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ORIGINAL ARTICLE

Disease activity and changes in the fibrosis-4 index in patients with rheumatoid arthritis treated with methotrexate for a short period

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ABSTRACT

Objectives: This study aims to investigate the relationship between disease activity and changes in the fibrosis-4 index (FIB-4) in patients with rheumatoid arthritis (RA) who received methotrexate as Phase I treatment for a short period.

Patients and methods: In this retrospective study, 144 patients (106 females, 38 males; median age: 68.05 years; range, 58.3 to 76.0 years) diagnosed with RA who had not received methotrexate before their diagnosis were included between April 2015 and September 2020. The patients' clinical data were recorded at baseline, six months, and 12 months. Patients with hepatitis, alcoholism, severe obesity, hypercholesterolemia, or overlapping autoimmune diseases and those receiving a maximum methotrexate dose of ≤10 mg/week were excluded. Multiple regression analysis was performed to identify predictors of the changes in FIB-4 values from baseline. Mediation analysis was employed to determine the association between Disease Activity Score-28 for RA with erythrocyte sedimentation rate (DAS28-ESR) and changes in FIB-4 values, with the cumulative methotrexate dose as a mediator.

Results: FIB-4 values increased significantly from baseline to 12 months after methotrexate initiation. The cumulative methotrexate dose did not independently influence changes in FIB-4 values. After adjusting for confounding factors, the factor independently influencing the change in fibrosis-4 values from baseline was DAS28-ESR at six and 12 months (β=0.107 and β=0.086, respectively). The cumulative methotrexate dose did not mediate the relationship between DAS28-ESR at baseline and changes in FIB-4 values, and it did not affect changes in FIB-4 values over a short period.

Conclusion: Rheumatoid arthritis disease activity before methotrexate administration independently affected changes in FIB-4 values. We suggest monitoring FIB-4 values in patients with RA with high disease activity, even for a short period after methotrexate administration, as FIB-4 values in these patients may be underestimated.

Keywords: Anti-rheumatic agents, fibrosis, mediation analysis, methotrexate, rheumatoid arthritis.

Methotrexate (MTX) causes liver dysfunction in a dose-dependent manner. Follow-up monitoring of aspartate aminotransferase (AST) and alanine transaminase (ALT) levels is routinely performed in patients with rheumatoid arthritis (RA).[1] MTX use is associated with long-term hepatic damage, with liver fibrosis reported to occur after long-term therapy;[2] patients with liver fibrosis have an increased risk of death compared to healthy individuals.[3,4] Unfortunately, monitoring AST and ALT levels in routine practice is unreliable for detecting liver fibrosis. Recently, the fibrosis-4 index (FIB-4) has been used as a simpler and more economical screening method for liver fibrosis.[5] It is easily accessible with routine laboratory testing and is superior to other noninvasive fibrosis markers.[6] FIB-4 is as accurate as transient elastography (TE) with FibroScan for assessing liver fibrosis severity, and it has proven to be a significant predictor of poor outcomes in chronic liver diseases.[7,8] A study suggested that the FIB-4 correlates with the cumulative MTX dose.[9] Another study reported an association between

liver fibrosis measured using FibroScan and the cumulative MTX dose.[10] However, these studies involved patients with RA treated with MTX over a long period. The longer MTX is administered for, the older the patient becomes and, as expected from the FIB-4 formula, the higher the FIB-4 value.

Therefore, studies of patients treated with MTX over long periods suggest that the FIB-4 value correlates with the cumulative MTX dose; however, the length of long-term use may be a confounding factor in this correlation. Furthermore, the higher disease activity in patients with RA may result in a higher cumulative MTX dose. Although there are reports of the FIB-4 and liver fibrosis correlating with the cumulative MTX dose, no study has reported an association with liver fibrosis after short-term MTX treatment, nor has an association between the FIB-4 and disease activity in RA been reported.

At our hospital, patients with RA and high disease activity are aggressively treated with high-dose MTX. Regardless of age, the target maximum MTX dose is 12 to 16 mg/week if the risk of side effects is low. On the basis of these issues and the status of MTX dosing for patients with RA at our hospital, we considered it necessary to investigate whether FIB-4 values correlate with cumulative MTX dose over short observation periods. Therefore, this study focused on the FIB-4 for assessing liver fibrosis in patients with RA treated with MTX during Phase I of the European League Against Rheumatism algorithm recommendations.[11] The primary objective of the study was to evaluate early changes in FIB-4 over a short period to identify potential early liver fibrosis associated with cumulative MTX exposure in this patient population.

PATIENTS AND METHODS

This single-center retrospective observational study used data obtained from patients' medical records at the Takarazuka City Hospital. In total, 163 patients diagnosed with RA between April 2015 and September 2020 who had not used MTX prior to their diagnosis were included. All patients fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism criteria for RA. MTX was administered as first-line treatment. Oral informed consent was obtained from the patients by agreement or opt-out in accordance with the regulations in Japan. The study protocol was approved by the Takarazuka City Hospital Clinical Research Ethics Review Committee (date: 15.11.2021, no: 20211101). This study was conducted in accordance with the principles of the Declaration of Helsinki.

