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ABSTRACT

Background: Liver fibrosis is an infrequent complication of methotrexate (MTX) treatment diagnosed by liver biopsy, an invasive procedure rarely used in clinical practice. **Objectives:** To evaluate liver fibrosis by transient elastography (TE, FibroScan®) in MTX treated rheumatic patients.

Methods: This was a cross-sectional study of rheumatic patients treated with MTX (n=70) and controls (rheumatic patients not on MTX, n=24). Liver fibrosis was assessed blindly by TE. Eleven patients had repeated measurements during MTX treatment. **Results:** No baseline differences were noted between the 2 groups. The mean cumulative MTX dose was 1807±1846mg while the median treatment duration was 28 months. The mean liver stiffness of MTX treated patients was 5.9±2.1kPa compared to 6.5±3.6kPa of controls (p=0.755). Liver stiffness >7.1kPa (significant liver fibrosis) was observed in 21.5% of patients and 37.5% of controls (p=0.174). There was no correlation between cumulative MTX dose and liver stiffness (Spearman rho p=0.668 r=0.46) and no difference between patients who had received >1.5g (5.7±2.0kPa) compared to those treated with

<1.5g of MTX (6±2kPa, p=0.244). Using multivariate analysis, only γ-GT levels were significantly associated with liver stiffness (p=0.01). In longitudinally followed patients, MTX caused a mild, non-significant increase in liver stiffness (from 6±2.3kPa to 6.7±2kPa, p=0.484).

Conclusions: Long-term MTX administration is not associated with significant liver fibrosis in rheumatic patients, as assessed by TE. This method could be a useful tool for the screening and monitoring of liver fibrosis during MTX treatment.

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Keywords: Methotrexate; liver fibrosis; rheumatoid arthritis; psoriatic arthritis; elastography.

INTRODUCTION

Methotrexate (MTX) is widely used for the treatment of a number of chronic inflammatory rheumatic diseases, such as rheumatoid arthritis (RA), spondyloarthritis (SpA) and vasculitides.^{1,2} The potential hepatotoxicity of prolonged treatment with MTX has been extensively studied for more than 20 years, mostly in retrospective studies. It has been established that MTX could induce liver fibrosis in a small proportion of patients after long-term treatment.³⁻⁶ Recent multi-national recommendations suggest regular monitoring (every 1-3 months) of serum aminotransferases (AST and ALT), while drug discontinuation is recommended in the presence of an increase in transaminases greater than 3 times the upper limit of normal (ULN) values, and liver biopsy is suggested in the case of persistently elevated aminotransferases after drug discontinuation.⁷

Although persistently normal ALT levels have a high negative predictive value for the presence of liver fibrosis, mild elevations of aminotransferases do not predict the development of liver fibrosis, which is accurately diagnosed only with liver biopsy. However, considering that the incidence of MTX-induced liver fibrosis seems to be much lower than previously believed and that liver biopsy is a costly, invasive procedure with potentially life-threatening complications, its use in daily clinical practice is limited.^{8,9} Furthermore, the procedure is poorly accepted and difficult to repeat in asymptomatic subjects while its accuracy for assessing fibrosis has also been questioned, due to sampling errors and intra- and inter-observer variability that may lead to over- or underestimation of the stage of fibrosis.¹⁰⁻¹³

Therefore, there is a need for accurate, non-invasive and easy-to-repeat methods of assessing liver fibrosis. Transient elastography (TE) is a recently developed, non-invasive method for assessing liver fibrosis and cirrhosis in various liver diseases, including chronic hepatitis B and C, non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease.¹⁴⁻¹⁹ Data on its performance in MTX-treated patients are limited so far.

The aim of our study was to evaluate the degree of liver fibrosis in rheumatic patients receiving MTX therapy by TE.

PATIENTS AND METHODS

Patients

This was a cross-sectional study of rheumatic patients treated with MTX who were followed at a Rheumatology Outpatient Clinic of a tertiary referral center (Clinical Immunology-Rheumatology Unit, 2nd Department of Medicine and Laboratory, National University of Athens, Hippokraton General Hospital, Athens, Greece) over a 3-year period. A subgroup of patients was followed prospectively during MTX treatment with repeated measurements of liver stiffness by TE.

All patients were older than 18 years and were on treatment with MTX for various rheumatic diseases. Control patients with rheumatic diseases - such as osteoarthritis (OA) or other diseases - who were not treated with MTX were also included. Patients with known chronic liver diseases such as acute or chronic viral hepatitis B or C, autoimmune hepatitis, chronic cholestatic diseases (such as primary biliary cirrhosis or primary sclerosing cholangitis) or with history of alcoholic liver disease or alcohol abuse (>12 drinks per week for women and >15 drinks per week for men) were excluded from the study.

