Lieve Vrouwe Gasthuis (OLVG) and Reade.

At baseline, demographic and clinical characteristics were collected by our paediatric rheumatologists/immunologists. Collected clinical characteristics included ILAR subtype, active joint count (AJC), limited joint count, visual analogue scale (VAS) for disease activity as assessed by the physician and a VAS for global wellbeing as assessed by the patient and/or their parents. Both VAS scores ranged from 0 to 10, with 0 corresponding to no disease activity/excellent wellbeing and 10 to maximal disease activity/very poor wellbeing. Blood samples for determination of MRP8/14 and NE levels and standard laboratory measures were obtained and included ESR, CRP, leucocyte and neutrophil counts, and presence of ANA, IgM RF and HLA-B27. During clinical follow-up, patients were assessed by their paediatric rheumatologist with intervals of 3–6 months. Clinical follow-up of patients was retrospectively inspected by the investigators using the medical files.

Patients were subdivided in two groups based on their disease activity at inclusion (supplementary Fig. S1, available at *Rheumatology* online). The first group consisted of 32 patients with DMARD-naïve arthritis (AJC ≥1) attributed to JIA ('early arthritis'). The second group consisted of 54 JIA patients in clinically inactive disease as defined following the Wallace criteria [14] (no joints with active arthritis, no fever, rash, serositis, splenomegaly or generalized lymphadenopathy attributable to JIA, no uveitis, normal ESR or CRP, and the physician's VAS of disease activity indicating no disease activity). We deemed VAS disease activity inactive when <1 was scored. In practice, physicians hesitate to score a zero, even when patients have inactive disease based on physical examination. Therefore, this VAS modification for categorization of inactive disease, as used by other researchers as well [2, 15], was implemented in our study.

In total, 120 patients could not be categorized as described above because of not being DMARD-naïve ($n = 58$), not eventually having JIA ($n = 39$), uncertain diagnosis ($n = 21$) or not fulfilling all Wallace criteria ($n = 2$; elevated ESR in both patients), and were therefore excluded from the study.

Cohort II

The second cohort consisted of 94 patients who participated in the investigator-initiated, multicentre single-blinded BeSt for Kids trial that investigated different treatment strategies in children with new-onset DMARD-naïve JIA [16]. Written informed consent was obtained from all parents and also from children, if ≥12 years of age. Institutional review board (IRB) approval was obtained from Leiden University Medical Center and the IRB of local centres where patients were included. After enrolment in the trial, laboratory and clinical follow-up data were prospectively collected every 3 months for a period of 24 months by a paediatric rheumatologist or trial nurse following the trial protocol. For determination of MRP8/14 and NE levels, samples acquired at

inclusion in the study and samples acquired 12 months after inclusion in the trial were analysed.

Similar to cohort I, cohort II was subdivided in two groups (supplementary Fig. S1, available at *Rheumatology* online): the first group ($n = 81$) consisted of patients with early (<18 months of complaints) DMARD-naïve arthritis ('early arthritis'). The second group consisted of 34 patients who, after 12 months of treatment, had attained inactive disease following the Wallace criteria.

In total, 13 patients included in the trial could not be analysed in this study because of failure of biomarker level determination ($n = 9$) or acquisition of a biomarker sample after initiation of a DMARD ($n = 3$), or not having JIA ($n = 1$).

Primary and secondary outcomes

In both cohorts, we studied similar primary outcomes at 6 and 12 months: treatment response in the early arthritis group and occurrence of a flare of arthritis in the group of patients with clinically inactive disease. For assessment of treatment response, we dichotomized the patients into responders [fulfilling ≥ adjusted ACRpedi50 (aACRpedi50) response [15, 17] and/or criteria for Wallace inactive disease [14]] or non-responders [<aACRpedi50 response and/or having active disease (i.e. not fulfilling Wallace criteria)]. The ACRpedi (American College of Rheumatology Pediatric) response is based on the percentage of change in the six ACR core outcome variables: physician global assessment of disease activity (on a 10-cm VAS), parent/patient assessment of overall well-being (on a 10-cm VAS), functional ability of the child as measured using the Childhood Health Assessment Questionnaire, the number of joints with active arthritis, the number of joints with limited range of motion and the ESR [15, 17]. For example, fulfilling the aACRpedi50 response requires a ≥50% improvement in at least three of the ACR outcome variables, with no more than one of the other variables showing a worsening of disease activity of 30%. We deliberately assessed the groups based on aACRpedi responses with the aim to correct for non-significant changes in scores that remained within normal limits: this concerned any changes in ESR measures between 0 and 16 mm/h and changes in the physician global assessment of disease activity that were between 0 and 1 cm. For the assessment of occurrence of a flare of arthritis, patients with clinically inactive disease at moment of inclusion were dichotomized into flare (AJC ≥1) or no flare (AJC 0).

Secondary outcomes for the early arthritis group included fulfillment of aACRpedi30/50/70/90/100 responses (data only available for cohort II), and attainment of inactive disease, i.e. fulfilling Wallace criteria within 6 or 12 months (data available for both cohorts).

