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BRIEF REPORT

Reduction of plasma IL-6 but not TNF-α by methotrexate in patients with early rheumatoid arthritis: a potential biomarker for radiographic progression

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Abstract
Objective To determine the effect of methotrexate (MTX) on plasma levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α and to investigate their associations with clinical and radiographic responses in patients with early rheumatoid arthritis (RA).
Methods Sixty-two untreated RA patients with the disease duration of ≤36 months in whom MTX was initiated were consecutively identified in our prospective RA cohort and included in this study. Concomitant use of prednisolone and synthetic disease-modifying anti-rheumatic drugs with MTX was allowed, but patients who used biological agents were excluded. Plasma IL-6 and TNF-α levels were measured at the time of diagnosis (baseline) and 1 year later. The relationships of the clinical and radiographic data with plasma levels of IL-6 and TNF-α were analyzed.
Results The median age of the patients was 57 years, 49 patients were female, and the median disease duration was 3 months. Forty-six (74.2 %) patients were anti-cyclic citrullinated protein antibody-positive. Serum C-reactive protein (CRP), plasma IL-6, and DAS28 decreased significantly (p <0.001) after MTX treatment, but plasma TNF-α did not. Radiographic progression was significantly correlated with disease activity score and plasma IL-6 levels but not with CRP or TNF-α after MTX treatment. Patients with plasma IL-6 level above 4.03 pg/ml showed clinically relevant radiographic progression with a sensitivity of 91.7 % and a specificity of 88.0 %.
Conclusion In this early RA cohort, we demonstrated a significant (p <0.001) reduction of plasma IL-6, but not TNF-α, during MTX treatment. The post-treatment IL-6 level was a strong indicator of radiographic progression.

Keywords Interleukin-6 · Methotrexate · Rheumatoid arthritis · Tumor necrosis factor-alpha

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Introduction

Methotrexate (MTX) is the anchor drug in the treatment of rheumatoid arthritis (RA), and its effectiveness is well established [1]. However, it is still unclear how MTX affects the complicated cytokine network involved in the pathology of RA [2, 3], and few studies have investigated changes in plasma cytokine levels during MTX treatment [4].

Tumor necrosis factor (TNF)-α and interleukin (IL)-6 play central roles in the pathogenesis of RA and are the main targets of biological agents [5]. MTX inhibits cytokine production induced by T cell activation in vitro [6, 7]. However, there are no reports regarding the effects of MTX on circulating cytokine levels in treatment-naive early RA patients. We performed longitudinal measurements of plasma TNF-α and IL-6 in patients in such a cohort and analyzed the relationship between cytokine levels and clinical effectiveness and radiographic progression.

Patients and methods

Patients

Systematic Cohort Analysis in Keio University–Rheumatoid Arthritis (SAKURA) is a prospective cohort consisting of 150 consecutive newly diagnosed RA patients at Keio University Hospital between August 2008 and March 2011. At inclusion in SAKURA, those patients had never been treated

with any disease-modifying antirheumatic drugs (DMARDs) or corticosteroids, and each rheumatologist in charge started treatment. Since some patients in SAKURA had already severe joint destruction with a long history of arthralgia at the time of diagnosis, we selected patients with disease duration (which we defined here as a time period from symptom onset to diagnosis) of up to 36 months in this current study in order to catch small changes in joint destruction. Concomitant use of classic DMARDs or low-dose prednisolone at a dose of no more than 10 mg/day was allowed. Clinical, radiographic, and plasma cytokine data was collected at baseline and 1 year later. Since the interval of the patient's visit was 1–3 months, 1-year data was obtained with 3-month allowance. Patients in whom a biological agent was started within 1 year were analyzed with data just before the commencement of it using last observation carried forward (LOCF) method. Although the time points of data collection at baseline were not necessarily exactly the same with the time of the MTX commencement, all the patients had been treated with MTX for more than 5 months during this study. Six patients assessed at 16 to 18 months due to patient's personal reasons were exceptionally included. This study was approved by the ethics committee at Keio University School of Medicine. Written informed consent was obtained from all patients.