Clinical data and complete medical history with past and current medications were recorded for each patient. After obtaining informed consent, all patients underwent TE (Fibroscan® - Echosens, Paris, France) and blood testing on the same day that included fasting alanine aminotransferase (ALT), γ -glutamyl transpeptidase (γ GT), total bilirubin, lipids (total cholesterol, HDL, LDL, triglycerides) and glucose.

According to the cumulative dose of MTX, 2 subgroups of patients were defined: Group 1 consisted of patients who had received more than 1500mg and Group 2 who had received less than 1500mg. The cumulative dose cutoff was determined according to the established guidelines for monitoring liver toxicity.²⁰

TRANSIENT ELASTOGRAPHY

We used TE, which is an established novel non-invasive method for the assessment of liver fibrosis by measuring liver stiffness.²¹ Results are expressed in kilopascals (kPa) and correspond, according to the manufacturer's recommendations, to the median of 10 repeated validated measurements. Liver stiffness values range from 2.5 to 75 kPa.²²

The validity of TE results depends on two important parameters: the interquartile range (IQR), which reflects the variability of the validated measures and should not exceed 30% of the median value, and the success rate (the ratio of the number of successful measurements to the total number of acquisitions), which should be at least 60%.^{23,24} Only patients with valid measurements (IQR<30% and success rate \geq 60%) of liver stiffness were included in the study.

Liver stiffness was assessed blindly, using TE by a single experienced operator in all patients. The cutoff value for significant liver fibrosis, severe fibrosis and cirrhosis was set at 7.1kPa, 9.5kPa and 12.5kPa, according to previous publications.²⁴

Statistical analysis

Statistical analysis was conducted using SPSS 16 Statistical Software (SPSS Inc., Chicago, Ill). Results are expressed as mean \pm 1 standard deviation (SD) or median (range), as appropriate. Comparisons of quantitative

data between groups were performed by Mann-Whitney rank-sum tests for non-parametric independent samples. For the group of patients who had repeated measurements of liver stiffness during MTX treatment, paired *t*-test statistical analysis was performed. Qualitative data were compared using chi-squared test and Fisher's exact test. Statistical correlations were performed after assessment of the nonparametric coefficient of Spearman (r_s). Univariate analysis was performed using linear regression test. A value of $p < 0.05$ was considered significant.

RESULTS

Patient characteristics

One hundred twelve patients were enrolled in the study. Ninety-four (84%) had valid measurements of liver stiffness and were included in the analysis. Among the 18 patients (16%) with invalid measurements, 7 had $IQR > 30\%$, 3 had success rate $< 60\%$ and 6 had both the previous parameters. In 2 patients, the measurement was not possible. Patients with valid or invalid measurements had no statistical difference in main characteristics such as gender, age and BMI.

Among these 94 patients, 70 were on MTX (designated as "cases") and 24 were included as "disease controls" (patients with rheumatic diseases who were not receiving MTX). Forty-six patients (66%) had RA, 15 (21%) had psoriatic arthritis and 9 (13%) had other rheumatic diseases. Among disease controls, 15 (62.5%) had OA, 6 (25%) had RA (without MTX treatment) and 3 (12.5%) had another rheumatic disease (see **Table 1**). The demographical and clinical characteristics of the 94 patients are presented in **Table 1**. The majority were women (75.7% of the cases and 87.5% of the controls) with a mean ± 1 SD age of 54 ± 15 years for cases and 57.7 ± 7.3 years for disease controls. There were no statistically significant differences in all characteristics between cases and disease controls.

Liver stiffness in the various groups

The mean value of liver stiffness of the whole population ($n=94$) was 6 ± 2.5 kPa while the values for cases and controls were 5.9 ± 2.1 and 6.5 ± 3.6 kPa, respectively ($p=0.755$). The majority of studied patients, 78.5% of the cases and 62.5% of the controls, had values of liver stiffness < 7.1 kPa; indicative of absent or mild

