Efficacy of MRP8/14 as a Marker of Disease Activity in Rheumatoid Arthritis

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Abstract

Objective: Early and accurate evaluation of the presence and activity of synovitis is extremely important in the diagnosis and treatment of rheumatoid arthritis. Myeloid related protein 8/14 (MRP8/14), also known as calprotectin or S100A8/A9 is considered as a sensitive marker for local inflammatory activity in rheumatoid arthritis. The aim of this study is to demonstrate the efficacy of MRP8/14 as a marker of disease activity in RA. Methods: Thirty-one patients with diagnosis of RA who received treatment without biological drugs at our institution were included in this study. Serum MRP8/14, CRP and MMP-3 were tested in all patients. Disease activity was evaluated using DAS28-CRP and SDAI. Ultrasonography was performed on the wrists and MCP joints of both hands using semi-quantitative scale of power Doppler signal. The sum of scales in joints was calculated as the PD score. The correlation of MRP8/14 with serum biomarkers, disease activity and ultrasonography examination was investigated. Result: Serum MRP8/14 was strongly correlated with CRP (r = 0.63) and MMP-3 (r = 0.69). A correlation was observed between serum MRP8/14 and DAS28-CRP (r = 0.53) and SDAI (r = 0.66). No significant correlation was found between PD scores and MRP8/14. Conclusion: This study demonstrated that MRP8/14 is correlated with evaluated disease activity and markers of serum inflammatory response in patients not using biological drugs. MRP8/14 is considered an effective new method for objective evaluation of synovitis in RA.

Keywords

Rheumatoid Arthritis, Myeloid Related Protein 8/14 (MRP8/14), Calprotectin, Disease Activity

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease which mainly causes inflammation of the joint synovium. Prolonged synovitis leads to progression of joint destruction and joint degeneration. Pain due to synovitis and limitations in the range of joint motion due to joint degeneration greatly limit patients' activities of daily living (ADL). In the early phase of RA, a period during which significant therapeutic efficacy can be expected by therapeutic intervention (the window of opportunity) exists. Once joint destruction has occurred, operative therapy is often needed for functional recovery and pain relief. Therefore, early intervention and adequate synovitis control are necessary to prevent joint destruction. Thus, early and accurate evaluation of the presence and activity of synovitis is extremely important in the diagnosis and treatment of rheumatoid arthritis.

Disease Activity Score (DAS) 28 and Simplified Disease Activity Index (SDAI) are currently used for evaluation of disease activity in RA. Each of these is a composite disease activity score that is a quantitative evaluation consisting of evaluation by an examiner, the patient's subjective evaluation and serum inflammatory response as an objective measure. Use of the DAS28 and SDAI improves control of RA [1]-[3], and is effective in determining whether treatment with a biological drug is necessary [4]. However, certain challenges remain, as for example, joint destruction may progress even in cases classified as remission by DAS28. In addition, although the use of joint ultrasonography and contrast-enhanced MRI for the evaluation of localized synovitis has been growing, the validity of joint ultrasonography evaluation may depend on the examiner's experience, and contrast-enhanced MRI has a high cost.

Myeloid related protein (MRP) 8/14 has attracted attention in recent years as an index of synovitis activity in rheumatoid arthritis patients. MRP8/14 is a calcium-binding protein belonging to the S100 protein family which is also known as calprotectin [5] [6] or S100A8/A9 [7]. MRP8 and MRP14 have an important role in the acute inflammatory reaction [5] [8] [9], as they function to mobilize inflammatory cells to the inflamed region [10]. MRP8/14 is released from activated granulocytes or mononuclear cells and macrophages which are present in the synovial membrane and joint fluid [11] [12]. C-reactive protein (CRP) is mainly produced in hepatocytes through the action of interleukins (tumor necrosis factor [TNF], interleukin [IL]-1 and IL-6). It has been reported that this process is affected by genetic factors [13]. On the other hand, MRP8/14 directly reflects the leukocyte counts within inflamed joints. Thus, MRP8/14 is considered a sensitive marker for localized inflammatory activity in RA [11] [12] [14].

We hypothesized that if the relationship between MRP8/14 as an index of synovitis and existing RA disease activity evaluation methods was adequately clarified, easy and objective evaluation of the RA condition using MRP8/14 would be feasible.

The purpose of this study is to demonstrate the efficacy of MRP8/14 as a marker of disease activity in RA.

2. Patients and Methods

2.1. Patient Characteristics

This study was conducted with the approval of the Ethical Review Board at our institution (RBMR-C-976-1). Out of the 106 patients with a diagnosis of rheumatoid arthritis based on the 1987 American College of Rheumatology (ACR) criteria [15] who received treatment without biological agents at our institution, and who were not using biological drugs, 31 patients (3 male and 28 female) from whom written consent was obtained were included in this study. The patient characteristics were shown in **Table 1**. Mean age was 62.2 ± 1.6 years, and mean disease duration was 141.1 ± 26.3 months. Twenty-eight patients used methotrexate with mean dosage of 7.10 ± 0.67 mg. One patient used prednisolone at 4 mg/day. Four patients used tacrolimus at 0.5 to 2 mg/day (mean 1.1 mg/day).

