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Original Article

Methotrexate therapy is not associated with increased liver stiffness and significant liver fibrosis in rheumatoid arthritis patients: A cross-sectional controlled study with real-time two-dimensional shear wave elastography

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ABSTRACT

Objective: To explore the significance of the association between treatment with methotrexate (MTX) and liver stiffness in rheumatoid arthritis (RA) patients.
Methods: We enrolled 140 consecutive RA patients under MTX treatment (MTX-treated RA; mean treatment duration: 6.2 years; mean MTX cumulative dose: 4.67 g), 33 RA patients naive to MTX (MTX-naive RA) and 100 age and sex-matched healthy blood donors (HD). Liver stiffness was assessed by real time two-dimensional shear wave elastography, with values ≥7.1 Kilopascals (kPa) defining significant liver fibrosis.
Results: kPa values in HD (4.32 ± 0.7) were lower than that in MTX-naive RA (4.92 ± 0.8) and MTX-treated RA (4.85 ± 0.9, $p < .0005$ for trend). On the contrary, the difference in kPa between MTX-naive and MTX-treated RA was not significant ($p = .89$). Similarly, liver stiffness was not significantly different across strata of cumulative MTX dose (4.95 ± 0.7 kPa in MTX < 1 g, 4.90 ± 1.1 kPa in MTX 1–3 g and 4.80 ± 0.9 in MTX > 3 g, $p = .610$). Significant liver fibrosis was diagnosed in 4 patients in the MTX-treated RA (highest kPa value = 7.6; no liver function test abnormalities or clinical signs of hepatic failure) and in none in both the MTX-naive RA and HD groups ($p = .145$).
Conclusion: Liver stiffness values, although within the normal range, are significantly higher in RA patients vs. controls, irrespective of MTX treatment. RA patients taking MTX do not have a higher prevalence of significant liver fibrosis when compared to MTX naive RA patients and the general population.

1. Introduction

Despite significant therapeutic advances over the last 20 years, methotrexate (MTX) is still regarded as the first-line drug in the treatment of rheumatoid arthritis (RA) [1]. In fact, MTX has proven to have a broad range of beneficial biological effects including immunomodulation, regulation of inflammatory response and atheroprotection [2,3]. However, MTX use has been linked with a broad spectrum of liver injuries including steatosis, cholestasis, portal inflammation, liver fibrosis and even cirrhosis [4,5]. However, the studies investigating this issue are relatively small, retrospective, and without appropriate control populations [6]. In a relatively old meta-analysis, 2.7% of RA patients developed severe liver fibrosis/cirrhosis after 55 months of treatment with low-dose MTX [7]. These results are in contrast with a more recent meta-analysis that failed to show an increased risk of a composite end-point of liver failure, fibrosis, and cirrhosis in RA patients taking low-dose MTX [8]. Moreover, MTX was not associated with an increased risk of cirrhosis in a population-based

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cohort of RA patients with HBV and HCV-related chronic [9,10].

Uncertainty also exists regarding whether MTX induces specific liver histological changes in RA patients [11]: some studies reported pre-treatment liver biopsy abnormalities in a large proportion of RA patients [4,12,13], whereas others described stable histopathological findings in repeated biopsies [5,14]. The scenario is further compounded by the evidence that concomitant conditions such as obesity, heavy alcohol intake and metabolic syndrome strongly influence the risk of liver fibrosis in RA patients under MTX [15]. As a consequence, the role of MTX as an independent risk factor for liver fibrosis and cirrhosis in RA patients is still debated.

We sought to address this issue by investigating liver stiffness and prevalence of significant liver fibrosis in a consecutive series of RA patients under MTX (MTX-treated RA patients), MTX-naive RA patients and healthy blood donors (HD) by a real time two-dimensional shear wave elastography technology mounted on Aixplorer supersonic imaging ultrasound scanner (2D.SWE.SSI.).

2D.SWE.SSI is a new technique that has shown good diagnostic accuracy for the staging of liver fibrosis in patients with chronic liver disease [16] and RA [17].