Clinical and radiological assessments

Clinical characteristics including matrix metalloproteinase-3 (MMP-3) which was routinely measured in our clinics were obtained from the medical charts. Radiographs of the hands and feet were taken at baseline and 1 year later (at the same time as the blood samples). Two blinded and independent readers (NN and YK) scored the images using the van der Heijde modified Total Sharp Score (mTSS) [8]. The smallest detectable change of the two in mTSS was 1.32. The mean of the two scores was used in the analysis. Estimated yearly progression of mTSS (ΔmTSS/year) was adjusted by the exact months from baseline to the second time of x-ray.

Plasma assays

Plasma samples were collected at baseline and 1 year later. All samples were stored immediately after collection at −30 °C in our laboratory. Plasma TNF-α and IL-6 levels were assessed using chemiluminescent enzyme immunoassays (R&D Systems Inc., Minneapolis, MN, USA) that had a threshold of detection of 0.55 and 0.30 pg/ml, respectively. The details of the measurement methods were reported previously [9].

Statistical analysis

Continuous data are presented as the median with interquartile range (IQR), mean with standard deviation, or as a number with percentage value as appropriate. The Wilcoxon signed rank test was used to examine changes between baseline and 1 year later. Correlation of two continuous variables was analyzed using Pearson's correlation. Receiver operating characteristics (ROC) curve analysis was used to evaluate the performance of the indices as a method for identifying clinically relevant radiographic progression (CRRP), defined as ΔmTSS/year >3 [10]. Multivariate analysis was performed using multiple regression models by parameters with p value <0.20 in preceding univariate analysis. All statistical analyses were performed with JMP 9 (SAS Institute Inc., Cary, NC, USA).

Table 1 Baseline characteristics and treatments during this study of rheumatoid arthritis patients (n=62)

Female, *n* (%)	49 (79.0)
Age, years; median (IQR)	57 (49–66)
Mean ± SD	56±14.5
Disease duration, months; median (IQR)	3 (2–7)
Mean ± SD	6.3±8.0
Serologic status, *n* (%)	
RF+/anti-CCP+	43 (69.4)
RF+/anti-CCP-	3 (4.8)
RF-/anti-CCP+	3 (4.8)
RF-/anti-CCP-	13 (21.0)
Steinbrocker Stage, I/II/III/IV, *n*	51/9/0/2
MTX use during this study, *n* (%)	62 (100)
dosage, mg/week; median (IQR)	8 (8–10)
dosage, mg/week; mean ± SD	8.7±2.3
Concomitant PSL use during this study, *n* (%)	6 (9.8)
Concomitant DMARD use during this study, *n* (%)	
BUC	6 (9.8)
SSZ	6 (9.8)
BUC and SSZ	1 (1.6)
TAC	1 (1.6)

Results are expressed as median (IQR), mean ± SD, or number (%) as appropriate

BUC bucillamine, *CCP* cyclic citrullinated protein, *DMARD* disease-modifying antirheumatic drug, *MTX* methotrexate, *PSL* prednisolone, *RF* rheumatoid factor, *SSZ* salazosulfapyridine; *TAC* tacrolimus

Results

Patient characteristics

Sixty-two patients were analyzed. Of the SAKURA cohort, 88 patients were excluded because of one of the following reasons: disease duration >36 months, a biological agent was started within 5 months, MTX was not used, or lost to follow-up. The median duration of observation was 11 (7–14) months. The median age was 57 (49–66) years, 49 (79.0 %) patients

were female, and the median disease duration was 3 (2–7) months. Patient characteristics and the treatment during this study are shown in Table 1. Nineteen (31 %) patients did not have 1-year data because of the commencement of a biologic agent or patient's personal reasons and their data were analyzed using LOCF methods.

Clinical assessments

The median Disease Activity Score 28 (DAS28) and the Health Assessment Questionnaire–Disability Index (HAQ-DI) significantly (p <0.001) decreased from baseline to 1 year later from 4.42 to 2.58 and from 0.625 to 0.125, respectively (Table 2). Changes in other parameters are shown in Table 2. Despite the clinical effectiveness of MTX, the mTSS showed a statistically significant increase (p <0.001) from 4 (0.75–8.25) to 7 (2–11.25). CRRP was observed in 12 (19.4 %) patients. Median ΔmTSS/year was 0.5 (0–2.5). Thirty-one (50 %) patients showed no radiographic progression (ΔmTSS/year <0.5).