2.2. Methods

CRP was measured as existing biomarkers of synovitis. In addition, matrix metalloproteinase-3 (MMP-3) was measured as a biomarker of cartilage destruction. Latex coagulating nephelometry was used to measure CRP and MMP-3. MRP8/14 concentration was assessed using a commercial sandwich enzyme-linked immunosorbent assay system (ELISA) development kit (BMA Biomedicals, Rheinstrasse, Switzerland). The specific monoclonal antibody for the MRP8/14 heterodimer (mAb 27E10) was used as primary antibody and a polyclonal antibody coupled with horseradish peroxidase was used as secondary antibody. The correlation of serum

Table 1. Characteristics of patients at enrollment in this study.

Number of patients	31
Age (year)	62
Gender (% female)	90.3
Disease duration (months)	141
Using methotrexate (%/mg)	90/7.1
CRP (mg/ml)	0.63
MMP-3 (mg/dl)	97.0
MRP8/14 (ng/dl)	6630.3
DAS28-CRP (remission/low/moderate/severe) (%)	46.4/25.8/22.6/3.2
SDAI (remission/mild/moderate/severe) (%)	32.2/35.5/29.0/3.2
Number of swollen joints	1.6
Number of tender joints	1.5
Visual analog scale for patient pain (mm)	21.4
Power Doppler score	5.6

MRP8/14 with CRP, and MMP-3 was evaluated.

Rheumatology specialist evaluated swelling and tenderness in 28 joints, and the physician's global assessment score. In addition, pain was evaluated using the Visual analogue scale (VAS) on a 100-mm VAS. Disease activity was evaluated using the DAS28-CRP [16] and the SDAI [17]. In DAS28-CRP, a score of less than 2.3 was defined as remission, a score of 2.3 to 3.2 was defined as low disease activity, a score of 3.3 to 5.1 was defined as moderate disease activity, and a score greater than 5.1 was defined as high disease activity. In SDAI, a score of less than 3.3 was defined as remission, a score of 3.3 to 11 was defined as mild disease activity, a score of 11 to 26 was defined as moderate disease activity, and a score greater than 26 was defined as severe disease activity. The relationship between DAS28-CRP and SDAI, and serum MRP8/14 was statistically evaluated.

Ultrasonography was performed on the wrists (radiocarpal joint, intercarpal joint, ulnocarpal joint) and metacarpophalangeal (MCP) joints of both hands. The intra-articular power Doppler (PD) signal was subjectively graded on a semi-quantitative scale ranging from 0 to 3 [18]. The sum of grades in 16 joints was calculated as the PD score. Therefore, the PD score ranged from 0 to 48. All ultrasonography examinations were performed using HI VISION Avius, (Hitachi Aloka Medical, Tokyo, Japan) with a 3 - 15 MHz convex probe (Hitachi Aloka). Correlations between PD scores and serum MRP8/14 values were investigated.

SPSS Statistics Version 21 (Chicago, IL, USA) was used for the statistical analysis. Quantitative values were shown as mean ± standard error. The correlation between MRP8/14 and clinical evaluation and hematological tests data was investigated using Pearson's correlation analysis. $P < 0.05$ was defined as statistically significant. The relationship between PD scores from hand joint ultrasonography examination and MRP8/14, DAS28-CRP, and SDAI was investigated using Spearman signed rank test.

3. Results

A strong correlation was observed between MRP8/14 and CRP, the serum markers of inflammatory response and MMP-3, a marker of cartilage destruction (**Table 2**).

According to the evaluation using DAS28-CRP, remission was observed in 15 cases (48.4%), low disease activity in 8 cases (25.8%), moderate disease activity in 7 cases (22.6%), and severe disease activity in 1 case (3.2%). According to the evaluation using SDAI, remission was observed in 10 cases (32.3%), mild disease activity in 11 cases (35.5%), moderate disease activity in 9 cases (29.0%), and severe disease activity in 1 case (3.2%).

A correlation was observed between serum MRP8/14 and DAS28-CRP and SDAI. In addition, MRP8/14 was correlated with each of the clinical evaluation components including swollen joint count (SJC) and VAS for patient pain (**Table 3**).

The mean PD score from joint ultrasonography was 4.4 ± 1.3. No significant correlation was found between

Table 2. Correlations between MRP8/14 and laboratory tests.

	CRP	MMP-3
Correlation coefficients	0.63	0.69
P values	P < 0.001	P < 0.001

Serum MRP8/14 strongly correlated with CRP and MMP-3.

Table 3. Correlations between MRP8/14 and clinical assessments.

	SDAI	DAS28-CRP	SJC	TJC	Patient pain VAS
Correlation coefficients	0.66	0.53	0.40	0.25	0.59
P values	P < 0.001	P < 0.01	P < 0.05	N.S.	P < 0.001

There was correlation between MRP8/14 and disease activity assessed with SDAI and DAS28-CRP.