2. Materials and methods

2.1. RA patients and healthy controls

We studied a consecutive series of RA patients attending our outpatient clinic. RA diagnosis was based on the 2010 ACR/European League Against Rheumatism (EULAR) criteria [18]. Patients were included in the MTX-treated RA group if they had received the drug for at least 6 consecutive months prior to the study. RA patients naive for MTX were enrolled in the MTX-naive RA group. Age and sex-matched healthy blood donors (HD) attending the local blood transfusion service, without musculoskeletal signs and symptoms suggestive of rheumatic disease, were enrolled as a control group.

Exclusion criteria for both RA patients and HD were the following: recent infection; history of hepatitis B and C virus infection; treatment with hepatotoxic drugs other than RA-specific drugs; alcohol abuse ($\geq$ 30 g/day in men and $\geq$20 g/day in women); cancer; Wilson's disease; α1-antitrypsin deficiency; genetic hemochromatosis; autoimmune liver diseases; diabetes; and pregnancy.

The following routine clinical and laboratory data were collected: age, weight, body mass index (BMI), waist circumference, total cholesterol, triglycerides, AST and ALT levels.

To assess potential correlations between liver stiffness and RA-specific features the following disease specific scores, disease descriptors and treatment data were collected: current steroid treatment; current treatment with synthetic or biological disease-modifying anti-rheumatic drugs (sDMARDs and bDMARDs) other than MTX; number of swollen joints; number of tender joints; C-reactive protein (CRP) concentrations, mg/dl; erythrocyte sedimentation rate (ESR), mm/h; DAS-28-ESR, disease activity score, 28 joints, calculated with ESR.

All subjects gave their written informed consent before starting the study. The study followed the ethical guidelines of the 1975 Declaration of Helsinki and was approved by our institutional review board (2351/CE_2017).

2.2. 2D.SWE.SSI

Liver stiffness was assessed by an investigator experienced in 2D.SWE.SSI (GV), using the Aixplorer ultrasound equipment (SuperSonic Imaging, S.A., Aix en Provence, France) after an overnight fast. 2D.SWE.SSI measures the elasticity of the tissue, by the speed of shear waves.

The elastography method of 2D.SWE.SSI is a dynamic system by which an acoustic radiation force induces the propagation of tissue displacement to determine the shear wave speed imaging. In particular, the acoustic radiation force focus is swept down the acoustic axis faster than the shear-wave speed, so as to generate tissue displacements (tens of μm) at all positions along the acoustic axis almost simultaneously. This produces a shear-wave in the shape of a cone with a shallow angle (and hence is almost cylindrical), known as a Mach cone, that travels away from the push line, which spreads less and thus decays less rapidly with distance than that from a single pushing focus. By this technology, shear waves are summed thus increasing their amplitude, and improving their propagation distance. The ultrafast imaging capability of the Aixplorer by which the system has a high frame rate with a few thousands of image per second, allow to follow the shear waves generated [19].

Liver stiffness is calculated by the speed of shear waves in m/s, which is converted into kilopascals (kPa). The shear waves speed is related to the liver parenchyma stiffness, with faster waves progression in stiffer tissue.

With this technology it is possible to provide a real time quantitative viscoelasticity imaging mode in a large bidimensional area, using a color-coded image superimposed on the top of the B-mode image. Stiffer tissues are coded in red, while softer tissues are coded in blue (Supplementary Fig. 1).

The size of the sample box (Q-box) is user adjustable. In our study, we used a sample box size of 3,5 × 2,5 cm, placed 1,5–2 cm from the Glisson's capsule, in an area of liver parenchyma free of large vessels. By placing a circular region of interest (ROI) in the Q-Box, the mean and standard deviation of the elasticity within the ROI were recorded.

Measurements were performed in the right liver lobe using the convex array broad band transducer (SC6–1) placed through the intercostal space, with the patient lying supine and keeping the right arm in abduction. The elasticity map of the liver was detected while the patient held his breath.

Measurement quality was represented by the stability index (scale between 0 and 100%). We accepted only measurements with stability index > 92%. Ten valid measurements of liver stiffness were performed in each patient to calculate the mean and standard deviation.

A kPa cut-off value $\geq$7.1 was used to define the presence of significant liver fibrosis (F $\geq$ 2), as proposed by Herrmann E [16].