Plasma cytokine concentrations

Plasma IL-6 and TNF-α were detectable in 59 (95.2 %) and 36 (58.1 %) patients at baseline, respectively, and in 57 (91.9 %) and 37 (59.7 %) patients 1 year later, respectively. The median plasma IL-6 level decreased from 4.72 to 1.04 pg/ml (p <0.001), while there was no significant difference in the level of TNF-α at baseline and 1 year later (0.87 and 0.83 pg/ml, respectively; p=0.14) (Table 2).

Association of radiographic progression with clinical parameters

The ΔmTSS/year correlated with DAS28 (r=0.469, p <0.01); simplified disease activity index (SDAI) (r=0.422, p <0.0006); MMP-3 (r=0.581, p <0.01); and IL-6 (r=0.673, p <0.01). However, it did not correlate with C-reactive protein (CRP); (r=0.221, p=0.08) or with TNF-α (r=0.158, p=0.22) (Fig. 1a–f). Plasma IL-6 level at 1 year discriminated between patients with CRRP and those without CRRP at a cut-off level of 4.03 pg/ml with a sensitivity of 91.7 % and a specificity of 88.0 %, while plasma TNF-α level at 1 year did not (Fig. 1k and l). DAS28, SDAI, CRP, and MMP-3 discriminated between these groups but less effectively than IL-6 (areas under the ROC curve were 0.810, 0.813, 0.792, and 0.675, respectively; Fig. 1g–j). We also performed the ROC analyses in the same way on the Sharp units of 0.5, 2, and 5 instead of 3 (CRRP), and got the almost same results (data not shown). The proportion of patients with CRRP paralleled with specified categories of disease activity or with parameter values, except for TNF-α (Fig. 1m–r). Moreover, we divided the patients by their DAS28 at 1 year into "remission and low disease activity (LDA)" group and "moderate disease activity (MDA) and high

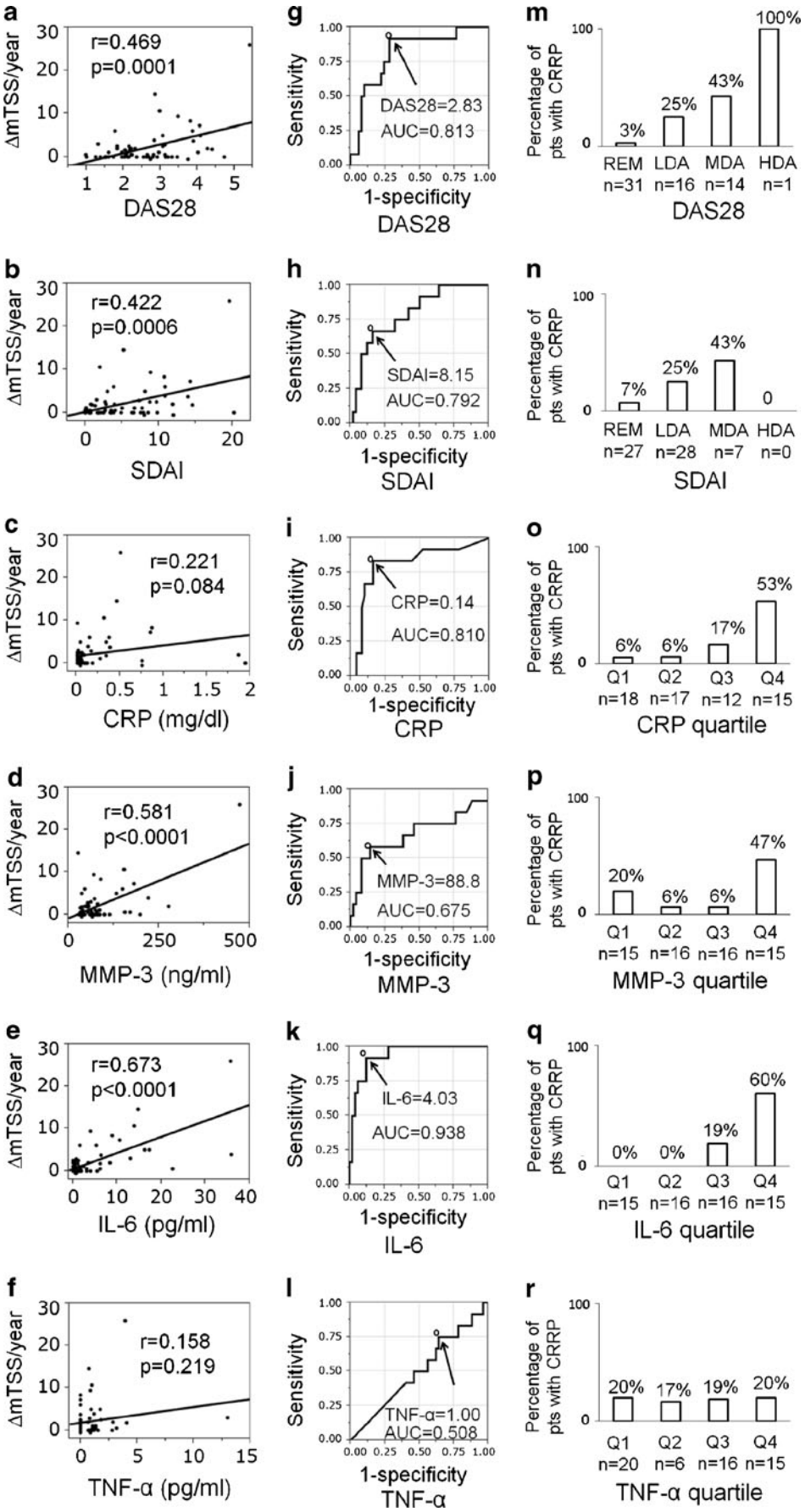
Table 2 Comparison of the clinical and biological characteristics of early rheumatoid arthritis patients at baseline and 1 year later