Laboratory analyses

MRP8/14 and NE levels were measured by ELISAs that were developed at Sanquin laboratory, The Netherlands. Additional details about the development of the ELISAs and measurement of MRP8/14 and NE can be found in supplementary File S1, available at *Rheumatology* online.

Statistical analyses

Normality of all variables was considered by histogram analysis; none of our variables followed a normal distribution. Patient characteristics were analysed using Mann–Whitney U tests, χ^2 tests or Fisher exact tests. The difference in MRP8/14 and NE levels between responders and non-responders, and patients with and without a flare, was analysed by Mann–Whitney U test. Similarly, differences in secondary outcomes as described above were analysed by Mann–Whitney U test.

The diagnostic performance of MRP8/14 and NE for prediction of treatment response or flare was studied using receiver operating characteristic curve analysis, from which the area under the curve (AUC) was calculated. If the AUC was >0.7, cut-off levels of MRP8/14 or NE were determined for further calculation of sensitivity, specificity and odds ratios to predict treatment response or the occurrence of flare.

Correlation of MRP8/14, NE and inflammatory laboratory markers (ESR, leukocytes and neutrophils) was analysed using Spearman's correlation coefficient. All statistical analyses were done using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). For analysis of differences in MRP8/14 and NE considering secondary outcomes (aACRpedi responses and attainment of inactive disease), a Bonferroni correction was used to account for multiple testing; thus, a *P*-value of <0.008 (0.05/6) was used. For all other analyses, a *P*-value of <0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics

Thirty-two patients in cohort I and 81 patients in cohort II had early arthritis (supplementary Fig. S1, available at *Rheumatology* online). Cohort II consisted of younger patients who had a shorter duration of symptoms, higher AJC and higher composite DAS. Patients in cohorts I and II did not differ regarding gender and the presence of ANA. Baseline characteristics of both groups are summarized in Table 1. In cohort I, 4 and 10 patients were categorized as responders to treatment after 6 and 12 months respectively. In cohort II, 50 and 66 patients were categorized as responders to treatment after 6 and 12 months, respectively (supplementary Fig. S1, available at *Rheumatology* online).

Clinically inactive disease was present in 54 patients in cohort I and 34 patients in cohort II (supplementary Fig. S1, available at *Rheumatology* online). As in the early arthritis cohorts, age and symptom duration were different between the cohorts. Moreover, the distribution of JIA subtypes was not equal, DAS were higher in cohort II and

Table 1 Description and comparison of patient characteristics of cohort I and II

	Early arthritis			Clinically inactive disease		
	Cohort I (n = 32)	Cohort II (n = 81)	P	Cohort I (n = 54)	Cohort II (n = 34)	P
Gender, female (n, %)	22 (69)	53 (65)	0.74	32 (59)	18 (53)	0.56
Median age, months (IQR)	161 (124–189)	108 (55–149)	<0.01	169 (151–183)	133 (73–165)	<0.01
Median months between onset of joint complaints and date of study (IQR)	14 (5–31)	8 (4–13)[a]	<0.01	84 (46–112)	20 (15–23)[b]	<0.01
JIA subtype (n, %)						
Oligoarticular JIA	11 (34)	10 (12)	<0.01	31 (57)	5 (15)	<0.01
Polyarticular RF− JIA	10 (31)	57 (70)	<0.01	18 (33)	24 (71)	<0.01
Polyarticular RF+ JIA	2 (6)	0 (0)	0.08	0 (0)	0 (0)	n/a
Undifferentiated JIA	0 (0)	0 (0)	n/a	3 (6)	0 (0)	0.28
Enthesis-related arthritis	5 (16)	0 (0)	<0.01	2 (4)	0 (0)	0.52
Psoriatic JIA	4 (13)	14 (17)	0.78	0 (0)	5 (15)	<0.01
DMARD treatment (n, %)						
MTX monotherapy	–	–	n/a	32 (59)	11 (32)	<0.05
Etanercept monotherapy	–	–	n/a	1 (2)	7 (21)	<0.01
Sulfasalazine monotherapy	–	–	n/a	1 (2)	0 (0)	n/a
MTX + anti-TNF	–	–	n/a	6 (11)	13 (38)	<0.05
No DMARD treatment	32 (100)	81 (100)	n/a	14 (26)	3 (9)	0.06
Median ESR, mm/h (IQR) (reference values = 0–16)	5 (2–10,3)	8 (2–17,3)[a]	0.10	5 (2–6)	5 (2–7,5)	0.37
Median neutrophils, 10^9 E/l (IQR) (reference values = 1.8–7.2)	3,3 (2,5–3,8)[c]	3,9 (2,7–4,9)[b]	<0.05	2,9 (2,2–4,3)[d]	Not measured	n/a
Median CRP[c], mg/l (IQR) (reference values = 0–5)	0,6 (0,3–2,5)	3 (3–3)[b]	<0.01	0,3 (0,0–1,0)[c]	Not measured	n/a
ANA+ (n, %)	10 (31)	31 (38)	0.48	17 (32)	14 (41)	0.35
Median AJC (IQR)	5 (2–10,5)	8 (5-13)	<0.01	0	0	1.00
Median JADAS-10 (IQR)	14 (7,9–18,1)[e]	17,5 (14,5–21,2)[a]	<0.01	0,25 (0,0–1,7)[d]	1,4 (0,4–3,3)	<0.01
Median JADAS-71 (IQR)	14 (7,9–20,0)[e]	18,4 (14,5–24,4)[a]	<0.01	0,25 (0,0–1,7)[d]	1,4 (0,4–3,3)	<0.01
Median MRP8/14, ng/ml (IQR)	5393 (1546–14 988)[f]	1855 (1164–3295)	<0.01	1183 (697–2100)[g]	1072 (621–2423)	0.79
Median elastase, ng/ml (IQR)	372 (199–538)[a]	252 (148–432)[f]	0.06	312 (186–483)[h]	170 (116–351)	<0.05

