Review of existing evidence demonstrates that methotrexate does not cause liver fibrosis

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ABSTRACT

It has long been believed that methotrexate in therapeutic doses causes progressive liver injury resulting in advanced fibrosis and cirrhosis. Historically, this was a common indication for serial liver biopsy. However, new evidence suggests that methotrexate may not be a direct cause of liver injury; rather the injury and fibrosis attributed to methotrexate may be mediated by other mechanisms, specifically non-alcoholic fatty liver disease. The recent widespread use of non-invasive assessment of liver fibrosis has provided new evidence supporting this hypothesis. Thus, we conducted a meta-analysis and systematic review to determine whether methotrexate is indeed a direct cause of liver injury. For the meta-analysis portion, a comprehensive literature search was performed to identify manuscripts relevant to the topic. Of the 138 studies examined, 20 met our inclusion criteria. However, only 3 studies had sufficient homogeneity to allow aggregation. Thus, the remainder of the study was dedicated to a critical review of all studies relevant to the topic with particular attention to populations examined, risk factors, and assessment of injury and/or fibrosis. Meta-analysis did not show a statistically significant association between methotrexate dose and liver fibrosis. Individual studies reported fibrosis related to confounding factors such as diabetes, obesity, pre-existing chronic liver disease but not methotrexate exposure. In conclusion, existing evidence demonstrates that advanced liver fibrosis and cirrhosis previously attributed to methotrexate are in fact caused by metabolic liver disease or other chronic liver diseases, but not by methotrexate itself. This observation should direct the care of patients treated with long-term methotrexate.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ It has long been accepted that methotrexate is a cause of progressive liver fibrosis, and prior medical standard of care included interval liver biopsy to assess liver fibrosis in patients receiving the drug. However, emerging evidence suggests that methotrexate use may be associated with liver fibrosis but not causal.

WHAT THIS STUDY ADDS

⇒ This systemic review shows that fatty liver disease, rather than methotrexate itself, causes progressive liver fibrosis and that development of cirrhosis is rare. Moreover, non-invasive assessment of liver fibrosis is sufficient to detect serial changes in this population.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The clinical implications for this are that serial monitoring of liver fibrosis in patients should be limited to those with risks for fatty liver disease and that monitoring should be non-invasive rather than via biopsy.

INTRODUCTION

Methotrexate (MTX) remains a first-line drug in management of chronic inflammatory diseases including rheumatoid arthritis (RA), spondyloarthritides and vasculitides.[1] Despite the advent of biological therapies, MTX is popular because of its low expense, ready availability and milder side effect profile.[2 3] MTX-related liver injury has been a topic of interest for years especially among dermatologists and hepatologists. Adverse effects attributed to MTX include mucositis, pneumonitis, pulmonary fibrosis, and liver injury. Specific liver injury proposed to be due to MTX includes aminotransferase elevations, steatohepatitis, hepatic fibrosis, and cirrhosis. However, there is reason to believe that liver injury attributed to MTX may be due to other factors. For instance, there may not be a dose–effect of the drug,[4] and confounding factors such as metabolic syndrome, alcoholism and obesity have not been excluded.[5]

The mechanism of action of MTX at a cellular level is complex. MTX is a dihydrofolate reductase inhibitor and reduces intracellular folate, thus affecting DNA and protein synthesis. This leads to reduced epidermal replication and affects T lymphocytes and macrophages. At therapeutic low doses (<0.4 mg/kg/wk), MTX causes changes in adenosine signaling (inhibition of 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase) rather than impaired folate metabolism. Interestingly, in the liver, adenosine is a profibrogenic signal that upregulates production of collagen and suppresses metalloproteinases during the process of wound healing in response to injury.[6]

