Original

Serum MMP-3 as a predictive factor for joint destruction in patients with rheumatoid arthritis who have achieved remission or low disease activity and the clinically relevant cut-off value for the structural remission

†Hiroe Konishi[*1], Mai Morimoto*[*1], Kosaku Murakami*[*2], Akira Onishi*[*3],*
Takayuki Fujii[*3,4], Koichi Murata*[*3,4], Masao Tanaka*[*3],*
Akio Morinobu[*5], Masayoshi Nakano*[*1,6], Masahiro Koshiba*[*1,7]*

†Department of Clinical Diagnosis and Laboratory Medicine, Hyogo Medical University School of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo, 663-8501, Japan
Tel: +81-798-45-6860 Ex. 2450; Fax: +81-798-45-6873;
E-mail: hiroe-k@hyo-med.ac.jp
Received October 30, 2025; accepted December 11, 2025
*1 Department of Clinical Diagnosis and Laboratory Medicine, Hyogo Medical University School of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo, 663-8501, Japan
*2 Division of Clinical Immunology and Cancer Immunotherapy, Center for Cancer Immunotherapy and Immunobiology, Kyoto University Graduate School of Medicine, Sakyo, Kyoto, 606-8501, Japan
*3 Department of Advanced Medicine for Rheumatic Diseases, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawahara-cho, Sakyo, Kyoto, 606-8507, Japan
*4 Department of Orthopaedic Surgery, Kyoto University Graduate School of Medicine, Sakyo, Kyoto, 606-8507, Japan
*5 Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Sakyo, Kyoto, 606-8507, Japan
*6 Clinical Laboratory Department, Baba Memorial Hospital, 4-244 Hamadera Funao-cho Higashi, Sakai, Osaka, 592-8341, Japan
*7 Shinsuma Clinic, 5-1-4 Suma-Murasame-cho, Kobe, Hyogo, 654-0046, Japan

ABSTRACT

Objectives

Joint destruction progression is often seen in RA patients achieving low disease activity (LDA) or clinical remission. In this study we evaluated whether increased MMP-3 (including prednisolone-induced elevation) is relevant to the joint destruction progression, and to explore the best cut-off value for the structural remission.

Methods

RA patients whose CRP levels normalized following methotrexate (MTX) monotherapy or DMARD combination therapy (MTX together with other DMARDs) were divided into two groups based on their MMP-3 positivity at the end of 1-year observation period, and joint destruction progression was retrospectively compared. Radiological joint destruction was assessed using the modified van der Heijde total sharp score (mTSS). The cut-off value of MMP-3 was determined by ROC analysis.

Results

Among MMP-3 positive patients who have achieved DAS28-ESR LDA or clinical remission, joint destruction progressed in 50.0% of prednisolone(-) and 45.7% of prednisolone(+) patients (p=1.00). Similarly, among MMP-3 positive patients who have achieved CDAI LDA or clinical remission, joint destruction progressed in 48.3% of

prednisolone(-) and 42.6% of prednisolone(+) patients (p=0.79). The ROC analysis in female patients revealed that the cut-off value of MMP-3 was 49.7 ng/mL (AUC 0.681; 95% CI 0.560–0.802, p<0.01) for the structural remission (ΔmTSS<0.5).

Conclusions

The data presented here suggest that prednisolone-induced serum MMP-3 increase contribute similarly to the joint destruction compared with the MMP-3 increase without prednisolone. Also indicated is that the current cut-off value of serum MMP-3 (59.7 ng/mL) is too high to achieve the structural remission in female RA patients.

〔Lab Med Int 2026; 5(1): 34-45〕

Key Words

C-reactive protein, joint destruction, matrix metalloproteinase-3, modified van der Heijde total sharp score, Rheumatoid arthritis

I. INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic synovitis and joint destruction, which leads to disability [1]. Clinical remission is the current therapeutic target for patients with RA, with low disease activity (LDA) considered the best possible alternative. A targeted treatment strategy should be employed when treating patients with RA, and treatment decisions should then be based on disease activity and other patient factors, such as comorbidities and the progression of structural damage [2)3)].

Methotrexate (MTX) is recommended as the first-line drug for the initial treatment of RA and remains the anchor drug in RA [4)5)]. MTX is not only an efficacious conventional synthetic (cs) disease-modifying antirheumatic drug (DMARD), it is also the basis for combination therapies with prednisolone (PSL), other csDMARDs, biological DMARDs (bDMARDs) or targeted synthetic DMARDs (tsDMARDs) [6]. While serum CRP levels often normalize (i.e. below the upper reference limit) after MTX therapy, joint destruction can still progress [7].

Matrix metalloproteinases (MMPs) are a family of enzymes that catalyze extracellular matrix degradation. Most MMPs are secreted as inactive preproteins that are activated when cleaved by extracellular proteinases [8]. MMP-3 is a proteinase secreted by synovial fibroblasts and chondrocytes within joints. In RA, joint destruction can be accelerated by active MMP-3, and the level of MMP-3 is elevated within the joints of RA patients [9]. This high MMP-3 level is believed to mediate the joint destruction seen in patients with RA [10)11)].

Increases in serum MMP-3 levels are observed from early to advanced stages in 80% 90% of patients with RA. Serum MMP-3 levels in these patients reflect the degree of synovial cell proliferation and may serve as a prognostic indicator of RA progression, particularly early after disease onset [12)]. Elevated or increasing serum MMP-3 levels in patients with RA are associated with rapid progression of joint destruction while, conversely, serum MMP-3 levels decline when the condition stabilizes in response to the therapeutic effect of DMARDs [13)]. Normal serum MMP-3 levels in combination with decreases in CRP levels or disease activity are reportedly useful for predicting clinical remission and normal physical function in patients with RA [14)]. However, progression of joint destruction is often seen in RA patients who are in remission or exhibiting LDA and normalized CRP levels [15)]. Those reports prompted us to observe RA patients with normalized CRP levels for 1 year by MTX monotherapy or combination therapy (MTX together with other DMARDs) to determine whether serum MMP-3 positivity correlates with joint destruction on these patients. Oral prednisolone administration reportedly increases serum MMP-3 levels [16)17)]. Currently, however, there are no reports examining whether PSL-induced elevation of serum MMP-3 is involved in joint destruction, which also made us to evaluate whether PSL-induced elevation of serum MMP-3 is truly associated with the progression of joint destruction. The data also enabled us to propose an adequate cut-off value for MMP-3 based on the relationship between serum MMP-3 levels and joint destruction by ROC analysis.