	Baseline	1 year	p Value
DAS28	4.42 (3.60–5.62)	2.58 (1.93–3.16)	<0.001*
HDA, *n* (%)	21 (33.9)	1 (1.6)	–
MDA, *n* (%)	35 (56.5)	14 (22.6)	–
LDA, *n* (%)	4 (6.5)	16 (25.8)	–
REM, *n* (%)	2 (3.2)	31 (50.0)	–
SDAI	16.2 (9.4–28.1)	3.86 (1.66–8.24)	<0.001*
HDA, *n* (%)	18 (29.0)	0 (0)	–
MDA, *n* (%)	23 (37.1)	7 (11.3)	–
LDA, *n* (%)	21 (33.9)	28 (45.2)	–
REM, *n* (%)	0 (0)	27 (43.5)	–
SJC	4 (2–9)	0 (0–2)	<0.001*
TJC	4 (1–8)	0 (0–1.3)	<0.001*
PtGA (mm)	38.5 (12–65)	15 (5–33)	<0.001*
PhGA (mm)	33.5 (25–51)	6 (1–15)	<0.001*
ESR (mm/h)	33 (20–68)	14 (8–20.5)	<0.001*
CRP (mg/dl)	0.55 (0.10–1.43)	0.06 (0.02–0.18)	<0.001*
MMP-3 (ng/ml)	87.4 (57.2–163.7)	69.9 (46.1–87.0)	<0.001*
HAQ-DI	0.625 (0.344–1.156)	0.125 (0–0.625)	<0.001*
mTSS	4 (0.75–8.25)	7 (2–11.25)	<0.001*
IL-6 (pg/ml)	4.72 (2–11.55)	1.04 (0.58–5.41)	<0.001*
TNF-α (pg/ml)	0.87 (<0.55–1.58)	0.83 (<0.55–1.18)	0.14

Results are expressed as median (IQR) or number (%) as appropriate. The Wilcoxon signed rank test was used to compare the variables

CRP C-reactive protein, *DAS* disease activity score, *ESR* erythrocyte sedimentation rate, *HAQ-DI* health assessment questionnaire-disability index, *HDA* high disease activity, *IL* interleukin, *LDA* low disease activity, *MDA* moderate disease activity, *MMP* matrix metalloproteinase, *mTSS* modified Total Sharp Score, *PhGA* physician global assessment, *PtGA* patient global assessment, *REM* remission, *SDAI* simplified disease activity index, *SJC* swollen joint count, *TNF* tumor necrosis factor, *TJC* tender joint count