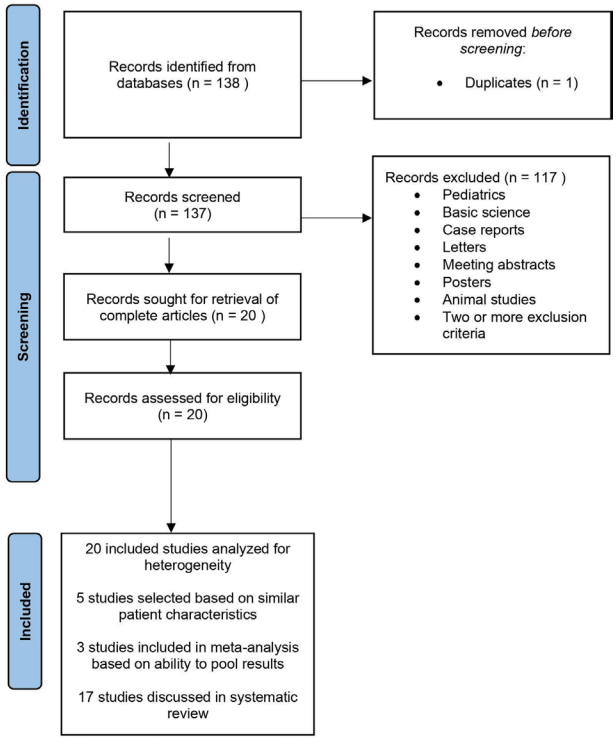

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow sheet.

In light of these contradicting data, we attempted to re-examine the possible role of MTX as a cause of liver injury via a thorough review of the literature. We found that several manuscripts had data sufficiently homogeneous to allow meta-analysis (please see figure 1 for a schematic of the methodology used); however, the majority of published studies were quite different and required individual analysis (Supplemental File 1). Specifically, meta-analysis was so limited in scope and source content that a thorough review of the literature was of greater value. The results of this approach are as follows.

Evidence provided by serial liver biopsy

Older dermatological literature described liver fibrosis in patients with psoriasis who were treated with MTX. Table 1 shows a summary of the histological changes attributed to MTX in the liver. Thus, the dermatology community had been careful about use of MTX, and recommendations to follow such patients with liver biopsy after a total cumulative dose of 1.5 g of MTX were standard. Biopsies were to be repeated after each additional 1 g cumulative dose. The studies cited, however, had important limitations, including lack of consideration of confounding variables such as alcohol, obesity, and chronic medical conditions.[7 8]

Table 1 Histological changes attributed to methotrexate use

	Details
Steatotic changes	Microvascular and macrovascular steatosis
Reactive changes	Hyperchromasia, anisocytosis of hepatocyte nuclei, patchy hepatocyte necrosis
Inflammatory changes	Portal inflammation
Fibrotic changes	Periportal fibrosis; portal to portal bridging; portal to central bridging; pericellular fibrosis

In an 8-year prospective cohort, 209 liver biopsy specimens were analyzed by electron and light microscopy. A mean of 6.3 liver biopsies per patient was obtained during the follow-up. Results showed no alteration in hepatic architecture on weekly dosing of MTX in patients with RA.[9] Thus, this trial suggests that patients with RA, even those who used large doses of MTX, did not develop advanced liver fibrosis or cirrhosis as assessed by liver biopsy. In a study by Tishler *et al*, in an evaluation of MTX-induced liver damage in 10 patients with RA with sequential biopsies, not a single patient showed evidence of advanced fibrosis or cirrhosis after 4 years of treatment.[10] The study that examines the largest number of patients by biopsy was performed by Whiting-O'Keefe *et al*[11] in 1991 using a systematic review of 636 patients from 15 studies. The authors observed that advanced fibrosis or cirrhosis was independent of cumulative MTX dose. In contrast, alcohol consumption was associated with fibrosis progression. Moreover, in this aggregate group, patients with psoriasis were more likely than patients with RA to have advanced fibrosis observed on biopsy.

Lastly, it is important to note that there is no pathognomonic histological finding suggesting MTX-induced liver injury. Rather, findings dovetail if not completely overlap with those of fatty liver disease, including mixed cellular infiltrate, Mallory's hyaline, apoptotic bodies, steatosis, and progressive fibrosis commensurate with duration of injury.[12]

Evidence provided by serial transient elastography

In our data analysis, 3 studies[13–15] compared the MTX cumulative dose for patients with liver fibrosis versus without liver fibrosis as measured by transient elastography (TE, FibroScan) using a liver stiffness measurement (LSM) >7.1 kPa as a threshold. Meta-analysis did not show a statistically significant association between MTX dose and liver fibrosis. Individual studies reported fibrosis related to confounding factors such as diabetes, obesity, pre-existing chronic liver disease rather than MTX exposure.

