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Risk of liver fibrosis associated with long-term methotrexate therapy may be overestimated

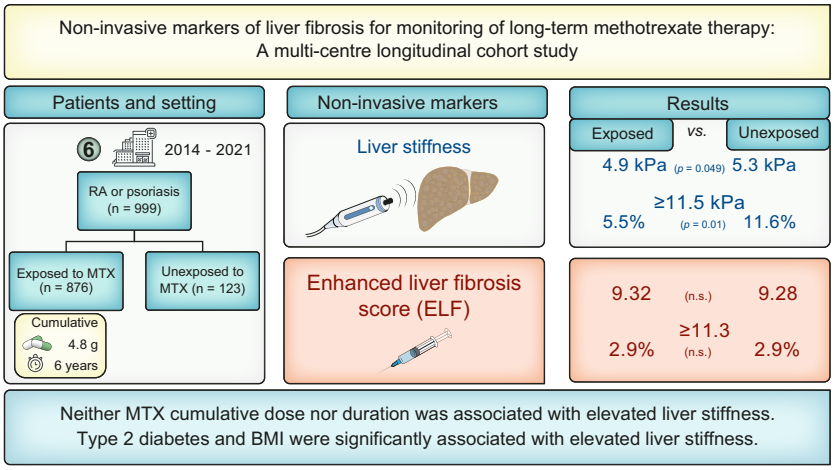
Authors

Edmond Atallah, Jane I. Grove, Colin Crooks, …, Richard J. Aspinall, Ruth Murphy, Guruprasad P. Aithal

Correspondence

guru.aithal@nottingham.ac.uk (G.P. Aithal).

Graphical abstract



Highlights

- Guidelines have recommended intensive monitoring based on the reported risk of liver fibrosis linked to methotrexate.
- Using non-invasive markers, we show that the risk of liver fibrosis linked to long-term methotrexate may have been overestimated.
- Our findings support the need to improve patients' metabolic risk factors, which are significantly associated with liver fibrosis.
- In patients with rheumatoid arthritis, transient elastography is more reliable to screen for liver fibrosis than ELF.

Impact and implications

Current guidelines recommend intensive (2-3 monthly) monitoring strategies for patients on long-term methotrexate therapy due to the potential risk of liver fibrosis. Evaluation of the association using two validated non-invasive markers of liver fibrosis, liver stiffness and enhanced liver fibrosis score, in a large cohort of patients with rheumatoid arthritis or psoriasis shows that the reported risk has previously been overestimated. The clinical focus should be to improve patients' metabolic risk factors, diabetes and BMI, that are independently associated with liver stiffness. There is a need to consider modifying current treatment monitoring guidelines for methotrexate.

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Risk of liver fibrosis associated with long-term methotrexate therapy may be overestimated

Edmond Atallah[1,2,†], **Jane I. Grove**[1,2,†], Colin Crooks[1,2], Esther Burden-Teh[3], Abhishek Abhishek[4], Sulleman Moreea[5], Kelsey M. Jordan[6], Aftab Ala[7,8,9], David Hutchinson[10], Richard J. Aspinall[11], Ruth Murphy[12], Guruprasad P. Aithal[1,2,*]

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Background & Aims: The risk of significant liver fibrosis from prolonged methotrexate (MTX) exposure has been estimated at around 5%, prompting intensive monitoring strategies. However, the evidence is derived from retrospective studies that under-reported risk factors for liver disease. We evaluated the risk of long-term MTX therapy on liver fibrosis in a longitudinal cohort study using two non-invasive markers.

Method: Between 2014-2021, adult patients diagnosed with rheumatoid arthritis (RA) or psoriasis for ≥2 years were recruited prospectively from six UK sites. The MTX group included patients who received MTX for ≥6 months, whereas the unexposed group included those who never received MTX. All patients underwent full liver profiling, with transient elastography (TE) and enhanced liver fibrosis (ELF) marker measurements.

Results: A total of 999 patients (mean age 60.8 ± 12 years, 62.3% females) were included. Of 976 with valid TE values, 149 (15.3%) had liver stiffness ≥7.9 kPa. Of 892 with a valid ELF, 262 (29.4%) had ELF ≥9.8. Age and BMI were independently associated with elevated liver stiffness and ELF. Neither MTX cumulative dose nor duration was associated with elevated liver stiffness. Diabetes was the most significant risk factor associated with liver stiffness ≥7.9 kPa (adjusted odds ratio = 3.19; 95% CI 1.95–5.20; p <0.001). Regular use of non-steroidal anti-inflammatory drugs showed the strongest association with ELF ≥9.8 (odds ratio = 1.76; 95% CI 1.20–2.56; p = 0.003), suggesting the degree of joint inflammation in RA may confound ELF as a non-invasive marker of liver fibrosis.

Conclusion: The risk of liver fibrosis attributed to MTX itself might have been previously overestimated; there is a need to consider modifying current monitoring guidelines for MTX.

Introduction

Methotrexate (MTX) has been widely used as a disease-modifying drug for the treatment of rheumatoid arthritis (RA) and psoriasis with or without arthritis for several decades. It is recommended by NICE (the National Institute of Clinical Excellence) in the UK as the first-line treatment for both newly diagnosed RA and adult patients with moderate to severe psoriasis who need systemic therapy.[1,2] MTX-induced liver injury has been described since the early 1970s,[3–5] and been investigated in multiple studies, mostly in retrospective cohorts.[6–8] This has led to intensive monitoring strategies and liver biopsies being recommended by numerous guidelines.[9,10]

The main clinical concern arises due to the potential risk of significant liver fibrosis with prolonged MTX exposure, which has been estimated to occur in approximately 5% of patients (range: 3.5-7%), with some reports linking fibrosis to total cumulative dose.[10,11] Systematic reviews in patients with psoriasis and RA highlight the discrepancy in the available evidence regarding the risk of significant fibrosis from long-term MTX therapy.[12–14] Furthermore, most studies that assessed the association between MTX and hepatotoxicity were at high risk of selection bias and under-reported the main risk factors for liver disease, *e.g.* obesity, diabetes, and alcohol use.[14] This limitation is crucial in this population, in particular because of their well-known high risk of metabolic syndrome,[15,16] and alcohol misuse.[17] Due to the lack of specific biomarkers, it is difficult to distinguish whether the liver injury is due to MTX exposure or other underlying risk factors of liver disease that can cause chronic liver injury and lead to fibrosis.