II. METHODS

1. Study design and patient selection

Enrolled in the study were patients who visited Hyogo College of Medicine Hospital (currently Hyogo Medical University Hospital) or Kyoto University Hospital between April 2011 and April 2021 and met the 1987 and/or 2010 RA classification criteria. Patients with RA (n = 182) whose CRP levels normalized following MTX monotherapy or DMARD combination therapy (MTX together with other DMARDs) were divided into two

groups based on their MMP-3 positivity at the end of the 1-year observation period, and progression of joint destruction was retrospectively compared using X-rays.

2. Assessment

The medical records of the patients were retrospectively reviewed, including information related to their visual analog scale (VAS), CRP, erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), serum MMP-3, anticitrullinated protein antibody (ACPA), 28 joint disease activity score (DAS28), and clinical disease activity index (CDAI). Patients who had severe renal dysfunction (eGFRcre < 30 mL/min/1.73 m^2), a contraindication to MTX administration, or were taking oral PSL > 25 mg/day due to complications were excluded. Patients with other forms of arthritis, such as ankylosing spondylitis, were also excluded because of their possible effect on MMP-3 levels. After the observation period, the patients were divided into MMP-3 positive (MMP-3(+)) and MMP-3 negative (MMP-3(−)) groups based on whether their MMP-3 levels were above or below the upper reference limit for each sex. Progression was assessed based on radiography of the hands, wrists and feet, and scored using the modified total sharp score (mTSS) method recommended by Bruynesteyn et al. (18). Radiographs of each patient's hands and feet were taken upon initiation of MTX therapy and after 1 year of therapy. Radiographic progression was evaluated independently by two rheumatologists (MM and KMurakami) trained on the mTSS scoring system and certified by Prof. van der Heijde (Leiden University). mTSS progression after 1 year (ΔmTSS) was then calculated from the mean progression determined by the two readers. If their calculations of ΔmTSS differed by ≥ 10, the two rheumatologists discussed the scores and reached a consensus. Patients were classified as exhibiting structural remission (ΔmTSS < 0.5) or radiographic evidence of progression (ΔmTSS ≥ 0.5).

3. Ethical approval

This study protocol was approved by the Ethics Committee of Hyogo Medical University (Protocol No. 3923). Because of the retrospective nature of the study, the requirement for individual informed consent was waived owing to the "opt-out" principle; that is, patients were allowed to "opt-out" of the database if they wanted. Use of clinical data collected at Kyoto University Hospital was approved by the Medical Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine (No. R0357). Written informed consent to participate in the study was obtained from all patients treated at Kyoto University Hospital. This study was performed in accordance with the Declaration of Helsinki.

4. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA). Demographic and baseline characteristics were analyzed using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Multivariate analysis was performed using multiple logistic regression analysis with variables for which the p values were <0.05 in the preceding univariate analysis. All reported p values are two-sided and not adjusted for multiple testing. Any difference with a p value <0.05 was considered statistically significant.

III. RESULTS

1. Clinical characteristics of patients upon initiation of MTX therapy

Of the patients who received MTX monotherapy or DMARD combination therapy for at least 1 year, 182 (including 142 females) were evaluated radiographically. Upon initiation of MTX therapy (at baseline), participants had median age was 60 years (interquartile range [IQR], 51–68 years), with median disease duration was 6 years (IQR 3–11 years). Among them, 62.7% were RF-positive, 64.3% were ACPA-positive and 56.0% were both RF- and ACPA-positive. The median DAS28-ESR was 2.56 (IQR 1.94–3.21), the median CDAI was 3.50 (IQR 1.15–6.65), the median MTX dose was 6.0 mg/week (IQR 4.0–8.0 mg/week) in 125 (68.7%) patients, and the median PSL dose was 4.0 mg/day (IQR 2.0–5.0 mg/day) in 69 patients (37.9%) (**Table 1**).

Table 1 Subject demographics and clinical characteristics at 0week

Parameter	
Patients, n	182
Age (year, median, IQR)	60 (51-68)
Female, n (%)	142 (78.0)
RA duration (year, median, IQR)	6 (3-11)
RF positive (%)	62.7
ACPA positive (%)	64.3
RF & ACPA positive (%)	56.0
DAS28 (ESR) (median, IQR)	2.56 (1.94-3.21)
CDAI (median, IQR)	3.50 (1.15-6.65)
MTX use at baseline, n (%)	125 (68.7)
MTX dose (mg/w, median, IQR)	6 (4-8)
PSL use at baseline, n (%)	69 (37.9)

RA, rheumatoid arthritis; RF, rheumatoid factor; ACPA, anti-cyclic citrullinated peptide antibodies; DAS28, Disease Activity Score 28-joint assessment; CDAI, Clinical Disease Activity Index; MTX, methotrexate; PSL, prednisolone.

2. Comparison of joint destruction progression at the end of the 1-year observation period

The clinical characteristics of the joint destruction(−) group (n = 124) and joint destruction(+) group (n = 58) are summarized in **Table 2**. The numbers of serum MMP 3(+) patients in the joint destruction(−) and joint destruction(+) groups were 47 (37.9%) and 35 (60.3%), respectively. The median DAS28-ESR were 2.25 (IQR 1.75–2.89) and 2.51 (IQR 2.11–3.01), the median CDAI were 2.10 (IQR 0.70–5.88) and 3.20 (IQR 1.35–6.10), and the PSL usage rates were 33.9% (n = 42) and 39.3% (n = 22), respectively. There were no significant differences in disease activity or PSL usage rates between the joint destruction(−) and the joint destruction(+) groups. However, there were significant differences in gender, ACPA positivity, and MMP-3 positivity between the joint destruction(−) and the joint destruction(+) groups. We performed multivariate logistic regression analysis on these factors. The results showed that only MMP-3 positivity (odds ratio, 2.20; 95% CI: 1.14–4.25, p = 0.018) was significantly associated with joint destruction.