2.3. Statistical analysis

Data are expressed as mean ± standard deviation, unless otherwise stated. One-way Welch ANOVA and Games-Howell post hoc analysis were conducted to determine statistical differences in liver stiffness (kPa) across the study groups. Fisher's exact test was used to determine if the probability distributions of fibrosis were significantly different between the study groups. Multiple linear regression analysis with stepwise forward method was performed to identified variables that were independently associated with liver stiffness in the RA population. Biologically plausible variables and variables showing significant association ($p \leq .05$) with liver stiffness in simple linear regression were included in the regression model.

Statistical analyses were performed using SPSS 24.0 Statistical Software (SPSS Inc., Chicago).

A value of $p \leq .05$ was considered statistically significant.

3. Results

3.1. RA patients and healthy controls

One hundred and forty MTX-treated, 33 MTX-naive RA patients and 100 HD were enrolled in the study. According to the study protocol mean age and gender distribution were similar across the groups (Table 1). Similarly, there were no significant between-group differences in mean BMI, waist circumference, total cholesterol, triglycerides, AST and ALT levels (Table 1).

MTX-naive patients had a significant shorter disease duration and

Table 1
Demographics, clinical and laboratory characteristics of RA patients and HD.

	MTX-treated RA	MTX-naive RA	HD	*p* value
	n = 140	*n* = 33	*n* = 100	
Age, yrs	60.6 ± 11.0	60.3 ± 12.7	57.0 ± 13.7	0.098
Female, n (%)	106(75.7)	21(63.6)	70(70)	0.310
BMI, kg/m^2	25.3 ± 4.2	26.5 ± 5.5	24.7 ± 3.3	0.159
Waist, cm	90.1 ± 12.0	92.1 ± 14.1	89.0 ± 10.6	0.477
Hypertension, n(%)	57(40.7)	10(30.3)	38(38)	0.539
Total cholesterol, mg/dL	206.5 ± 38.2	202.7 ± 36.4	211.3 ± 32.5	0.430
Triglycerides, mg/dL	94.4 ± 40.4	87.1 ± 32.8	82.2 ± 36.4	0.076
AST, U/L	19.9 ± 6.5	17.9 ± 5.9	19.1 ± 4.8	0.221
ALT, U/L	21.7 ± 13.9	19.2 ± 16.5	18.5 ± 7.3	0.090

MTX, methotrexate; BMI, body mass index; Difference between means has been calculated by ANOVA. Difference between proportions has been calculated with Chi-square.

higher HAQ compared to MTX-treated patients. As expected, according to the EULAR guidelines for the treatment of RA, patients naive to MTX were less frequently under bDMARDs at the time of the study (see TNF inhibitors, Abatacept, Tocilizumab and Rituximab rows in Table 2) compared to MTX-treated patients.

In the MTX-treated group of RA patients, the mean cumulative dose of MTX was 3715 ± 3560 mg and the mean time of treatment exposure was 71.3 ± 66.4 months (Table 2).

3.2. Liver stiffness

Liver stiffness (kPa) was significantly different across the groups, Welch's F (2, 91.223) = 14.488, $p < .0005$. kPa values were significantly lower in HD (4.32 ± 0.7) when compared to MTX-naive RA [4.92 ± 0.8, mean difference 0.6 (95% CI, 0.2 to 0.9) kPa, $p = .001$] and MTX-treated RA patients [4.85 ± 0.9, mean difference 0.5 (95% CI, 0.2 to 0.7) kPa, $p = .0005$]. By contrast, the difference in kPa between the MTX-naive and the MTX-treated RA groups was not statistically significant [0.07 (95% CI, −0.32 to 0.47), $p = .89$] (Fig. 1, Panel A).

In multiple linear regression in the whole sample, diagnosis of RA [B (95%CI) = 0.521(0.318–0.724, p < .0005], older age [B (95%CI) = 0.009(0.001–0.017, $p = .023$] and male sex [B(95%CI) = 0.688(0.469–0.906, p < .0005] were independently associated with higher liver stiffness values.

We also performed a multiple group comparison stratifying RA patients according to cumulative MTX dose into 3 categories: MTX cumulative dose < 1 g ($n = 53$), MTX cumulative dose between 1 and 3 g ($n = 30$) and MTX cumulative dose > 3 g ($n = 90$) (Fig. 1, Panel B). Liver stiffness was not significantly different across the groups (4.95 ± 0.7 kPa in MTX < 1000 mg, 4.90 ± 1.1 kPa in MTX 1000–3000 mg and 4.80 ± 0.9 in the MTX > 3000 mg group; Welch's F(3, 72.820) = 0.498, $p = .610$), suggesting that higher cumulative doses of MTX were not significantly associated with higher liver stiffness values.