The influence of the underlying disease itself on liver fibrosis and clinical outcome in MTX-exposed patients has been investigated in multiple studies. A recent population-based study in patients treated with MTX showed that cutaneous psoriasis or psoriatic arthritis (PsA) were independently associated with liver disease events and cirrhosis compared to

Keywords: Methotrexate; hepatotoxicity; rheumatoid arthritis; psoriasis; liver fibrosis; transient elastography; liver stiffness; Enhanced Liver Fibrosis.
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* Corresponding author. Address: Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham, UK.
E-mail address: guru.aithal@nottingham.ac.uk (G.P. Aithal).
† Joint first authors.
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RA.[18] However, whether there was an effect of MTX on the liver disease, and to what degree, was not determined. Over a 24-year period, only 0.07% of adult liver transplantation listings for liver failure in the USA were attributed, wholly or partly, to MTX therapy.[19]

Although liver biopsy remains the gold standard test to quantify and stage liver fibrosis, it is an invasive procedure. It carries significant risks, including bleeding and hospitalisation, with an overall rate of bleeding up to 7 days after biopsy of 6.5 per 1,000 biopsies (95% CI 5.8–7.1).[20] Moreover, sampling variability may lead to misdiagnosis and inaccurate staging of liver fibrosis.[21,22] Therefore, multiple non-invasive markers of liver fibrosis have emerged, including liver stiffness measurement through transient elastography (TE) and measurement of the enhanced liver fibrosis (ELF) blood biomarker panel.[23,24] These non-invasive markers are used to select patients for further assessment by biopsy. TE for liver stiffness has a high-performance characteristic for detecting advanced fibrosis in non-alcoholic fatty liver disease (NAFLD).[25,26] ELF score combines the quantitative measurements of three serological markers, procollagen type III N-terminal peptide (PIIINP), tissue inhibitor of matrix metalloproteinase 1 (TIMP1) and hyaluronic acid (HA), in an algorithm to produce an ELF score.[27,28] The ELF score has been validated in large cohorts of patients with chronic liver diseases and showed a high accuracy to predict mortality and liver-related clinical outcomes.[29–31]

Therefore, we aimed to establish the association between MTX exposure and liver fibrosis in a large cohort study of patients with RA or psoriasis using two validated non-invasive surrogate measures of liver fibrosis, liver stiffness by TE and ELF score.

Patients and methods

Study population and design

From June 2014 to September 2021, eligible adult patients with RA and/or psoriasis were recruited from six different sites in the UK (Bradford, Brighton, Cornwall, Nottingham, Portsmouth, and Surrey). Each site independently elected to participate and enrol patients through the UK Clinical Research Network following adoption of the current study into the portfolio of the National Institute for Health and Care Research. Eligible patients were at least 18 years old and had established diagnoses of RA or psoriasis (with or without PsA) based on clinical, immunological and radiological changes for at least two years. All patients followed the standard of care pathway with weekly MTX, and folic acid supplementation as directed by their care team where appropriate. Patients were classified into two groups based on their exposure to MTX. The MTX group included patients receiving MTX for more than six months prior to recruitment. The unexposed group included patients who had never received MTX (no-MTX). Patients with other dermatological or rheumatological conditions or pre-existing liver disease, except for NAFLD or alcohol-related fatty liver disease, were excluded. The study was conducted according to the Declaration of Helsinki (Hong Kong Amendment) and Good Clinical Practice (European guidelines), with all participants providing written informed consent. The study protocol was approved by the East Midlands Health Research authority (REC Ref: 14/EM/0145) in April 2014. Clinical data, age, sex, weight, height, BMI, waist circumference, diabetes, hyperlipidaemia, hypertension, alcohol consumption and detailed medication history were recorded at enrolment. The study did not include investigations to screen for hepatic steatosis; however, patients who are at risk of metabolic dysfunction-associated fatty liver disease (MAFLD) were identified using the international expert consensus criteria.[32]

In patients who were receiving MTX, dose and duration were recorded. Changes in dose over time were taken into account based on patients' MTX monitoring charts and medication records and the total cumulative dose was calculated as the sum of all doses taken.

Liver investigations

On the day of recruitment, all patients had liver stiffness measurement through TE, and blood tests were taken for a full serological liver profile and ELF markers. The liver profile includes liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma glutamyltransferase, total bilirubin), and complete metabolic, virology and autoimmune serology (full blood count, urea and electrolytes, clotting profile, lipids, HbA1C, ferritin, alpha-1 antitrypsin, caeruloplasmin, HBsAg, anti-HCV, autoantibodies and immunoglobulins). 'Elevated ALT' was defined as above the upper limit of normal (ULN), 45 IU/L.

Liver stiffness was estimated using TE (FibroScan, Echosens, Paris, France) as previously described.[33] All patients had 10 validated measures and IQR <30% of median liver stiffness. The cut-off of 7.9 kPa was used to rule out advanced fibrosis, and 11.5 kPa to rule in cirrhosis, based on previous work in patients with biopsy-proven NAFLD.[34] Assays of HA, PIIINP, and TIMP-1 were performed on an Immuno-1 autoanalyser at Nottingham University Hospitals using the manufacturer's reagents, and ELF score was calculated in accordance with the manufacturer's instructions (Siemens Healthineers). We used the manufacturer's thresholds, 9.8 to rule out advanced fibrosis and 11.3 to rule in cirrhosis, that have been shown to correlate with clinical outcomes in a large cohort of patients with mixed chronic liver disease over an up to 7-year follow-up.[31,35]

Statistical analysis

Demographic and clinical data were described using descriptive statistics, mean ± SD for continuous measurements that are normally distributed, median (IQR) for non-normally distributed continuous variables and n (%) for categorical data. Patients' pathological and clinical characteristics were compared using the Chi-square test for categorical variables or Fisher's exact test when one or more expected cell counts were less than five. For continuous variables, Student's *t* test was applied. For continuous outcome variables exhibiting a skewed distribution, they were transformed using the natural logarithms before *t* tests were conducted to satisfy the prerequisite assumptions of normality. p <0.05 was considered statistically significant. The correlation between liver fibrosis markers was determined using Spearman's rank correlation. Multivariable logistic regression analysis was performed, including all variables that showed statistically significant association in the univariable analysis. We considered age, sex, diabetes, BMI and alcohol >14 units/week as *a priori* confounders, which were included in the final models regardless of their effect. Multivariable linear regression models were performed (fibrosis

markers as continuous variables) in the exposed and unexposed groups using box-cox transformation of the dependent variables. Multivariable analyses were performed using MTX cumulative dose and MTX duration as independent variables in separate models. To study the independent influence of the diagnosis, we excluded patients with both RA and psoriasis from the multivariable analysis. Separate regression analyses were performed wherein MAFLD was considered a single metabolic risk factor based on its diagnostic criteria. All analyses were conducted using R programme version 4.0.3.[36]

Results

The total number of patients recruited was 1,024. Twenty-five patients (2.4%) were excluded from the analysis as they did not meet the inclusion criteria at the time of enrolment (11 patients in the unexposed group [no-MTX] previously received MTX and 14 patients in the MTX group had less than 6 months of exposure prior to recruitment). After exclusion, 999 patients were included in the analysis (876 exposed to MTX and 123 unexposed), as shown in Fig. 1. Distribution of patients recruited across the sites is summarised in Table S1.

