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Myeloid-related Protein 8/14 Levels in Rheumatoid Arthritis: Marker of Disease Activity and Response to Methotrexate

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ABSTRACT. ***Objective.*** Myeloid-related proteins (MRP) 8/14 belong to a family of calcium-binding proteins produced by myeloid cells. Baseline serum levels of MRP8/14 have been shown to predict response to biologicals in rheumatoid arthritis (RA). Because methotrexate (MTX) is the first-line therapy in RA, we studied whether MRP8/14 levels can predict response to MTX.

Methods. Patients with active RA disease who were naive to disease-modifying antirheumatic drugs were enrolled. All patients were treated with MTX only, to a maximum of 25 mg/week or the maximal tolerated dose. At 4 months, the European League Against Rheumatism response was assessed. All patients who needed rescue therapy after 2 months or who did not respond at 4 months were classified as nonresponders.

Results. Ninety patients were enrolled, of whom 3 discontinued MTX within 4–6 weeks, so 87 patients were analyzed [74 women, median (interquartile range; IQR) for the Disease Activity Score at 28 joints (DAS28) was 4.43 (4.1–5.1)]. The median (IQR) serum MRP8/14 level at baseline was 19.95 μg/ml (11.49–39.06). The serum MRP8/14 had good correlation with DAS28-C-reactive protein (CRP; r = 0.35, p = 0.001). The MRP8/14 levels fell significantly after 4 months of treatment (10.28 μg/ml, 5.95–16.05, p < 0.001). Among 87 patients, 69 were responders. The median (IQR) baseline level of MRP8/14 was higher among responders compared with nonresponders: 23.99 μg/ml (15.39–42.75) versus 9.58 μg/ml (6.11–24.93, p = 0.00250). The levels declined in the responders, from 23.99 μg/ml (15.39–42.75) to 10.41 μg/ml (5.83–15.61, p < 0.001), but not in the nonresponders, from 9.58 μg/ml (6.11–24.93) to 9.19 μg/ml (7.74–21.96, p = 0.687). Receiver-operation characteristic analysis showed that MRP8/14 was a better predictor of response than CRP and erythrocyte sedimentation rate, especially with early disease onset (< 1-yr duration).

Conclusion. MRP8/14 is a good marker of disease activity in RA, and higher levels predict response to MTX. (First Release February 1 2016; J Rheumatol 2016;43:731–7; doi:10.3899/jrheum.150998)

Key Indexing Terms:

RHEUMATOID ARTHRITIS | INFLAMMATORY ARTHRITIS | METHOTREXATE
MRP8/14 | DRUG RESPONSE | BIOMARKER

Rheumatoid arthritis (RA) is a common chronic immune-mediated arthritis characterized by synovial inflammation, and progressive cartilage and bone destruction[1]. Early RA represents a "window of opportunity" for the prevention of joint damage by early drug intervention[2]. Thus, a better understanding of biomarkers, which can predict response to conventional disease-modifying antirheumatic drugs (DMARD), would be helpful in choosing the right drug for each patient.

Though in the past, only the adaptive immune system was thought to be involved in the pathogenesis of RA; in the last decade, the involvement of an innate immune system is becoming more evident. Macrophage activation through Toll-like receptors (TLR) either by microbial triggers or by endogenous ligands results in the release of proinflammatory cytokines such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor (TNF)[3]. Further, the activated macrophages promote development of autoreactive CD8+T cells and Th17 cells that further mediate bone and cartilage loss. Among the known endogenous activators of TLR4 are myeloid-related proteins (MRP) 8 (S100A8) and 14 (S100A9)[4].

Methotrexate (MTX) has been the first-line DMARD for RA since the 1980s, and has a good efficacy to toxicity ratio

From the Department of Clinical Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India.

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P.S. Patro, DM Senior Resident, MD Internal Medicine, Department of Clinical Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences; A. Singh, PhD Student, MSc Biotechnology, Department of Clinical Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences; R. Misra, Professor, FRCP, Department of Clinical Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences; A. Aggarwal, Professor, DM Clinical Immunology, Department of Clinical Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences.

Address correspondence to Prof. Amita Aggarwal, Department of Clinical Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India 226014. E-mail: aa.amita@gmail.com, amita@sgpgi.ac.in.

