

# Liver injury in long-term methotrexate treatment in psoriasis is relatively infrequent

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## SUMMARY

### Background

Methotrexate-induced liver damage in psoriasis has led to dermatologic guidelines that stipulate monitoring of liver injury by means of serial liver biopsies. Recent literature data suggest that methotrexate may be considerably less hepatotoxic than previously assumed.

### Aim

To evaluate prevalence and development of liver injury in methotrexate treated psoriasis.

### Methods

Retrospective chart review (1976–2005).

### Results

Hundred and twenty-five patients (F58/M67; mean age 45.0, SD 12.7 years) received a median cumulative methotrexate dose of 2113 mg (range 180–20 235) over a median period of 228 weeks (range 16–1763). Two hundred and seventy eight liver biopsies were analysed and 71% were classified as Roenigk grade I, 14% as Roenigk II, 14% grade IIIa, 2% grade IIIB and 2% grade IV. Liver injury was not associated with cumulative dose, weekly prescribed dose, age or duration of treatment. Obesity and diabetes were significant risk factors for liver injury. A total of 68 patients had multiple biopsies, 3% improved, 72% did not change and in 25% liver histology deteriorated. The majority of cases (84%) that progressed to Roenigk 2 had a cumulative dose of 1500–6000 mg.

### Conclusions

Methotrexate-related liver injury is less frequent than previously thought and mostly occurred at cumulative dose of <6000 mg. Diabetes and being overweight are significantly correlated with liver injury.

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## INTRODUCTION

Methotrexate (MTX) is the most commonly prescribed systemic therapy for severe psoriasis. MTX, a folate antagonist, competitively inhibits dihydrofolate reductase, an enzyme necessary for methionine, purine and thymidylate synthesis and, ultimately, DNA synthesis. MTX possesses potent anti-inflammatory effects on T-cell mediated immune responses as it inhibits proliferation or induces apoptosis in activated T-cells and blocks the abnormal rapid epidermal cell proliferation, both responsible for the characteristic skin lesions in psoriasis.<sup>1</sup> Long-term, weekly low-dose methotrexate therapy is associated with some serious adverse reactions as myelosuppression, interstitial pneumonitis and hepatotoxicity. The pathogenesis of MTX-induced hepatic damage is poorly understood, but intra hepatocellular accumulation of a polyglutamated metabolite of MTX might be responsible for liver toxic effects. Several studies have suggested that a pre-existent liver disease, overweight, diabetes mellitus (DM) or alcohol use carries an increased risk of hepatotoxicity.<sup>2</sup>

Early studies in MTX treated psoriatic patients reported a very high prevalence of hepatotoxicity with fibrosis occurring in up to 50% and cirrhosis in up to 20%.<sup>3–5</sup> Concerns about liver injury in psoriasis patients has led to dermatologic guidelines that stipulate monitoring periodically after every 1500 mg cumulative dose, in order to identify liver injury by means of serial liver biopsies. Liver biopsy is considered as the gold standard method in the assessment of histological changes,<sup>6</sup> but its application is complicated by some important limitations. A liver biopsy is an invasive procedure and carries inherent side effects such as pain and localized bleeding.<sup>7, 8</sup> However, recent literature suggests that MTX might be significantly less hepatotoxic and reports that liver fibrosis and cirrhosis are notably less prevalent than previously assumed.<sup>9, 10</sup> These data suggest that reconsideration of our current monitoring strategies for patients on MTX may be warranted. Recent literature proposes that procollagen III aminopeptide (PIIINP) measurements may be of additional value in some patients.<sup>11, 12</sup>

In the present study, we reviewed the clinical details and liver biopsies of 125 patients on long-term, low-dose MTX therapy for psoriasis seen in a large tertiary referral centre from 1976–2005. Our aim was to establish the prevalence of MTX-induced

liver injury and to delineate potential contributing factors.