A critical analysis of each of the studies showed interesting findings. Bafna *et al*[13] followed the patients with RA who had been on an MTX dose range of 2.4–22 g for at least 3 years. These patients had an average body mass index (BMI) of 24.8±3.9. LSM score of 7.1 kPa was used as the cut-off for any fibrosis, >9.5 kPa for severe fibrosis and >12.5 kPa for cirrhosis. Eight patients had an LSM score of 7.1–9.5 kPa, 3 patients had an LSM score of 9.5–12.5 kPa, and 1 patient had an LSM score more than 12.5 kPa. To summarize, factors associated with elevated LSM were obesity, steatosis, and waist circumference.

In 2019, Erre *et al*[14] compared 140 MTX-treated patients with RA to 33 MTX-naïve patients with RA. One hundred healthy blood donors were used as controls. MTX-treated patients were on a dose up to 7.2 g for around 11 years. Four patients had LSM values from 7.1 to 7.6 kPa. None of the treated patients were in the moderate fibrosis or cirrhosis range. Liver stiffness in MTX-treated patients was not significantly higher than that of naïve patients. Also, liver stiffness was not significantly different across different cumulative doses of MTX. In multivariate regression

Table 2 Association of presence of fibrosis by TE with cumulative MTX dose as assessed by meta-analysis (p=0.15)

LSM value (kPa)	Subjects (n)	Mean MTX dose (mg)	SD
TE>7.1 (any fibrosis)	45	5030 (2929–7133)	7198
TE≤7.1 (no fibrosis)	311	3680 (3582–4479)	448

APRI, AST to platelet ratio index; BARDS, BMI, AST/ALT ratio and Diabetes Score; FBG, fibrinogen; FIB-4, Fibrosis-4; LSM, liver stiffness measurement; MTX, methotrexate; TE, transient elastography; VCTE, vibration-controlled transient elastography.

analysis, length of exposure to MTX and cumulative dose were not significantly associated with increase in liver stiffness in this cohort of patients with RA.

Lertnawapan *et al*[15] analyzed the MTX dose of 1.8–3 g and used LSM >7.1 kPa as the cut-off for liver fibrosis in a group of 108 patients with RA who had a BMI of 24±5. Only 16 patients were found to have liver fibrosis using this approach. Factors associated with liver fibrosis included impaired fasting blood glucose, fatty liver, hyperlipidemia, prescribed statin, cumulative MTX dose, and prolonged duration of treatment.

In a cross-sectional study done by Neema *et al*[16] on 82 patients with plaque psoriasis, an LSM cut-off of 7 kPa was used to indicate liver fibrosis. These patients had an average BMI of 25±5. All of these patients had received a minimum cumulative dose of 1.5 g of MTX for approximately 10 years. Twenty-three patients had LSM scores >7 kPa. This study was large enough to allow regression analysis. Variables shown to be significant at the multivariate level included: age of patient, waist circumference, diastolic blood pressure, fasting and postprandial blood sugar, elevated liver function tests, presence of metabolic syndrome, and severity of psoriasis itself. However, the cumulative dose of MTX was not associated with liver fibrosis at the cut-off defined by the authors.

Arena *et al*[17] included 100 patients with RA who were on MTX therapy for 3–11 years. This study is noteworthy because patients suspected of liver fibrosis (LSM >7 kPa) also underwent liver biopsy. The enrolled patients had an average BMI of 25±4.3, and MTX dose ranged from 1.5 to 13 g. A total of 5 patients underwent liver biopsy. In 2 patients with LSM score ranging from 9.8 to 11.6 kPa, liver biopsy showed mild to moderate perisinusoidal fibrosis. In 4 patients, biopsy showed minimal signs of lobular inflammation. It is important to note that no patient had evidence of cirrhosis as assessed by LSM or biopsy irrespective of cumulative MTX dose.

Thus, taken together, there are limited data linking use of MTX or cumulative MTX dose received with liver fibrosis. It is critical to note that an LSM threshold of 7 or 7.1 kPa to define liver fibrosis is very low, as this threshold has not been associated with clinical outcomes. In contrast, there are data from studies examining serial elastography that demonstrate that known risk factors of metabolic liver disease are also associated with liver fibrosis. However, in these cohorts, no patients met elastography cut-offs for cirrhosis, casting doubt on the concept that even large cumulative doses of MTX are associated with cirrhosis development in patients with RA or psoriasis/psoriatic arthritis.