PD scores and MRP8/14. Moreover, no significant correlation was found between PD scores and either DAS28-CRP or SDAI.

4. Discussions

Evaluation of synovitis activity in RA treatment is extremely important for determining the initiation of RA treatment and selecting or changing medication. Adequate control of synovitis activity connects to the prevention of joint destruction, and therefore to the prevention of a decrease in ADL and QOL.

Sunahori *et al.* compared MRP8/14 levels in joint fluid between osteoarthritis patients and RA patients, and demonstrated that MRP8/14 levels are significantly higher in RA patients [19]. Kane *et al.* demonstrated that MRP8/14 expression is increased in macrophages in the inflamed synovial membrane, and MRP8/14 in joint fluid of RA patients correlates with serum MRP8/14. These findings suggest that MRP8/14 is suitable for the evaluation of synovitis due to RA.

In previous studies, a relationship has been reported between MRP8/14 and disease activity [20] [21], and the evaluation of localized synovitis [20] [21], or more specifically a relationship with joint destruction observed with plain radiography [21] [22] in rheumatoid arthritis patients. Moreover, a relationship has been suggested between MRP8/14 and serum inflammatory response biomarkers including CRP, and between MRP8/14 and various autoantibodies, such as anti-CCP antibody [20] [23].

In this study, MRP8/14 was correlated with CRP, MMP-3, and disease activity assessed with DAS28-CRP and SDAI. In addition, MRP8/14 was also correlated with the swollen joint count (SJC) and VAS for patient pain, which are elements of these composite disease activity scores. DAS28 and SDAI make easy evaluation possible in the clinical setting. As the development of these evaluation methods has improved control of rheumatoid arthritis, they are currently widely used in evaluating RA disease activity. However, as evaluation of SJC and TJC is relatively strongly subjective, it is considered that there are issues with reproducibility between examiners [24]. Therefore, inter-examiner error is likely to be generated in DAS28 and SDAI including those evaluations. Since MRP8/14 reflects both objective and subjective evaluation endpoints, the possibility of evaluating RA disease activity more objectively and quantitatively was shown.

In the clinical course of RA, there are cases in which joint destruction progresses despite negative CRP tests [25]. This can be explained by persistent localized synovitis in small joints which is not reflected by CRP levels, yet causes progression of joint destruction. Joint ultrasonography is used to evaluate localized inflammation, and is becoming a standard evaluation method in RA [26]. Hammer *et al.* compared the relationship between joint ultrasonography scores and MRP8/14 in 20 RA patients. They demonstrated a strong correlation between MRP8/14 and ultrasonography scores. On the other hand, Dejaco *et al.* [27] found no relationship between joint ultrasonography scores and composite measures such as DAS28 or SDAI, although there was a relationship with CRP and morning stiffness. The ultrasonography scores evaluated in this study did not show a significant correlation with either MRP8/14 or disease activity. The difference between our study and the report by Hammer *et al.* is that while in our study and in the report by Dejaco *et al.*, evaluation of hands and hand joints was conducted, Hammer *et al.* included large joints such as shoulder joints, elbow joints, and hip joints in their evaluation. Therefore, evaluation limited to the hand joints and fingers was found to be insufficient when joint ultrasono-

graphy is used for evaluation of RA disease activity. When joint ultrasonography is used for evaluation of systemic synovitis activity due to RA, it may be necessary to select joints to be evaluated and to establish a specific gravity of score by joint size.

In synovitis due to RA, various inflammatory mediators act to induce infiltration of inflammatory cells or proliferation of synovial fibroblasts, however the accurate pathological process, including pathogenesis of RA, has not been completely elucidated. In addition, some of the functions of MRP8/14 in vivo have not been explained. Thus, in order to collect basic data on the relationship between MRP8/14 and disease activity, inflammatory biomarkers, and localized synovitis evaluated using joint ultrasonography in RA patients, patients not using biologic drugs were included in this study. As a result, patients with relatively low disease activity were included and the sample size was relatively small. However, the efficacy of MRP8/14 in evaluating RA disease activity in patients with low disease activity was demonstrated in this study.

This study has some limitations. Currently, methotrexate, steroids, and various biological drugs with different mechanism of action are used in RA treatment; however, the effect of these medications on the dynamic state of MRP8/14 in vivo is unknown. This study was limited to patients who did not use biological drugs, however there were some patients using steroids or immunosuppressive drugs, and these drugs may have affected the results of the study. To analyze the effect of these medications on MRP8/14, additional data collection is considered necessary. It is necessary to establish a more objective method for evaluation of disease activity in RA by combining various medical factors including MRP8/14 in the future.

5. Conclusion

The results of this study demonstrated that MRP8/14 correlated with disease activity and serum inflammatory biomarkers in patients not using biological drugs. Therefore, MRP8/14 is considered an effective new method for objective evaluation of synovitis in RA.

Acknowledgements

Disclosures

All authors declare no conflict of interest.

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