Table 1. Patient characteristics

	Cases (n=70)	Controls (n=24)	p
Rheumatoid arthritis	46 (66%)	6 (25%)	
Psoriatic arthritis	15 (21%)	0	
Other rheumatic diseases	9 (13%)	3 (12.5%)	
Osteoarthritis	0	15 (62.5)	
Age, years	54 ± 15	57.7 ± 7.3	NS
Gender, male/female	53/17	21/3	NS
BMI (Kg/m ²)	26.9 ± 5.1	27.4 ± 3.8	NS
Diabetes mellitus	7 (10%)	1 (4.2%)	NS
Hypertension	15 (21.7%)	7 (29.2%)	NS
Steroid treatment	41 (59.4%)	5 (21.7%)	0.002
Daily steroid dose (mg)	5.6 ± 2	5.7 ± 1.5	NS
Anti-TNF	27 (38.6%)	0	0.000
ALT (IU/L)	25 ± 15	27.5 ± 15.4	NS
ALT > 1x ULN	5 (7%)	2 (8%)	NS
γ GT (IU/L)	20.6 ± 10	22.3 ± 13	NS
Cholesterol (mg/dl)	202 ± 41	210 ± 46.6	NS
Triglycerides (mg/dl)	126 ± 69.8	147 ± 93	NS

The characteristics in the table are shown as number (percentage) of patients: n (%) and mean value ± 1 standard deviation (SD). BMI: body mass index, TNF: tumor necrosis factor, ALT: alanine aminotransferase, γ GT: γ -glutamyl transpeptidase, ULN: upper limit of normal. p values < 0.05 are shown in bold.

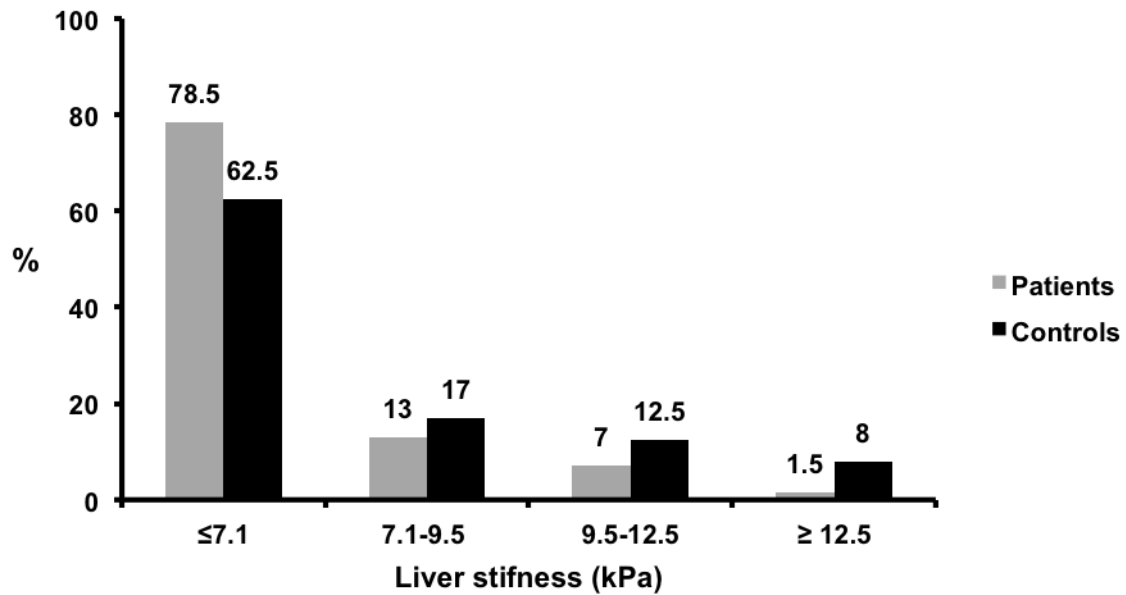


Figure 1. Graph showing the liver stiffness values according to different cutoffs in patients (black columns) and controls (gray columns). Values <7.1kPa indicate absence of liver fibrosis, 7.1-9.5kPa significant fibrosis, 9.5-12.5kPa severe fibrosis and >12.5 kPa cirrhosis.