The demographic and clinical characteristics of the 999 patients analysed are summarised in Table 1. A summary of medications taken in each group is shown in Table S2. Patients who received MTX were older (p <0.001), predominantly females (p <0.01), and more often diagnosed with RA (p <0.001). In contrast, the unexposed group were more likely to drink alcohol >14 units/week (p <0.001) and have received regular non-steroidal anti-inflammatory drugs (NSAIDs) (p = 0.01) and metformin (p = 0.02). There was no significant difference in ethnicity; most participants were white. The difference in the metabolic risk factors between groups (type 2 diabetes, dyslipidaemia, hypertension, BMI and MAFLD) was not statistically significant.

Liver enzymes and AST/ALT ratio

There was no significant difference in liver enzymes or AST/ALT ratio between the groups, as shown in Table 2. The distribution of ALT in exposed and unexposed patients is illustrated in Fig. S1.

Out of 989 with ALT reported, 134 patients (13.5%) had elevated ALT, >45 IU/L (ULN). In the MTX group, 112 out of 866 (12.9%) had elevated ALT compared to 22 out of 101 in the unexposed group (17.9%), p = 0.13. In the MTX group, patients with PsA were more likely to have elevated ALT >45 IU/L compared to RA (19 out of 99 PA [19.2%] compared to 65 out of 615 RA [10.6%], p = 0.01). However, there was no significant association between the type of arthritis and elevated ALT in multivariable analysis, Table S3.

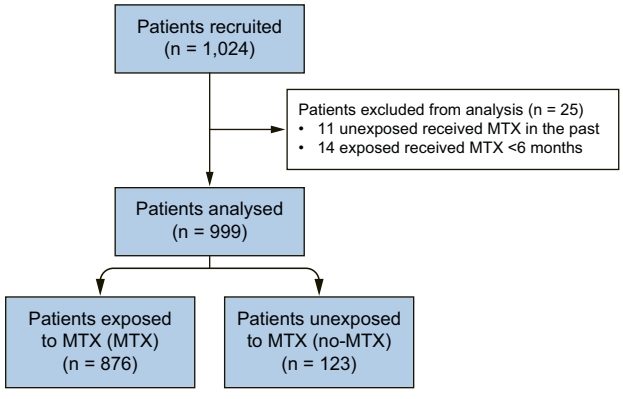

Fig. 1. Flow diagram of recruitment. MTX, methotrexate.

Non-invasive markers of liver fibrosis

Liver stiffness using TE

Liver stiffness from 23 patients (2.3%) could not be reliably obtained, so they were excluded from the analysis. Among the 976 patients with reliable liver stiffness, the median value of liver stiffness was 4.9 kPa (IQR 3.9–6.5), and 149 patients had liver stiffness ≥7.9 kPa (15.3%). Patients who were unexposed to MTX had higher median liver stiffness than those exposed (p = 0.049). Although a higher proportion of unexposed patients had liver stiffness ≥7.9 kPa, this difference did not reach statistical significance p = 0.08 (Table 3). Nonetheless, 14 unexposed (11.6%) met the cut-off for cirrhosis compared to 47 exposed (5.5%), p = 0.01.

In univariable analysis, factors that were significantly associated with elevated liver stiffness ≥7.9 kPa were male sex, psoriasis, BMI, diabetes, hyperlipidaemia and hypertension, with MTX duration showing a protective effect (Table 4, Table S4). The use of metformin was not independently associated with elevated liver stiffness after adjusting for diabetes status. In multivariable analyses, neither MTX cumulative dose nor duration had a significant association (Table 4, Table S4). Diabetes showed the strongest independent association with liver stiffness ≥7.9 kPa (adjusted odds ratio [OR] = 3.19; 95% CI 1.95–5.20; p <0.001). Other factors that showed significant association were age (p = 0.04), male sex (p = 0.02) and BMI (p <0.001). When the risk of MAFLD was used as a single metabolic predictor in regression models, neither MTX cumulative dose nor duration were associated with elevated liver stiffness (Table S5 and S6). MAFLD showed the strongest association with elevated liver stiffness (adjusted OR = 2.73; 95% CI 1.58–5.08; p <0.001). In this model, the association between psoriasis and elevated liver stiffness was statistically significant (adjusted OR = 1.76; 95% CI 1.19–2.60; p = 0.004).

ELF fibrosis score

There was no statistically significant difference in PIIINP, HA or ELF score between exposed and unexposed patients (Table 5). ELF score showed a weak correlation with liver stiffness (Spearman's rank correlation rho = 0.22; 95% CI 0.16–0.29; p <0.001).

Out of 892 patients with ELF score results, 28.6% of exposed patients had ELF ≥9.8 compared to 35.2% in the unexposed group, and 2.9% of patients from each group had ELF ≥11.3 suggesting cirrhosis. However, there was no significant difference between groups (Table 5).

In the univariable analysis, factors that were associated with elevated ELF ≥9.8 were MTX cumulative dose, MTX duration, age, RA, hypertension and regular use of NSAIDs. In multivariable analysis, regular use of NSAIDs showed the strongest association with elevated ELF (p = 0.003), Table 6. When MTX duration was used as the independent variable, factors that were associated with elevated ELF were age, BMI and regular NSAIDs (Table S7).

Table 1. Demographics and clinical features of exposed (MTX) and unexposed (no-MTX) patients.

Characteristics	MTX (n = 876)	No-MTX (n = 123)	*p* value
Age (years), mean (SD)	61.6 (11.6)	55.6 (13.5)	<0.001
Female, n (%)	560 (63.9)	62 (50.4)	0.004
Diagnosis, n (%)			
RA	615 (70.2)	55 (44.7)	
Psoriasis	241 (27.5)	67 (54.5)	
Both	20 (2.3)	1 (0.8)	<0.001*
Ethnicity n (%)			
White	825 (94.2)	118 (95.9)	
Black	6 (0.7)	0	
Mixed Asian	0	1 (0.8)	
South Asian	32 (3.7)	3 (2.5)	
Asian	0	1(0.8)	
Other	10 (1.1)	0	
Unknown	3 (0.3)	0	0.09*
Type 2 diabetes, n (%)	100 (11.5)	21 (17.1)	
Unknown	5 (0.6)	0	0.08
Hyperlipidaemia, n (%)	225 (25.9)	28 (22.8)	
Unknown	7 (0.8)	0	0.46
Hypertension, n (%)	296 (33.8)	36 (29.3)	0.32
BMI (kg/m^2), mean (SD)	29.9 (6.7)	30.9 (7.5)	0.19
Waist circumference (cm), mean (SD)	99 (16.6)	103.1 (17.4)	0.01
MAFLD, n (%)	686 (78.3)	101 (82.1)	0.33
Alcohol >14 units/week, n (%)	83 (9.5)	25 (20.3)	
Not reported	5 (0.7)	0	<0.001
MTX exposure, median (IQR)			
Dose (mg)	15 (12.5–20)	NA	
Duration (months)	72 (36–132)	NA	
Total cumulative dose (g)	4.8 (2.16–7.95)	NA	

p values were derived from Pearson's Chi-squared for categorical variables and Student's *t* test for continuous variables.
MAFLD, metabolic dysfunction-associated fatty liver disease; MTX, methotrexate; RA, rheumatoid arthritis.
*Fisher's Exact test was applied because one or more expected cell counts in the cross-tabulation were less than 5.