Patients were subdivided in early arthritis patients (second and third column) and patients with clinically inactive disease as defined following the Wallace criteria [14] (fifth and sixth column).
[a]One missing value; [b]four missing values; [c]two missing values; [d]six missing value; [e]three missing values; [f]10 missing values; [g]20 missing values; [h]seven missing values. AJC: active joint count; JADAS: juvenile arthritis DAS; n/a: not applicable.

treatment regimens were different (Table 1). In cohort I, six and eight patients had a flare within 6 and 12 months, respectively. In cohort II, 8 and 15 patients suffered a flare within 6 and 12 months, respectively.

MRP8/14 levels and response to treatment or prediction of flare

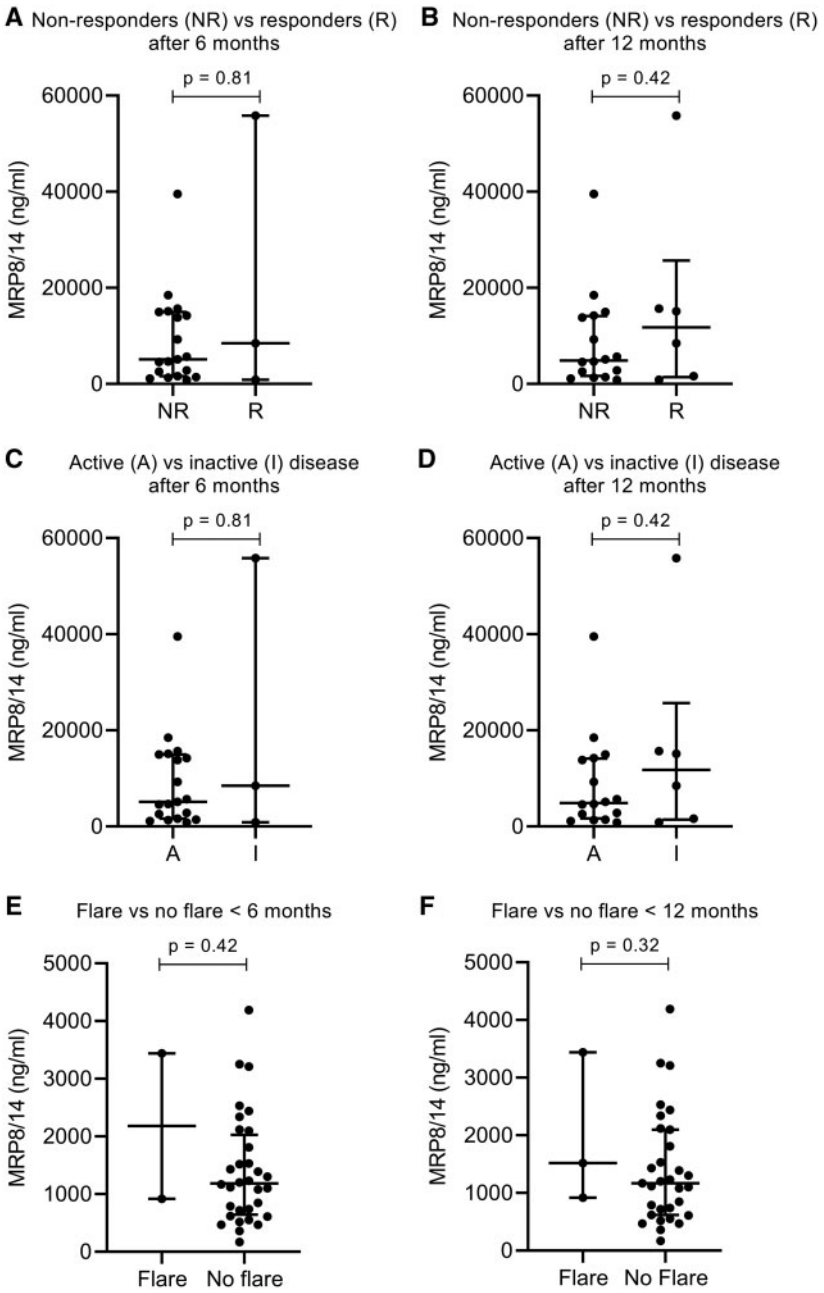
In the patients with early arthritis in both cohorts we found no differences in baseline MRP8/14 levels in responders as compared with non-responders after 6 and 12 months of treatment (Figs 1 and 2; supplementary Table S1, available at *Rheumatology* online). Also, when treatment response was analysed using achievement of aACRpedi responses as well as inactive disease as secondary outcomes, no differences in MRP8/14 levels were found (Figs 1 and 2; supplementary Table S1, available at *Rheumatology* online).