MTX use in patients with pre-existing liver disease

Several studies have examined the effect of MTX treatment in patients with pre-existing liver disease, as such patients may be expected to develop more severe liver injury and/or more advanced fibrosis if MTX were an independent cause of injury. Tang *et al* examined 2 separate retrospective cohorts of patients with RA with chronic hepatitis B or chronic hepatitis C not on any treatment.[18 19] Exclusion criteria included alcoholic and biliary cirrhosis, coexistent hepatitis B and C infection, and other chronic liver diseases. Patients in these cohorts received cumulative MTX doses of 1.5–3 g and received therapy for 5–9 years. In the hepatitis B cohort, the incidence of cirrhosis between MTX users and non-users was comparable (6.2% vs 7% respectively). There was no increased risk of cirrhosis in patients who received a higher cumulative dose of MTX (>1.5 g). Fifty-six patients who were given a cumulative dose of more than 3 g did not develop liver cirrhosis after 97 months of treatment. In the hepatitis C cohort, a total of 55 patients developed cirrhosis: 19 MTX users and 36 non-MTX users. Out of the 19 MTX users who developed liver cirrhosis, 17 had a cumulative dose of <1.5 g, and only 2 had a cumulative dose of 1.5–3 g. Of note, no cirrhosis was identified among the 43 MTX users with cumulative dose of >3 g.

Similar results were reported by Tang *et al* in 2018 in a retrospective cohort in patients with psoriasis.[20] A total of 3544 patients with chronic hepatitis B or chronic hepatitis C were followed over the course of 9 years. The average cumulative MTX dose was 3.9±5.8 g after a mean of 123 months in MTX users with chronic hepatitis B, and 4.4±7.9 g after a mean of 122 months in MTX users with chronic hepatitis C. In this population, there was no increased risk of cirrhosis in patients with viral hepatitis with long-term use of MTX.

Taken together, these large cohort trials indicate that MTX does not increase the risk of cirrhosis in patients with chronic hepatitis B or hepatitis C. In fact, 1 study demonstrated that patients with chronic hepatitis C may be protected from cirrhosis by MTX, although this is a single observation that would have to be repeated to be clinically meaningful. In any case, these data are a powerful argument that MTX does not augment fibrosis in patients with pre-existing liver disease and provide insight supporting the concept that MTX does not independently cause liver injury or fibrosis.

MTX use in patients with pre-existing fatty liver disease or metabolic risk factors

A variety of population studies using distinct methods have been performed on patients with either known fatty liver disease or with metabolic risk factors, and these provide insight into the relative roles of fatty liver disease and MTX as causes of liver injury and fibrosis. Mori *et al* followed 289 patients using liver to spleen ratio with CT scan to assess in hopes of assessing whether pre-existing fatty liver disease altered the development of fibrosis in patients receiving MTX.[21] Patients with RA on median cumulative MTX dose of 1.1 g for at least 29 months were followed, and patients with elevations of aminotransferases were studied in detail. Of the 44 patients with aminotransferase elevations, 24 underwent liver biopsy, with the majority of patients

Table 3 Cross-sectional studies considered for meta-analysis

First author, year	Sample size	Study population	Exclusion criteria	MTX indication	MTX cumulative dose g (range)	MTX duration y (range)	Testing modality	BMI (mean±SD)	Patients with cirrhosis
Bafna, 2021[13]	75	92% female; 45% obese; 57% >10y MTX exposure; 97% on hydroxychloroquine	BMI >30, viral hepatitis, autoimmune hepatitis, heavy alcohol use, hepatotoxin use, ascites, elevated transaminases	RA	6.3 (2.4–17.4)	>3 (2.7–17.4)	VCTE; liver biopsy	24.8±3.9	1
Erre, 2019[14]	140	MTX treated=140; MTX naïve=33; healthy donors=100	Chronic liver disease, other autoimmune diseases	RA	4.7 (0.2–7.2)	6.2 (0.5–11.4)	VCTE	26±5	0
Lertnawapan, 2019[15]	108	Large number of patients with high FBG	Chronic liver disease, elevated aminotransferases, alcohol use disorder	RA	1.0 (1.8–3)	4 (1.5–9)	VCTE; AST/ALT ratio; APRI; NAFLD fibrosis score; BARDS score; FIB-4	24±5	0
Neema, 2020[16]	82		Viral hepatitis, alcohol use, hepatotoxin use	Psoriasis	1.5	10	VCTE	25.1±3.9	0
Feuchtenberger, 2021[33]	110	MTX >1 y=65; MTX naïve at initiation=54	Chronic liver disease, autoimmune hepatitis, alcoholic liver disease, NASH, obesity	RA	3.6	4.39 (1.0–18)	ARFI	25±2	0
Mahajan, 2020[31]	134	Large number of patients in both groups with alcohol use, DM, HTN	Viral hepatitis, HIV infection	Psoriasis	0.89 (0–5.2)	2	VCTE; APRI; NAFLD fibrosis score; FIB-4	25±4.3	0
Pongpit, 2016[32]	162	Majority with metabolic syndrome and/or hyperlipidemia	Chronic liver disease, alcohol use disorder, pregnancy	Psoriasis	Majority <1.5	6 (4–28)	VCTE; liver biopsy	24.8±4.7	0