## METHODS AND MATERIALS

### Subjects

This study is a retrospective chart review over a period between 1976 and 2005. The strategy we followed to identify subjects was as follows. We identified records of all patients who had had a liver biopsy from the pathology files and searched for patients who were known at the Department of Dermatology. Subjects eligible for our study were all psoriasis patients, receiving a weekly dosage MTX and underwent single or serial follow-up liver biopsies for monitoring liver injury.

The clinical records were scrutinized for details regarding age and sex, duration and onset of MTX therapy, cumulative dose at the time of liver biopsy, maximum prescribed weekly dosages, date and reason for MTX-discontinuation, information about the liver biopsy and histological outcomes, presence of risk factors for liver damage (DM, alcohol use and obesity) and other relevant facts from the clinical history. We also recorded laboratory and liver enzyme test results; alanine transaminase (ALAT), aspartate transaminase (ASAT), alkaline phosphatase (AP),  $\gamma$ -glutamyl-transpeptidase ( $\gamma$ -GT), total bilirubin level, platelets and leucocytes counts. Elevated levels were defined as those above the upper limit of normal.

All information was entered in a computerized database. Samples of obtained data were structurally verified to achieve maximum data exactness.

The Department of Dermatology is a large referral centre for psoriasis and we have implemented a monitoring process that closely follows the US guidelines for MTX treatment. Briefly, pre-MTX evaluation includes history and physical examination, laboratory tests consisting of complete blood cell count with differential and platelet count, renal function, liver chemistry and a chest X-ray. In case significant risk factors are absent, a liver biopsy is not necessary until the patient has been treated with 1.5 g of MTX. Monitoring during MTX-treatment includes complete blood cell count with differential and platelet count and liver chemistry every 4–8 weeks, and renal function every 3 months. A follow-up liver biopsy is recommended after every 1.5 g MTX. If there is clinical

suspicion of liver injury the patient should be referred to a hepatologist.<sup>13</sup>

### Histology

Percutaneous liver biopsy was performed via right intercostal approach with local lidocaine anaesthesia. The biopsy specimen was immersed in 2% formaldehyde and subsequently fixed with paraffin. Haematoxylin and eosin-stained sections of liver tissue were examined for steatosis, lobular and portal tract inflammation, hepatocyte necrosis and nuclear variability. A von Gieson stain for collagen was assessed for the presence of pericellular and perivenular fibrosis, as well as the expansion of the portal tracts. All liver biopsies, sampled as part of the monitoring process of MTX-induced hepatic injury were, graded according to the Roenigk classification (Grade 1, normal tissue, no/mild fatty change, no/mild nuclear pleomorphism, no fibrosis, mild portal inflammation; Grade 2, moderate/severe fatty changes, moderate/severe nuclear pleomorphism, no fibrosis, moderate/severe portal inflammation; Grade 3a, mild fibrosis, portal fibrotic septa, extending in the lobuli, portal tract enlargement; Grade 3b, moderate/severe fibrosis; Grade 4 = cirrhosis, regenerating noduli and bridging of the portal tracts). We defined a baseline biopsy when it had been performed either at start of MTX treatment and/or before reaching a cumulative dose of 600 mg.

### Statistical analysis

Data from the computerized database were analysed using SAS statistical software, version 8.2 (SAS Institute Inc, Cary, NC, USA). Frequency tables were provided for demographic and histological variables. Risk factors for liver injury (presence of DM, overweight and use of alcohol) were analysed over histology, expressed as Roenigk classification and dichotomously analysed using Pearson's chi-square analysis (Fisher's exact test was used where appropriate). The same procedure was used for deviant concentrations of liver enzymes.

Percentage of patients with liver injury, defined as Roenigk classification beyond 1, was plotted per cumulative MTX dose, both for the total patient population and subdivided by the risk factors, mentioned above. Finally, discontinuation of therapy was also analysed per cumulative dose of MTX treatment, using the same type of plots.