Table 2. Liver enzymes in exposed (MTX) and unexposed (no-MTX) patients.

Liver enzymes, median (IQR)	MTX (n = 876)	No-MTX (n = 123)	*p* value
ALT[§]	22.5 (17–33)	21 (16–37)	0.96
AST[¥]	24 (20–30)	21 (16–31)	0.23
AST/ALT ratio	1.05 (0.81–1.31)	0.93 (0.78–1.22)	0.35
ALT >ULN, n (%)	112 (12.9)	22 (17.9)	0.13

p values were derived from Pearson's Chi-squared for categorical variables and Student's *t* test for the natural logarithms of continuous variables.
ALT, alanine transaminase; AST, aspartate aminotransferase; MTX, methotrexate; ULN, upper limit of normal (45 IU/L).
[§]Missing data in 10 exposed.
[¥]Missing data in 77 exposed and 8 unexposed.

Table 3. Liver stiffness in exposed (MTX) and unexposed (no-MTX) patients.

TE results	MTX (n = 855)	No-MTX (n = 121)	*p* value
Liver stiffness (kPa), median (IQR)	4.9 (3.9–6.3)	5.3 (3.9–6.8)	0.049
Liver stiffness groups, n (%)			
Low <7.9 kPa	731 (85.5)	96 (79.3)	
High ≥7.9 kPa	124 (14.5)	25 (20.7)	0.08
Cirrhosis (≥11.5 kPa)	47 (5.5)	14 (11.6)	0.01

p values were derived from Student's *t* test for the natural logarithm of liver stiffness and Pearson's Chi-squared for categorical variables.
MTX, methotrexate; TE, transient elastography.

Because ELF score has been shown to significantly differ between patients with RA and psoriasis,[37] a sensitivity analysis was performed wherein patients with RA and psoriasis were analysed separately. It showed that MTX cumulative dose, duration and regular NSAIDs were associated with elevated ELF >9.8 only in patients with RA, which suggests that the association seen may be due to active arthritis rather than liver fibrosis (Table S8-11). When MAFLD was used as a single metabolic risk factor in regression models, it was not associated with elevated ELF whereas regular NSAIDs had the strongest association (Table S12 and S13).

Secondary analysis

We have performed a secondary analysis using linear regression models in each cohort to avoid potential selection bias that could have been generated due to an imbalance between the groups. Multivariable linear regression models in patients exposed to MTX showed results consistent with previous findings in all patients (Table S4-17). Age and BMI showed a significant linear relationship with liver stiffness and ELF in patients exposed to MTX. In the unexposed group, BMI and diabetes were significantly associated with liver stiffness but not with ELF (Table S18-19).

Table 4. Factors associated with elevated liver stiffness ≥7.9 kPa.

Factors	Unadjusted OR	*p* value	Adjusted OR	95% CI	*p* value
MTX cumulative dose	0.96	0.06	0.99	0.95–1.03	0.68
Age	1.003	0.63	1.02 *	1.00–1.04	0.04
Sex (male)	1.56*	0.01	1.62 *	1.07–2.45	0.02
Psoriasis	1.74 **	0.003	1.51	0.98–2.32	0.06
BMI	1.13***	<0.001	1.13 ***	1.10–1.17	<0.001
Type 2 diabetes	5.25***	<0.001	3.19 ***	1.95–5.20	<0.001
Hyperlipidaemia	1.97***	<0.001	1.23	0.77–1.94	0.37
Hypertension	2.33***	<0.001	1.34	0.87–2.06	0.18
Alcohol (>14 units/wk)	0.76	0.37	0.68	0.33–1.32	0.28

Univariable and multivariable logistic regression model of liver stiffness ≥7.9 kPa in the whole population (MTX cumulative dose was used as the independent variable). *p <0.05; **p <0.01; ***p <0.001.
MTX, methotrexate; OR, odds ratio.

Table 5. ELF scores in exposed (MTX) and unexposed (no-MTX) patients.

ELF fibrosis score	MTX (n = 876)	No-MTX (n = 123)	*p* value
PIIINP (μg/L), mean (SD) (Values missing for 70 exposed and 14 unexposed)	8.42 (4.24)	8.74 (4.06)	0.44
HA (μg/L), median (IQR) (Values missing for 85 exposed and 18 unexposed)	51.89 (30.79, 89.92)	49.21 (26.11, 109.07)	0.76
ELF score, mean (SD) (Values missing for 89 exposed and 18 unexposed)	9.32 (0.98)	9.28 (0.96)	0.1
ELF groups, n (%)			
Low <9.8	562 (71.4)	68 (64.8)	
High ≥9.8	225 (28.6)	37 (35.2)	0.16
Cirrhosis (≥11.3)	23 (2.9)	3 (2.9)	0.97

p values were derived from Student's *t* test for PIIINP, HA and ELF scores, and the Chi-squared test for ELF groups. *p <0.05; **p <0.01; ***p <0.001.
ELF, enhanced liver fibrosis; HA, hyaluronic acid; MTX, methotrexate; PIIINP, procollagen type III N-terminal peptide.

Table 6. Factors associated with elevated ELF score ≥9.8.

Factors	Unadjusted OR	*p* value	Adjusted OR	95% CI	*p* value
MTX cumulative dose	1.05***	<0.001	1.04 *	1.01–1.07	0.02
Age	1.06***	<0.001	1.07 ***	1.05–1.09	<0.001
Sex (male)	1.17	0.30	1.15	0.83–1.60	0.39
Psoriasis	0.63**	0.007	0.87	0.60–1.26	0.47
BMI	1.003	0.72	1.03 *	1.01–1.06	0.01
Type 2 diabetes	1.49	0.07	1.25	0.78–1.99	0.35
Hyperlipidaemia	1.27	0.14			
Hypertension	1.66***	<0.001	1.09	0.77–1.54	0.62
Alcohol >14 units	0.74	0.24	0.75	0.44–1.26	0.29
Regular NSAIDs	1.47*	0.02	1.76 **	1.20–2.56	0.003

Univariable and multivariable logistic regression model of ELF score ≥9.8 in the whole population (MTX cumulative dose as the independent variable). *p <0.05; **p <0.01; ***p <0.001.
ELF, enhanced liver fibrosis; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio.