3. Comparison of progression of joint damage in MMP-3(−) and MMP-3(+) patients

The incidences of progression of joint destruction in MMP-3(−) (n = 100) and MMP-3(+) patients (n = 82) were 23.0% (n = 23) and 42.7% (n = 35), respectively (p < 0.01). The mean changes in the mTSS from baseline (ΔmTSS) were 0.46 ± 1.12 and 0.97 ± 1.99 (p = 0.03) (**Figure 1A**), and the incidences of nonprogression (ΔmTSS < 0.5) were 77.0% and 57.3% (**Figure 1B**). At baseline, the median MMP-3 levels were 55.0 ng/ml (IQR 44.2–84.6) and 119.0 ng/ml (IQR 78.0–159.6) (p < 0.01) and the PSL usage rates were 17.0% (n = 17) and 57.3% (n = 47) (p < 0.01). Progression of joint destruction was then further examined in the MMP-3(−) (n = 77) and MMP-3(+) (n = 36) patients not receiving PSL to exclude the effect of PSL for the serum MMP-3 increase. Among those patients, the incidences of joint destruction progression were 24.7% (n = 19) and 47.2% (n = 17) in the MMP-3(−) and MMP-3(+) groups, respectively (p = 0.03). The ΔmTSS values were 0.51 ± 1.22 and 1.03 ± 1.90 (p = 0.09) (**Figure 1C**), and the incidences of nonprogression were 75.3% and 52.8% (**Figure 1D**).

Notably, however, even when CRP levels had normalized after a year of treatment, it did not necessarily indicate the absence of inflammation. We sometimes encountered patients with a few small joint swellings but normalized CRP levels. We therefore assessed the progression of joint destruction in the MMP-3(−) (n = 58) and MMP-3(+) (n = 39) patients who exhibited no joint swelling at 1 year. Among this group, the incidences of joint destruction progression were 20.7% (n = 12) and 43.6% (n = 17) in the MMP-3(−) and MMP-3(+) patients, respectively (p = 0.02). The ΔmTSS values were

Table 2 Joint destruction (-) or (+) groups analysis at 1-year

	Joint destruction	Joint destruction		Multivariate	
	(-)	(+)	*p*	OR (95% CI)	*p*
Age (year, median, IQR)	60 (50-67)	61 (53-69)	0.4		
Female / Male	91 / 33	51 / 7	0.03	2.32 (0.94-5.71)	0.0681
RA duration (year, median, IQR)	6 (3-11)	6 (3-13)	0.25		
RF positive (%)	61.9	76.5	0.07		
ACPA positive (%)	57.9	77.8	0.04	1.44 (0.75-2.79)	0.2753
RF & ACPA positive (%)	51.4	65.7	0.22		
MMP-3 (+) group, n (%)	47 (37.9)	35 (60.3)	< 0.01	2.20 (1.14-4.25)	0.0182
DAS28 (ESR) (median, IQR)	2.25 (1.75-2.89)	2.51 (2.11-3.01)	0.17		
CDAI (median, IQR)	2.10 (0.70-5.88)	3.20 (1.35-6.10)	0.97		
MTX use, n (%)	104 (83.9)	45 (77.6)	0.31		
MTX dose (mg/w, median, IQR)	6 (4.5-8)	6 (6-10)	0.34		
PSL use, n (%)	42 (33.9)	22 (39.3)	0.61		
PSL dose (mg, median, IQR)	4 (2-5)	3 (2-5)	0.48		

Data are median (IQR).
RA, rheumatoid arthritis; RF, rheumatoid factor; ACPA, anti-cyclic citrullinated peptide antibodies; MMP-3, matrix metalloproteinase-3; DAS28, Disease Activity Score 28-joint assessment; CDAI, Clinical Disease Activity Index; MTX, methotrexate; PSL, prednisolone.