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in comparison with other oral DMARD[5]. Evidence from animal studies and *in vitro* studies of MTX has shown multiple mechanisms of action, including inhibition of DNA synthesis, increase in adenosine levels, and inhibition of cytokine production by T cells and monocytes[6,7]. The response rate with MTX is 60%–65%, with one-third of patients achieving a low disease activity state[8]. Thus, if nonresponders can be identified using baseline variables, alternative treatments can be started for them. This identification also offers the possibility of early control of disease activity and a reduction in longterm damage. Multiple variables have been studied including clinical variables, genetic markers, and immunological markers.

Among the clinical variables, these were associated with poorer response to MTX treatment: lower age, female sex, high baseline disease activity, higher disability, longer duration of symptoms, and current smoking[9]. Various cytokines including high ratio of IL-1 receptor antagonist/IL-1β and low TNF-α had good correlation with response to treatment with MTX at 6 months, while serum IL-1β, IL-6, IL-8, IL-10, and IL-12 levels, and expression of multidrug resistance protein (encoded by MDR1) in peripheral blood mononuclear cells, did not correlate with response to MTX[10,11].

Among the genetic factors, 13 genes important for the MTX mechanism of action, including purine and pyrimidine synthesis pathways, were studied and a model was generated that included sex, smoking status, rheumatoid factor (RF) presence, disease activity, and polymorphisms (adenosine monophosphate deaminase, aminoimidazolecarboxamide ribonucleotide transformylase, inosine triphosphate pyrophosphatase, and methylene-tetrahydrofolate reductase) in 4 genes. This model had good predictive value for clinical response; however, it was costly and the assay may not be widely available[12].

MRP8 and MRP14 belong to the family of S100 proteins, are calcium-binding proteins produced mainly by monocytes, macrophages, and neutrophils, and are secreted upon activation at local sites of inflammation[13]. MRP8/MRP14 complex stimulation of TLR4 leads to the activation of nuclear factor-κB and p38 MAPK pathways[14]. MRP8/14 also promotes the development of Th17 cells as well as autoreactive T cells, thus contributing to joint damage[15]. Its pathogenic involvement in experimental models of arthritis has also been demonstrated[16].

MRP8/14 levels are increased locally at sites of inflammation as well as in the serum of patients with RA[17,18]. MRP8/14 levels independently correlate with joint inflammation and damage in patients with RA[19]. More importantly, among patients with established RA, MRP8/14 was found to be a good prognostic biomarker for longterm radiographic joint progression[20]. Recently, higher MRP8/14 levels at baseline have been shown to predict response to biological therapy[21].

To our knowledge, there are no studies on the use of MRP8/14 in predicting response to MTX alone in RA, and MTX is the most commonly used DMARD in patients with RA. In addition, MRP8/14 can be measured by simple ELISA that can be done easily in clinical practice. We studied the relationship between serum levels of MRP8/14 at baseline and the clinical response to treatment with MTX among DMARD-naive patients with RA in a prospective, open design.

MATERIALS AND METHODS

Patients. All consecutive patients of Asian Indian ethnicity with RA of age > 18 years and fulfilling the American College of Rheumatology/European League Against Rheumatism (EULAR) 2010 classification criteria for RA[22] were screened between February 2014 and May 2015. Patients were enrolled in the study if they had active disease [defined as a Disease Activity Score at 28 joints (DAS28)[23] > 3.2], had not received prior DMARD or corticosteroid treatment, had no contraindications to MTX therapy, and consented to participate. During this period, a total of 326 patients were screened. The study was approved by the institutional ethics committee and all patients gave informed consent.