In patients with arthritis (RA or PsA) on prolonged MTX therapy, the type of inflammatory arthritis was not associated with elevated liver stiffness or ELF in multivariable logistic regression models (Table S20 and S21).

Liver biopsy

All recruited patients with elevated liver stiffness ≥7.9 kPa or ELF ≥9.8 were offered a liver biopsy to establish the histological fibrosis stage when suitable. However, most patients declined or were considered unsuitable for liver biopsy due to frailty. In addition, some patients underwent a liver biopsy as part of clinical care to investigate elevated liver enzymes. In total, liver biopsy was performed in 26 patients (22 exposed and four unexposed), as described in Table 7. In unexposed patients, the histology was in keeping with non-alcoholic steatohepatitis in two patients, and autoimmune hepatitis and seronegative primary biliary cholangitis in each of the others. Among the 22 patients exposed to MTX who had a liver biopsy, histology showed features of non-alcoholic steatohepatitis in all patients. Out of these, 12 patients had at least fibrosis grade ≥F3 according to the Metavir score,[38] and four patients had established cirrhosis (F4).

Discussion

In this multicentre large longitudinal cohort study involving about 1,000 patients with psoriasis or RA, we have demonstrated that neither MTX cumulative dose (median 4.8 g) nor duration of exposure (median of 6 years) was associated with liver fibrosis using two non-invasive markers, liver stiffness and ELF score. Our results are consistent with two other studies that showed no association between MTX cumulative dose and elevated liver stiffness.[39,40] Laharie's cohort study involved patients with a variety of inflammatory diseases, and their median liver stiffness was 4.6 kPa compared to 4.9 kPa in our study population.[39] Furthermore, the latter study included 390 patients exposed to a median dose of only 1.3 g over 1.8 years and reported 6% of

Table 7. Clinical and histological details of exposed (MTX) and unexposed (no-MTX) patients who underwent liver biopsy.

Study Group	Diagnosis	Cumulative MTX dose (grams)	BMI	Type 2 Diabetes	Indication for biopsy	LSM (kPa)	ELF score	Mode of biopsy	HVPG (mmHg)	Histological Diagnosis	Fibrosis stage (Metavir score)
No-MTX	PS	n.a.	28	No	Elevated liver enzymes	6.4	9.9	PC		AIH	F1/F2
No-MTX	PS	n.a.	40	No	Raised LSM	73.5	11.6	TJ	8	NASH	F3
No-MTX	RA	n.a.	35	No	Elevated liver enzymes	5.6	9.5	PC		PBC	F1/F2
No-MTX	PS	n.a.	61	Yes	Raised LSM	10.5	8.6	TJ	3	NASH	F1
MTX	PS	2.4	26	No	Elevated liver enzymes	4.4	8.5	PC		NASH	F1
MTX	PS	10.8	35	Yes	Raised LSM	20.5	9.6	PC		NASH	F4
MTX	RA + PS	3.08	46	No	Elevated PIIINP and failed LSM	n.a.	n.a.	TJ	2	NASH	F2
MTX	RA + PS	3.78	34	Yes	Raised LSM	41.6	11.1	TJ	5	NASH	F4
MTX	RA	6	47	No	Raised LSM	21.3	10.4	TJ	14	NASH	F3
MTX	RA	5.4	33	No	Elevated liver enzymes	4.2	n.a.	TJ	4	NASH	F1
MTX	PS	6.24	43	No	Raised LSM	12.9	12.2	TJ	9	NASH	F3/F4
MTX	PS	0.24	45	No	Raised LSM	9.5	10.3	PC		NASH	F2
MTX	RA	16.32	33	No	Raised LSM	8.7	12.5	TJ	5	NASH	F3/F4
MTX	PS	6	27	Yes	Raised LSM	9.3	10.2	PC		NASH	F3
MTX	PS	10.14	38	Yes	Raised LSM	38.6	9.2	TJ	5	NASH	F4
MTX	RA	3.84	41	No	Elevated liver enzymes	7.2	9.2	PC		NASH	F3
MTX	PS	0.41	49	No	Raised LSM	9.4	8.9	TJ	2	NASH	F3
MTX	PS	1.35	39	No	Elevated liver enzymes	8.6	10.2	TJ	2	NASH	F2
MTX	RA	10.8	41	No	Raised LSM	21.8	10.1	TJ	4	NASH	F3
MTX	PS	2.88	37	No	Raised LSM	11.1	9.5	PC		NASH	F3
MTX	PS	6.86	27	No	Raised LSM	8.8	11.03	PC		NASH	F3
MTX	PS	7.65	37	No	Raised LSM	8.8	9.8	TJ	4	NASH	F1
MTX	PS	7.68	41	Yes	Raised LSM	21.5	10.05	TJ	9	NASH	F4
MTX	PS	0.72	47	No	Raised LSM	9.7	9.5	PC		NASH	F1
MTX	PS	9.6	43	Yes	Raised LSM	10.1	n.a.	TJ	5	NASH	F2
MTX	PS	1.23	41	No	Elevated liver enzymes	9.6	n.a.	PC		NASH	F0

AIH, autoimmune hepatitis; ELF, enhanced liver fibrosis; HVPG, hepatic venous pressure gradient; LSM, liver stiffness measurement; MTX, methotrexate; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cholangitis; PC, percutaneous; PS, psoriasis; RA, rheumatoid arthritis; TJ, transjugular.

their patients had significant fibrosis, based on liver stiffness >7.9 kPa, compared to 14.7% in our study using the same threshold. However, our included patients were older and had significantly more risk factors for liver disease (BMI, type 2 diabetes, and higher alcohol intake) which might explain the higher proportion of patients with elevated liver stiffness. Diabetes was the most significant independent risk factor associated with elevated liver stiffness in our study, in addition to age, male sex, and BMI. In contrast, only BMI and alcohol consumption were associated with elevated liver stiffness in the study by Laharie *et al.*.[39] The lack of association between MTX cumulative dose and liver fibrosis was previously observed in retrospective studies involving patients with psoriasis exposed to MTX, using histology (n = 71)[41] and non-invasive markers (n = 61).[42] Single centre studies involving patients on MTX for RA and inflammatory bowel disease have reported similar findings, although all of these studies enrolled a very small number of patients (n = 46-185).[43–45] The insufficient sample size of these previous studies and the lack of unexposed groups limited their power to identify independent risk factors associated with fibrosis through multivariable modelling.