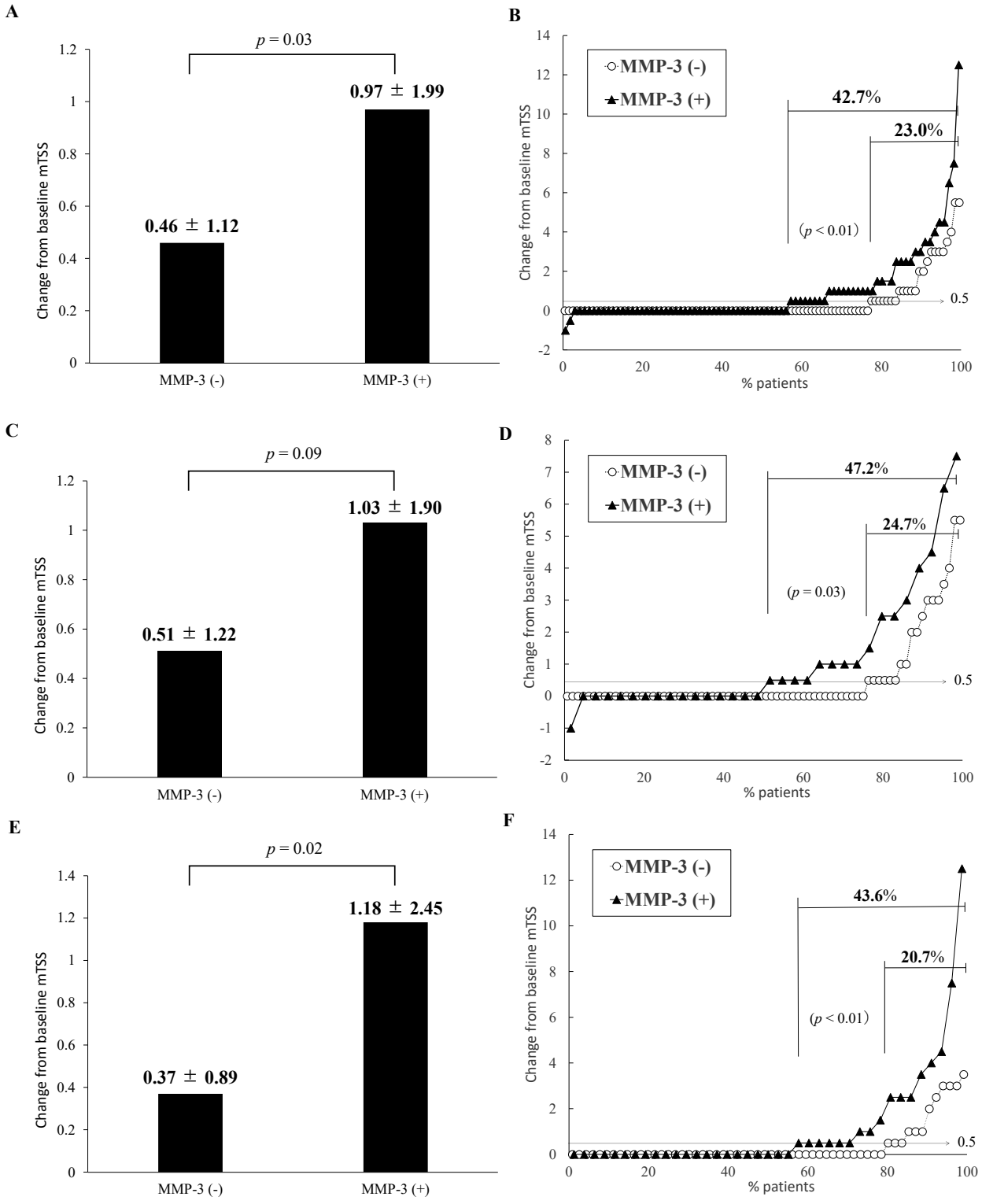

Figure 1 Progression of joint destruction in the MMP-3(−) and MMP-3(+) patients with RA after 1 year of treatment (A, B) Changes in the modified total sharp score from baseline (ΔmTSS) (A) and plot of cumulative mTSS probability (B) for the total RA patient sample (n = 182). (C, D) ΔmTSS (C) and cumulative mTSS probability (D) for RA patients not receiving PSL (n = 113). (E, F) ΔmTSS (E) and cumulative mTSS probability (F) for patients without swollen joints at 1 year (n = 97). Values in (A) (C) (E) indicate the mean (SD) at each time point and in the MMP-3(−) or MMP-3(+) group. Percentages in (B) (D) (F) indicate incidences of joint destruction progression (ΔmTSS > 0.5) in the MMP-3(−) or MMP-3(+) group.

0.37 ± 0.89 and 1.18 ± 2.45 (p = 0.02) (**Figure 1E**), the incidences of nonprogression were 79.3% and 56.4% (**Figure 1F**), and the PSL usage rates at baseline were 15.5% (n = 9) and 48.7% (n = 19) (p < 0.01) (**Table 3**).

4. Comparison of joint destruction progression between MMP-3(−) and MMP-3(+) patients with normal CRP levels at baseline

We also examined the progression of joint destruction in the 142 RA patients (MMP 3(−), n = 79; MMP-3(+), n = 63) who had normal CRP levels at baseline. Progression of joint destruction was detected in 24.1% (n = 19) and 47.6% (n = 30) (p < 0.01) of the MMP 3(−) and MMP 3(+) patents, respectively, while the ΔmTSS values were 0.47 ± 1.17 and 1.13 ± 2.21 (p = 0.03), the incidences of nonprogression (ΔmTSS < 0.5) were 75.9% and 52.4%, and the PSL usage rates at baseline were 15.2% (n = 12) and 57.1% (n = 36) (p < 0.01). This suggests that progression of joint destruction is more likely when MMP-3 levels remain positive, even when baseline CRP is normal.

5. Comparison of the progression of joint damage estimated by radiography in the MMP-3(−) and MMP-3(+) groups with LDA or clinical remission (DAS28-ESR < 2.6 or CDAI ≦ 2.8)

Clinical remission is the therapeutic target for patients with RA, with LDA being the best possible alternative. Thus, the study enrolled 109 of 183 patients with RA (MMP-3(−), n = 63; MMP-3(+), n = 46) who had achieved LDA or clinical remission (DAS28-ESR < 2.6). The progression of joint destruction was found in 25.4% (MMP-3(−)) and 50.0% (MMP-3(+)) (p < 0.01); the ΔmTSS values were 0.42 ± 1.02 and 1.21 ± 2.41 (p = 0.02); and the nonprogression rates (ΔmTSS < 0.5) were 74.6% and 50.0%, respectively. The progression of joint destruction was further examined in 70 of these 109 patients (MMP-3(−), n = 48; MMP-3(+), n = 22)

Table 3 MMP-3 (−) or (+) patients analysis at 1-year

	MMP-3 (−)	MMP-3 (+)	*p*
Age (year, median, IQR)	57 (49-65)	63 (53-70)	0.01
Female / Male	73 / 27	69 / 13	0.07
RA duration (year, median, IQR)	5 (3-9)	7 (4-15)	0.17
RF positive (%)	52.6	76.9	< 0.01
ACPA positive (%)	54.4	75.0	0.02
RF & ACPA positive (%)	43.9	69.2	< 0.01
MMP-3 baseline (week 0) (ng/ml, median, IQR)	55.0 (44.2-84.6)	119.0 (78.0-159.6)	< 0.01
DAS28 (ESR) (median, IQR)	2.14 (1.75-2.59)	2.57 (2.12-3.31)	0.02
CDAI (median, IQR)	2.00 (0.70-5.88)	3.50 (1.40-6.60)	0.52
PSL use, n (%)	17 (17.0)	47 (57.3)	< 0.01
mTSS baseline (week 0) (median, IQR)	8.0 (2.0-33.0)	18.5 (7.0-52.0)	0.1
ΔmTSS	0.46 ± 1.12	0.97 ± 1.99	0.03
0.5 ≦ Δ baseline, n	23	35	
progression ratio	23.0	42.7	< 0.01
Without PSL use at baseline, n	77	36	
Δ mTSS	0.51 ± 1.22	1.03 ± 1.90	0.09
0.5 ≦ Δ baseline, n	19	17	
progression ratio	24.7	47.2	0.03
no swollen joints group, n	58	39	
ΔmTSS	0.37 ± 0.89	1.18 ± 2.45	0.02
0.5 ≦ Δ baseline, n	12	17	
progression ratio	20.7	43.6	0.02
PSL use, n (%)	9 (15.5)	19 (48.7)	< 0.01

Data are mean ± S.D. or median (IQR)
RA, rheumatoid arthritis; RF, rheumatoid factor; ACPA, anti-cyclic citrullinated peptide antibodies; MMP-3, matrix metalloproteinase-3; DAS28, Disease Activity Score 28-joint assessment; CDAI, Clinical Disease Activity Index; mTSS, modified Total Sharp Score; PSL, prednisolone.