## RESULTS

### Demographics

Hundred twenty-five-psoriasis patients (58 female and 67 male) with an average age of 45 years (s.d. = 13) at start of MTX therapy underwent one or more monitoring liver biopsies. Ninety-two patients carried pre-existent risk factors for MTX induced liver injury at time of the first biopsy. Thirty-nine patients (31%) were overweight (defined as body mass index  $>25 \text{ kg/m}^2$ ). Some 49% of patients ( $n = 61$ ) were consuming alcohol, and we categorized 11 (8%) as excessive consumers ( $>14 \text{ U/week}$ ). Nine patients (7%) had DM. Twenty-six of them only had a baseline biopsy, forty-seven had only one or more follow-up biopsies and 19 had both.

The median maximum prescribed weekly dosage MTX was 12.5 mg/week (range 2.5–35 mg/week). Patients received a median cumulative MTX dose of 2113 mg (180–20 235 mg) during a median follow-up period of 228 weeks (range 16–1763 weeks).

The 92 patients with risk factors also received a maximum prescribed weekly dosage MTX of 12.5 mg/week (range 0–35) with a median cumulative MTX dose of 2256 mg (0–20 235) during a median follow-up period of 219 weeks (range 16–1763 weeks) (this includes baseline biopsies).

### Histology

Patients underwent between 1 and 9 (median 2) biopsies per person, and collectively 278 liver biopsies were sampled during the study period. Fifty-six biopsies were performed at baseline. Table 1 lists the distribution of the various Roenigk scores among all liver biopsies taken as well as those taken at baseline. Eighty-eight patients (71%) did not develop histological abnormalities during treatment (Roenigk grade I), 18 patients (14%) had Roenigk grade II, 15 had (12%) grade IIIa, two had (2%) grade IIIB and two (2%) had grade IV. (Table 1.) Characteristics about the patients without histological abnormalities during treatment are shown in Table 2.

A total of 69 patients had two or more liver biopsies. Intra-individual comparison between first and last biopsy demonstrated that the histological findings remained unchanged in 72%, improved in 3% of patients but deteriorated in a further 25%.

	Roenigk (%)					<i>P</i> *
	1	2	3a	3b	4	
<b>(a) Liver biopsies</b>						
Baseline† biopsy ( <i>n</i> = 55)	44 (80)	8 (15)	2 (4)	0	1 (2)	0.11
Follow-up biopsy ( <i>n</i> = 223)	154 (69)	31 (14)	33 (15)	4 (2)	1(0,4)	
Total biopsies ( <i>n</i> = 278)	198	39	35	4	2	
<b>(b) Patients</b>						
No risk factors ( <i>n</i> = 34)	29 (85)	5 (15)	0 (0)	0	0	0.19
Overweight ( <i>n</i> = 38)‡	24 (63)	10 (26)	2 (5)	1 (3)	1 (3)	0.01
Alcohol use ( <i>n</i> = 62)	49 (79)	8 (13)	4 (6)	0	1 (2)	0.67
DM ( <i>n</i> = 9)	6 (67)	1 (11)	0	1 (11)	1 (11)	0.42

MTX, methotrexate; DM, diabetes mellitus.

\* Chi-square: Roenigk = 1 vs. Roenigk > 1 (Fisher's exact where appropriate).

† Baseline = cum dose MTX ≤ 600 mg.

‡ Missing values = 20.

	Patients without histologic injury ( <i>n</i> = 88) (71%)	<b>Table 2. Characteristics of patients with no histologic abnormalities during treatment</b>
Median maximum weekly dosage MTX	12.5 mg (range 0–25)	
Median cumulative dosage MTX	2352 mg (range 0–16722)	
Median duration follow-up period	214 weeks (range –238–1761)	
<b>Risk factors</b>		
Alcohol	37	
Alcohol > 14U/week	5	
DM	8	
Adipositas	25	

MTX, methotrexate; DM, diabetes mellitus.

Table 1 also lists the distribution of Roenigk score per risk factor. Those with overweight and/or DM had higher Roenigk scores in comparison to patients without risk factors. In contrast, we did not observe an adverse effect of alcohol use on histological scores.