For many years PIIINP has been used to determine the presence of liver fibrosis in those receiving MTX[46] and in 2016 it was implemented as a screening and monitoring test for liver fibrosis by the British Association of Dermatologists.[9] PIIINP is one of the biomarkers for fibrosis released during collagen synthesis;[10] this marker forms one of the three components of the ELF score. While ELF score has been recommended by NICE for the non-invasive detection of advanced fibrosis in NAFLD,[47] PIIINP on its own has been validated for the detection and assessment of non-alcoholic steatohepatitis.[48] Important limitations of PIIINP as a diagnostic test on its own include its lack of specificity to the liver and its association with arthritis and disease activity.[49] Our study showed no significant difference in PIIINP levels between patients exposed and unexposed to MTX, raising the question of its role in monitoring of liver fibrosis in these groups of patients and the cost-effectiveness of serial measures every three months.

In fact, a retrospective cohort study of patients with psoriasis treated with MTX, of whom 27 underwent liver biopsy, showed that serial ELF score measurements had possibly superior diagnostic accuracy than serial PIIINP measures to detect fibrosis.[50] However, we found ELF scores were similar among patients exposed and unexposed to MTX. In multivariable analyses, the cumulative dose of MTX was associated with an increase in the ELF score. However, in sensitivity analysis,

the association between MTX cumulative dose and ELF was only apparent in patients with RA. This is consistent with a recent study in RA patients, and a cross-sectional study that showed the highest proportion of increased ELF score was seen in RA patients.[37,44] Nonetheless, the association seen might reflect disease severity and inflammation at joints (rather than liver fibrosis) due to increased collagen turnover in inflammatory arthritis and hence, an increase of PIIINP. Although disease severity scores for psoriasis and RA were not captured consistently as part of our study, regular use of NSAIDs probably reflects disease activity in our study population. Regular use of NSAIDs was the most significant independent risk factor associated with elevated ELF ≥9.8, in addition to age and BMI. Severe disease activity has been shown to correlate with ELF (adjusted OR 5.850; 95% CI 1.740–19.673) in a cross-sectional study of patients with psoriasis and RA, with no significant difference between different medication subgroups, including MTX;[37] however, the particular study did not evaluate liver fibrosis using liver stiffness. Similarly, a recent study in a RA cohort showed an association between cumulative dose of MTX and ELF, but not with liver stiffness. Furthermore, the DAS-28 (disease activity score for 28 joints) scale had the strongest correlation with ELF (Pearson correlation coefficient $r = 0.51$, $p < 0.001$).[44] Inflammatory markers, such as high-sensitivity C-reactive protein, were not included in this study; however, their association with PIIINP and ELF biomarkers could be investigated in future studies.

In multivariable analysis, using all risk factors, the type of disease was not significantly associated with liver fibrosis using both non-invasive fibrosis markers. Even in patients with arthritis exposed to MTX, the type of arthritis (RA compared to PsA) did not influence significant liver fibrosis using non-invasive markers. However, when metabolic risk factors were combined according to MAFLD criteria,[32] psoriasis was independently associated with elevated liver stiffness but not with ELF. This could be explained by merging all the metabolic risk factors into one variable, MAFLD status, it is assumed that they have a similar effect on elevated liver stiffness. However, the degree of association between the different metabolic risk factors and liver stiffness varied, as shown in Table 4. A large population-based study showed that the diagnosis differentially influenced liver disease risk in the setting of MTX use independent of risk factors; patients with psoriasis were at high risk of cirrhosis and liver-related events compared to those with RA.[18] Nonetheless, the study did not adjust for BMI, which is a crucial risk factor and was associated with elevation of both liver fibrosis markers in our data.

The existing evidence indicates that prolonged MTX exposure does not lead to worse clinical outcomes. A meta-analysis of 32 randomised controlled trials of MTX *vs*. comparator in adults with RA, psoriasis and inflammatory bowel disease showed that exposure to MTX was not associated with risk of liver failure, cirrhosis, or death (relative risk 0.12; 95% CI 0.01–1.09).[51] Moreover, in a population-based cohort of patients with RA and chronic hepatitis B, there was no increased risk of cirrhosis with long-term MTX use over more than six years of follow-up.[52]

Our study was the largest such study to investigate the association between MTX exposure and liver fibrosis. In addition, our study has multiple strengths, including study design and a detailed characterisation of risk factors for liver disease that was lacking in previous studies. We used both liver stiffness and ELF score, two of the most validated non-invasive biomarkers of liver fibrosis accessible internationally, to investigate the association between MTX and liver fibrosis.

Despite its strengths, our study has a few limitations. The cut-off points used for liver stiffness and ELF score are not validated specifically in patients with psoriasis/RA, but instead were extrapolated from the literature. Because the recruitment of patients in multiple centres was not consecutive, this might have generated selection bias, especially in the unexposed group which was smaller than the exposed group. Clinicians may have referred patients unexposed to MTX with risk factors of liver disease to obtain a liver fibrosis assessment (referral bias). We tried to correct for these potential biases by adjusting for risk factors of liver disease in the multivariable analysis and performing a secondary analysis on each group which demonstrated similar results.

Our study included a low number of liver biopsies. Biopsies are generally performed in only a selected patient subgroup when a non-invasive marker stratifies patients as being at high risk of having severe liver fibrosis. The use of a surrogate fibrosis marker such as liver stiffness can be considered a valuable alternative approach in a large population.

In conclusion, we found no association between MTX cumulative dose or duration and liver stiffness in patients with RA or psoriasis. This indicates that the risk of liver fibrosis due to MTX itself might have been overestimated in this population who is at higher risk of metabolic syndrome and NAFLD. Hence, this supports the current evidence on the need to improve patients' metabolic risk factors that are associated with liver fibrosis. MTX cumulative dose and duration were associated with the ELF score in the RA subgroup, which may reflect arthritis activity rather than liver fibrosis. The degree of inflammation, especially in those who have RA, may confound ELF as a marker to detect fibrosis; therefore, TE would be a more reliable tool to screen for significant fibrosis in this group. Guidelines for monitoring patients on MTX should be revisited to compare non-invasive tests to the current reliance on liver enzymes and PIIINP, and an evaluation of the cost-effectiveness of regular assessments in this population should be considered in future studies.

Affiliations

[1]Nottingham Digestive Diseases Centre, Translational Medical Sciences, School of Medicine, University of Nottingham, Nottingham, UK; [2]National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, UK; [3]Centre of Evidence Based Dermatology, School of Medicine, University of Nottingham, Nottingham, UK; [4]Academic Rheumatology, Clinical Sciences Building, City Hospital, Nottingham NG5 1PB; [5]Bradford Teaching Hospitals NHS Foundation Trust, Bradford, UK; [6]University Hospitals Sussex NHS Foundation Trust, Brighton, UK; [7]Dept of Gastroenterology and Hepatology, Royal Surrey NHS Foundation Trust, Surrey, UK; [8]Department of Clinical and Experimental Medicine, FHMS, University of Surrey, Surrey, UK; [9]Institute of Liver Studies, Kings College Hospital NHS Foundation Trust. London, UK; [10]Royal Cornwall Hospitals NHS Trust, Cornwall, UK; [11]Portsmouth Liver Centre, Portsmouth Hospitals University NHS Trust, Portsmouth, UK; [12]Sheffield University Teaching Hospitals, Sheffield, UK

Abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELF, enhanced liver fibrosis; HA, hyaluronic acid; MAFLD, metabolic dysfunction-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; PIIINP, procollagen type III N-terminal peptide; PsA, psoriatic arthritis; RA, rheumatoid arthritis; TE, transient elastography; ULN, upper limit of normal.