who were PSL-free. Among these patients, progression of joint destruction was observed in 27.1% and 54.5% (p = 0.03), the ΔmTSS values were 0.49 ± 1.15 and 1.39 ± 2.23 (p = 0.03), and the nonprogression rates were 72.9% and 45.5%, respectively. Similarly, 128 of 182 patients with RA (MMP-3(−), n = 70; MMP-3(+), n = 58) who had achieved LDA or clinical remission (CDAI ≦ 2.8) were compared. The progression of joint destruction was found in 24.3% (MMP-3(−)) and 48.3% (MMP-3(+)) (p < 0.01), the ΔmTSS values were 0.36 ± 0.83 and 1.19 ± 2.23 (p < 0.01), and the nonprogression rates (ΔmTSS < 0.5) were 75.7% and 51.7%, respectively. We further examined the progression of joint destruction in 81 of these 128 patients (MMP-3(−), n = 52; MMP-3(+), n = 29) who were PSL-free. Among these patients, we observed progression of joint destruction in 26.9% and 48.3% (p = 0.08); the ΔmTSS values were 0.42 ± 0.93 and 1.19 ± 2.03 (p = 0.02); and the nonprogression rates were 73.1% and 51.7%, respectively. Joint destruction was more severe in the MMP-3(+) group than in the MMP-3(−) group (**Table 4**), indicating that neither the achievement of LDA nor clinical remission is sufficient to prevent the progression of joint destruction, particularly when the serum MMP-3 levels remain positive.

6. Effect of PSL-induced elevation in MMP-3 on progression of joint destruction

Within the MMP-3(+) group, progression of joint de-

Table 4 In LDA and clinical remission group, MMP-3 (−) or (+) patients analysis at 1-year

	MMP-3 (−)	MMP-3 (+)	*p*
DAS-28 :			
LDA and clinical remission group, n	63	46	
DAS28 (ESR) (median, IQR)	2.09 (1.71-2.44)	2.31 (1.88-2.79)	0.04
mTSS baseline (week 0) (median, IQR)	9.5 (3.1-33.0)	14.5 (5.3-44.0)	0.37
ΔmTSS	0.42 ± 1.02	1.21 ± 2.41	0.02
0.5 ≦ Δ baseline, n	16	23	
PSL use at baseline, n (%)	15 (23.8)	24 (52.2)	
progression ratio	25.4	50.0	< 0.01
DAS-28 (without PSL use at baseline) :			
LDA and clinical remission group, n	48	22	
DAS28 (ESR) (median, IQR)	2.04 (1.70-2.43)	2.31 (1.81-2.79)	0.17
mTSS baseline (week 0) (median, IQR)	8.0 (2.5-31.0)	8.75 (5.3-17.0)	0.42
ΔmTSS	0.49 ± 1.15	1.39 ± 2.23	0.03
0.5 ≦ Δ baseline, n	13	12	
progression ratio	27.1	54.5	0.03
CDAI :			
LDA and clinical remission group, n	70	58	
CDAI (median, IQR)	1.90 (0.63-3.50)	3.10 (1.33-4.80)	0.08
mTSS baseline (week 0) (median, IQR)	10.0 (3.0-38.0)	16.0 (5.3-44.0)	0.72
ΔmTSS	0.36 ± 0.83	1.19 ± 2.23	< 0.01
0.5 ≦ Δ baseline, n	17	28	
PSL use at baseline, n (%)	18 (25.7)	29 (50.0)	
progression ratio	24.3	48.3	< 0.01
CDAI (without PSL use at baseline) :			
LDA and clinical remission group, n	52	29	
CDAI (median, IQR)	1.90 (0.68-3.80)	2.80 (1.20-4.50)	0.36
mTSS baseline (week 0) (median, IQR)	8.5 (3.3-42.8)	8.0 (5.0-19.1)	0.19
ΔmTSS	0.42 ± 0.93	1.19 ± 2.03	0.02
0.5 ≦ Δ baseline, n	14	14	
progression ratio	26.9	48.3	0.08

Data are mean ± S.D. or median (IQR)
DAS28, Disease Activity Score 28-joint assessment; ESR, erythrocyte sedimentation rate; mTSS, modified Total Sharp Score; LDA, low Disease Activity; CDAI, Clinical Disease Activity Index; PSL, prednisolone.

struction was assessed in patients treated with (n = 47) and without (n = 35) PSL. Progression of joint destruction was observed in 51.4% (n = 18) and 40.4% (n = 19) (p = 0.37) in the PSL(−) and PSL(+) groups, ΔmTSS values were 1.13 ± 1.93 and 0.85 ± 2.05 (p = 0.54) (**Figure 2A**), and the incidences of nonprogression were 48.6% and 59.6% (**Figure 2B**).

We then examined whether PSL-induced increases in MMP-3 were associated with progression of joint destruction in MMP-3-positive RA patients who achieved low disease activity or clinical remission (DAS28-ESR < 3.2 or CDAI ≤ 10.0). In patients who achieved LDA or clinical remission based on a DAS28-ESR, progression of joint destruction was observed in 52.2% (n = 11) and 47.8% (n = 12) (p = 1.00) in the PSL(−) and PSL(+) groups, and the incidences of nonprogression were 47.8% and 52.2%. Similarly, of patients who achieved LDA or clinical remission based on a CDAI, progression of joint destruction was observed in 50.0% (n = 15) and 44.4% (n = 12) (p = 0.79) in the PSL(−) and PSL(+) groups, and the incidences of nonprogression were 50.0% and 55.6%. These results indicate that achieving LDA or clinical remission is not sufficient to prevent the progression of joint destruction when MMP-3 remain positive. This suggests the increases in MMP-3 contributed similarly to the progression of joint destruction whether or nor they were related to PSL administration.

