Non-invasive markers of liver fibrosis for monitoring of long-term methotrexate therapy: A multi-centre longitudinal cohort study

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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

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Data Transparency Statement

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

Abbreviations

ALP, alkaline phosphatase

ALT, alanine aminotransferase

AST, aspartate aminotransferase

CI, confidence interval

ELF, enhanced liver fibrosis score

GGT, gamma-glutamyl transpeptidase

HA, hyaluronic acid

IQR, inter-quantile range

MAFLD, metabolic dysfunction-associated fatty liver disease

MTX, methotrexate

NAFLD, non-alcoholic fatty liver disease

NASH, non-alcoholic steatohepatitis

OR, odds ratio

PsA, psoriatic arthritis

PIIINP, procollagen type III N-terminal peptide

RA, rheumatoid arthritis

SD, standard deviations

TE, transient elastography

TIMP1, tissue inhibitor of matrix metalloproteinase 1

ULN, upper limit of normal

Keywords

Methotrexate, hepatotoxicity, rheumatoid arthritis, psoriasis, liver fibrosis, transient elastography, liver stiffness, Enhanced Liver Fibrosis

Impact and implications

- Current guidelines recommend intensive (2-3 monthly) monitoring strategies for 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 been previously 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 methotrexate therapy monitoring guidelines.

Abstract

Background: The risk of significant liver fibrosis from prolonged methotrexate (MTX) exposure has been estimated in around 5% of patients, which has led to intensive monitoring strategies. However, the evidence is derived from retrospective studies that underreported risk factors of 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. MTX group included patients who received MTX for ≥6 months, whereas unexposed group included those who never received MTX. All patients underwent full liver profiling, enhanced liver fibrosis (ELF) markers, and transient elastography (TE).

Results: 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 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 OR = 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 (OR = 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 MTX therapy monitoring guidelines.

Introduction

Methotrexate (MTX) has been widely used as a disease-modifying drug in the treatment of rheumatoid arthritis and psoriasis with or without arthritis for several decades. It is recommended by the National Institute of Clinical Excellence (NICE) as the first-line treatment for both newly diagnosed rheumatoid arthritis (RA) and adult patients with moderate to severe psoriasis who need systemic therapy [1, 2]. Methotrexate-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 methotrexate 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 were independently associated with liver disease events and cirrhosis compared to 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 adults with liver failure who had been listed for liver transplantation 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 1000 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 enhanced liver fibrosis blood biomarker panel (ELF) [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]. Latter 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 the two validated non-invasive surrogate measures of liver fibrosis, liver stiffness and ELF score.

Methods

Patients included and methods

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 Sussex). 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 psoriatic arthritis) 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. MTX group included patients receiving MTX for more than six months prior 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, body mass index (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 by 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 transaminase level (AST), alanine transaminase level (ALT), gamma glutamyl-transpeptidase level (GGT), 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 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 ten 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 biopsy-proven NAFLD patients [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 with up to 7-year follow up [31, 35].

Statistical Analysis

Demographic and clinical data were described using descriptive statistics, mean ± standard deviation (SD) for continuous measurements that are normally distributed, median and inter-quantile range (IQR) for non-normally distributed continuous variables and frequencies and percentiles 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 5. 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. Correlation between liver fibrosis markers was done using Spearman's rank correlation. Multivariable 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 priori confounders and were included in the final models regardless of their effect. Linear regression models were performed using box-cox transformation of the dependent variables. Multivariable analysis was 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 taking the risk of MAFLD as a single metabolic risk factor following its diagnostic criteria. All analyses were conducted using R programme version 4.0.3 [36].

Results

The total number of patients recruited was 1024. 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 Figure 1. Distribution of patients recruited across the sites is summarised in Supplementary Table 1.

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 Supplementary Table 2. 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. Distribution of ALT in exposed and unexposed patients is illustrated in Supplementary Figure 1.

Out of 989 with ALT reported, 134 patients (13.5%) had elevated ALT, >45 IU/L (ULN). In 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 psoriatic arthritis (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, Supplementary Table 3.

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, Supplementary Table 4). 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, Supplementary Table 4). Diabetes showed the strongest independent association with liver stiffness ≥ 7.9 kPa (adjusted 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 (Supplementary Tables 5 and 6). 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 procollagen type III N-terminal peptide (PIIINP), hyaluronic acid (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, 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 (Supplementary Table 7).

Because ELF score has been shown to significantly differ between patients with RA and psoriasis [37], sensitivity analysis on patients with RA and psoriasis separately was performed. 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 (Supplementary Tables 8-11). 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 (Supplementary tables 12 and 13).

Secondary analysis

We have performed a secondary analysis on 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 logistic regression in all patients (Supplementary Tables 14-17). 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 (Supplementary Tables 18-19).