In multiple linear regression (Table 3) increasing age and male sex, but not treatment with and cumulative dose of MTX, were independently associated with increasing liver stiffness in the RA population with age and sex explaining 12% of liver stiffness variability (model $R^2 = 0.12$).

Analyses performed to investigate whether specific factors may significantly affect kPa across strata of cumulative dose of MTX did not show significant differences in demographic and clinical characteristics, with the exception of increasing disease duration, duration of exposure to MTX, use of DMARDs and use of TNFi with increasing cumulative dosage of MTX (Table 4).

3.3. Liver fibrosis

Using the proposed cut-off of 7.1 kPa, only 4 out of 173 RA patients analysed were classified as having significant liver fibrosis (kPa values ranging from 7.1 to 7.6). All 4 patients with significant liver fibrosis were in the MTX-treated RA group (4/140 = 2.8%) with consequently no subjects in the MTX-naive RA ($p = .145$). Patients classified as having significant liver fibrosis do not have liver function test abnormalities or clinical sign of hepatic failure.

In multiple logistic analysis neither MTX treatment nor cumulative dose of MTX were independently associated with liver fibrosis (Table 5).

4. Discussion

The development of liver fibrosis is of significant concern with long-

Table 2
Demographics and disease descriptors of RA population.

	All RA	MTX-treated RA	MTX-naive RA	p value
	n = 173	n = 140	n = 33	
Disease duration, months	115.2 ± 120.9	134 ± 123	32.1 ± 59.6	< **0.001**
ACPA positivity, %	68.9	66.1	80	0.141
RF positivity, %	72.8	72.3	75	0.754
ESR, mm/h	25.5 ± 17	24.7 ± 17.4	28.6 ± 18.8	0.261
CRP, mg/dL	0.78 ± 0.9	0.71 ± 0.9	1.08 ± 1.1	0.084
DAS28-ESR	4.0 ± 0.8	4.07 ± 0.8	4.09 ± 0.8	0.942
Steroid use, %	30.1	36.4	28.6	0.380
Steroid dose, mg/day	1.47 ± 3.0	1.21 ± 2.3	2.58 ± 4.9	0.134
Steroid cumulative dose, mg	44.1 ± 92	36.3 ± 71.3	77.2 ± 149.3	0.134
MTX use, %	80.9	100	/	/
MTX cumulative dose, mg	3715 ± 3,56	3715 ± 3,56	/	/
MTX time of exposition, months	71.3 ± 66.4	71.3 ± 66.4	/	/
sDMARDs use, %	86.7	100	30.3	< **0.001**
TNF inhibitors use, %	17.3	21.4	0	**0.003**
Abatacept use, %	1.2	3	0.7	0.263
Tocilizumab use, %	1.2	4.3	0	0.226
Rituximab use, %	3.5	1.4	0	0.490

MTX, methotrexate; ACPA, anti-citrullinated peptide antibodies; RF, rheumatoid factor; BMI, body mass index; CRP, C-reactive protein concentrations, mg/dl; ESR, erythrocyte sedimentation rate, mm/h; DAS-28-ESR, disease activity score, 28 joints, calculated with ESR; Steroid cumulative dose, cumulative dose of steroids during the last month. sDMARDs, synthetic disease-modifying anti-rheumatic drugs; TNF, tumor necrosis factor; In bold significant *p* values.