### Association between cumulative dose MTX and liver injury

We observed that histological progression to Roenigk grade 2 or higher, mostly occurred when the cumulative MTX dose was between 1500 and 6000 mg. Higher dosages did not lead to increased liver injury as the prevalence of progression to a higher Roenigk

score levelled beyond a cumulative MTX dosage of 6000 mg (Figure 1.)

### Risk factors, cumulative doses and development of liver damage

Next, we assessed whether biopsies taken from patients with risk factors (*n* = 190) progressed faster to higher Roenigk score in comparison to those biopsies from patients without known risk factors (*n* = 88) (Figure 2.) The presence of obesity, and/or DM led to progression to a higher Roenigk score (Roenigk grade >1), at earlier cumulative MTX dosages, while alcohol did not.

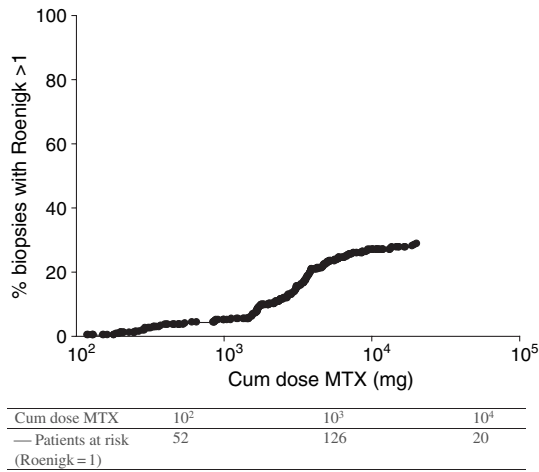


Figure 1. Percentage of biopsies with Roenigk >1.

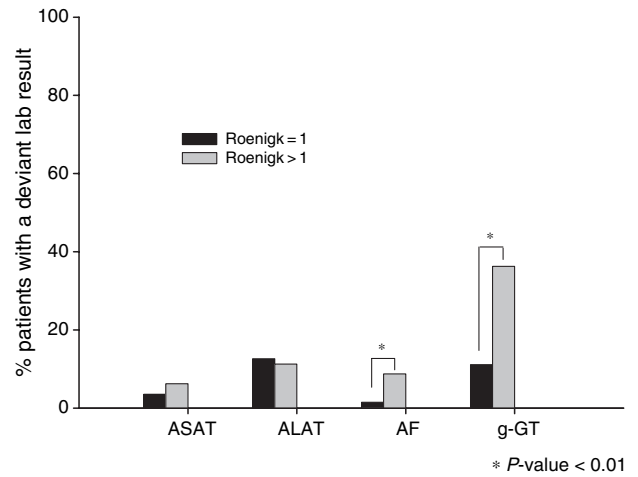


Figure 3. Deviant lab results.

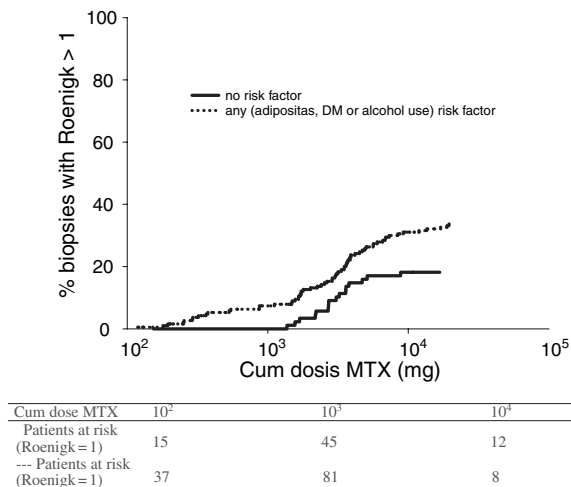


Figure 2. Percentage of biopsies with liver injury (any risk factor vs. none).