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 (Supplementary Tables 20 and 21).

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 4 unexposed), as described in Table 7. In unexposed patients, the histology was in keeping with non-alcoholic steatohepatitis (NASH) in two patients, and autoimmune hepatitis and seronegative primary biliary cholangitis (PBC) in each of the others. Among the 22 patients exposed to MTX who had a liver biopsy, histology showed features of NASH 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 and conclusion

In this multicentre large longitudinal cohort study involving about 1000 patients with psoriasis or rheumatoid arthritis, 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 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 Laharie et al. [39]. The lack of association between MTX cumulative dose and liver fibrosis has been previously observed in retrospective studies involving patients with PS exposed to MTX using histology (n=71) [41], and non-invasive markers (n=61) [42]. Single centre studies involving patients with RA as well as inflammatory bowel disease (IBD) on MTX have described similar findings although all of these enrolled a very small number of patients (n=46-185) [43-45]. The insufficient sample size of these previous studies and lack of unexposed group limited their power to identify independent risk factors associated with fibrosis through multivariable modelling.

PIIINP has been used for determining liver fibrosis in methotrexate therapy for many years [46] and has been implemented by the British Association of Dermatologists

since 2016 as a screening and monitoring test for liver fibrosis [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 the National Institute for Health and Care Excellence 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 and raises 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 which 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 score was similar among patients exposed and unexposed to MTX. In multivariable analyses, cumulative dose of MTX was associated with increase of 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 increase 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 psoriasis and RA patients 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 Disease Activity Score for 28 joints (DAS-28) scale had the strongest correlation with ELF (Pearson correlation coefficient r = 0.51, P<0.001) [44]. Inflammation markers, such as high-sensitivity C-reactive protein (hs-CRP), were not priori markers 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 in one variable, MAFLD status, it is assumed that they have 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 diagnosis differentially influence liver disease risk in the setting of methotrexate 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 influence worse clinical outcomes. In a meta-analysis of 32 randomised controlled trials of MTX versus comparator in adults with rheumatoid arthritis, psoriasis and inflammatory bowel disease showed that exposure to MTX was not associated with risk of liver failure, cirrhosis, or death (RR: 0.12, 95%CI 0.01–1.09) [51]. Moreover, in a population-based cohort of RA patients with chronic hepatitis B with more than six years of follow up, there was no increased risk of cirrhosis with long-term MTX use [52].

Our study included the largest number of patients from multiple centres to date to assess 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 sub-group 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 previously 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 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, transient elastography 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.

Appendix A

Supplementary materials.

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Tables

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

Characteristics	MTX (n = 876)	No-MTX (n = 123)	P
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** ○ **Psoriasis** ○ **Both**	 615 (70.2) 241 (27.5) 20 (2.3)	 55 (44.7) 67 (54.5) 1 (0.8)	**<0.001***
Ethnicity n (%) ○ **White** ○ **Black** ○ **Mixed Asian** ○ **South Asian** ○ **Asian** ○ **Other** ○ **Unknown**	 825 (94.2) 6 (0.7) 0 32 (3.7) 0 10 (1.1) 3 (0.3)	 118 (95.9) 0 1 (0.8) 3 (2.5) 1(0.8) 0 0	0.09*
Type 2 Diabetes n (%) **Unknown**	100 (11.5) 5 (0.6)	21 (17.1) 0	0.08
Hyperlipidaemia n (%) **Unknown**	225 (25.9) 7 (0.8)	28 (22.8) 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 (%) ○ **Not reported**	83 (9.5) 5 (0.7)	25 (20.3) 0	**<0.001**
MTX exposure, median (Q1, Q3) ○ **Dose (mg)** ○ **Duration (months)** ○ **Total cumulative dose (g)**	 15 (12.5, 20) 72 (36, 132) 4.8 (2.16, 7.95)	 NA NA NA	

Table 1. P values were derived from Pearson's Chi-squared for categorical variables and Student's t-test for continuous variables. *Fisher's Exact Test was applied because one or more expected cell counts in the cross-tabulation were less than 5. **Abbreviations:** BMI: body mass index, MAFLD: metabolic dysfunction-associated fatty liver disease, MTX: methotrexate, RA: rheumatoid arthritis.

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

Liver enzymes, median, (Q1, Q3)	MTX (n = 876)	No-MTX (n = 123)	P
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

Table 2. P values were derived from Pearson's Chi-squared for categorical variables and Student's t-test for the natural logarithms of continuous variables. **Abbreviations:** ALT: alanine transaminase, AST: aspartate aminotransferase, 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
Liver stiffness (kPa), median, (Q1, Q3)	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)	0.08
○ **High ≥ 7.9 kPa**	124 (14.5)	25 (20.7)	
○ **Cirrhosis (≥ 11.5 kPa)**	47 (5.5)	14 (11.6)	**0.01**

Table 3. P values were derived from Student's t-test for the natural logarithm of liver stiffness and Pearson's Chi-squared for categorical variables.