During the screening, all patients were tested for hepatitis B virus surface antigen, surface antibody, core antibody, and deoxyribonucleic acid (HBV-DNA), as well as hepatitis C virus antibody (HCV Ab). The patients' clinical and functional data were recorded at baseline and at all subsequent visits (6 and 12 months). Data on clinical characteristics before the initiation of MTX therapy were used as the baseline data. Patients were excluded from the study if they were HBV-DNA or HCV Ab positive, severely obese (body mass index [BMI] >35 kg/m^2), had hypercholesterolemia, consumed alcohol (chronic alcohol consumption for at least five to 10 years with a daily or weekly intake exceeding 60 g), or had a history of autoimmune liver disease or overlapping autoimmune diseases (e.g., Sjögren's syndrome). Patients who could not continue MTX treatment for more than 12 months, those without valid laboratory findings at baseline or any follow-up time point (6 and 12 months), and those who used a maximum MTX dose of <10 mg/week during the observation period were also excluded. Among the 163 patients, 19 were excluded on the basis of the aforementioned criteria. Hence, 144 patients (106 females, 38 males; median age: 68.05 years; range, 58.3 to 76.0 years) were included in the final analyses.

The study period comprised the 12 months after the initiation of MTX therapy. All patients were followed up throughout this period. We collected the following data on clinical characteristics and laboratory findings at baseline from the patients' medical records: laboratory data, clinical composite measures of RA such as the Disease Activity Score-28 for RA with erythrocyte sedimentation rate (DAS28-ESR) and simplified disease activity index (SDAI), duration from onset to diagnosis,

weight, and BMI. The duration from onset to diagnosis was defined as the period from the onset of the patient's joint symptoms to the date of diagnosis at the hospital. The AST, ALT, platelet count, and FIB-4 values were also measured at baseline and after six and 12 months. The FIB-4 is a marker of fibrosis, which can be an extended process that exceeds the 12-month study period. However, given that we aimed to assess short-term changes in the FIB-4, we selected the six- and 12-month time points. Data on therapeutic medications for RA (MTX dose and cumulative MTX dose) were collected after six and 12 months. We confirmed that 5 mg/week of folic acid was concomitantly prescribed with a high dose of MTX (12 to 16 mg/week).

The FIB-4 was calculated using the following equation: [age (years) × AST level (U/L)] / [platelet count (10^9/L) × √ALT level (U/L)]. The study population was stratified according to FIB-4 values: FIB-4 <1.30 was defined as a low risk for advanced fibrosis, FIB-4 >2.67 as a high risk, and FIB-4 between 1.30 and 2.67 as an intermediate risk.[12]

Statistical analysis

Data were analyzed using R version 4.1.0 (The R Foundation for Statistical Computing, Vienna, Austria) and IBM SPSS version 26.0 software (IBM Corp., Armonk, NY, USA). Patient characteristics and history of RA treatment were reported as descriptive statistics. All results were expressed as medians and interquartile ranges for continuous variables and as frequencies and percentages for categorical variables. AST, ALT, FIB-4, and platelet values were compared between baseline and each observation period using the Wilcoxon signed-rank test. The association between the change in the FIB-4 values from baseline to each observation period and the cumulative MTX dose was analyzed using multiple linear regression analysis. To identify the predictors of the changes in FIB-4 values from baseline, we conducted a multiple linear regression analysis to evaluate the relationship between the factors involved in RA. Pearson correlation coefficients were calculated to determine the correlation between the DAS28-ESR at baseline and changes in the FIB-4 value from baseline to each observation period. In addition, a mediation analysis[13] was conducted to evaluate the association between the DAS28-ESR and changes in FIB-4 values, considering the cumulative MTX dose as a mediator. The level of statistical significance was set at $p<0.05$.

RESULTS

Baseline patient characteristics and history of RA treatment over 12 months

The baseline characteristics of the patients are presented in Table 1. The median BMI was 21.5 kg/m^2, and the median weight was 51.5 kg before the initial administration of MTX. Rheumatoid factor (RF) and anti-citrullinated protein autoantibodies (ACPA) were detected in 54.2% and 46.5% of the patients, respectively. At diagnosis, the median SDAI, DAS28-ESR, modified health assessment questionnaire (mHAQ) score, and C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and matrix metalloproteinase-3 (MMP-3) levels were 25.35, 4.89, 0.5, 2.28 mg/dL, 68.5 mm/h, and 204.45 ng/mL, respectively. The median AST, ALT, and platelet levels used to calculate the FIB-4 values at baseline were 17.0 U/L, 12.0 U/L, and 32.25×10^3/μL, respectively. The median FIB-4 value at baseline was 0.96. Table 1 shows the MTX and cumulative MTX doses at six and 12 months. The median MTX doses were 15.0 and 14.0 mg/week, while the median cumulative MTX doses were 388.0 and 753.5 mg during each observation period (6 and 12 months, respectively).