At baseline visit, blood was drawn and MTX (10 mg/week) and folic acid (10 mg/week) were started. The physician's and patient's global assessments, Health Assessment Questionnaire (HAQ), and DAS28 score were assessed at baseline and at 2 monthly visits for 4 months. Corticosteroids were not permitted during the study and patients maintained treatment with a stable dose of nonsteroidal antiinflammatory drugs. MTX dose was escalated by 5 mg/month to a maximum of 25 mg/week after assessing for disease response and toxicity every month. Patients were contacted by phone at months 1 and 3, whereas at months 2 and 4 they were assessed in the clinic. At the 4-month visit, another blood sample was drawn for MRP8/14 analysis. The EULAR criteria for response were used to assess response at 4 months[24]. The patients were classified as good responders, moderate responders, and nonresponders. Briefly, good responders had a DAS28 ≤ 3.2 plus a > 1.2 decrease in DAS28, and EULAR moderate response refers to a reduction in DAS28 of > 1.2 with the baseline DAS28 being > 5.1, or a reduction of > 0.6 if the baseline DAS28 is > 3.2 and < 5.1, or DAS28 ≤ 3.2 plus a > 0.6 and ≤ 1.2 decrease in DAS28. Nonresponders were defined as having a < 0.6 decrease in DAS28 or a DAS28 > 5.1 plus a ≤ 1.2 decrease in DAS28. In addition, patients who required steroid or alternative therapy other than MTX for control of disease activity after 2 months of MTX treatment were also classified as nonresponders. Those who stopped MTX treatment before the 2-month followup because of side effects or other reasons were excluded from our study.

Blood samples were immediately centrifuged and serum stored at –80°C until analysis. The levels of serum MRP8/14 were measured by sandwich ELISA (BMA Biomedical) following the manufacturer's instructions. Absorbance was detected using the ELISA reader with 450 nm as the primary wave length. C-reactive protein (CRP) and immunoglobulin M (IgM) RF were measured by nephelometry. Anticitrullinated peptide antibodies were estimated using ELISA.

Statistical analysis. The results are expressed as median ± interquartile range (IQR). Intergroup comparison was done using nonparametric tests. The Mann–Whitney U test was used to compare continuous variables. The Wilcoxon signed-rank test was used for analyzing the differences in MRP8/14 levels at 2 paired timepoints. For correlation with other known markers of disease activity such as CRP, the erythrocyte sedimentation rate (ESR) was identified using the Spearman rank correlation. Receiver-operation characteristic (ROC) curve for response prediction was generated. P value < 0.05 was considered significant.

RESULT

Baseline characteristics. Ninety patients were enrolled in the

study, of whom 3 were excluded because of drug intolerance within 4–6 weeks. Among the remaining 87 patients, there were 74 women, and the mean duration of disease was 28 months (Table 1). At baseline, 19 patients had highly active disease (mean DAS28 > 5.1) and 68 patients had moderate disease activity (mean DAS28 3.2 to ≤ 5.1). Forty-seven patients had erosions at baseline. None of the patients were receiving corticosteroids at baseline. There were no significant differences in clinical and laboratory variables among responders and nonresponders at baseline (Table 1).

Baseline serum MRP8/14 levels. The median (IQR) baseline serum MRP8/14 levels were 19.95 μg/ml (11.49–39.06). The baseline serum MRP8/14 levels had significant correlation with DAS28-ESR (r = 0.323, p = 0.003; Figure 1A) and DAS28-CRP (r = 0.351, p = 0.001; Figure 1B).

Clinical response to MTX treatment. On followup, 11 patients required additional or a change of medication after 2 months of MTX therapy because of a lack of disease control: 2 patients received prednisolone, 3 received an additional DMARD, and 6 switched to an alternative medication. These were classified as nonresponders.

After 4 months of treatment with MTX, a significant reduction in disease activity was observed [median (IQR) tender joint count (TJC) 10 (7–14) to 3 (2–6), swollen joint count (SJC) 7 (5–11) to 2 (1–0.7), CRP mg/dl 2.2 (0.9–4.85) to 0.48 (0.32–1.5), DAS28-CRP 4.43 (4.1–5.1) to 2.86 (2.4–3.7); p < 0.001 for all comparisons]. Forty-three patients achieved good response, 26 patients showed moderate response, and 7 were nonresponders. Overall, 18 patients did not respond to MTX monotherapy. No linear relationship was found between response and duration of disease prior to treatment.

Table 1. Baseline characteristics of patients with RA (n = 87). No significant differences were seen between responders and nonresponders at baseline. Values are median (interquartile range) or n (%) unless otherwise specified.