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

Factors	Unadjusted OR	P	Adjusted OR	95% CI	P
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/week)	0.76	0.37	0.68	0.33 - 1.32	0.28
*** p<0.05 ** p<0.01 *** p<0.001**					

Table 4. Univariable and multivariable logistic regression model of liver stiffness ≥ 7.9 kPa in the whole population (MTX cumulative dose as the independent variable). **Abbreviations**: BMI: body mass index, CI: confidence interval, 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
PIIINP (ug/L), mean (SD) (Values missing for 70 exposed and 14 unexposed)	8.42 (4.24)	8.74 (4.06)	0.44
HA (ug/L), median (Q1, Q3) (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)	0.16
○ **High ≥ 9.8**	225 (28.6)	37 (35.2)	
○ **Cirrhosis (≥ 11.3)**	23 (2.9)	3 (2.9)	0.97

Table 5. P values were derived from Student's t-test for PIIINP, HA and ELF scores, and the chi-square test for ELF groups. **Abbreviations**: ELF: enhanced liver fibrosis, HA: hyaluronic acid, PIIINP: procollagen type III N-terminal peptide.

Table 6. Factors associated with elevated ELF score ≥ 9.8

Factors	Unadjusted OR	P	Adjusted OR	95% CI	P
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**
*** p<0.05 ** p<0.01 *** p<0.001**					

Table 6. Univariable and multivariable logistic regression model of ELF score ≥ 9.8 in the whole population (MTX cumulative dose as the independent variable). **Abbreviations:** BMI: body mass index, CI: confidence interval, MTX: methotrexate, NSAIDs: non-steroidal anti-inflammatory drugs, OR: odds ratio.

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	NA	28	No	Elevated liver enzymes	6.4	9.9	PC		AIH	F1/F2
No-MTX	PS	NA	40	No	Raised LSM	73.5	11.6	TJ	8	NASH	F3
No-MTX	RA	NA	35	No	Elevated liver enzymes	5.6	9.5	PC		PBC	F1/F2
No-MTX	PS	NA	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	NA	NA	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	NA	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	NA	TJ	5	NASH	F2
MTX	PS	1.23	41	No	Elevated liver enzymes	9.6	NA	PC		NASH	F0

Table 7. Abbreviations: BMI: Body mass index; ELF: Enhanced liver fibrosis; LSM: liver stiffness measurement, HVPG: hepatic venous pressure gradient, PC: percutaneous, TJ: trans jugular, AIH: autoimmune hepatitis, PBC: primary biliary cholangitis, NASH: non-alcoholic steatohepatitis.

Figures

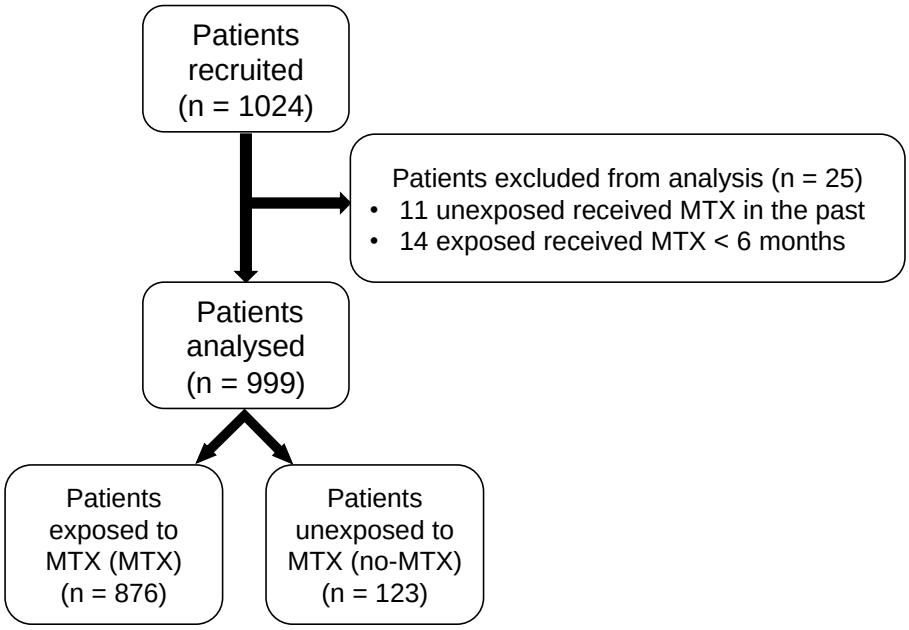

Figure. 1. Flow diagram of recruitment