AST, ALT, platelet, and FIB-4 values after MTX administration

Figure 1 shows that the median AST (Figure 1a), ALT (Figure 1b), FIB-4 (Figure 1c), and platelet (Figure 1d) values increased from baseline to six and 12 months after the introduction of MTX ($p<0.001$). Figure 2 shows the frequency of each group stratified by FIB-4 values at each observation period. The frequencies of FIB-4 values >2.67 were 0.70% at baseline, 4.9% at six months, and 9.0% at 12 months. The frequencies of FIB-4 values <1.3 were 79.2% at baseline, 45.8% at six months, and 36.8% at 12 months. A longer period of

Table 1. Clinical characteristics of patients at baseline (n=144) and MTX dose and cumulative dosage at each observation period

Variables	n	%	Median	Min-Max
Age (year)			68.05	58.3-76.0
Sex Female	106	73.6		
Body mass index (kg/m^2)			21.50	19.70-23.60
Weight (kg)			51.50	46.33-59.55
Rheumatoid factor (positive)	78	54.2		
ACPA (positive)	67	46.5		
SDAI			25.35	17.90-35.70
DAS28-ESR			4.89	4.22-5.69
mHAQ			0.50	0.25-1.00
Duration from onset to diagnosis (day)			110	75-190
C-reactive protein level (mg/dL)			2.28	0.80-5.83
Erythrocyte sedimentation rate (mm/h)			68.50	40.00-89.00
AST level (U/L)			17.0	14.0-20.0
ALT level (U/L)			12.0	9.5-18.5
Platelet count (×10^3/μL)			32.25	26.13-38.38
FIB-4 index			0.96	0.69-1.27
MTX dose (mg/week) 6 months 12 months			 15.0 14.0	 14.0-16.0 12.0-15.3
Cumulative MTX dosage (mg) 6 months 12 months			 388.00 753.50	 351.50-417.00 678.75-820.00

MTX: Methotrexate; ACPA: Anti-cyclic citrullinated peptide; SDAI: Simplified disease activity index; DAS28-ESR: Disease Activity Score-28 for rheumatoid arthritis with erythrocyte sedimentation rate; mHAQ: Modified health assessment questionnaire; AST: Aspartate aminotransferase; ALT: Alanine transaminase; FIB-4: Fibrosis-4 index.

MTX treatment increased the frequency of a FIB-4 value >2.67 and decreased that of a FIB-4 value <1.3.[12]

Effect of the cumulative MTX dose and factors involved in RA on changes in the FIB-4

To examine the association between the cumulative MTX dose and the change in the FIB-4 from baseline to six and 12 months, multiple regression analysis was performed after adjusting for mHAQ scores, DAS28-ESR, duration from onset to diagnosis, sex, BMI, and FIB-4, CRP, RF, MMP-3, and ACPA values at baseline. The cumulative MTX dose did not independently influence the change in the FIB-4 over either six months (95% confidence interval −0.246 to 0.058, p=0.223) or 12 months (95% confidence interval −0.017 to 0.347, p=0.076). There was no association between the cumulative MTX dose and changes in the FIB-4 over a short period.

Therefore, we examined factors involved in RA other than the cumulative MTX dose to identify predictors of changes in the FIB-4 from baseline. Table 2 shows the results of the multiple linear regression analysis conducted to investigate the independent influence of individual variables on the change in the FIB-4 from baseline to six and 12 months and the beta coefficient of each variable for each period. We performed multiple linear regression analysis adjusted for sex, BMI, duration from onset to