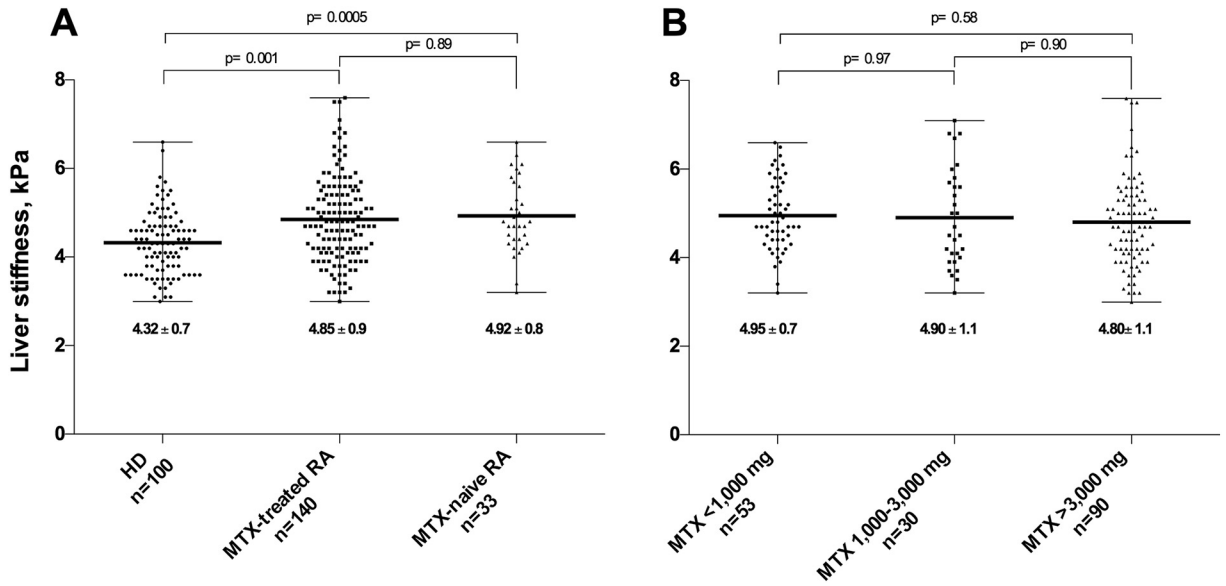

Fig. 1. Liver stiffness according to MTX use and dosage.
Distribution of kilopascals values across Methotrexate (MTX)-treated RA, MTX-naive RA and healthy blood donors groups (Panel A), and across cumulative dose of MTX strata (Panel B).

Table 3
Independent determinants of liver stiffness in RA population.

Independent variable	Bivariate correlation	Univariate linear regression	Multiple linear regression
	rho *p*	B coefficient (95%IC) *p*	B coefficient (95%IC) *p*
Age, yrs	0.222 **0.003**	0.016(0.004–0.028) **0.011**	0.016(0.004–0.028) **0.008**
Male sex	0.284 < **0.0005**	0.608(0.301–0.915) < **0.0005**	0.611(0.309–0.912) < **0.0005**
BMI	0.142 0.063		
Waist	0.124 0.105		
Cholesterol	−0.077 0.319		
Triglycerides	−0.029 0.712		
Hypertension	0.077 0.313		
AST	0.116 0.128		
ALT	0.084 0.270		
RA duration, months	−0.024 0.751		
CRP, mg/dL	0.089 0.245		
ESR, mm/h	0.066 0.392		
DAS-28 ESR	0.120 0.422		
ACPA positivity	−0.064 0.436		
RF positivity	−0.128 0.436		
MTX use, n(%)	−0.054 0.477		
MTX cumulative dose, mg	−0.063 0.411		
MTX length of exposure, months	−0.041 0.593		
Steroid use, n(%)	−0.104 0.171		
Steroid cumulative dose, mg	−0.101 0.187		
TNF inhibitors use	0.020 0.798		

CRP, C-reactive protein concentrations, mg/dl; ESR, erythrocyte sedimentation rate, mm/h; DAS-28-ESR, disease activity score, 28 joints, calculated with ESR; ACPA, anti-citrullinated peptide antibodies; RF, rheumatoid factor; MTX, methotrexate; TNF, tumor necrosis factor; A linear regression for multiple variables (stepwise method) was performed including into the models variables showing significant association ($p < .05$) with the dependent variable kPa at the univariate regression analysis. In bold significant p.

term, 'low-dose' MTX therapy in chronic inflammatory diseases such as RA. Earlier studies reported a prevalence of significant liver fibrosis and cirrhosis of up to 30% [5], while more recent studies reported figures between 0 and 1.3% [8,20]. However, uncertainty still exists regarding the independent role of MTX in the development of liver fibrosis. Therefore, in this study we specifically evaluated liver stiffness and liver fibrosis in RA patients according to treatment with MTX, comparing i) MTX treated RA patients to MTX naïve RA patients and healthy controls and ii) three groups of MTX-treated RA patients stratified on the basis of MTX cumulative dose.