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Conflict of interest

GP Aithal has received consulting fees from Pfizer, GlaxoSmithKline, Clinicpace, Servier Pharmaceuticals, NuCANA Plc, AstraZeneca and BenevolentAI paid to the University of Nottingham. A Abhishek reports institutional research grants from AstraZeneca and Oxford Immunotec, personal author royalties from UpTodate and Springer, personal consulting fees from Inflazome and NGM Biopharmaceuticals, and personal payments for lectures from Menarini Pharmaceuticals and Cadilla Pharmaceuticals, in the past 36 months and unrelated to the current work. RJ Aspinall has received consulting fees and speaker honoraria from Intercept, Novartis UK, Falk Pharma and Norgine UK.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

EA: writing original draft, formal analysis, resources and visualisation; JIG: writing original draft, data curation, resources, project management and supervision; CC: formal analysis, review and editing; EB-T: resources, review and editing; AA: resources, review and editing, SM: review and editing, resources, project management and supervision; KJ: review and editing, resources, project management and supervision; AA: review and editing, resources, project management and supervision; DH: review and editing, resources, project management and supervision; RJA: review and editing, resources, project management and supervision; GPA: conceptualization, funding and writing original draft.

Data availability statement

The data that support the findings of this study are available on reasonable request to the corresponding author.

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Supplementary data

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References

Author names in bold designate shared co-first authorship

[1] Rheumatoid arthritis in adults: management. National Institute for Health and Care Excellence. NICE guideline 2020 October 2020; Available from: www.nice.org.uk/guidance/ng100.

[2] Psoriasis: assessment and management. 2017. Available from: https://www.nice.org.uk/guidance/cg153.

[3] Weinstein GD, Cox JW, Suringa DWR, Millard MM, Kaiser M, Frost P. Evaluation of possible chronic hepatotoxicity from methotrexate for psoriasis. Arch Dermatol 1970;102(6):613–618.

[4] Roenigk HH, Bergfeld WF, Jacques RS, Owens FJ, Hawk WA. Hepatotoxicity of methotrexate the treatment of psoriasis. Arch Dermatol 1971;103(3):250–261.

[5] Dahi MGC, Gregory MM, Scheuer PJ. Liver damage due to methotrexate in patients with psoriasis. Br Med J 1971;1(5750):625–630.

[6] Kremer JM, Alarcon GS, Lightfoot Jr RW, Willkens RF, Furst DE, Williams HJ, et al. Methotrexate for rheumatoid arthritis. Suggested guidelines for monitoring liver toxicity. American College of Rheumatology. Arthritis Rheum 1994;37(3):316–328.

[7] Kremer JM, Lee JK. The safety and efficacy of the use of methotrexate in long-term therapy for rheumatoid arthritis. Arthritis Rheum 1986;29(7):822–831.

[8] Lanse SB, Arnold GL, Gowans JD, Kaplan MM. Low incidence of hepatotoxicity associated with long-term, low-dose oral methotrexate in treatment of refractory psoriasis, psoriatic arthritis, and rheumatoid arthritis. An acceptable risk/benefit ratio. Dig Dis Sci 1985;30(2):104–109.

[9] Warren RB, Weatherhead SC, Smith CH, Exton LS, Mohd Mustapa MF, Kirby B, et al. British Association of Dermatologists' guidelines for the safe and effective prescribing of methotrexate for skin disease 2016. Br J Dermatol 2016;175(1):23–44.

[10] Aithal GP. In: Kaplowitz DLN, editor. Hepatotoxicity related to methotrexate, in Drug-induced liver disease. London, UK, Walthan, MA and San Diego, CA: Elsevier; 2013.

[11] Whiting-O'Keefe QE, Fye KH, Sack KD. Methotrexate and histologic hepatic abnormalities: a meta-analysis. Am J Med 1991;90(1):711–716.

[12] **Maybury CM**, **Jabbar-Lopez ZK**, Wong T, Dhillon AP, Barker JN, Smith CH. Methotrexate and liver fibrosis in people with psoriasis: a systematic review of observational studies. Br J Dermatol 2014;171(1):17–29.

[13] Salliot C, van der Heijde D. Long-term safety of methotrexate monotherapy in patients with rheumatoid arthritis: a systematic literature research. Ann Rheum Dis 2009;68(7):1100–1104.

[14] Azzam A, Jiyad Z, O'Beirne J. Is methotrexate hepatotoxicity associated with cumulative dose? A systematic review and meta-analysis. Australas J Dermatol 2021;62(2):130–140.

[15] Rodriguez-Zuniga MJM, Garcia-Perdomo HA. Systematic review and meta-analysis of the association between psoriasis and metabolic syndrome. J Am Acad Dermatol 2017;77(4):657–666 e8.

[16] Mori S, Arima N, Ito M, Ueki Y, Abe Y, Aoyagi K, et al. Incidence, predictive factors and severity of methotrexate-related liver injury in rheumatoid arthritis: a longitudinal cohort study. Rheumatol Adv Pract 2020;4(2).

[17] McAleer MA, Mason DL, Cunningham S, O'Shea SJ, McCormick PA, Stone C, et al. Alcohol misuse in patients with psoriasis: identification and relationship to disease severity and psychological distress. Br J Dermatol 2011;164(6):1256–1261.

[18] **Gelfand JM**, **Wan J**, Zhang H, Shin DB, Ogdie A, Syed MN, et al. Risk of liver disease in patients with psoriasis, psoriatic arthritis, and rheumatoid arthritis receiving methotrexate: a population-based study. J Am Acad Dermatol 2021;84(6):1636–1643.

[19] Dawwas MF, Aithal GP. End-stage methotrexate-related liver disease is rare and associated with features of the metabolic syndrome. Aliment Pharmacol Ther 2014;40(8):938–948.

[20] West J, Card TR. Reduced mortality rates following elective percutaneous liver biopsies. Gastroenterology 2010;139(4):1230–1237.

[21] **Ratziu V**, **Charlotte F**, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 2005;128(7):1898–1906.

[22] Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38(6):1449–1457.

[23] Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29(12):1705–1713.

[24] Rosenberg WM, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, et al. Serum markers detect the presence of liver fibrosis: a cohort study. Gastroenterology 2004;127(6):1704–1713.