*p <0.05 when compared with baseline value


a
ΔmTSS/year
r=0.469
p=0.0001
DAS28
g
Sensitivity
DAS28=2.83
AUC=0.813
1-specificity
DAS28
m
Percentage of pts with CRRP
3%
25%
43%
100%
REM n=31
LDA n=16
MDA n=14
HDA n=1
DAS28
b
ΔmTSS/year
r=0.422
p=0.0006
SDAI
h
Sensitivity
SDAI=8.15
AUC=0.792
1-specificity
SDAI
n
Percentage of pts with CRRP
7%
25%
43%
0
REM n=27
LDA n=28
MDA n=7
HDA n=0
SDAI
c
ΔmTSS/year
r=0.221
p=0.084
CRP (mg/dl)
i
Sensitivity
CRP=0.14
AUC=0.810
1-specificity
CRP
o
Percentage of pts with CRRP
6%
6%
17%
53%
Q1 n=18
Q2 n=17
Q3 n=12
Q4 n=15
CRP quartile
d
ΔmTSS/year
r=0.581
p<0.0001
MMP-3 (ng/ml)
j
Sensitivity
MMP-3=88.8
AUC=0.675
1-specificity
MMP-3
p
Percentage of pts with CRRP
20%
6%
6%
47%
Q1 n=15
Q2 n=16
Q3 n=16
Q4 n=15
MMP-3 quartile
e
ΔmTSS/year
r=0.673
p<0.0001
IL-6 (pg/ml)
k
Sensitivity
IL-6=4.03
AUC=0.938
1-specificity
IL-6
q
Percentage of pts with CRRP
0%
0%
19%
60%
Q1 n=15
Q2 n=16
Q3 n=16
Q4 n=15
IL-6 quartile
f
ΔmTSS/year
r=0.158
p=0.219
TNF-α (pg/ml)
l
Sensitivity
TNF-α=1.00
AUC=0.508
1-specificity
TNF-α
r
Percentage of pts with CRRP
20%
17%
19%
20%
Q1 n=20
Q2 n=6
Q3 n=16
Q4 n=15
TNF-α quartile

◀ **Fig. 1** Association of disease activity and biomarkers after methotrexate (MTX) treatment with radiographic progression in early rheumatoid arthritis patients. Association of **a** DAS28, **b** SDAI, **c** CRP, **d** MMP-3, **e** IL-6, and **f** TNF-α with ΔmTSS/year. The ROC curve discriminated between ΔmTSS/year >3 (CRRP) and ΔmTSS/year ≤3 for **g** DAS28, **h** SDAI, **i** CRP, **j** MMP-3, **k** IL-6, and **l** TNF-α. The percentage of patients who had CRRP according to disease activity category: **m** DAS28 and **n** SDAI or quartiles of **o** CRP, **p** MMP-3, **q** IL-6, and **r** TNF-α. *CRP* C-reactive protein; *CRRP* clinically relevant radiographic progression; *DAS* disease activity score; *HDA* high disease activity; *IL* interleukin; *LDA* low disease activity; *MDA* moderate disease activity; *MMP* matrix metalloproteinase; *pts* patients; *Q* quartile; *REM* remission; *ROC* receiver operating characteristics; *SDAI* simplified disease activity index; *TNF* tumor necrosis factor; ΔmTSS/year, estimated yearly progression of modified Total Sharp Score

disease activity (HDA)" group, and examined the association between cytokines and radiographic changes. While in remission and LDA group, patients with the high IL-6 level were disposed to show CRRP, in MDA and HDA group no patients with low IL-6 level showed CRRP. In regard to TNF-α, we could not find any relationships (data not shown).

Baseline characteristics for prediction of RA patients with radiographic progression

Among the parameters, sex and the plasma level of TNF-α at baseline were statistically significant (p <0.05) but weakly correlated with ΔmTSS/year in multivariate analysis (Table 3). Thus, female patients with higher plasma TNF-α level at baseline may be predisposed to worse radiographic progression.