Characteristics	Total	Responders	Nonresponders
Female/male, n	74/13	59/10	15/3
Age, yrs	40 (33–50)	40 (33–48)	40 (34–52)
TJC28	10 (7–14)	10 (8–14)	10 (7–13)
SJC28	7 (5–11)	7 (5–10.5)	9 (5–12)
HAQ	1.5 (1.0–1.9)	1.5 (1–1.97)	1.5 (1.1–1.8)
CRP, mg/dl	2.2 (0.9–4.8)	2.54 (1.02–5.1)	1.9 (0.6–3.6)
DAS28-CRP	4.43 (4.1–5.1)	4.52 (4.1–5.11)	4.31 (3.9–4.53)
ESR, Westergren method	56.5 (37–85)	56 (38–85)	58 (35–86)
DAS28-ESR	5.93 (5.4–6.5)	5.95 (5.34–6.48)	5.92 (5.63–6.58)
IgM-RF–positive	71 (82)	56 (81)	15 (83)
ACPA-positive	69 (79)	54 (79)	15 (82)

RA: rheumatoid arthritis; TJC28: tender joint count at 28 joints; SJC28: swollen joint count at 28 joints; HAQ: Health Assessment Questionnaire; CRP: C-reactive protein; DAS28: Disease Activity Score at 28 joints; ESR: erythrocyte sedimentation rate; IgM: immunoglobulin M; RF: rheumatoid factor; ACPA: anticitrullinated protein antibodies.

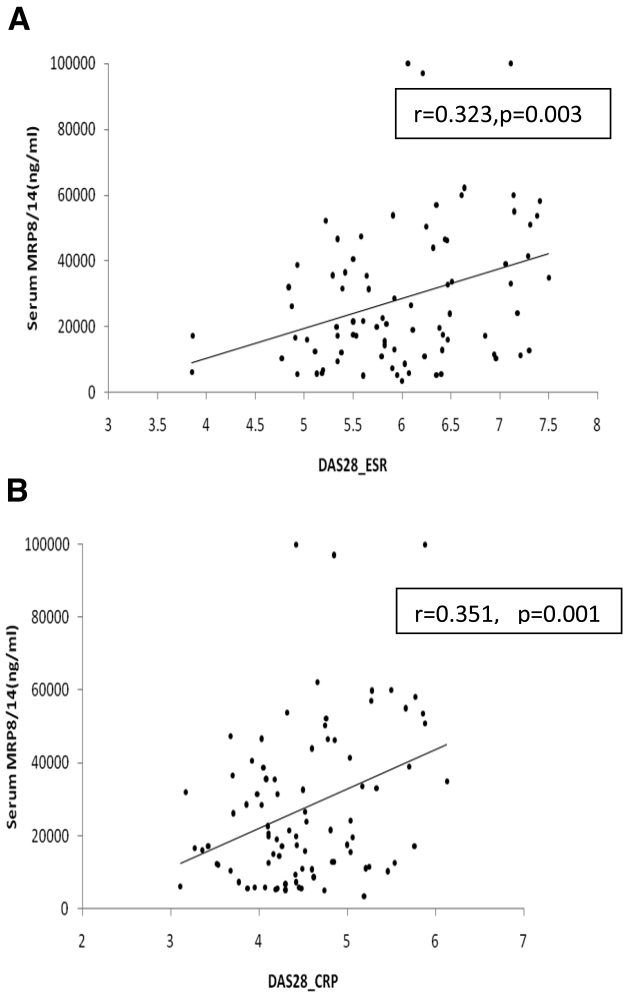

Figure 1. Scatterplots showing correlations between serum levels of MRP8/14 and the disease activity score at baseline. A. Serum MRP8/14 and DAS28-ESR. B. Serum MRP8/14 and DAS28-CRP. MRP: myeloid-related proteins; DAS28: Disease Activity Score at 28 joints; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

Decrease of MRP8/14 serum complex levels in responders to treatment. At the end of 4 months of treatment with MTX, serum MRP8/14 levels reduced significantly from 19.95 μg/ml (11.49–39.06) to 10.28 μg/ml (5.95–16.05, p < 0.001). Comparing the fall in responders and nonresponders separately, the levels reduced in the responders from 23.99 μg/ml (15.39–42.75) to 10.41 μg/ml (5.83–15.61, p < 0.001; Figure 2A), but not in the nonresponders, from 9.58 μg/ml (6.11–24.93) to 9.19 μg/ml (7.74–21.96, p = 0.687; Figure 2B). The change of MRP8/14 moderately correlated with changes in levels of CRP (r = 0.29, p = 0.01) and changes in ESR (r = 0.25, p = 0.03). Among the correlations between changes in levels of TJC, SJC, CRP, DAS28, and baseline MRP8/14, only the change in SJC showed significant correlation with baseline MRP8/14 serum levels (r = 0.32, p = 0.007).