To overcome the risks and limitations of liver biopsy we measured liver stiffness by 2D.SWE.SSI, a recent non-invasive method for the assessment of liver stiffness showing high accuracy in the diagnosis of fibrosis in a broad range of liver diseases [21–23]. The mean proposed cut-off value of 2D.SWE.SSI, which differentiates Metavir fibrosis stage 1 and 2, is 7.1 kPa [16].

We found that liver stiffness in MTX-treated patients is not significantly higher than that of MTX naïve patients and that liver stiffness is not significantly different across strata of cumulative dose of MTX. Moreover, in regression analyses, use of MTX, cumulative dose of MTX and length of exposure to MTX were not significantly associated with increased liver stiffness in RA patients.

Collectively taken, these data clearly suggest that MTX does not significantly influence liver stiffness in RA, in line with the results of a

Table 4
RA demographic and clinical features according to methotrexate cumulative dose strata.

	MTX < 1 g	MTX 1–3 g	MTX > 3 g	*p* value
	n = 53	n = 30	n = 90	
Age, yrs	60.6 ± 11.8	61.3 ± 11.7	60.3 ± 11.0	0.918
Female, n (%)	39(73.6)	22(73.3)	66(73.3)	0.999
BMI, kg/m^2	26.3 ± 5.2	24.1 ± 3.1	25.6 ± 4.3	0.092
Waist, cm	91.2 ± 13	86.7 ± 12.3	91.3 ± 12.0	0.195
Hypertension, n (%)	18(34.0)	11(36.7)	38(42.2)	0.599
Total cholesterol, mg/dL	206 ± 35	209 ± 40	204 ± 38	0.827
Triglycerides, mg/dL	95 ± 38	85 ± 34	94 ± 41	0.479
AST, U/L	20.0 ± 8.1	18.8 ± 5.8	19.5 ± 5.4	0.714
ALT, U/L	24.9 ± 22.5	20.9 ± 9.3	19.1 ± 8.1	0.066
Disease duration, months	38.4 ± 71.7	73.5 ± 62.3	174.3 ± 127.9	< **0.0005**
ACPA positivity, %	76	63.3	66.2	0.396
RF positivity, %	69.2	70	75.9	0.649
ESR, mm/h	28.5 ± 19.0	20.3 ± 13.2	25.4 ± 17.9	0.126
CRP, mg/dL	1.0 ± 1.0	0.6 ± 0.7	0.7 ± 0.9	0.150
DAS28-ESR	4.1 ± 0.9	4.0 ± 1.2	4.0 ± 0.7	0.983
Steroid use, %	37.7	16.7	30	0.132
Steroid cumulative dose, mg	67.2 ± 127.6	20.5 ± 51.7	38.4 ± 74.4	0.059
MTX time of exposition, months	3.63 ± 5.2	47.9 ± 17.7	118.9 ± 56.6	< **0.0005**
sDMARDs use, %	56.6	100	100	< **0.0005**
TNF inhibitors use, %	1.9	20	25.6	**0.001**
Abatacept use, %	1.9	0	1.1	0.741
Tocilizumab use, %	0	3.3	5.6	0.215
Rituximab use, %	0	0	0.2	0.393

ACPA, anti-citrullinated peptide antibodies; RF, rheumatoid factor; BMI, body mass index; CRP, C-reactive protein concentrations, mg/dl; ESR, erythrocyte sedimentation rate, mm/h; DAS-28-ESR, disease activity score, 28 joints, calculated with ESR; Steroid cumulative dose, cumulative dose of steroids during the last month. MTX, methotrexate; sDMARDs, synthetic disease-modifying anti-rheumatic drugs; TNF, tumor necrosis factor; In bold significant p values.

study performed with 2D.SWE.SSI in 185 Korean RA patients [17]. In this study, liver stiffness was not significantly different across strata of cumulative dosage of MTX. Similar results were also reported by a systematic review from Rouhi A. et al. [21] of studies reporting the assessment of fibrosis by transient elastography [22–28] or SWE [17] in RA patients under MTX. The role of MTX as a risk factor for fibrosis in RA patients was explored by six studies [22–25] included in the meta-analysis and, apart from one [24], MTX was not a risk factor for fibrosis. This meta-analysis also suggests that factors other than MTX (such as alcohol intake [22] and higher BMI [17,22] are likely to be involved in liver stiffness and fibrosis in RA.