Discussion

This study demonstrated that in early RA patients, the plasma IL-6 level significantly decreased during MTX treatment, while the plasma TNF-α level did not change. Radiographic progression correlated with higher levels of plasma IL-6 after MTX treatment, suggesting that inhibition of plasma IL-6 may be critical for preventing radiographic progression.

Most of the patients analyzed in this study were in the early stages of RA and had mild to moderate disease activity. Although the overall clinical response to MTX was excellent, one-fifth of the patients exhibited CRRP, which is one of the criteria that typically prompt consideration of treatment changes [10]. In fact, there was a significant correlation between the DAS28 score at 1 year and radiographic progression. However, it should be noted that one-fourth of the patients with LDA resulted in CRRP according to DAS28 category. In this regard, the plasma IL-6 level after MTX treatment showed the strongest association with radiographic progression. For these patients, the measurement of plasma IL-6 might be able to provide additional clinical information that we may as well consider the treatment strategy more carefully in those patients with plasma IL-6 levels greater than 4.03 pg/ml to suppress joint destruction even if they were in remission or LDA.

MTX appeared to have a greater effect in terms of suppressing circulating IL-6 than on suppressing circulating TNF-α. It is likely that the effect of MTX on cytokines in RA is achieved mainly via inhibition of IL-6 production. In this context, it makes sense that TNF inhibitors have a greater effect when used in combination with MTX than when used as monotherapy [11] which is not the case with the IL-6 receptor inhibitor tocilizumab [12, 13]. Moreover, the result that the patients with higher TNF-α at baseline showed worse radiographic progression indicated that TNF-α could be used as a biomarker to consider the treatment strategy. Since our study showed that MTX have little effect on TNF-α, we may be able to suggest that anti-TNF agents be an option at an early stage in patients with high TNF-α.

Table 3 Association of baseline parameters of early rheumatoid arthritis patients with ΔmTSS/year

	Univariate analysis		Multivariate analysis
	r value	p value	p value
Age	0.099	0.44	–
Sex	0.220	0.09	0.03*
Disease duration	0.096	0.46	–
Seropositivity	0.171	0.18	0.98
Erosions	0.084	0.52	–
MTX dosage	0.085	0.51	–
DAS28	0.059	0.65	–
SDAI	0.012	0.92	–
SJC	0.002	0.99	–
TJC	0.060	0.65	–
PtGA	0.085	0.51	–
PhGA	0.117	0.37	–
ESR	0.220	0.09	0.32
CRP	0.008	0.95	–
MMP-3	0.006	0.97	–
HAQ-DI	0.004	0.98	–
IL-6	0.049	0.70	–
TNF-α	0.472	0.0001	0.0006*

Pearson's correlation coefficient (r value) and p value are shown. Dichotomous variables (i.e., sex, seropositivity, and erosion) were scored as 0 or 1:0, male, seronegative, or the absence of initial erosion; 1, female, seropositive, or the presence of initial erosion. Baseline markers with p values less than 0.20 in univariate analysis were analyzed in multivariate analysis.

*p <0.05

ΔmTSS/year estimated yearly progression of modified Total Sharp Score, *CRP* C-reactive protein, *DAS* disease activity score, *HAQ-DI* health assessment questionnaire-disability index, *IL* interleukin, *ESR* erythrocyte sedimentation rate, *MMP* matrix metalloproteinase, *PhGA* physician global assessment, *PtGA* patient global assessment, *SDAI* simplified disease activity index, *SJC* swollen joint count, *TJC* tender joint count, *TNF* tumor necrosis factor

Our study has several limitations. First, since the study population was small, the results of this study may not be valid for all RA patients. For example, the results showing that IL-6 was affected by MTX might be different if the patients showed an inadequate response to MTX. Second, we examined only two cytokines. Since multiple cytokines are involved in the pathogenesis of RA [14], other cytokines need to be investigated as well. Third, the patients showed a good response to MTX when a lower dose of MTX was used rather than the global recommended dose [15]. Ethnic differences between Caucasians and Japanese might account for the difference in the effectiveness of MTX.

In conclusion, this study is the first to demonstrate that MTX reduces IL-6 but not TNF-α in vivo and to show that the plasma IL-6 concentration after MTX was the parameter that was most associated with radiographic progression. Further investigations in a larger population are needed to confirm these findings.

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