In both cohorts, MRP8/14 levels in patients with clinically inactive disease were not different in patients who flared within 6 or 12 months as compared with patients who did not flare within 6 or 12 months (Figs 1 and 3; supplementary Table S1, available at *Rheumatology* online).

NE levels and response to treatment or prediction of flare

In patients with early arthritis in both cohorts I and II, no significant differences in baseline NE levels were found between responders and non-responders after 6 and 12 months of treatment (Figs 4 and 5; supplementary Table S1, available at *Rheumatology* online). When

Fig. 1 Dot plots of MRP8/14 levels in cohort I for the primary outcomes



(**A**) Treatment response after 6 months. (**B**) Treatment response after 12 months. (**C**) Active *vs* inactive disease after 6 months. (**D**) Active *vs* inactive disease after 12 months. (**E**) Occurrence of a flare within 6 months. (**F**) Occurrence of a flare within 12 months.

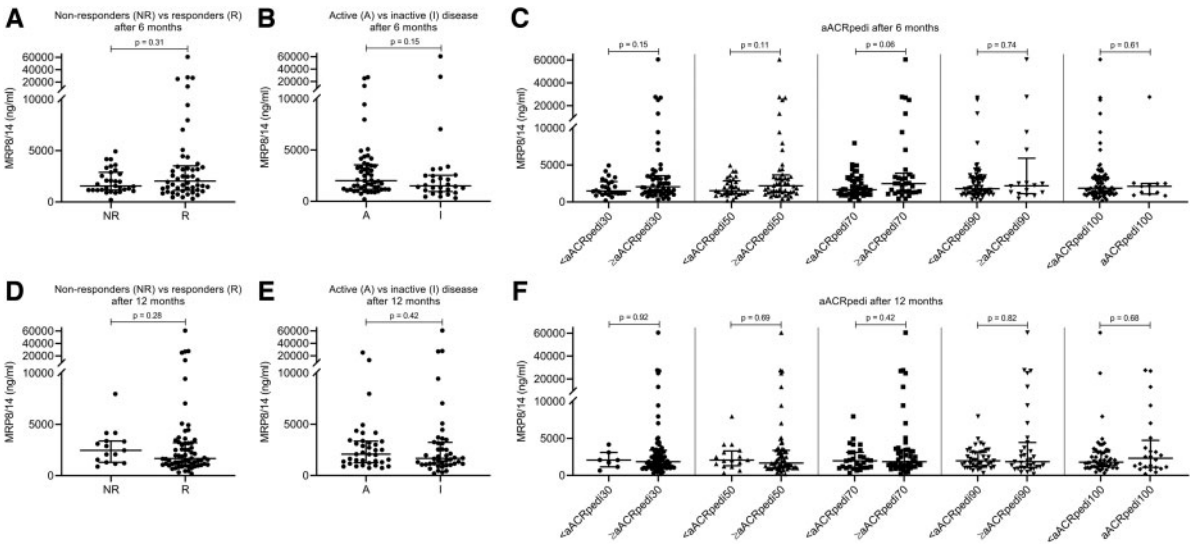
treatment response was analysed using aACRpedi responses as well as inactive disease as secondary outcomes, no differences in baseline NE levels were found (Figs 4 and 5; supplementary Table S1, available at *Rheumatology* online).

For both cohorts, no differences were found in NE levels between patients with inactive disease who did or did not suffer from a flare of arthritis within 6 or 12 months (Figs 3 and 4; supplementary Table S1, available at *Rheumatology* online).

Diagnostic performance of MRP8/14 and NE

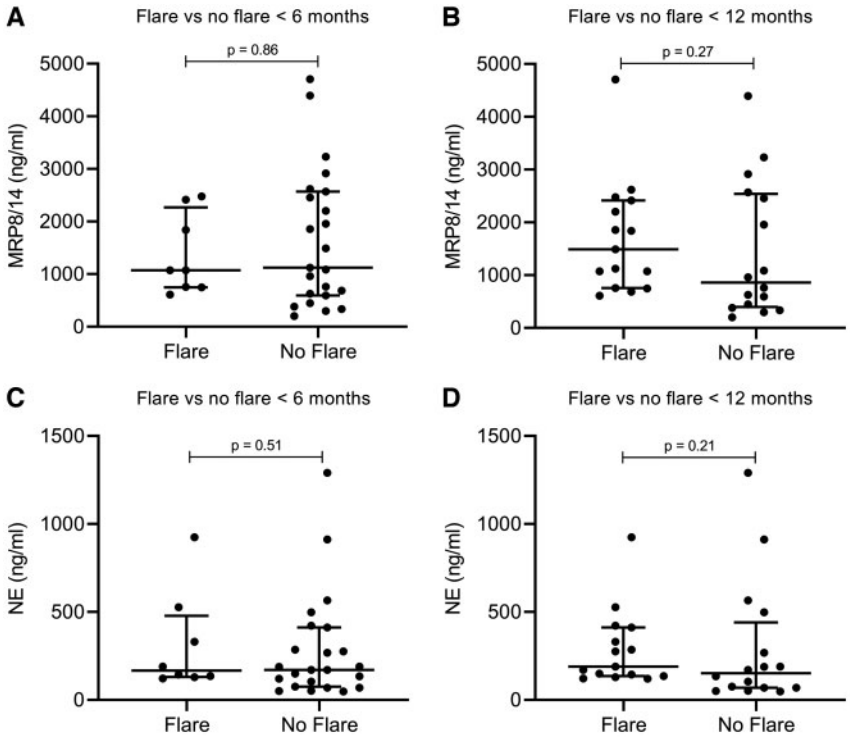
With receiver operating characteristic curve analyses, the diagnostic performance of MRP8/14 and NE levels for prediction of treatment response or flare was studied