Baseline MRP8/14 serum levels in responders versus non-

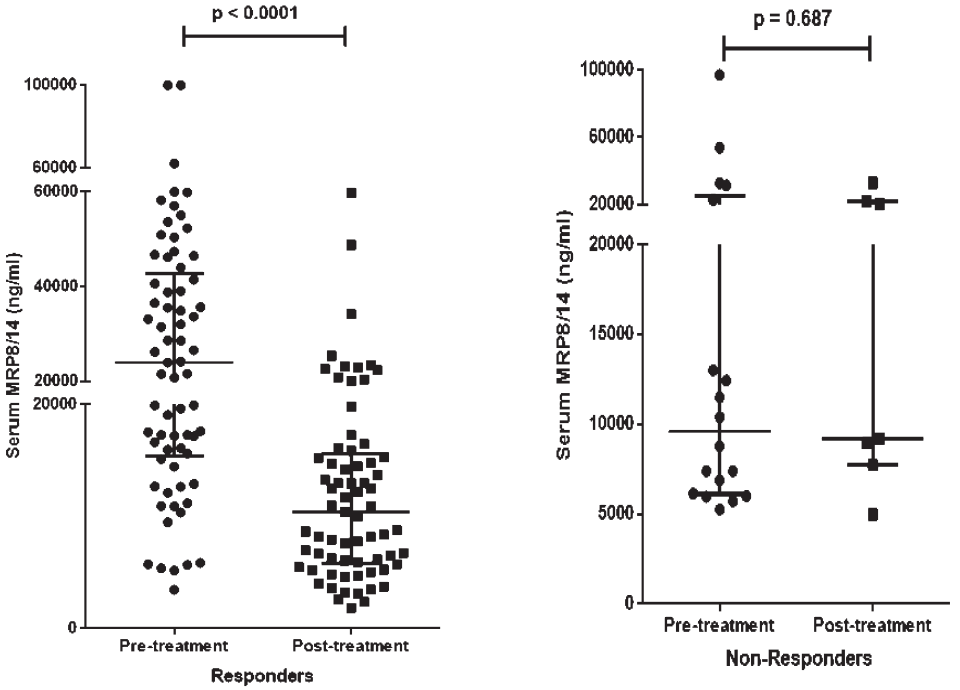

Figure 2. Changes in MRP8/14 levels in patients with rheumatoid arthritis after treatment with MTX. Serum MRP8/14 levels in (A) responders (good and moderate EULAR response, n = 69) and (B) nonresponders (n = 18) before and after MTX treatment. Each dot represents a patient. Comparisons were made using the Wilcoxon matched pairs signed-rank test. MRP: myeloid-related proteins; MTX: methotrexate; EULAR: European League Against Rheumatism.

responders to MTX. Baseline MRP8/14 serum levels were higher in responders compared with nonresponders to MTX: 23.99 μg/ml (15.39–42.75) versus 9.58 μg/ml (6.11–24.93, p = 0.0025; Figure 3). At baseline, the median DAS28-CRP in responders [4.52 (4.1–5.11)] and nonresponders [4.31 (3.9–4.53), p = 0.094] was not different. Baseline characteristics were not different between responders and nonresponders to MTX treatment (Table 1).

Baseline MRP8/14 serum levels predict clinical response to treatment. ROC analysis (Figure 4) showed that MRP8/14 was a good predictor of response, with an area under the curve (AUC) of 0.705 (95% CI 0.549–0.862), better than that of baseline CRP (AUC 0.590) and baseline ESR (AUC 0.520). Using a threshold of MRP8/14 levels of 13.70 μg/ml, the sensitivity was 79%, specificity was 69%, and the OR for prediction of response to MTX therapy was 9.2 (95% CI 4.8–17.5), with a positive predictive value of 73%.