Of interest, although within the normal range, in our study liver stiffness in RA patients was significantly higher than in controls. This difference was not explained by age, sex and BMI in the regression analysis performed in the whole sample, and might depend on disease-specific factors.

Non-alcoholic fatty liver disease (NAFLD) is an umbrella term that includes different types of liver alterations, caused by the increase of fat liver content. In particular, it encompasses steatosis, non-alcoholic steatohepatitis (NASH, a condition in which the fat liver storage favours ongoing inflammation), advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC) [29]. Given their unfavourable metabolic and cardiovascular profile RA patients are likely to develop liver steatosis as well as other features of NAFLD [30–32]. In addition, RA specific factors, such as overproduction of pro-inflammatory cytokines (tumor necrosis factor alpha, interleukin-1 and interleukin-6), as well as the use of specific medications such as steroids [29] are likely to increase the risk of hepatic steatosis in this group. Of note, Quintin E. et al. reported a high prevalence of NAFLD-like lesions in a cross-sectional study on 41 liver biopsies of RA patients with elevated liver enzymes [11]. Therefore, although not being specifically evaluated in our study, we hypothesize that liver steatosis may be an important independent contributor to increased liver stiffness in RA.

Moreover, it is conceivable that RA itself, as autoimmune disorder, might contribute to liver damage and increased mean liver stiffness. Autoimmune liver involvement is generally not recognized as a significant extra-articular feature of RA [33]. However, a high prevalence of sub-clinical autoimmune liver damage has been reported by Quintin E. et al. [11]. In this study, 17 out of 41 liver biopsies performed in RA patients taking MTX showed autoimmune hepatitis-like (AIH-like) lesions. Of interest, increased liver stiffness was associated with older age and male gender in regression analysis performed in the whole sample as well as in the RA sample.

Association between male gender and higher liver stiffness is consistent with results from a 2D.SWE.SSI study performed in 500 healthy subjects in which liver stiffness was greater in men than in women [34]. Moreover, older age (> 50 years) has proved to be an independent factor for increased liver stiffness in a large study on the reliability of 2D.SWE.SSI measures in chronic liver diseases [35]. Further studies aimed at evaluating gender and age specific cut-offs for 2D.SWE.SSI are warranted both in the general and in the RA populations.

Despite significant liver fibrosis was diagnosed only in the group of MTX-treated RA patients, we found no significant differences in the prevalence of significant liver fibrosis in MTX-treated RA patients with respect to MTX naïve RA and healthy controls: prevalence of significant liver fibrosis was 2.8% in MTX-treated RA patients, 2% in the whole sample of RA and 0% in the healthy population. At this regard, we should acknowledge that this difference, although not statistically significant (as a consequence of the relatively low prevalence of fibrosis and of the small sample size in this study) it could be biologically (and clinically) relevant. Our findings are in line with those of some studies (3–5%) [17,22,23], but significantly lower than that reported in others (10–23%) [24–27], as systematically reviewed by Rouhi A. et al. [21]. It is possible that heterogeneity in clinical and demographic (ethnicity, sample sizes and characteristics of RA patients) as well as methodological factors (liver stiffness cut-off for the diagnosis of liver fibrosis and methodology used to assess liver stiffness) may partly account for these differences.

One of the main results of our study was the lack of association, in logistic regression analysis, between MTX use and significant liver fibrosis in RA patients, suggesting that MTX does not increase the risk of liver fibrosis.

Moreover, in the context of a low prevalence of fibrosis in RA patients, we found no association between the presence of significant liver fibrosis and RA-specific descriptors in logistic regression analysis. In particularly, there were no significant associations between disease activity, autoantibody positivity and type of DMARDs and significant liver fibrosis in RA patients.

Our study has a number of limitations. First, we did not have histological confirmation of hepatic fibrosis, however this would have been hard to justify from an ethical point of view. In fact, the higher values of liver stiffness found in our patients (7.6 kPa, corresponding to a METAVIR stage F2) were not high enough to suggest the presence of advanced chronic liver disease, requiring confirmation with liver biopsy.