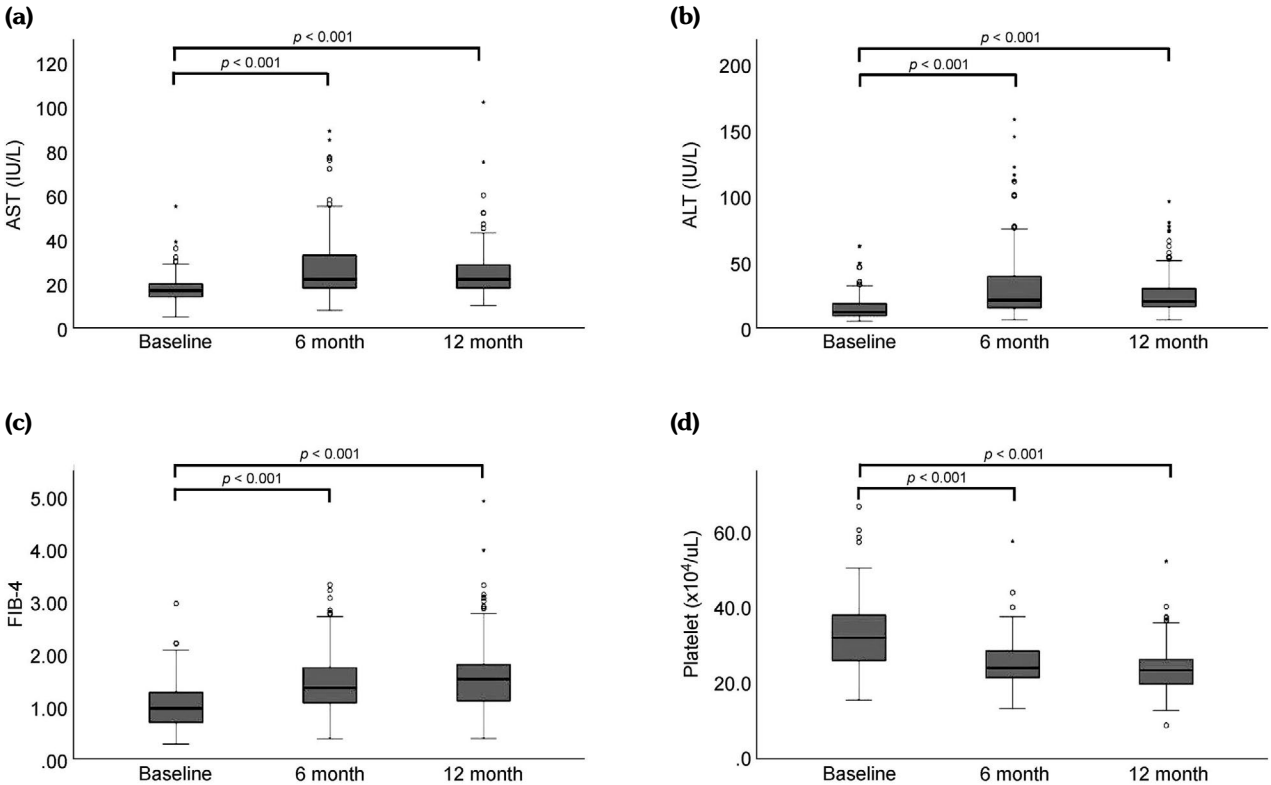

Figure 1. Median levels of AST **(a)**, ALT **(b)**, FIB-4 **(c)**, and platelets **(d)** at each observation period. The top and bottom of each box are the 25th and 75th percentiles. The line through the box is the median, and the error bars are the 5th and 95th percentiles. Outlier values are shown as an open circle and cross. Wilcoxon signed-rank test: p<0.001 for the difference between the value at baseline and each observation period.

AST: Aspartate aminotransferase; ALT: Alanine transaminase; FIB-4: Fibrosis-4 index.

diagnosis, mHAQ score, and CRP, MMP-3, RF, ACPA, DAS28-ESR, and FIB-4 values at baseline, defining the change in the FIB-4 from baseline as the dependent variable. ESR and SDAI were excluded from the analysis due to their strong correlation with DAS28-ESR and CRP levels. Factors that independently influenced the change in the FIB-4 were DAS28-ESR (β=0.107) at six months and CRP levels and DAS28-ESR (β=0.035 and β=0.086, respectively) at 12 months. The DAS28-ESR at baseline had an impact on the change in the FIB-4 score from baseline to both observation periods.

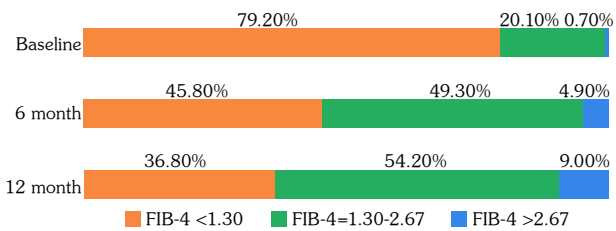

Figure 2. Percentage of patients exhibiting a change in the FIB-4. The percentage of patients was stratified according to FIB-4 value as follows: FIB-4 <1.30, FIB-4=1.30-2.67, and FIB-4 >2.67 at each observation period.

FIB-4: Fibrosis-4 index.

To analyze whether and to what extent the DAS28-ESR at baseline affected the increase in the FIB-4, the correlation between the change in FIB-4 values from baseline to each period and the DAS28-ESR at baseline was determined (Figure 3). The results revealed a significant correlation between the change in FIB-4 scores from baseline to each period and the DAS28-ESR. Table 3 presents the result of the mediation analysis. The average causal mediation effect (ACME), which represents the indirect effect, showed the indirect effect of the

Table 2. Rheumatoid arthritis-related factors associated with changes in FIB-4 values using multiple regression analysis