Table 2. Univariate and multivariate analysis using linear regression for the association of each variable with liver stiffness assessed by transient elastography

Variables	Beta	95% CI	p value
Univariate analysis			
Age	0.021	-0.017 – 0.059	0.277
Gender	0.855	-0.413 – 2.122	0.184
BMI	0.06	-0.050 – 0.169	0.283
Cumulative MTX dose	5.310E-5	0.00 – 0.00	0.731
Diabetes mellitus	1.612	-0.235 – 3.46	0.086
Glucose	0.008	-0.009 – 0.025	0.358
ALT	0.038	0.005 – 0.071	0.026
γGT	0.082	0.031 – 0.132	0.002
Cholesterol	-0.001	-0.016 – 0.13	0.878
triglycerides	0.003	-0.006 – 0.11	0.541
Anti-TNF therapy	-0.530	-1.682 – 0.623	0.364
Multivariate analysis			
ALT	0.017	-0.020 – 0.054	0.367
γGT	0.073	0.018 – 0.127	0.010

BMI: body mass index, MTX: methotrexate, ALT: alanine aminotransferase, γGT: γ-glutamyl transpeptidase, TNF: tumor necrosis factor. p values<0.05 are depicted in bold.

liver fibrosis. The patients with significant liver fibrosis (stiffness >7.1kPa) represented 21.5% of cases and 37.5% of controls (**Figure 1**). There was no statistically significant difference between cases and controls ($p=0.174$). The patients with severe liver fibrosis (liver stiffness: 9.5-12.5kPa) were 7.1% of cases and 12.5% of controls, while there were 3 patients with cirrhosis (liver stiffness >12.5kPa); 1 case (1.5%) and 2 disease controls (8.3%) (**Figure 1**). More specifically, 1 patient with liver stiffness of 12.9kPa had a BMI of 34kg/m² and diabetes mellitus, while among the 2 disease controls, the first had a liver stiffness of 15.3kPa, a BMI of 34kg/m² and diabetes mellitus and the second had liver stiffness of 14kPa and a BMI of 28.7kg/m².

Factors associated with increased liver stiffness

The association between liver stiffness and other factors, such as age, gender, BMI, cumulative dose of MTX, diabetes mellitus, glucose, ALT, γ GT, cholesterol, triglycerides and previous treatment with anti-TNF, was assessed with linear regression analysis. Both γ GT and ALT were significantly associated with liver stiffness by univariate analysis ($p=0.002$ and $p=0.026$, respectively) but in multivariate linear regression analysis, only γ GT showed a statistically significant association ($p=0.01$, **Table 2**).

Anti-TNF treatment effect on liver stiffness

In a separate analysis, we examined the potential effect of anti-TNF treatment administered concurrently with MTX in liver stiffness. The mean value of liver stiffness of patients treated with anti-TNF agents ($n=27$) was 6.4 ± 2.5 kPa, while for those who did not receive such treatment, the mean value was 5.5 ± 1.7 kPa ($p=0.755$). Anti-TNF treatment had no such protective effect either in RA or psoriatic arthritis ($p=0.461$).

Correlation between MTX use and liver stiffness

The mean cumulative MTX dose was 1807 ± 1846 mg (median=1360mg) and the mean duration of therapy was 34.5 ± 29 months (median=28 months). There was no correlation between the cumulative MTX dose and liver stiffness (Spearman's rho $p=0.668$ $r=0.46$) (**Figure 2**). Thirty-three cases (47%) (Group 1) had received more than 1500mg of MTX and 37 cases (53%) (Group 2) less than 1500mg of MTX. The mean value of liver stiffness in these two groups was 5.7 ± 2 kPa and 6 ± 2 kPa respectively ($p=0.244$, **Figure 3**).

Longitudinal study of MTX treated patients

We assessed the liver stiffness longitudinally in 11 patients, with mean interval between the first and second assessment of 25 ± 7 months (range: 12-36). Although a small increase in the mean value of liver stiffness from