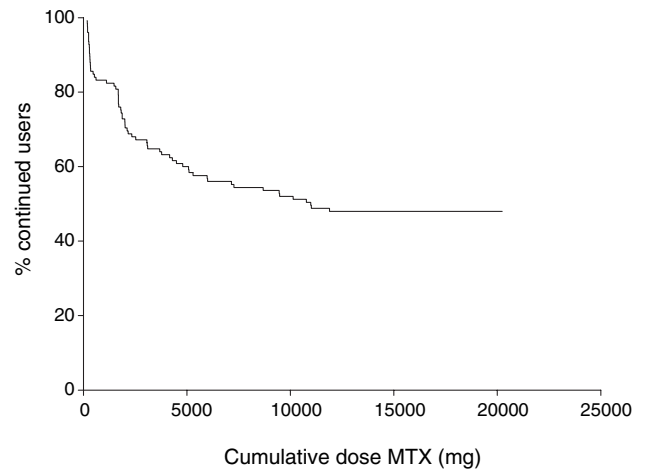


Figure 4. Percentage of continued users.

### Liver enzymes and histological changes

Next, we examined the effect of the Roenigk score on the levels of four different liver enzyme tests ( $\gamma$ -GT, AP, ASAT and ALAT). We observed that a higher proportion of patients who had had a liver biopsy with a Roenigk classification  $\geq 2$  had  $\gamma$ -GT beyond the upper limit of normal (Odds ratio 1.80; 95% CI 1.30–2.49) (Figure 3.) This contrasted with ASAT and ALAT serum concentrations that did not correlate with Roenigk scores. On the other hand, patients with Roenigk  $\geq 2$  had significantly higher AP and ASAT compared with

those with Roenigk = 1, but values were well within normal range. All other laboratory tests did not correlate with the histological findings.

### Continuation of therapy

Sixty patients (48%) still continued MTX at the end of the studied period. The remaining 52% (65 patients) had stopped using MTX. Most often MTX was stopped relatively early after initiation of MTX, and most patients who had stopped did so at cumulative doses of <5000 mg. (Figure 4). Three patients died during therapy but no deaths were attributed to use of MTX or liver disease related. Reasons mentioned for discontinuation are summarized in Table 3.

**Table 3.** Reasons for discontinuation methotrexate (MTX) treatment

Reasons to stop MTX therapy	<i>n</i> = 65 (%)
Persisting abnormal liver function tests	13 (20)
Dissatisfying results on MTX therapy	9 (14)
Strongly improved skin	9 (14)
Subjective side-effects	7 (11)
Histological abnormalities	6 (9)
Others	21 (32)

## Clinical outcome

### Biopsies

Pathology results have a limited effect on clinical treatment. Fibrosis/cirrhosis (Roenigk  $\geq$  3a) was detected in 41 biopsies from 24 patients at some point during MTX treatment. Despite these pathological findings, MTX therapy was continued in 26/41 biopsies. Histological results led to discontinuation in only 6/41 biopsies. In only 9/41 biopsies, treatment was stopped regardless of the results from pathological examination of the liver biopsy. Repeated liver biopsy in Roenigk 3 patients was useful in the clinical treatment.

### Patients

In 16 patients, liver biopsy was repeated after finding significant abnormalities at earlier assessments (average of 14 months). Histology did not change in five, while it improved in 10 patients (in five patients to a stage without fibrosis). The liver histology worsened in a single patient.

## DISCUSSION

One of our aims was to establish the prevalence of MTX-induced liver injury because the large variation in previous literature.<sup>2, 9, 10</sup> In this substantial cohort of 278 liver biopsies taken over 30 years MTX-induced liver fibrosis ( $\geq$ Roenigk grade 3a) was only seen in 15% (19 patients) of our population. Progression to a higher stage of liver injury mostly occurred at a cumulative dose-range between 1500 and 6000 mg, which translates to at least 2 years of MTX treatment at 15 mg weekly. Progressive development of liver damage was infrequent above 6000 mg MTX (after about 8 years with 15 mg weekly) when patients have

proven to tolerate high cumulative dosages MTX without previously obtaining pathological hepatic differences. In contrast to our data, others found that the cumulative probability of developing advanced hepatic fibrosis doubled after 5000 mg.<sup>9, 10</sup>

We carefully monitored our patients for MTX induced toxicity by regular liver biopsies. As such our practice mirrors the international guidelines and as such the data can be viewed as a field study for implementation of these guidelines.<sup>13, 14</sup> As a consequence, it is unlikely that we missed patients who were treated with MTX and actually developed MTX-hepatotoxicity.