Fig. 2 Dot plots of MRP8/14 levels in cohort II for treatment response



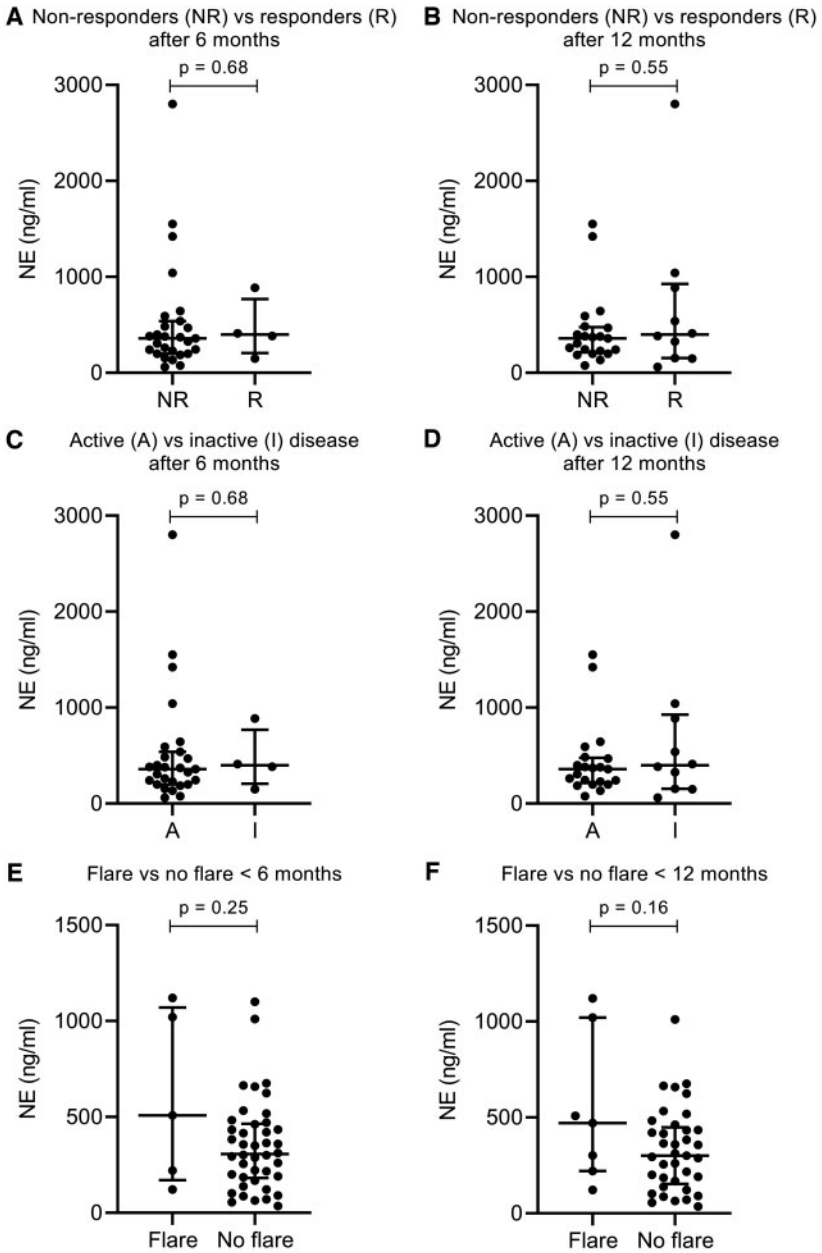
(**A**) Treatment response after 6 months. (**B**) Active *vs* inactive disease after 6 months. (**C**) Adjusted ACRpedi30/50/70/90/100 responses after 6 months. (**D**) Treatment response after 12 months. (**E**) Active *vs* inactive disease after 12 months. (**F**) Adjusted ACRpedi30/50/70/90/100 responses after 12 months.

Fig. 3 Dot plots of MRP8/14 and NE levels in cohort II for flare prediction



(**A**) MRP8/14, occurrence of a flare within 6 months. (**B**) MRP8/14, occurrence of a flare within 12 months. (**C**) NE, occurrence of a flare within 6 months. (**D**) NE, occurrence of a flare within 12 months. NE: neutrophil elastase.