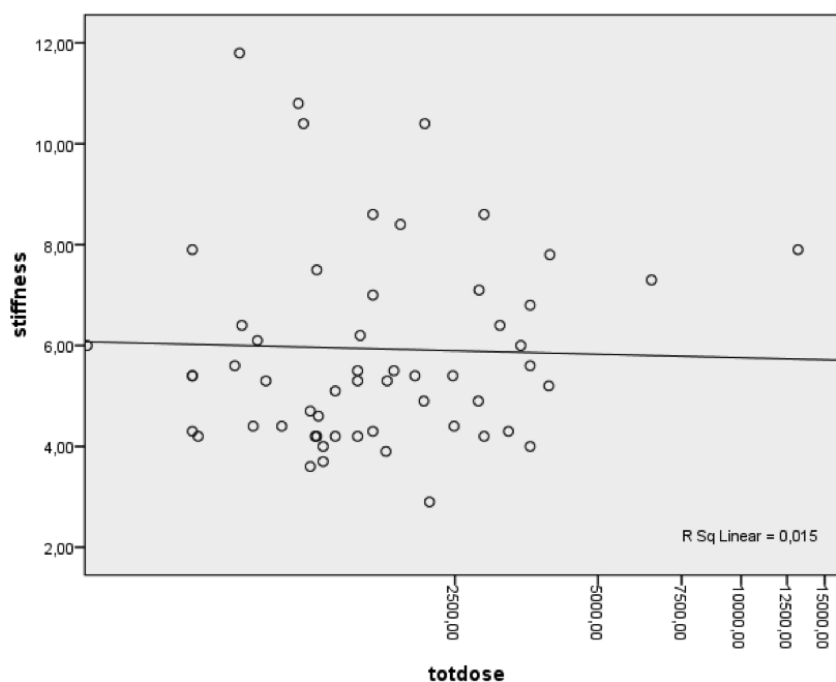


Figure 2. Graph showing the liver stiffness values according to different cutoffs in patients (black columns) and controls (grey columns). The correlation between the total methotrexate (MTX) dose (in mg) and liver stiffness (in kPa) in MTX treated patients is depicted.

6±2.3kPa to 6.7±2kPa was noted, this difference was not statistically significant (p=0.484, by paired t-test, **Figure 4**).

DISCUSSION

This is one of the largest studies in the literature assessing liver fibrosis by non-invasive methods such as TE in rheumatic patients under MTX therapy. The study found no statistically significant difference in the frequency of liver fibrosis in patients treated with MTX compared to patients with osteoarthritis or with other rheumatic diseases not receiving MTX therapy. Multivariate analysis of various factors potentially associated with increased liver stiffness (indicative of liver fibrosis) did not identify MTX use as a significant risk factor. Furthermore, a longitudinal study in a subgroup of patients receiving MTX over a period of approximately 2 years did not show a statistically significant increase in liver stiffness.

Methotrexate use in patients with various rheumatic diseases (RA, psoriatic arthritis) has been associated with liver toxicity manifested by elevation of aminotransferases (AST/ALT) and liver fibrosis.³⁻⁵ While MTX-induced ALT elevations are not uncommon (up to 17% in a recent systematic review of 18 studies in RA patients), the rate of MTX-related severe fibrosis and cirrhosis is much lower.²⁵ Visser et al., reviewing the available published studies in RA and psoriatic arthritis, found a pooled prevalence of advanced fibrosis and cirrhosis of 1.8% and 2.8%, respectively.²⁵ These results, though, should be interpreted with caution, since prospective data from serial liver biopsies including pretreatment liver histology are limited.

Factors that are associated with the development of liver fibrosis in these patients have not been extensively studied: amongst them, a high cumulative dose of MTX, serially elevated aminotransferase (AST/ALT) levels, obesity and type 2 diabetes have been identified.²⁶ In the review by Visser et al.²⁵ in RA patients, the prevalence of severe fibrosis/cirrhosis was 0.7%, 2.2% and 5.9% in patients that had received a cumulative dose of <1.5, 1.5-3 and >3g of MTX, respectively. Older data had led to guidelines which suggested that a liver biopsy should be performed in psoriatic patients who had received 1.5g of cumulative MTX dose and repeated with every additional 1.5g.²⁷ However, the 1.5g cutoff was solely based on expert opinion. Conversely, recent guidelines for monitoring MTX in patients with arthritis are not recommending liver biopsy, regardless of the cumulative MTX dose or duration of treatment.²⁸

Nevertheless, since a small proportion of patients (especially those with concomitant risk factors for liver fibrosis such as obesity, diabetes, alcohol use, etc.), may develop liver fibrosis during long-term MTX use, there is a pressing need to develop non-invasive meth-

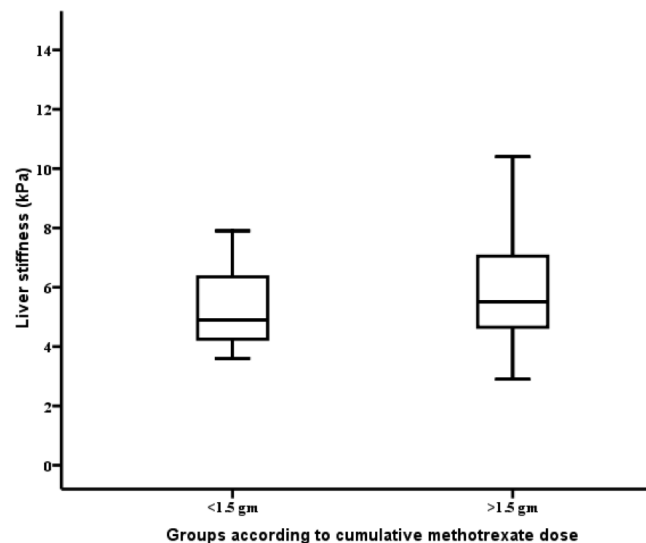


Figure 3. The liver stiffness values in the two groups of methotrexate treated patients according to the cumulative methotrexate dose (>1.5 or <1.5g) they have received.