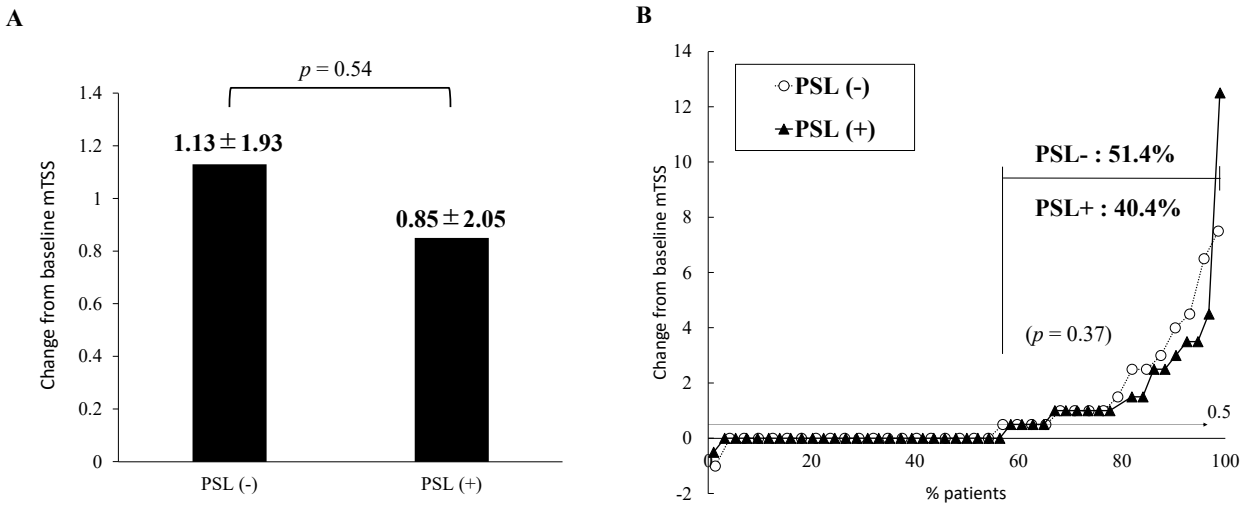

Figure 2 Progression of joint destruction in MMP-3+ patients after 1 year of treatment with or without PSL
(A) ΔmTSS. Values are the mean (SD) at each time point and in the PSL(−) or PSL(+) group. (B) mTSS cumulative probability plot. Percentages indicate the incidences of joint destruction progression (ΔmTSS > 0.5) in each treatment group. (n = 82)

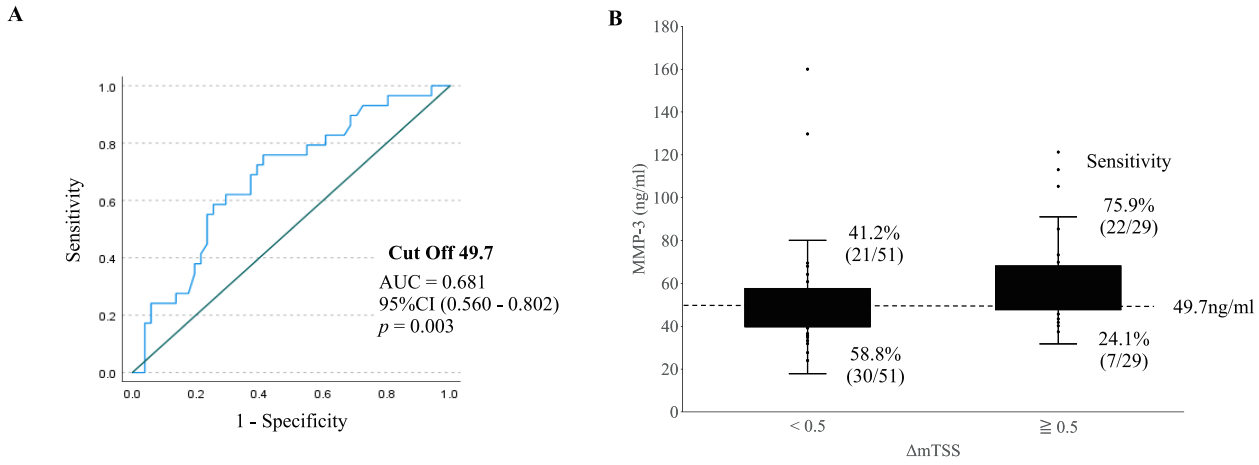

Figure 3 ROC analysis to determine the cut-off value of serum MMP-3 levels
(A) The ROC analysis revealed the cut-off value of the serum MMP-3 was 49.7 ng/mL (area under the curve: 0.681, 95%CI: 0.560–0.802, p < 0.01) to achieve ΔmTSS < 0.5. (B) In the group of patients with advanced joint destruction (ΔmTSS ≥ 0.5), patients had serum MMP-3 levels more than 49.7 ng/mL.

7. ROC analysis to determine the cut-off value of MMP-3 levels

Because of the suggested association between MMP-3 and the rate of joint destruction progression as above, we examined the optimal MMP-3 cut-off value using the ROC curve with the Youden index to determine a practical value for clinical criteria. PSL-free female patients were selected because serum MMP-3 levels are affected by sex (the upper limits of the currently used reference range for male and female are 121.0 and 59.7 ng/mL, respectively) and treatment with or without PSL.

As a result, The ROC analysis revealed that the adequate cut-off value of serum MMP-3 level was 49.7 ng/mL (area under the curve, 0.681; 95% CI 0.560–0.802, $p < 0.01$) (**Figure 3A**) to achieve structural remission (ΔmTSS < 0.5). In patients with advanced joint destruction (ΔmTSS ≥ 0.5), few patients had serum MMP-3 levels <49.7 ng/mL (**Figure 3B**). Therefore, in female patients with RA, lowering the serum MMP-3 level to <49.7 ng/mL is desirable to prevent the progression of joint destruction.

IV. DISCUSSION

Progressive joint destruction is often observed during RA treatment, despite normalized serum CRP levels [7]. The authors of an earlier study proposed that residual MMP-3 activity is one of the responsible factors [19]. Our findings indicate there is greater progression of joint destruction in MMP-3(+) than MMP-3(−) patients, despite their RA being well controlled by MTX therapy, as evidenced by normalized CRP levels and the absence of swollen joints after 1-year of treatment. Moreover, in this study, there were no significant differences in disease activity between the joint destruction(+) and the joint destruction(−) groups. These results suggest that persistently elevated MMP-3 levels lead to joint destruction in patients with RA, including those in remission or with LDA.