In subgroup analysis, among patients with < 1 year of disease duration, MRP8/14 (AUC 0.84) was a significantly better predictor of response to MTX than was baseline CRP (AUC 0.74) and ESR (AUC 0.60). There were no significant differences for predictor of response among patients with disease duration of > 1 year (AUC of MRP8/14 0.62, CRP 0.50, ESR 0.49; Supplementary Figure 1 available from the authors on request). Multivariate logistic regression was done including baseline MRP8/14, CRP, ESR, number of swollen joints, number of tender joints, age, and disease duration, and

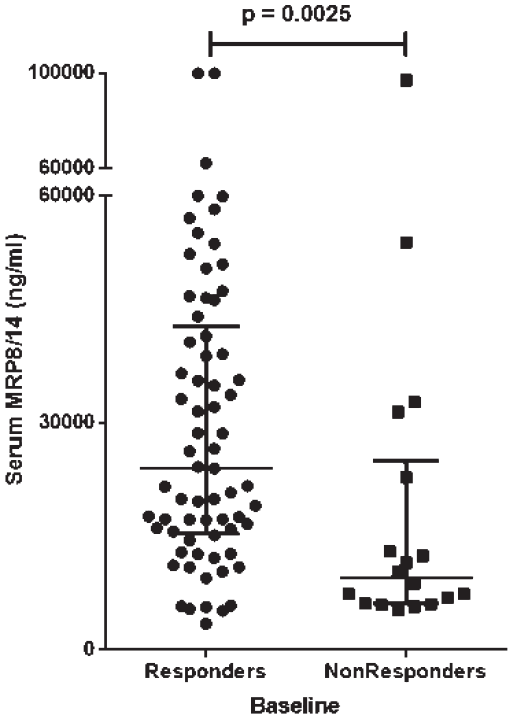

Figure 3. Comparison of serum MRP8/14 levels at baseline among responders and nonresponders. Baseline MRP8/14 levels were significantly higher in responders than in nonresponders. Each dot represents a patient. Significance of the comparison is determined by the Mann–Whitney U test. MRP: myeloid-related proteins.

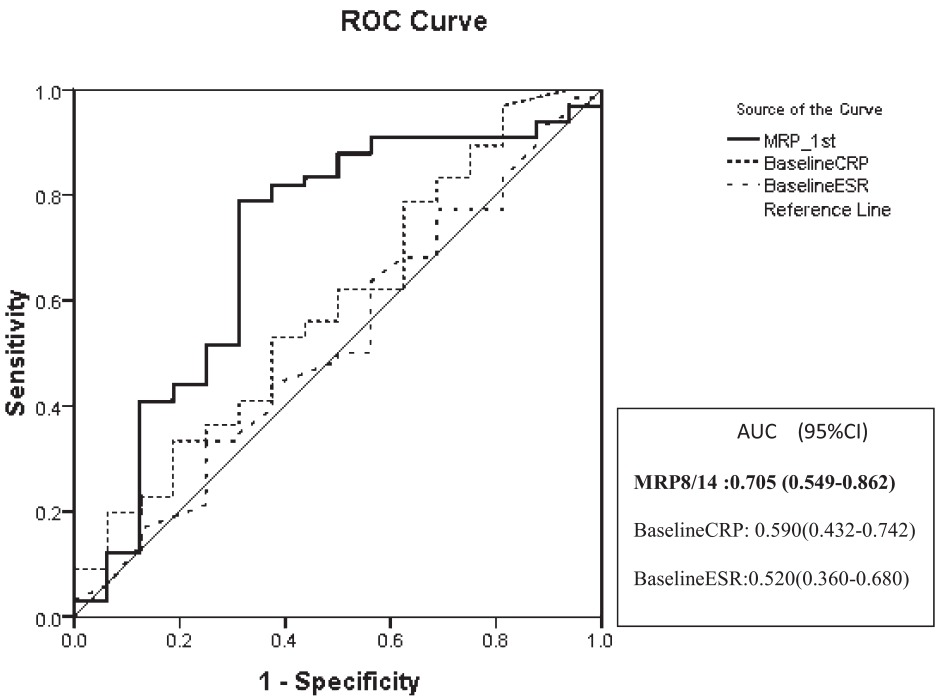

Figure 4. ROC curve analyses for achievement of EULAR good or moderate response after treatment with methotrexate. The increasing area under the curve (AUC) corresponds to a higher diagnostic test yield. The diagnostic accuracy of baseline MRP8/14 levels, CRP, and ESR are presented. ROC: receiver-operating characteristic; EULAR: European League Against Rheumatism; MRP: myeloid-related proteins; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