Most long-term observational studies in rheumatoid arthritis indicate that toxicities, rather than lack of efficacy, are the most common cause of discontinuing MTX.<sup>15</sup> This appears to be in contrast with the situation in psoriasis. We and others found that low-dose, long-term MTX treatment seems to be relatively safe and effective, and that undesired side effects lead to discontinuation in a minority.<sup>16</sup>

Another aim of our study was to delineate potential contributing factors to the development of MTX-induced liver injury. Risk factors play an important role in the development of liver fibrosis. At any given cumulative MTX dosage, patients with obesity and or DM had a significantly worse liver histology compared with patients without these risk factors. The presence of fatty infiltration (steatosis) of the liver is highly prevalent among obese and or diabetic patients, and probably plays a role. This reminds us of the situation in non-alcoholic steatohepatitis (NASH) where the presence of steatosis is associated with hepatitis with necroinflammation and pericellular fibrosis.<sup>17</sup> Indeed, a recent case series showed that the presence of NASH in psoriatic patients contributes to the hepatotoxicity of MTX.<sup>18</sup> These data suggest that histological monitoring of MTX toxicity could be tailored to obese patients with or without DM. Other researchers have emphasized the role of alcohol consumption as a risk factor for MTX-induced fibrosis.<sup>10</sup> Surprisingly, we could not confirm this finding in our study and is possible that the association is indeed absent in our cohort. On the other hand, the retrospective nature of our study carries inherent limitations, which may hamper the interpretation of the data on alcohol use. The retrospective nature of our study could have resulted in the under-reporting of risk factors. But even so, we found a significant correlation between the percentage of patients with liver disease and the presence of any risk factors. On the other hand, we cannot rule

out the possibility of entry bias, e.g. that physicians do not consider MTX as a therapeutic option for psoriatic patients with a history of alcohol (ab)use.

We detected a correlation between grades of histological change and  $\gamma$ -GT. In 40% of patients with Roenigk >1,  $\gamma$ -GT was significantly elevated. In contrast, ASAT, ALAT, and AP serum concentrations were not elevated beyond the normal range for any of the Roenigk grades, which fits with other literature data.<sup>10, 19</sup> One of the clinical consequences is that as a whole, normal liver enzymes do not exclude progression of liver injury. Conversely, elevated liver enzymes do not necessarily correlate with MTX induced hepatotoxicity. In this respect, liver enzymes serve as a poor predictor for MTX induced liver injury. One exception may be  $\gamma$ -GT levels, as our data are consistent with the notion that they may serve as a marker for MTX hepatotoxicity. Regular PIIINP measurements might be of additional value in monitoring these patients.<sup>11, 12</sup>

In our study, patients with advanced MTX-induced fibrosis and cirrhosis (Roenigk  $\geq$  3a) ran a benign course. Liver histology did not deteriorate (and some-

times even improved) under ongoing MTX therapy. Our relatively large series that documented a large number of biopsies over a 20-year period confirms those of two small studies.<sup>9, 10</sup>

In conclusion, MTX-induced fibrosis occurs in a minority of patients. Liver histology deterioration is seen mostly at cumulative MTX dosages between 1500 and 6000 mg, which call for close monitoring at this stage. Our study confirms that normal liver function tests do not guarantee normal liver histology. Furthermore, risk factors play an important role in the development of liver injury and they should be taken into account before the decision is made to start MTX. Obese patients with or without DM should be monitored closely by regular liver biopsies. From our studies it is difficult to indicate a correct interval, but it is probably more often than stated in the guidelines.

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