ALT, alanine aminotransferase; APRI, AST to platelet ratio index; ARFI, acoustic range force impulse; AST, aspartate aminotransferase; BARDS, BMI, AST/ALT ratio and Diabetes Score; BMI, body mass index; DM, diabetes mellitus; FBG, fibrinogen; FIB-4, Fibrosis-4; HTN, hypertension; MTX, methotrexate; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; RA, rheumatoid arthritis; VCTE, vibration-controlled transient elastography.

Table 4 Cohort studies considered for meta-analysis

First author, year	Sample size	Study population	Exclusion criteria	MTX indication	MTX cumulative dose g (range)	MTX duration y (range)	Testing modality	BMI (mean±SD)	Patients with cirrhosis
Arena, 2012[17]	100	Unspecified	Chronic liver disease; abnormal liver tests; DM; metabolic syndrome	RA	1.5 (1.5–13)	7 (3–11)	VCTE; biopsy	25±4.3	0
Barbero-Villares, 2011[26]	53	3.8% chronic alcohol use; 3 patients with chronic viral hepatitis	Obesity; advanced liver disease	RA, IBD, psoriasis	1.8 (3.0–5.7)	3.4 (1.0–10)	VCTE	26±1.0	1
Mansour-Ghanaei, 2017[25]	101	44% obese; possible under-reported alcohol use	Unspecified	RA, psoriasis	2.0 (2.0–5.0)	Unknown	VCTE	28.2±5.4	0
Maybury, 2019[27]	333	37% overweight; 37% obese; majority insulin resistant	Unspecified	Psoriasis	0.45	0.6	VCTE	Unknown	0
Mori, 2020[21]	289	22% obese; 30% HTN	Chronic liver disease; viral hepatitis; abnormal liver tests	RA	1.1	Unknown	Liver/spleen ratio; liver tests; biopsy	Unknown	0
Rattanakaemakorn, 2021[22]	160	No exclusion of viral hepatitis, DM, or obesity	Unspecified	Psoriasis	2.08 (0.5–5.6)	Unknown	VCTE	Unknown	
Tang, 2018[20]	3544	Varied	HBV/HCV coinfection; chronic liver disease	Psoriasis	3.9 for HBV; 4.4 for HCV	10	n/a	Unknown	Yes
Tang, 2016[18]	450	Chronic HBV	Cirrhosis; alcohol; HBV/HCV coinfection	RA	Majority <3.0 g	9	n/a	Unknown	Yes
Tang, 2016[19]	450	Chronic HCV	Cirrhosis; alcohol; HBV/HCV coinfection	RA	Majority <3.0 g	5	n/a	Unknown	Yes
Yeo, 2013[23]	59	25% DM; 25% HTN; 15% hyperlipidemia; 8% social alcohol use	Viral hepatitis; abnormal liver tests	Psoriasis	2.5 (0.8–14)	7	Biopsy	Unknown	0

BMI, body mass index; DM, diabetes mellitus; HBV, hepatitis B virus; HCV, hepatitis C virus; HTN, hypertension; IBD, inflammatory bowel disease; MTX, methotrexate; n/a, not applicable; RA, rheumatoid arthritis; VCTE, vibration-controlled transient elastography.