Variables	β	95% CI	*p*	β	95% CI	*p*
MMP-3 level	0.0040	−0.011 to 0.016	0.631	0.0070	−0.010 to 0.025	0.416
mHAQ	0.0300	−0.079 to 0.139	0.586	−0.0200	−0.134 to 0.130	0.974
DAS28-ESR	0.1070	0.041 to 0.172	0.002	0.0860	0.006 to 0.165	0.035
CRP level	0.0030	−0.016 to 0.023	0.749	0.0350	0.012 to 0.059	0.004
Sex Female	−0.0030	−0.143 to 0.138	0.968	−0.0090	−0.0260 to 0.081	0.298
BMI	0.0100	−0.011 to 0.031	0.336	0.0120	−0.013 to 0.037	0.346
RF (+)	−0.1030	−0.314 to 0.107	0.334	−0.0520	−0.307 to 0.204	0.689
ACPA (+)	−0.1410	−0.348 to 0.068	0.187	−0.1410	−0.392 to 0.112	0.275
Duration from onset to diagnosis	−0.0240	−0.058 to 0.089	0.173	−0.0010	−0.042 to 0.041	0.973
FIB-4 index at baseline	−0.0590	−0.206 to 0.089	0.432	0.176	−0.003 to 0.354	0.054

FIB-4: Fibrosis-4 index; CI: Confidence interval; MMP-3: Matrix metalloproteinase 3; mHAQ: Modified health assessment questionnaire; DAS28-ESR, Disease Activity Score-28 for Rheumatoid Arthritis with erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: Body mass index; RF: Rheumatoid factor; ACPA: Anti-cyclic citrullinated peptide.

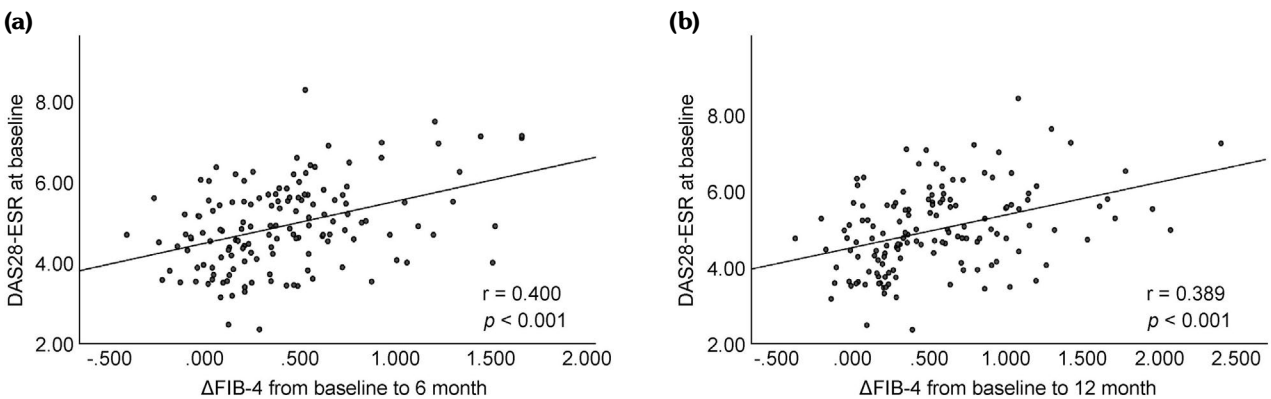

Figure 3. Changes in FIB-4 values from baseline to six and 12 months and the baseline DAS28-ESR. **(a)** Significant correlation between changes in FIB-4 values from baseline to six months (r=0.400, p<0.001). **(b)** Significant correlation between changes in FIB-4 values from baseline to 12 months (r=0.389, p<0.001).

FIB-4: Fibrosis-4 index; DAS28-ESR, Disease Activity Score-28 for rheumatoid arthritis with erythrocyte sedimentation rate.

DAS28-ESR mediated by the cumulative MTX dose on the change in the FIB-4, whereas the average direct effect (ADE) showed the direct effect of the DAS28-ESR on the changes in FIB-4 values. The total effect is the sum of the indirect and direct effects. The ADE and total effects for both periods showed that the DAS28-ESR at baseline affected the change in the FIB-4. The ACME results indicated that the cumulative MTX dose did not mediate the relationship between the DAS28-ESR at baseline and the change in the FIB-4 during either period.

DISCUSSION

The findings revealed that AST and ALT levels, which are routinely monitored, as well as FIB-4, were elevated within 12 months of commencing MTX treatment in patients with RA. First, even over a short period, FIB-4 values were higher after the initial introduction of MTX, and the proportion of patients at risk of advanced liver fibrosis (FIB-4 ≥2.67) increased. Few studies have reported a correlation between the cumulative MTX dose and indicators of liver stiffness in patients with RA treated

Table 3. Direct, indirect, and total effect of DAS28-ESR on changes in FIB-4 values with the cumulative dosage of MTX

	Effect	Lower 95% CI	Upper 95% CI	*p*
6 months				
ACME	–0.002	–0.013	0.011	0.842
ADE	0.108	0.034	0.181	0.008
Total effect	0.107	0.033	0.179	0.006
12 months				
ACME	0.002	-0.005	0.015	0.66
ADE	0.084	0.01	0.163	0.016
Total effect	0.086	0.016	0.164	0.010

DAS28-ESR, Disease Activity Score-28 for rheumatoid arthritis with erythrocyte sedimentation rate; FIB-4: Fibrosis-4 index; MTX: Methotrexate; CI: Confidence interval; ACME: Average causal mediation effect (indirect effect); ADE: Average direct effect.

with MTX.[9,10,14] However, these studies were conducted over several years.