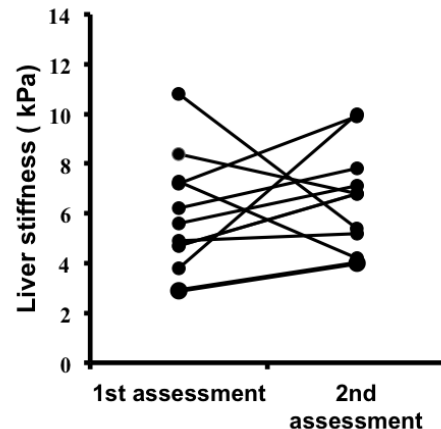


Figure 4. The changes in liver stiffness in eleven patients who were followed longitudinally during methotrexate treatment are shown. The interval between the 1st and 2nd assessment was 25 ± 7 months. No statistical difference was observed (p=0.484, by paired t-test).

ods for the early diagnosis and monitoring of fibrosis in these patients. Transient elastography currently represents the best imaging method for the non-invasive assessment of liver fibrosis. Its utility has been established in various chronic liver diseases such as NAFLD

and chronic hepatitis B or C infections.²⁹ For example, in patients with NAFLD, the negative and positive predictive value of TE for liver fibrosis was 97% and 52%, respectively.³⁰

So far, data on the performance of TE in rheumatic patients treated with MTX are limited. Laharie et al.³¹ have published one of the largest studies evaluating liver fibrosis in patients treated with MTX and showed that severe fibrosis is a rare event (31/518 or 6%, cutoff value=7.9kPa) in these patients and was not related to the cumulative MTX dose. The main differences between our study and theirs were that their patient population also included non-rheumatic patients (Crohn's disease, ulcerative colitis) and their control population consisted only of patients before MTX treatment. Furthermore, they did not exclude patients with alcoholic or other chronic liver diseases from the study. In our study, the definition of significant, severe fibrosis and cirrhosis was set at 7.1, 9.5 and 12.5kPa, respectively (according to previous publications),²⁴ while in the Laharie et al. study only one cutoff point was selected (7.9kPa for severe fibrosis).³¹ There have also been a few other studies with heterogeneous or small patient populations which demonstrated that MTX treatment does not induce liver fibrosis as it is evaluated with TE.³²⁻³⁶

In contrast to our findings, a recent study by Arena et al.³⁷ found a positive correlation between liver stiffness and cumulative MTX dose. In this study, 100 RA patients were included who had received much higher cumulative MTX doses (mean±SD/range: 3595±1938/1530-13000mg) compared to our study. However, the mean value of liver stiffness (4.93kPa) in their population was within the normal range; and liver biopsy, which was performed in 5 patients with liver stiffness >7.1kPa did not reveal significant fibrosis.

In a large Asian study of RA patients treated with MTX and other DMARDs (including leflunomide, sulfasalazine, hydroxychloroquine, corticosteroids) for more than 6 months, only the cumulative dose of leflunomide (and not MTX) was an independent predictor of abnormal liver stiffness.³⁸ Seitz et al.³⁹ have suggested that anti-TNF therapy may have a protective role in the development of liver fibrosis in MTX-treated psoriatic arthritis patients. In our study, anti-TNF treatment did not have such a protective effect in either RA or psoriatic arthritis patients.

So far, there have not been any longitudinal studies assessing liver fibrosis in MTX-treated patients by TE. We prospectively followed a small subgroup of patients treated with MTX with repeated measurements of liver stiffness by TE. Although there was a trend for increased stiffness during MTX treatment, this did not reach statistical significance. Further studies including larger number of patients followed for a longer period of time are needed to confirm these initial findings.

The main limitation of our study was that liver biopsies were not performed, since most of our patients were asymptomatic without ALT elevations.

In conclusion, severe liver fibrosis as assessed by TE is rare in patients treated with MTX. Non-invasive methods such as TE should be strongly considered for screening and monitoring of rheumatic patients treated with MTX. Further longitudinal prospective studies are mandatory in order to confirm our findings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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