Table 5 Studies included in meta-analysis

First author, year	Study design	Sample size	Study population	MTX indication	MTX cumulative dose g (range)	MTX duration y (range)	Testing modality
Maybury, 2014[28]	Systematic review	429	Aggregate of 8 observational studies	Psoriasis	Unknown (1.7–5.1)	2 (2–5)	Biopsy
Montaudié, 2011[29]	Systematic review	53	Unaggregated examination of 7 distinct studies	Psoriasis	Unknown	Unknown	Varied
Lladó, 2021[30]	Case–control	84	37% overweight; 9% obese; 5% type 2 DM; 13% hyperlipidemia	Crohn's disease	1.5 (0.7–2.7)	2.5	VCTE; APRI

APRI, AST to platelet ratio index; DM, diabetes mellitus; MTX, methotrexate; VCTE, vibration-controlled transient elastography.

having histology compatible with non-alcoholic steatohepatitis (NASH) (n=19) or benign steatosis (n=3), with the remainder demonstrating interface hepatitis (n=2). Of the 21 patients with any fibrosis, 19 had evidence of NASH, and 2 had interface hepatitis. Importantly, only 5 cases had bridging fibrosis, and none had cirrhosis.

Rattanakaemakorn *et al* compared MTX monotherapy and MTX-acitretin (ACI) in patients with psoriasis to assess the risk of hepatic fibrosis.[22] In this retrospective cohort, 160 patients who received MTX dose in the range of 1–4 g were followed. This study did not exclude patients with viral hepatitis or type 2 diabetes. Cumulative incidence of hepatic fibrosis at 5 years was identical in both groups (16% for MTX-ACI; 16% for MTX alone). The only factor that predicted liver fibrosis in either group was the presence of type 2 diabetes mellitus (DM2) or obesity. MTX dose was not a predictor of liver fibrosis.

Yeo *et al* performed a cohort study on patients with psoriasis receiving long-term MTX.[23] Fifty-nine patients receiving MTX in a range of 0.8–14 g were followed for 7 years. Thirty-four of these 59 patients had no risk factors for liver disease, and 25% had type 2 diabetes, 25% had hypertension, 15% had hyperlipidemia, 8% consumed alcohol (deemed by the authors to be 'social use'). Patients with hepatitis B and C and aminotransferase elevations prior to the study were excluded. Ninety-eight biopsies were acquired on 59 patients. Biopsies were scored using Roenigk system, which takes into account both stage and grade.[24] In this group, fibrosis was staged as follows: stage 1 (6 patients), stage 2 (23 patients), stage 3 (7 patients), stage 4 (0 patient). Thus, in this study examining liver fibrosis via liver biopsy, advanced fibrosis was rare, and high cumulative MTX dose was not a risk for this. Moreover, no patients were found to have cirrhosis.

Mansour-Ghanaei *et al* carried out a retrospective cohort with 101 patients with a variety of rheumatological disorders.[25] These patients had an average BMI of 28.2±5, and 44% of the patient population was obese. History of alcohol use was present in only 3% of the cases. MTX dose range was 2–5 g with duration of treatment from 2 to 5 years. Liver stiffness and steatosis was measured by using TE. Statistically significant risk factors for liver fibrosis included BMI and waist circumference; however, MTX dose and duration were not risk factors for liver fibrosis.

In a cross-sectional study including patients with inflammatory bowel disease, Barbero-Villares *et al* assessed liver stiffness by TE.[26] The MTX dose range was 1–3 g over a course of 2 years. Cut-off for liver fibrosis was >7.1 kPa. In this group, 35, 8, and 3 patients had TE values of <7, 7–9, and >9.5 kPa, respectively. No cirrhosis was reported. This study was limited in description of patient population and reporting of confounding factors.

A cohort study was performed by Maybury *et al* in 2019 in 333 patients with severe chronic plaque psoriasis with a mean dose of 15 mg/wk of MTX with average treatment duration of 0.6 years.[27] In this study, 85% of patient population had already been exposed to MTX, 53% were insulin resistant, 37% overweight, 30% obese, 22% had type 2 diabetes, 50% had non-alcoholic fatty liver disease, and 66% population were taking another biologic. LSM results showed 14.1% patients with TE values >8.3 kPa. Importantly, most of these patients were not in the MTX group.