During RA treatment, PSL administration is considered to provide a good therapeutic effect when used at appropriate doses [6)20)]. However, MMP-3 levels tend to increase via an unknown mechanism in patients receiving PSL [16)17)]. In our study, there was a positive correlation between PSL dose and MMP-3 value ($r = 0.53$). If this observed PSL-induced elevation in MMP-3 is not related to the pathophysiology of RA, the MMP-3 levels truly derived from the disease pathophysiology should be lower in PSL(+) than in PSL(−) patients, and greater progression of joint destruction should be seen in PSL(−) than PSL(+) patients when their MMP-3 levels are similar. However, our results show that there was no significant difference in mean ΔmTSS and/nor incidences of joint destruction progression between PSL(+) and PSL(−) patients with similar MMP-3 levels. To exclude the possibility that PSL was administered more frequently to patients with high disease activity, we evaluated the progression of joint destruction in MMP-3-positive RA patients who achieved low disease activity or clinical remission (DAS28-ESR < 3.2 or CDAI ≤ 10.0) and found no significant difference in mean ΔmTSS and/nor incidences of joint destruction progression between PSL(−) and PSL(+) patients. Taken together, these findings suggest that PSL-induced increases in MMP-3 levels are also involved in the progression of joint destruction. To our best knowledge, this is the first report to show that PSL induced increases in MMP-3 levels contribute to joint destruction and that PSL is a possible confounding factor in the treatment of RA.

Interestingly, when we selected patients in remission or with LDA at baseline, the MMP-3(+) group had significantly higher mean ΔmTSS and incidences of joint destruction progression than the MMP-3(−) group. Although joint destruction reportedly correlates with disease activity [21)], our results suggest that MMP-3 levels are a more important indicator of radiographic progression of joint destruction. There was no correlation between DAS28 and MMP-3 or between CDAI and MMP-3 in patients in remission or exhibiting LDA (data not shown). Those results also suggest that MMP-3 levels serve as an indicator of joint destruction and imply that even with reduced inflammation and achievement of LDA or remission, joint destruction may continue to progress unless MMP-3 levels are appropriately managed.

Previous studies have reported cut-off values for serum MMP-3 levels to prevent joint destruction [22)23)]. These studies included RA patients with positive CRP values, high disease activity, and receiving PSL treatment. Furthermore, these studies calculated the cut-off value from a patient cohort comprising both sexes, despite different upper limit values of the reference range. In the present study, we analyzed the cut-off values for PSL-free female patients with negative CRP values and in remission or LDA. In the ROC analysis, the appropriate cut-off value of serum MMP-3 levels was found to be 49.7 ng/mL for preventing the progression of joint destruction in these patients; this value falls below the upper reference limit (59.7 ng/mL). Serum MMP-3 levels may be a better indicator of whether joint destruction is likely to progress and suggest treatment intensification, even if disease activity and CRP levels have remained low for up to 1

year. The current reference range of serum MMP-3 was determined based on reference individuals who met the following conditions: negative CRP values, negative RF values, normal fasting blood sugar levels, and normal liver enzyme test results. However, these conditions do not exclude potential RA patients. Thus, it is not surprising that our cut-off value was lower than the upper reference limit of serum MMP-3 [24].

MMP-3 can activate several other MMPs, including MMP-1, MMP-7, and MMP-9, thereby increasing connective tissue matrix proteolysis [25]. It is thought that these proteases contribute to joint destruction, either directly or indirectly, by degrading the cartilage extracellular matrix [9,11]. Thus, it is difficult to achieve structural remission unless MMP-3 levels are reduced to an appropriate level. However, there are currently no therapeutic agents that directly lower MMP-3 levels. In our in vitro experiment, TNF-alpha-induced increase in MMP-3 levels from RA synoviocytes was inhibited by PSL (data not shown); however, oral PSL administration increases MMP-3 levels in vivo [17]. It is necessary to elucidate the mechanism of the PSL-induced increase in MMP-3 levels to develop new therapeutic agents that lower MMP-3 levels in future.

Limitations

This study has some limitations. The retrospective design limits the ability to draw causal inferences. The sample size is relatively small, and the sample is from two university hospitals in the Kansai area of Japan, which may limit generalizability. Joint destruction occurring over a period longer than 1 year was not estimated.

Conclusion

Our study indicates that residual MMP-3 activity may lead to progression of joint destruction in RA patients, even when they achieve clinical remission or LDA through successful treatment with MTX, and that PSL-induced serum MMP-3 increase contribute similarly to the joint destruction compared with the MMP-3 increase without PSL administration. It is also suggested that the clinically relevant cut-off value to achieve the structural remission in female RA patients is 49.7 mg/ml, well under the upper limit of reference range. The development of therapeutic strategies to regulate MMP-3 expression may be necessary to achieve both clinical and structural remission in RA management.

Funding

Open access funding provided by the Hyogo Medical University.

Statements and Declarations:

Competing Interests

The Department of Advanced Medicine for Rheumatic Diseases in Kyoto University is supported by Nagahama City, Shiga, Japan, Toyooka City, Hyogo, Japan, and four pharmaceutical companies (Chugai Pharmaceutical Co. Ltd, UCB Japan Co. Ltd, AYUMI Pharmaceutical Co., and Asahi Kasei Pharma Corp.). The abovementioned pharmaceutical companies were not involved in the study design, data collection and analysis, manuscript writing, or manuscript submission.

KMurakami received speaker fees and/or consulting fees from Eisai Co. Ltd., Chugai Pharmaceutical Co. Ltd., Pfizer Inc., Bristol-Myers Squibb, UCB Japan Co. Ltd., and Astellas Pharma Inc.

AO received research grants and/or speaker fees from Pfizer Inc., Bristol-Myers Squibb., Advantest, Asahi Kasei Pharma Corp., Chugai Pharmaceutical Co. Ltd., Eli Lilly Japan K. K., Ono Pharmaceutical Co., UCB Japan Co., Eisai Co. Ltd., AbbVie Inc., Takeda Pharmaceutical Co. Ltd.

TF received speaker fees from AbbVie, Asahi Kasei, Jansen, and Eisai.

KMurata received speaker and/or consulting fees from AbbVie G.K., Eisai Co., Ltd., Pfizer Inc., Chugai Pharmaceutical Co., Ltd., Pfizer Inc., Bristol-Myers Squibb, and Asahi Kasei Pharma Corp.

MT received research grants and speaker fees from AbbVie G.K., Asahi Kasei Pharma Corporation, Astellas Pharma Inc., Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd., Eli Lilly and Company, Pfizer Inc., UCB Japan Co., Ltd., Janssen Pharmaceutical K.K., Kyowa Kirin Co., Ltd., Taisho Pharma Co., Ltd., and Teijin Pharma, Ltd.