Fig. 4 Dot plots of NE levels in cohort I for the primary outcomes



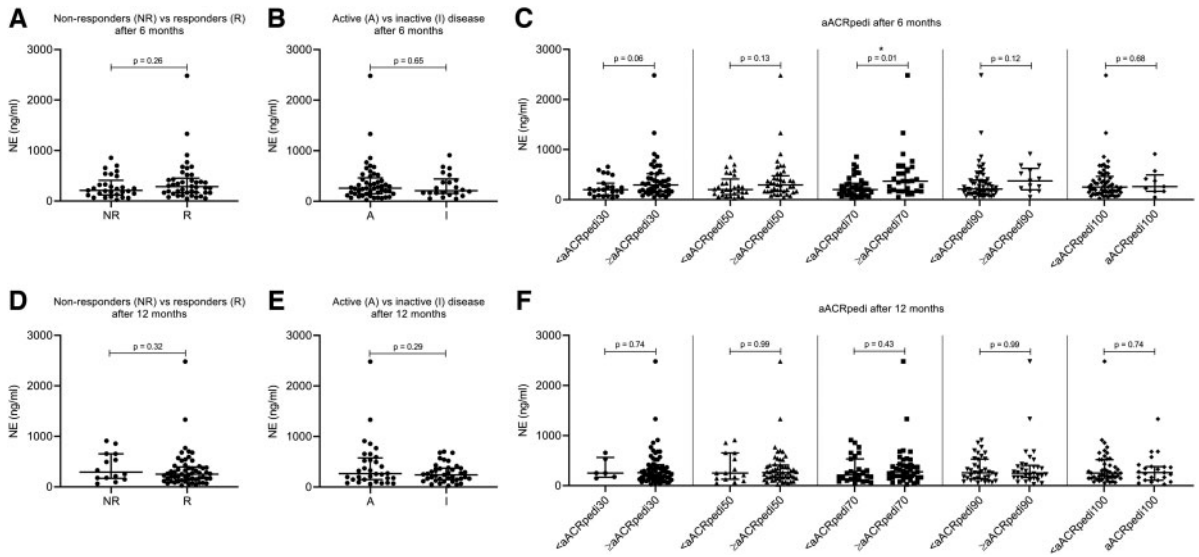
(**A**) Treatment response after 6 months. (**B**) Treatment response after 12 months. (**C**) Active *vs* inactive disease after 6 months. (**D**) Active *vs* inactive disease after 12 months. (**E**) Occurrence of a flare within 6 months. (**F**) Occurrence of a flare within 12 months. NE: neutrophil elastase.

using the AUC for both cohorts. All calculated AUCs were <0.7, which was our cut-off level for further calculation of sensitivity, specificity and odds ratios to predict treatment response or the occurrence of flare.

Correlation of MRP8/14 with laboratory markers of inflammation

In the early arthritis patients in cohort I, no correlations were found between MRP8/14 and ESR, leucocyte counts and neutrophil counts (supplementary Table S2, available at *Rheumatology* online). In cohort II, MRP8/14 and ESR were correlated (ρ 0.38; $P < 0.01$). Similarly, MRP8/14 levels and neutrophil counts showed correlation (ρ 0.46; $P < 0.01$). In patients with inactive disease in cohort I, MRP8/14 levels were not correlated with markers of inflammation, while in cohort II, a significant correlation was found between MRP8/14 and ESR (ρ 0.43; $P = 0.01$). When combining the patients with

FIG. 5 Dot plots of NE levels in cohort II for treatment response



(**A**) Treatment response after 6 months. (**B**) Active *vs* inactive disease after 6 months. (**C**) Adjusted ACRpedi30/50/70/90/100 responses after 6 months. (**D**) Treatment response after 12 months. (**E**) Active *vs* inactive disease after 12 months. (**F**) Adjusted ACRpedi30/50/70/90/100 responses after 12 months. NE: neutrophil elastase.

early arthritis of cohort I and II, MRP8/14 and ESR (ρ 0.27; $P < 0.05$) as well as MRP8/14 and neutrophil counts (ρ 0.30; $P < 0.01$) were correlated. Taking all patients of cohort I and II together, without distinction by disease activity, MRP8/14 levels showed correlation with ESR (ρ 0.16; $P < 0.05$), leucocyte counts (ρ 0.18; $P < 0.05$) and neutrophil counts (ρ 0.33; $P < 0.01$) (supplementary Table S2, available at *Rheumatology* online).

Correlation of NE with laboratory markers of inflammation

In the early arthritis patients in both cohorts, NE levels significantly correlated with neutrophil counts (cohort I: ρ 0.59; $P < 0.01$; cohort II: ρ 0.27; $P = 0.03$) (supplementary Table S2, available at *Rheumatology* online). Also, NE levels correlated with leucocyte counts in early arthritis patients in cohort I (ρ 0.48; $P < 0.01$). In patients with inactive disease in both cohorts, no correlations were found between NE levels and other markers of inflammation (supplementary Table S2, available at *Rheumatology* online). When combining the patients with early arthritis of cohorts I and II, NE correlated with neutrophil counts only (ρ 0.31; $P < 0.01$). Taking all patients of cohorts I and II together, without distinction by disease activity, NE levels showed correlation with leucocyte counts (ρ 0.16; $P < 0.05$) and neutrophil counts (ρ 0.27; $P < 0.01$) (supplementary Table S2, available at *Rheumatology* online).