These findings indicate that central obesity as well as insulin resistance, rather than MTX, in this cohort of patients was the main factor related to liver fibrosis.

In a 2014 systematic review by Maybury *et al*, 8 observational studies were analyzed to assess the risk of liver fibrosis with MTX.[28] The risk difference (RD) for developing any fibrosis was 0.22 with 95% CI 0.04 to 0.41. The RD for cirrhosis was 0.04 with 95% CI of 0.02 to 0.07. This study showed that there was no clear association between cumulative dose of MTX and fibrosis. In these studies, liver biopsy data did show disease progression associated with MTX use. However, in the included observational studies, confounding factors such as obesity, diabetes, alcohol use and metabolic syndrome were underreported. This study was limited by selection bias and small study population.

Montaudié *et al* in 2011 performed a systematic review in patients with psoriasis on MTX therapy.[29] The analysis assessed the diagnostic performance of procollagen III as a biomarker of liver fibrosis as compared with the commercial FibroTest assay and TE. All 3 modalities had comparable performance, and all demonstrated that type 2 diabetes and obesity but not MTX dose were major risk factors for developing liver fibrosis in patients with psoriasis. In the studies cited, cirrhosis was not reported.

Llaó *et al* carried out a case–control study in patients with Crohn's disease.[30] Fifty-six patients who were being treated with MTX versus 28 patients not treated with MTX were compared. Thirty-seven per cent were reported to be overweight, 5% with type 2 diabetes, and 13% with hyperlipidemia. These basal characteristics were similar between the 2 groups. MTX dose range was 0.7–2.7 g for 29 months of therapy. Liver fibrosis as defined by LSM of 7.9 kPa was detected in 3 cases and 4 controls. In multivariate analysis, alcohol consumption, type 2 diabetes, and mean age, but not MTX use, were associated with liver fibrosis.

Mahajan *et al* in 2020 compared MTX-receiving and non-MTX-receiving patients with plaque psoriasis.[31] Patients with chronic hepatitis B, hepatitis C, and HIV were excluded. The patient population included patients with alcohol use disorder, smoking, type 2 diabetes, and essential hypertension. Average BMI of patient population was 25±4.34. MTX dose range was 3–4.5 g over a period of 2 years. In multivariate analysis, this study also associates metabolic syndrome, central obesity, and female gender to liver fibrosis. There was no statistical difference between MTX-exposed and non-exposed groups in liver fibrosis.

Pongpit *et al* performed a cross-sectional study with 162 patients with chronic plaque psoriasis.[32] Mean cumulative dose of MTX was >1.5 g for at least 12 months. In this population, 50% of patients had metabolic syndrome, 53% had hyperlipidemia, 33% had essential hypertension, and 18% had type 2 diabetes. Average BMI was 24.8±4.7. Patients with chronic liver disease, alcohol abuse, and pregnancy were excluded. Results showed LSM >7 kPa (n=18), >9.5 kPa (n=11), and >13 kPa (n=4). Two patients with LSM of 8.9 and 11 kPa underwent liver biopsy, which demonstrated a Metavir stage of F2 and F3, respectively. Multivariate analysis showed waist circumference, type 2 diabetes, and aspartate aminotransferase level were independently associated with liver fibrosis. Of note, patients with LSM >7 kPa had a BMI of 30.7±5.7.

Feuchtenberger *et al* in 2021 performed a cross-sectional study in patients with RA.[33] Sixty-five patients with MTX exposure and 54 patients without MTX exposure were assessed using acoustic range force impulse (ARFI), which employs principles of shear wave elastography during diagnostic ultrasound.[34] The average BMI of these patients was 25±2. Patients with chronic liver disease, autoimmune and alcoholic liver disease, NASH, or pre-existing cirrhosis were excluded. Mean cumulative dose of MTX was 3.6 g with mean treatment duration of 4.3 years. For ARFI measurements, mean value equated to liver fibrosis stage 2 was 1.34 m/s or higher. In this study, MTX group had an average value of 1.11 m/s versus MTX-naïve patients with a mean value of 1.06 m/s. There was no statistical difference between the 2 groups. However, patients with metabolic diseases had higher mean ARFI values.