Age has a significant impact on the FIB-4 because it is a component of the FIB-4 formula. The duration of MTX use in the aforementioned studies is a possible confounding factor for the correlation between the cumulative MTX dose and indicators of liver stiffness. In contrast, our study was a short-term observational study conducted over 12 months following the initiation of MTX treatment, and changes in age would not have significantly affected changes in FIB-4. Second, our results revealed that the cumulative MTX dose did not affect the change in FIB-4 values. The study population included patients treated with high-dose MTX during each observation period, with a median dose of 14 to 15 mg/week. The mean MTX dose commonly reported in Japan is 12 mg/week, while the maximum dose approved in Japan is 16 mg/week.[15] Although this study included patients with RA who were administered high doses of MTX, the multiple regression analysis revealed that the cumulative MTX dose did not affect the change in FIB-4 values. Recently, it has been suggested that liver fibrosis associated with MTX may be related not only to the drug itself but also to factors such as alcohol consumption and obesity.[16] Therefore, our study included patients from cohorts that specifically excluded individuals with chronic alcohol consumption or severe obesity.

A potential reason for the cumulative MTX dose not affecting the change in FIB-4 values may be that this was a short-term observational study. Thus, the change in the age of the patients (the length of MTX use) was not a confounding factor for the association between the change in FIB-4 and the cumulative MTX dose. Therefore, we hypothesize that the cumulative MTX dose did not affect the change in FIB-4 values, even in patients treated with high-dose MTX for only a short period. Takahashi et al.[17] reported that higher MTX-polyglutamate (MTX-PG) levels lead to higher AST and ALT levels after MTX administration. Their study also identified BMI as a factor that significantly affects MTX-PG levels. In contrast, our study did not identify BMI as a factor that independently influences the change in FIB-4. The correlation between the FIB-4 values and MTX-PG concentrations was not determined because we did not measure MTX-PG levels in this study; however, this may be a topic for future research.

Recently, FIB-4 has been reported to predict not only liver-related adverse clinical outcomes but also non-liver-related clinical outcomes, such as mortality and risk of exacerbation of disease, in patients with cardiovascular disease, coronavirus disease, and RA.[18-20] However, the association between the FIB-4 and disease activity in patients with RA has not been investigated. Importantly, our findings revealed

that factors involved in RA disease activity, such as the DAS28-ESR, which is not associated with liver dysfunction, were associated with changes in the FIB-4. Additionally, significant correlations were observed between the change in the FIB-4 from baseline to 12 months and the DAS28-ESR at baseline. These results indicate that a higher disease activity of RA before MTX administration is associated with a greater change in FIB-4 values after MTX treatment. However, on the basis of clinical experience, higher disease activity before MTX treatment may result in higher administered doses of MTX. Therefore, DAS28-ESR at baseline may affect the cumulative MTX dose. In the multiple regression analysis (results shown in Table 2), cumulative MTX dose was not included as an adjusted factor. Accordingly, we needed to further investigate the influence of the DAS28-ESR at baseline on the change in the FIB-4 and whether the cumulative MTX dose is involved.

To this end, we performed a mediation analysis to investigate whether the cumulative MTX dose mediated the association between the DAS28-ESR and the change in the FIB-4, considering the cumulative dose of MTX as a mediator (Table 3). The total effect and ADE results in Table 3 are the same as those in Table 2, indicating that the DAS28-ESR at baseline affects the change in the FIB-4. However, from the ACME results, we found that the cumulative MTX dose did not mediate the relationship between the DAS28-ESR at baseline and the change in the FIB-4 during either period. Moreover, results of the multiple regression analysis revealed no association between the cumulative MTX dose and the changes in FIB-4 values. The results in Table 3 indicate that the cumulative MTX dose did not mediate the effect of the DAS28-ESR at baseline on changes in FIB-4 values in either period. This suggests that the DAS28-ESR at baseline is the independent factor that most influences the change in the FIB-4 value and proves that the cumulative MTX dose had no effect.

We hypothesize that interleukin (IL)-6 is involved in the relationship between FIB-4 and RA disease activity. IL-6 is produced in the liver in response to liver injury through several mechanisms, and it plays a multifaceted role in the liver in addition to its role as an inflammatory cytokine.[21,22] Additionally, IL-6–induced thrombocytosis occurs via thrombopoietin.[23] Conversely, if IL-6 production is suppressed, hepatoprotection may decline, and the number of platelets may decrease. Patients with RA and high disease activity before the initial administration of MTX have a high inflammatory state and increased IL-6 production. When these patients are treated with MTX, hepatoprotection may decline, AST and ALT levels may increase, and platelet production may decrease owing to the suppression of IL-6 production and MTX-induced hepatotoxicity. Herein, we did not measure IL-6 levels. However, one data point that proves these findings is an increase in AST, ALT, and FIB-4 levels, as well as the presence of thrombocytopenia after MTX administration (Figure 1a, b, d), indicating that patients with RA with more active disease have greater changes in FIB-4 values after MTX administration than their counterparts. Therefore, we suggest that monitoring the FIB-4 in patients with RA with high disease activity, even over a short period after MTX administration, is important because these patients are more likely to have unexpectedly high FIB-4 values before MTX administration.