Second, according to the European Federation of Societies for Ultrasound in Medicine and Biology guidelines and the recommendations on the clinical use of US elastography, liver stiffness might be influenced by the specific cause of chronic liver disease [36]. Hence, further studies should be performed in order to assess the most appropriate cut-off value of 2D.SWE.SSI in RA patients.

Table 5
Factors associated with significant liver fibrosis in the RA sample.

Factor	Fibrosis	No fibrosis	Binary logistic analysis
	$n = 4$	$n = 169$	OR(95%IC) p
Age, yrs	68.0 ± 8.4	60.4 ± 11.3	1.070(0.966–1.185) 0.192
Male sex, %	50	26	2.841(0.388–20.780) 0.304
BMI, kg/m^2	24.2 ± 2.9	25.6 ± 4.5	0.926(0.719–1.193) 0.553
Waist, cm	85.5 ± 6.5	90.6 ± 12.5	0.963(0.879–1.055) 0.417
Cholesterol, mg/dL	207.5 ± 41.9	205.6 ± 37.8	1.001(0.975–1.028) 0.925
Triglycerides, mg/dL	94.0 ± 25.0	93.2 ± 39.6	1.000(0.976–1.026) 0.970
ALT, UI/l	26.2 ± 11.4	21.1 ± 14.5	1.016(0.970–1.065) 0.492
AST, UI/l	23.0 ± 0.8	19.4 ± 6.4	1.067(0.947–1.203) 0.286
Hypertension, %	25	39.1	0.520(0.053–5.107) 0.575
RA duration, months	212.5 ± 160.6	112.9 ± 119.5	1.004(0.999–1.009) 0.127
CRP, mg/dL	0.69 ± 1.0	0.79 ± 0.9	0.888(0.269–2.933) 0.845
ESR, mm/h	17.5 ± 12.1	25.7 ± 17.7	0.966(0.895–1.042) 0.366
DAS-28	3.9 ± 0.7	4.0 ± 0.8	0.819(0.079–8.461) 0.867
ACPA positivity, %	50	69.4	0.441(0.060–3.231) 0.421
RF positivity, %	72.7	75	1.125(0.114–11.098) 0.920
MTX use, %	100	80.5	47,513,996,1 (0.000-.) 0.998
MTX cumulative dose, mg	5740 ± 3411	3677 ± 3559	1.000(1.000–1.000) 0.259
MTX length exposition, months	98.5 ± 52.0	70.6 ± 66.7	1.005(0.993–1.018) 0.412
Steroid use, %	0	30.8	/
Steroid cumulative dose, mg	0	45.2 ± 93.1	/
TNF inhibitors use, %	0	17.8	/

ACPA, anti-citrullinated peptide antibodies; RF, rheumatoid factor; BMI, body mass index; CRP, C-reactive protein concentrations, mg/dl; ESR, erythrocyte sedimentation rate, mm/h; DAS-28-ESR, disease activity score, 28 joints, calculated with ESR; Steroid cumulative dose, cumulative dose of steroids during the last month. MTX, methotrexate; sDMARDs, synthetic disease-modifying anti-rheumatic drugs; TNF, tumor necrosis factor. Odds ratio (OR) is based on the risk of the dependent variable (liver fibrosis), given the presence of the independent variable. 95% CI, 95% confidence interval.

Third, the possibility of a selection bias, due to the exclusion of RA patients that stopped MTX due to increased liver enzymes, according to clinical practice, could not be ruled out.

Lastly, due to the cross-sectional design of this study, causality or temporality between factors associated to liver fibrosis and fibrosis itself cannot be established. A prospective study would provide more insights on the time-dependent changes of liver stiffness both in the non-MTX and MTX-treated RA populations. Moreover, it would allow to gain firmer information about risk factors for liver fibrosis and phenotype of patients at “higher risk” for liver fibrosis.

5. Conclusions

Although remaining in the normal range, liver stiffness values are higher in RA patients than in the general population. However, significant liver fibrosis and liver stiffness in RA patients appear to be independent of MTX use.

2D.SWE.SSI technique could be a promising tool to assess the severity of and to follow-up liver stiffness in RA patients and other chronic inflammatory conditions under MTX treatment.

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Declaration of Competing Interest

All Authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejim.2019.08.022.

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