AM received honorarium from AbbVie G.K., Chugai Pharmaceutical Co. Ltd., Eli Lilly Japan K.K., Eisai Co. Ltd., Pfizer Inc., Bristol-Myers Squibb., Astellas Pharma Inc., and Gilead Sciences Japan.

AM received research grants from AbbVie G.K., Asahi Kasei Pharma Corp., Chugai Pharmaceutical Co. Ltd., and Eisai Co. Ltd. for work outside the scope of this study.

HK, MM, MN, and MK declare no potential conflicts of interest.

Contributions

H.K., M.M., and M.K. contributed to design, data acquisition and analysis, statistical calculations and manuscript writing. K. Murakami, A.O., T.F., K. Murata, M.T., A.M., and M.N. contributed to data analysis. All

authors have read and approved the final manuscript.

Acknowledgements

The authors thank the patients and medical staff of both hospitals for their contribution to this study. We would like to extend our heartfelt gratitude to Michie Asada, Haruo Horii, Naohiro Ito, and Masatoshi Fujii for their generous (financial) support for the KURAMA cohort. The funders were not involved in the study design, data collection, analysis, interpretation of the data, writing of the manuscript, or in any decision to publish the results. We also thank MSTEditing Company (https://www.mstediting.com) for the English language review.

References

1) Koch AE. The pathogenesis of rheumatoid arthritis. Am J Orthop (Belle Mead NJ). 2007; 36: 5-8.
2) Smolen JS, Aletaha D, Bijlsma JWJ, et al. Treating rheumatoid arthritis to target: Recommendations of an international task force. Ann Rheum Dis. 2010; 69 (4): 631-7.
3) Smolen JS, Breedveld FC, Burmester GR, et al. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. Ann Rheum Dis. 2016; 75 (1): 3-15.
4) Cronstein BN. Low-dose methotrexate : a mainstay in the treatment of rheumatoid arthritis. Pharmacol Rev. 2005; 57 (2): 163-72.
5) Pincus T, Yazici Y, Sokka T, et al. Methotrexate as the "anchor drug" for the treatment of early rheumatoid arthritis. Clin Exp Rheumatol. 2003; 21 (5 Suppl 31): S179-85.
6) Smolen JS, Landewé RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis. 2020; 79 (6): 685-99.
7) Smolen JS, Van Der Heijde DM, St Clair EW, et al. Predictors of joint damage in patients with early rheumatoid arthritis treated with high-dose methotrexate with or without concomitant infliximab: Results from the ASPIRE trial. Arthritis Rheum. 2006; 54 (3): 702-10.
8) Malemud CJ. Matrix metalloproteinases (MMPs) in health and disease: an overview. Fron Biosci. 2006; 11: 1696-701.
9) Yoshihara Y, Nakamura H, Obata K, et al. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. Ann Rheum Dis. 2000; 59 (6): 455-61.
10) Cawston T. Matrix metalloproteinases and TIMPs: Properties and implications for the rheumatic diseases. Mol Med Today 1998; 4 (3): 130-7.
11) Okada Y. Matrix-degrading metalloproteinases and their roles in joint destruction. Mod Rheumatol. 2000; 10 (3): 121-8.
12) Lerner A, Neidhöfer S, Reuter S, et al. MMP3 is a reliable marker for disease activity, radiological monitoring, disease outcome predictability, and therapeutic response in rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2018; 32 (4): 550-62.
13) Kobayashi A, Naito S, Enomoto H, et al. Serum levels of matrix metalloproteinase 3 (stromelysin 1) for monitoring synovitis in rheumatoid arthritis. Arch Pathol Lab Med. 2007; 131 (4): 563-70.
14) Hattori Y, Kida D, Kaneko A. Normal serum matrix metalloproteinase-3 levels can be used to predict clinical remission and normal physical function in patients with rheumatoid arthritis. Clin Rheumatol. 2019; 38 (1): 181-7.
15) Yoshimi R, Hama M, Takase K, et al. Ultrasonography is a potent tool for the prediction of progressive joint destruction during clinical remission of rheumatoid arthritis. Mod Rheumatol. 2013; 23 (3): 456-65.
16) Hattori Y, Kida D, Kaneko A. Steroid therapy and renal dysfunction are independently associated with serum levels of matrix metalloproteinase-3 in patients with rheumatoid arthritis. Mod Rheumatol 2018; 28 (2): 242-8.
17) Ribbens C, Martin y Porras M, Franchimont N, et al. Increased matrix metalloproteinase-3 serum levels in rheumatic diseases: Relationship with synovitis and steroid treatment. Ann Rheum Dis. 2002; 61 (2): 161-6.
18) Bruynesteyn K, Boers M, Kostense P, et al. Deciding on progression of joint damage in paired films of individual patients: Smallest detectable difference or change. Ann Rheum Dis. 2005; 64 (2): 179-82.
19) Konishi H, Kanou SE, Yukimatsu R, et al. Adenosine inhibits TNFα-induced MMP-3 production in MH7A rheumatoid arthritis synoviocytes via A_{2A} receptor signaling. Sci Rep. 2022; 12 (1): 6033.
20) Kirwan JR. The effect of glucocorticoids on joint destruction in rheumatoid arthritis. The Arthritis and Rheumatism Council Low-Dose Glucocorticoid Study Group. N Engl J Med. 1995; 333 (3): 142-6.
21) Tsuji H, Yano K, Furu M, et al. Time-averaged disease activity fits better joint destruction in rheumatoid arthritis. Sci Rep. 2017; 7 (1): 5856.
22) Mamehara A, Sugimoto T, Sugiyama D, et al. Serum matrix metalloproteinase-3 as predictor of joint destruction in rheumatoid arthritis, treated with non-biological disease modifying anti-rheumatic drugs. Kobe J Med Sci. 2010; 56 (3): E98-107.
23) Maksymowych WP, Landewé R, Conner-Spady B, et al. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. Arthritis and Rheumatism 2007; 56 (6): 1846-53.
24) Nakata Y, Tanaka Y, Nogami S, et al. Basic study of reagents

for measurement of matrix metalloproteinase-3. Japanese Journal of Medical Technology. 2014; 63(5): 579-85. (in Japanese)

25) Ogata Y, Enghild JJ, Nagase H. Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. J Biol Chem. 1992; 267(6): 3581-4.