[25] **Selvaraj EA**, **Mózes FE**, **Ajmer Jayaswal AN**, Zafarmand MH, Vali Y, Lee JA, et al. Diagnostic accuracy of elastography and magnetic resonance imaging in patients with NAFLD: a systematic review and meta-analysis. J Hepatol 2021;75(4):770–785.

[26] Mózes FE, Lee JA, Selvaraj EA, Jayaswal ANA, Trauner M, Boursier J, et al. Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients

with NAFLD: an individual patient data meta-analysis. Gut 2021. gutjnl-2021-324243.
[27] Vali Y, Lee J, Boursier J, Spijker R, Löffler J, Verheij J, et al. Enhanced liver fibrosis test for the non-invasive diagnosis of fibrosis in patients with NAFLD: a systematic review and meta-analysis. J Hepatol 2020;73(2):252–262.
[28] ELF physician brochure. 2016. Available from: https://cdn0.scrvt.com/39b415fb07de4d9656c7b516d8e2d907/1800000003470658/6d7021e5e01d/elf_physician_brochure_a91dx-160435-xc1-4a00_final-03470658_1800000003470658.pdf.
[29] Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology 2008;47(2):455–460.
[30] Parkes J, Roderick P, Harris S, Day C, Mutimer D, Collier J, et al. Enhanced liver fibrosis test can predict clinical outcomes in patients with chronic liver disease. Gut 2010;59(9):1245–1251.
[31] Day J, Patel P, Parkes J, Rosenberg W. Derivation and performance of standardized enhanced liver fibrosis (ELF) test thresholds for the detection and prognosis of liver fibrosis. J Appl Lab Med 2019;3(5):815–826.
[32] **Eslam M**, **Newsome PN**, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol 2020;73(1):202–209.
[33] Laharie D, Zerbib F, Adhoute X, Boue-Lahorgue X, Foucher J, Castera L, et al. Diagnosis of liver fibrosis by transient elastography (FibroScan) and non-invasive methods in Crohn's disease patients treated with methotrexate. Aliment Pharmacol Ther 2006;23(11):1621–1628.
[34] Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 2010;51(2):454–462.
[35] Enhanced liver fibrosis (ELF) testing Service. 2020. Available from: https://cdn0.scrvt.com/39b415fb07de4d9656c7b516d8e2d907/18c383ce2b038500/92e4f640da92/US-MD-SHL-ELF-Testing-Service-Brochure-0620-FINAL.pdf.
[36] R Core Team; R. A language and environment for statistical computing. 2020.
[37] van der Voort EAM, Wakkee M, Veldt-Kok P, Darwish Murad S, Nijsten T. Enhanced liver fibrosis test in patients with psoriasis, psoriatic arthritis and rheumatoid arthritis: a cross-sectional comparison with procollagen-3 N-terminal peptide (P3NP). Br J Dermatol 2017;176(6):1599–1606.
[38] Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996;24(2):289–293.
[39] Laharie D, Seneschal J, Schaeverbeke T, Doutre MS, Longy-Boursier M, Pellegrin JL, et al. Assessment of liver fibrosis with transient elastography and FibroTest in patients treated with methotrexate for chronic inflammatory diseases: a case-control study. J Hepatol 2010;53(6):1035–1040.
[40] Turner L, Bland M, Millson C, Veysey M, Hutchinson J. O11 Methotrexate: an innocent bystander in the development of liver fibrosis, findings of the STrATIFY study. Gut 2020;69(Suppl 1):A6–A7.
[41] Rosenberg P, Urwitz H, Johannesson A, Ros A-M, Lindholm J, Kinnman N, et al. Psoriasis patients with diabetes type 2 are at high risk of developing liver fibrosis during methotrexate treatment. J Hepatol 2007;46(6):1111–1118.
[42] Lee JHM, Loo CH, Tan WC, Lee CK, Jamil A, Khor YH. Comparison of noninvasive screening tools for hepatic fibrosis, association with methotrexate cumulative dose, and risk factors in psoriasis patients. Dermatol Ther 2022;35(1):e15203.
[43] **Kim TY**, **Kim JY**, Sohn JH, Lee H-S, Bang S-Y, Kim Y, et al. Assessment of substantial liver fibrosis by real-time shear wave elastography in methotrexate-treated patients with rheumatoid arthritis. J Ultrasound Med 2015;34(9):1621–1630.
[44] Frankowski M, Świerkot J, Gomułkiewicz M, Korman L, Skoczyńska M, Starba A. Usefulness of noninvasive diagnostic procedures for assessment of methotrexate hepatotoxicity in patients with rheumatoid arthritis. Rheumatol Int 2022;42(4):631–638.
[45] Barbero-Villares A, Jiménez-Ridruejo JM, Taxonera C, López-Sanromán A, Pajares R, Bermejo F, et al. Evaluation of liver fibrosis by transient elastography (Fibroscan®) in patients with inflammatory bowel disease treated with methotrexate: a multicentric trial. Scand J Gastroenterol 2012;47(5):575–579.
[46] Khan S, Subedi D, Chowdhury MMU. Use of amino terminal type III procollagen peptide (P3NP) assay in methotrexate therapy for psoriasis. Postgrad Med J 2006;82(967):353.
[47] NICE. Assessment of non-alcoholic fatty liver disease (NAFLD)- Advanced liver fibrosis risk scores Clinical Knowledge Summaries 2021 [cited 2022 18/05/2022]; Available from: https://cks.nice.org.uk/topics/non-alcoholic-fatty-liver-disease-nafld/diagnosis/assessment/#advanced-liver-fibrosis-risk-scores.
[48] Tanwar S, Trembling PM, Guha IN, Parkes J, Kaye P, Burt AD, et al. Validation of terminal peptide of procollagen III for the detection and assessment of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease. Hepatology 2013;57(1):103–111.
[49] Zachariae H, Aslam HM, Bjerring P, Sogaard H, Zachariae E, Heickendorff L. Serum aminoterminal propeptide of type III procollagen in psoriasis and psoriatic arthritis: relation to liver fibrosis and arthritis. J Am Acad Dermatol 1991;25(1 Pt 1):50–53.
[50] Martyn-Simmons CL, Rosenberg WM, Cross R, Wong T, Smith CH, Barker JN. Validity of noninvasive markers of methotrexate-induced hepatotoxicity: a retrospective cohort study. Br J Dermatol 2014;171(2):267–273.
[51] Conway R, Low C, Coughlan RJ, O'Donnell MJ, Carey JJ. Risk of liver injury among methotrexate users: a meta-analysis of randomised controlled trials. Semin Arthritis Rheum 2015;45(2):156–162.
[52] Tang K-T, Hung W-T, Chen Y-H, Lin C-H, Chen D-Y. Methotrexate is not associated with increased liver cirrhosis in a population-based cohort of rheumatoid arthritis patients with chronic hepatitis B. Scientific Rep 2016;6(1):22387.