In our own limited meta-analysis on studies with sufficient similarity and rigor (see figure 1 for specifics), the presence of liver fibrosis was not associated with cumulative MTX dose (table 2). In contrast, metabolic syndrome, DM2, waist circumference, and established fatty liver disease were related to liver fibrosis independent of MTX. Taken together, studies examining the relationship of MTX and fatty liver disease on liver fibrosis progression show that metabolic risk factors produce stronger fibrosis risk, and only some studies support the concept that MTX is an independent risk for liver fibrosis in such at-risk patients. Tables 3 and 4 show the studies considered for meta-analysis, and table 5 identifies the studies ultimately included.

DISCUSSION

MTX is an effective and easily available medication for treatment of chronic inflammatory disorders. Although MTX was once accepted as an actual cause of liver fibrosis/cirrhosis, recent observations call this into question. There has been an evolution of the literature, in large part due to the move from biopsy to non-invasive assessment of liver fibrosis via elastography. For example, Aithal *et al*[35] in 2004 reviewed 121 liver biopsies from 66 subjects to establish the relationship between MTX and fibrosis in patients with psoriasis. They showed that risk of fibrosis ranged from 0% in patients with cumulative MTX dose of 1.5 g to 8.2% in patients with cumulative dose of 6 g (although no patients developed cirrhosis). This would suggest at first glance a dose–response relationship between MTX and liver fibrosis. In contrast, a 2016 trial by Kranidioti *et al*[36] assessed liver fibrosis by TE in patients with RA treated with MTX. This study showed that long-term MTX administration is not associated with liver fibrosis. Thus, technological advances may have improved our understanding of the concept of MTX as a cause rather than uninvolved cofactor in the progression of liver fibrosis. Based on the preponderance of evidence, it is very unlikely that MTX, even at high cumulative doses, causes liver fibrosis (and certainly not cirrhosis) in patients without liver disease risk factors.

What about patients with known chronic liver diseases using MTX? Thankfully, there are sufficient numbers of studies in which MTX was used to treat patients with chronic liver diseases. Interestingly, in some cases, MTX has no effect (or in 1 case a beneficial effect) on populations with chronic viral hepatitis.[18–20] In contrast, alcohol and

metabolic risks are relevant causes of progressive fibrosis in patients using MTX. Especially in the case of metabolic risks, the relative contribution of MTX as a cause of liver fibrosis is still a bit out of focus. Some studies demonstrate that MTX has no causal relationship with liver fibrosis in such patients, but others show the opposite. At present, it is prudent to conclude MTX may exacerbate liver fibrosis in patients with metabolic liver disease, although the metabolic risks are almost certainly more relevant to disease progression. Lastly, it is important to note that the relationship of MTX with fibrosis in patients with less common causes of liver disease, such as primary biliary cholangitis, autoimmune hepatitis, and hemochromatosis, is unknown.

Interestingly, societal guideline statements are beginning, although slowly, to catch up to current observations. For example, current guidelines of the American Association of Dermatology recommend evaluating all patients before initiation of MTX therapy with basic lab work including complete blood count, liver function tests, and serology for hepatitis B and C viruses to exclude pre-existing disease. They recommend liver biopsy only in patients with previously diagnosed liver disease, abnormal type III procollagen amino terminal propeptide levels, or cumulative MTX dose exceeding 3.5–4 g of MTX. However, no consideration of non-invasive fibrosis measurement has been made. Of note, neither the American Association for the Study of Liver Disease nor the American Gastroenterological Association has guidelines or guidance statements directly addressing this question.

What can be recommended to the physician asked to aid with assessment of fibrosis risk and development in patients prescribed MTX? Several options may be considered. Perhaps, the simplest but not most cost-effective would be just to perform TE prior to initiation of therapy and after every 1–2 g of cumulative dose of MTX. A more refined approach may be to perform a formal assessment of metabolic risk prior to treatment in all patients prior to MTX with referral of all patients showing evidence of any metabolic risk for a hepatology assessment including elastography and liver tests. The appropriateness of either approach could be determined by long-term observational studies using elastography or other non-invasive assessment approaches in large numbers of patients, especially cohorts with large numbers of patients with metabolic risks. Lastly, one thing is clear—data do not support interval biopsy in patients receiving MTX. Severe bleeding is the most important adverse event in patients undergoing percutaneous liver biopsy, and its risk is 0.2% in patients with normal platelet count[37]; however, this is still a likelier event than detection of cirrhosis in patients treated with MTX.

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