Calculated across all patients of cohorts I and II, MRP8/14 and NE levels were significantly correlated (ρ 0.63; $P < 0.01$) (supplementary Table S2, available at *Rheumatology* online).

Discussion

In this study, we analysed data of two Dutch JIA cohorts including a total of 113 patients with early arthritis and 88 patients with clinically inactive JIA. We were not able to replicate the findings by other authors who demonstrated that increased levels of MRP8/14 were predictive of good response to treatment [5–7] and/or future flares in JIA [6, 8, 9, 18]. No differences were found in MRP8/14 levels in any of our primary outcomes: treatment response after 6 and 12 months and occurrence of a flare within 6 or within 12 months. We also did not find any differences in MRP8/14 levels for our secondary outcomes: aACRpedi responses and fulfillment of Wallace criteria for inactive disease. For an alternative neutrophil-derived biomarker of inflammation, NE, we also found no evidence that elevated levels might be associated with good treatment response or prediction of flare.

A detailed overview of published data on MRP8/14 for predicting treatment response and flare in non-systemic JIA is presented in supplementary Table S3, available at *Rheumatology* online. Our results contradict the earlier published data on MRP8/14 levels as a predictive marker for treatment response [5–7]. The cohorts we studied were heterogeneous concerning drug use; in all other studies, response to the start of one class of drugs (MTX [5] or anti-TNF [6, 7]) was studied, whereas in our first cohort (I), treatment choice was left to the paediatric rheumatologist and in our second cohort (II), treatment depended on trial randomization [15]. Moreover, JIA subtypes in the other studies were differently distributed as compared with

our cohorts of early arthritis patients: they included more polyarticular RF positive (poly-RF+) patients (4% [7] to 15% [6]) as compared with our first (6%) and second cohort (0%). Our cohort II, on the other hand, consisted of 70% poly-RF– patients, compared with 26% [7] to 40% [5] in other studies. Nonetheless, our cohort I was more similar to the other studies: it consisted of 31% poly-RF– patients. Potentially, the higher proportion of poly-RF+ patients, the subtype with most aggressive disease and highest inflammatory response, resulted in more pronounced results for prediction of treatment response in the papers from the other research groups.

Although the results of our study regarding MRP8/14 for prediction of flare contradict the findings of several other research groups, our data concur with the data of a recent paper that included 137 JIA patients with inactive disease [10]. It is likely that a multitude of factors account for these discrepant findings between studies. First, an important difference is that in our cohorts, medication was not specifically withdrawn at inclusion in the study, whilst the other studies were all MTX or anti-TNF withdrawal studies. In our cohort I, 26% of patients were DMARD-free at time of inclusion, 59% were using MTX monotherapy and 11% used both MTX and etanercept. Second, the composition of the reported patient groups varies: e.g. a few systemic JIA patients were included in the paper by Foell [8]. Also, three papers included a small proportion of patients with poly RF+ JIA (4% [6] to 12% [10]), while we did not include any poly-RF+ or systemic JIA patients. Our two cohorts of patients with clinically inactive disease consist of a majority of poly-RF– and oligoarticular JIA patients. Third, the definitions for flare or relapse differ between studies. We used an AJC $\geq$1 for definition of a flare, while most papers used the 2002 preliminary criteria for flare in JRA [19], which define a flare as at least 40% worsening of two of the six core response variables without concomitant improvement of more than one of the remaining core response variables by 30% or more, the core response variables being AJC (which has to be $\geq$2 in order to fulfill flare definition), limited joint count, physician VAS, patient/parent VAS, CRP or ESR and Childhood Health Assessment Questionnaire. We deliberately chose an isolated AJC $\geq$1 because we aimed at studying a flare of arthritis that could also include just one large joint in oligoarticular JIA patients.

Limitations of our study include, firstly, the relatively low number of patients in the cohorts, which could have led to type II errors in our analyses. For example, regarding response to therapy, few patients could be categorized as responders to treatment in cohort I (10 out of 32 patients after 12 months of treatment), although in cohort II, 66 out of 81 patients were categorized as responders to therapy after 12 months. These numbers clearly demonstrate that the patients in cohort I and II were not similar: the latter cohort consisted of patients participating in a multicentre medication strategy trial [16]. These patients were younger in age, mainly from the poly-RF– subtype and had a shorter symptom duration and higher disease activity as compared with cohort I, which consisted of non-systemic JIA patients that were seen on our outpatient department and consented to participate in the study. Another drawback is the retrospective nature of the determination of treatment response and flare in cohort I. At baseline, all patient characteristics were acquired in a standardized manner, but clinical follow-up by the paediatric rheumatologist was not standardized and the investigators had no influence on the timing and data collection of follow-up. This limitation is compensated by cohort II, which consisted of patients participating in the trial; thus all data were prospectively acquired, following the standardized trial protocol [16], and our data show comparable results for both cohorts.