This study had certain limitations. The retrospective design and single-institution setting may limit the generalizability of our findings. Notably, during the preparation of our manuscript, Avouac et al.[24] reported similar results indicating that the cumulative MTX dose did not affect the FIB-4. Collectively, these findings from two independent, single-institution, retrospective studies provide further evidence suggesting that the cumulative MTX dose does not affect the change in the FIB-4. Additionally, Avouac et al.[24] did not address the association between RA disease activity and the FIB-4; thus, to our best knowledge, our study is the first to report this association. Unfortunately, the current gold standard method for evaluating liver fibrosis is a liver biopsy, and the FIB-4 alone cannot accurately evaluate liver fibrosis. Nonetheless, performing a liver biopsy each time the FIB-4 score is high is not feasible and is unethical. Thus, it is necessary to use noninvasive methods other than the FIB-4 to evaluate liver fibrosis. The best results were

obtained with liver stiffness measurements by TE using FibroScan or with serum fibrosis markers such as FibroTest-ActiTest and FIBROSpect II.[25,26] In the future, to investigate liver fibrosis following MTX administration, corroboration with other indices, such as FibroScan, would be required. Therefore, noninvasive methods other than the FIB-4 (e.g., TE) should be performed in future studies to accurately reveal liver fibrosis given that FIB-4 levels in patients with RA may be elevated for short periods after MTX administration.

In conclusion, our results suggest that the FIB-4 is elevated in patients with RA, even within 12 months of MTX administration. The change in the FIB-4 value was not affected by the cumulative MTX dose but by high disease activity before MTX administration. FIB-4 should be monitored in patients with RA with high disease activity, even for a short period after MTX administration, as FIB-4 values in these patients may be underestimated.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, design, writing the article: N.N.; Control/supervision, critical review: K.M.; Data collection and/or processing: N.N., K.K., T.H.; Analysis and/or interpretation: N.N., K.M., K.T.; Literature review: N.N., K.M.; References and fundings, materials: N.N., K.K., T.H., K.T., K.M.

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REFERENCES

1. Whiting-O'Keefe QE, Fye KH, Sack KD. Methotrexate and histologic hepatic abnormalities: A meta-analysis. Am J Med 1991;90:711-6.
2. O'Rourke RA, Eckert GE. Methotrexate-induced hepatic injury in an adult. A case report. Arch Intern Med 1964;113:191-4. doi: 10.1001/archinte.1964.00280080027006.
3. Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, Castellanos M, Aller-de la Fuente R, Metwally M, et al. Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: A multi-national cohort study. Gastroenterology 2018;155:443-57.e17. doi: 10.1053/j.gastro.2018.04.034. .
4. Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis. Gastroenterology 2020;158:1611-25.e12. doi: 10.1053/j.gastro.2020.01.043.
5. Kim BK, Kim DY, Park JY, Ahn SH, Chon CY, Kim JK, et al. Validation of FIB-4 and comparison with other simple noninvasive indices for predicting liver fibrosis and cirrhosis in hepatitis B virus-infected patients. Liver Int 2010;30:546-53. doi: 10.1111/j.1478-3231.2009.02192.x.
6. Suh B, Park S, Shin DW, Yun JM, Yang HK, Yu SJ, et al. High liver fibrosis index FIB-4 is highly predictive of hepatocellular carcinoma in chronic hepatitis B carriers. Hepatology 2015;61:1261-8. doi: 10.1002/hep.27654.
7. Durazzo M, Marzari L, Bonetto S, Ferro A, Ghigo MC, Belci P, et al. Noninvasive diagnosis of fibrosis in non-alcoholic fatty liver disease: Diagnostic accuracy of different scores. Minerva Gastroenterol Dietol 2020;66:301-6. doi: 10.23736/S1121-421X.20.02753-1.
8. Kim SU, Kim BK, Park JY, Kim DY, Ahn SH, Song K, et al. Transient elastography is superior to FIB-4 in assessing the risk of hepatocellular carcinoma in patients with chronic hepatitis B. Medicine (Baltimore) 2016;95:e3434. doi: 10.1097/MD.0000000000003434.
9. Miyata M, Kuroda M, Unakami M, Tasaki K, Migita K, Ohira H. Validation of the Fibrosis-4 (FIB-4) index in the diagnosis of liver disease of rheumatoid arthritis patients treated with methotrexate. Mod Rheumatol 2019;29:936-42. doi: 10.1080/14397595.2018.1542962.
10. Lertnawapan R, Chonprasertsuk S, Siramolpiwat S. Association between cumulative methotrexate dose, non-invasive scoring system and hepatic fibrosis detected by fibroscan in rheumatoid arthritis patients receiving methotrexate. Int J Rheum Dis 2019;22:214-21. doi: 10.1111/1756-185X.13442.
11. Smolen JS, Landewé RBM, Bergstra SA, Kerschbaumer A, Sepriano A, Aletaha D, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 Update. Ann Rheum Dis 2023;82:3-18. doi: 10.1136/ard-2022-223356.
12. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7:1104-12. doi: 10.1016/j.cgh.2009.05.033.
13. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. J Stat Softw 2014;59:1-38.