it was found that only MRP8/14 showed association with response ($p < 0.008$).

DISCUSSION

Serum MRP8/14 levels were correlated with disease activity at baseline and reduced on treatment with MTX in patients who responded to treatment. In addition, higher baseline MRP8/14 levels were associated with response to MTX treatment.

Our data of increased serum MRP8/14 in RA are similar to those in previous studies[25]. MRP8/14 is produced by myeloid lineage cells and the macrophages infiltrating the synovium in RA express MRP8/14; this expression is highest at the cartilage-pannus junction, the site for destruction of cartilage and bone erosion[18]. The synovial fluid levels of MRP8/14 are nearly 10-fold higher than those of paired serum[26]. Thus, elevated serum MRP8/14 levels may suggest release of these proteins from activated macrophages within synovium. This is further supported by a correlation between serum levels of MRP8/14 and measures of disease activity such as the DAS28. This association with disease activity has also been previously shown in RA[25].

MTX therapy led to a reduction in MRP8/14 levels. This effect could be related to the decrease in disease activity or to a specific action of MTX on myeloid cells, the producers of MRP8/14. Significant reduction of MRP8/14 levels occurred in responders and not in nonresponders, and the correlation with the fall in ESR and CRP suggests that the MRP8/14 reduction could be related to inflammation control. In another study, significant decrease of MRP8/14 levels was observed in the responders to biologicals after 4 weeks of treatment, irrespective of their mechanism of action[21]. A decrease in the levels of MRP8/14 in patients who responded to treatment and the lack of such a pattern among nonresponders suggests that it may be a good biomarker for followup.

Despite the extensive clinical use of MTX, identifying clinical response predictors has not been easy. The mean time to initial response with MTX is 9.5 weeks[27], thus assessment at 4 months is sufficient to identify patients who would respond to MTX. Composite disease activity measures are simple and have been used to predict response to MTX[9,28]. However, baseline DAS28 was not different between responders and nonresponders to MTX therapy in our study, a finding similar to earlier data in patients with early RA[29].

Baseline serum MRP8/14 levels were higher in responders compared with nonresponders to MTX; further, MRP8/14 levels had better ROC characteristics than other routine acute-phase protein variables such as ESR and CRP, suggesting that MRP8/14 levels may be useful in predicting response to MTX at baseline. MRP8/14 is a relatively stable protein and can be measured easily, unlike cytokines. In subgroup analysis, MRP8/14 has better prediction response to MTX in early RA (< 1 yr) as compared with later onset of

disease (> 1 yr). In established RA, MRP8/14 protein has been demonstrated to predict 10-year radiographic progression[20]. The levels of MRP8/14 measured in our study were higher compared with previous studies[30,31], which can be explained by the use of different kits in different studies and the higher subclinical infection load in developing countries such as ours. Indeed, we saw similar higher levels of MRP8/14 in our previous work on juvenile arthritis[32].

The strengths of our study are that the patients treated with MTX monotherapy as the initial DMARD as per the current standard of care[33] were from a single center, the low dropout rate, and the measurement of MRP8/14 both at baseline and at 4 months. The limitations include the lack of a validation group and the short-term response.

A large study is needed to assess the effect of baseline MRP8/14 levels on response to MTX at 3 and 6 months, to see its potential in differentiating patients with early and late response. A longterm study is also needed to assess the potential of MRP8/14 levels in predicting radiological erosions.

Our study shows that measurement of MRP8/14 serum levels at baseline might be useful in prediction of response to MTX treatment in patients with RA. Patients who have low levels may be treated with alternative drugs.

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