In conclusion, we found no evidence that either MRP8/14 or NE levels could serve as a reliable marker for treatment response or flare prediction. The biomarkers were not able to predict which patients would or would not respond to treatment with a DMARD; in addition, when patients had inactive disease, neither marker could make accurate predictions for upcoming flares. The two cohorts that we studied are representative for Dutch day-to-day JIA care but were not equal regarding age, subtype and disease activity markers. Therefore, we report the cohorts separately, aiming at the reporting of all data to reinforce the discussion regarding the value of MRP8/14 levels in JIA.

Acknowledgements

This work was supported by an individual MD/PhD grant from the Academic Medical Center/University of Amsterdam, awarded to A.M.B.

Funding: No specific funding was received from any funding bodies in the public, commercial or not-for-profit sectors to carry out the work described in this manuscript.

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

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Thierry S, Fautrel B, Lemelle I, Guillemin F. Prevalence and incidence of juvenile idiopathic arthritis: a systematic review. Joint Bone Spine 2014; 81:112–7.

2 Guzman J, Oen K, Tucker LB *et al*. The outcomes of juvenile idiopathic arthritis in children managed with contemporary treatments: results from the ReACCh-Out cohort. Ann Rheum Dis 2015;74:1854–60.

3 Bulatović M, Heijstek MW, Van Dijkhuizen EH *et al*. Prediction of clinical non-response to methotrexate treatment in juvenile idiopathic arthritis. Ann Rheum Dis 2012; 71:1484–9.

4 Aquilani A, Marafon DP, Marasco E *et al*. Predictors of flare following etanercept withdrawal in patients with rheumatoid factor-negative juvenile idiopathic arthritis who reached remission while taking medication. J Rheumatol 2018;45:956–61.

5 Moncrieffe H, Ursu S, Holzinger D *et al*. A subgroup of juvenile idiopathic arthritis patients who respond well to methotrexate are identified by the serum biomarker MRP8/14 protein. Rheumatology (Oxford) 2013;52: 1467–76.

6 Anink J, Van Suijlekom-Smit LW, Otten MH *et al*. MRP8/14 serum levels as a predictor of response to starting and stopping anti-TNF treatment in juvenile idiopathic arthritis. Arthritis Res Ther 2015;17:200.

7 Alberdi-Saugstrup M, Nielsen S, Mathiessen P, Nielsen CH, Müller K. Low pretreatment levels of myeloid-related protein-8/14 and C-reactive protein predict poor adherence to treatment with tumor necrosis factor inhibitors in juvenile idiopathic arthritis. Clin Rheumatol 2017; 36:67–75.

8 Foell D. Methotrexate treatment in juvenile idiopathic arthritis: when is the right time to stop? Ann Rheum Dis 2004;63:206–8.

9 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.

10 Hinze CH, Foell D, Johnson AL *et al*. Serum S100A8/A9 and S100A12 levels in children with polyarticular forms of juvenile idiopathic arthritis: relationship to maintenance of clinically inactive disease during anti-tumor necrosis factor therapy and occurrence of disease flare after discontinuation of therapy. Arthritis Rheumatol 2019;71:451–9.

11 Austermann J, Spiekermann C, Roth J. S100 proteins in rheumatic diseases. Nat Rev Rheumatol 2018;14: 528–41.

12 Polverino E, Rosales-Mayor E, Dale GE, Dembowsky K, Torres A. The role of neutrophil elastase inhibitors in lung diseases. Chest 2017;152:249–62.

13 Kyriakidi KS, Tsianos VE, Karvounis E *et al*. Neutrophil anti-neutrophil cytoplasmic autoantibody proteins: bactericidal increasing protein, lactoferrin, cathepsin, and elastase as serological markers of inflammatory bowel and other diseases. Ann Gastroenterol 2016;29:258–67.

14 Wallace CA, Ruperto N, Giannini E; Childhood Arthritis and Rheumatology Research Alliance; Pediatric Rheumatology International Trials Organization; Pediatric Rheumatology Collaborative Study Group. Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis. J Rheumatol 2004;31:2290–4.

15 Hissink Muller PC, Brinkman DM, Schonenberg D *et al*. A comparison of three treatment strategies in recent onset non-systemic juvenile idiopathic arthritis: initial 3-months results of the BeSt for Kids-study. Pediatr Rheumatol Online J 2017;15:11.

16 Hissink Muller P, Brinkman DMC, Schonenberg-Meinema D *et al*. Treat to target (drug-free) inactive disease in DMARD-naive juvenile idiopathic arthritis: 24-month clinical outcomes of a three-armed randomised trial. Ann Rheum Dis 2019;78:51–9.

17 Giannini EH, Ruperto N, Ravelli A *et al*. Preliminary definition of improvement in juvenile arthritis. Arthritis Rheum 1997;40:1202–9.

18 Schulze zur Wiesch A, Foell D, Frosch M *et al*. Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis. Clin Exp Rheumatol 2004;22:368–73.

19 Brunner HI, Lovell DJ, Finck BK, Giannini EH. Preliminary definition of disease flare in juvenile rheumatoid arthritis. J Rheumatol 2002;29:1058–64.

20 Frosch M, Strey A, Vogl T *et al*. Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. Arthritis Rheum 2000;43:628–37 [Cited in Supplementary].