14. Arena U, Stasi C, Mannoni A, Benucci M, Maddali-Bongi S, Cammelli D, et al. Liver stiffness correlates with methotrexate cumulative dose in patients with rheumatoid arthritis. Dig Liver Dis 2012;44:149-53. doi: 10.1016/j.dld.2011.08.013.
15. Atsumi T, Yamamoto K, Takeuchi T, Yamanaka H, Ishiguro N, Tanaka Y, et al. The first double-blind, randomised, parallel-group certolizumab pegol study in methotrexate-naive early rheumatoid arthritis patients with poor prognostic factors, C-OPERA, shows inhibition of radiographic progression. Ann Rheum Dis 2016;75:75-83. doi: 10.1136/annrheumdis-2015-207511.
16. Labadie JG, Jain M. Noninvasive tests to monitor methotrexate-induced liver injury. Clin Liver Dis (Hoboken) 2019;13:67-71. doi: 10.1002/cld.765.
17. Takahashi C, Kaneko Y, Okano Y, Taguchi H, Oshima H, Izumi K, et al. Association of erythrocyte methotrexate-polyglutamate levels with the efficacy and hepatotoxicity of methotrexate in patients with rheumatoid arthritis: A 76-week prospective study. RMD Open 2017;3:e000363. doi: 10.1136/rmdopen-2016-000363.
18. Kim SU, Kim BK, Park JY, Kim DY, Ahn SH, Park YB, et al. Fibrosis-4 index at diagnosis can predict all-cause mortality in patients with rheumatoid arthritis: A retrospective monocentric study. Mod Rheumatol 2020;30:70-7. doi: 10.1080/14397595.2018.1558760.
19. Saito Y, Okumura Y, Nagashima K, Fukamachi D, Yokoyama K, Matsumoto N, et al. Impact of the fibrosis-4 index on risk stratification of cardiovascular events and mortality in patients with atrial fibrillation: Findings from a Japanese multicenter registry. J Clin Med 2020;9:584. doi: 10.3390/jcm9020584.
20. Ibáñez-Samaniego L, Bighelli F, Usón C, Caravaca C, Fernández Carrillo C, Romero M, et al. Elevation of liver fibrosis index FIB-4 is associated with poor clinical outcomes in patients with COVID-19. J Infect Dis 2020;222:726-33. doi: 10.1093/infdis/jiaa355.
21. Taub R, Greenbaum LE, Peng Y. Transcriptional regulatory signals define cytokine-dependent and -independent pathways in liver regeneration. Semin Liver Dis 1999;19:117-27. doi: 10.1055/s-2007-1007104.
22. Ozaki M, Haga S, Zhang HQ, Irani K, Suzuki S. Inhibition of hypoxia/reoxygenation-induced oxidative stress in HGF-stimulated antiapoptotic signaling: Role of PI3-K and Akt kinase upon rac1. Cell Death Differ 2003;10:508-15. doi: 10.1038/sj.cdd.4401172.
23. Kaser A, Brandacher G, Steurer W, Kaser S, Offner FA, Zoller H, et al. Interleukin-6 stimulates thrombopoiesis through thrombopoietin: Role in inflammatory thrombocytosis. Blood 2001;98:2720-5. doi: 10.1182/blood.v98.9.2720.
24. Avouac J, Degrave R, Vergneault H, Combier A, Wanono S, Boisson M, et al. Risk of liver fibrosis induced by methotrexate and other rheumatoid arthritis medications according to the Fibrosis-4 Index. Clin Exp Rheumatol 2022;40:150-7. doi: 10.55563/clinexprheumatol/usddjr.
25. Friedman SL. Preface. Hepatic fibrosis: Pathogenesis, diagnosis, and emerging therapies. Clin Liver Dis 2008;12:13-14. doi: 10.1016/j.cld.2008.07.009.
26. Patel K, Nelson DR, Rockey DC, Afdhal NH, Smith KM, Oh E, et al. Correlation of FIBROSpect II with histologic and morphometric evaluation of liver fibrosis in chronic hepatitis C. Clin Gastroenterol Hepatol 2008;6:242-7. doi: 10.1016/j.cgh.2007.11.009.