Liver International ISSN 1478-3223

CLINICAL STUDIES

Biochemical and biophysical assessment of MTX-induced liver fibrosis in psoriasis patients: Fibrotest predicts the presence and Fibroscan® predicts the absence of significant liver fibrosis

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Keywords
liver fibrosis and non-invasive methods – methotrexate – psoriasis

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Received 26 September 2006
accepted 22 January 2007

DOI:10.1111/j.1478-3231.2007.01489.x

Abstract

Background: Methotrexate (MTX) use is associated with hepatic fibrosis in psoriasis patients. To monitor this serial liver biopsies were performed. The Fibroscan® and the Fibrotest are two novel, non-invasive methods that might be able to assess MTX-induced hepatic fibrosis. *Aim:* Evaluating the accuracy and feasibility of the Fibroscan® and Fibrotest to detect *significant* MTX-induced liver fibrosis in psoriasis patients. *Methods:* We assessed 24 psoriasis patients who had a recent liver biopsy during MTX use. The results from the Fibroscan® and Fibrotest were compared with liver histology. *Results:* Fibroscan® values ($n = 20$) ranged between 3.3 and 18.4 kPa (median value 6.4 kPa) and correctly identified 88% of the patients without significant liver fibrosis (Metavir score $<$ F2, Fibroscan® $\leq$ 7.1 kPa). The Fibrotest identified 83% of the patients *with* significant liver fibrosis (Metavir score $\geq$F2, Fibrotest >0.31). *Conclusion:* In this population, Fibrotest accurately predicted the presence of significant liver fibrosis while the Fibroscan® accurately predicted the *absence* of significant liver fibrosis in MTX users. This suggests that a combination of Fibrotest and Fibroscan® should prospectively be evaluated in monitoring and detecting significant MTX-induced liver fibrosis in psoriasis patients.

Methotrexate (MTX) is the most commonly prescribed systemic drug for severe psoriasis. There is substantial evidence to suggest that it acts by inhibiting DNA synthesis. Probably as a consequence, it possesses potent anti-inflammatory effects on T-cell mediated immune responses as it inhibits proliferation or induces apoptosis in activated T-cells and blocks abnormal rapid epidermal cell proliferation, both responsible for the characteristic skin lesions in psoriasis (1). Low-dose treatment with MTX is regarded as an effective therapy for psoriasis.

However, one of the dreaded long-term side effects includes liver fibrosis and cirrhosis because of MTX hepatotoxicity. Therefore, frequent evaluation of liver enzymes and periodic liver biopsy are recommended during therapy. Specifically, dermatologic guidelines call for a liver biopsy at every 1500 mg cumulative dose (2). Although liver biopsy is considered as the golden standard for the assessment of histological changes (3), complications such as postprocedural pain and bleeding limit its clinical use, with a 0.01–0.1% risk of mortality (4).

Therefore, there is a pressing need for alternative, non-invasive and reliable methods to monitor MTX-induced liver injury in psoriasis patients. Non-invasive tools like blood tests and Fibroscan® are being used in patients with chronic liver diseases (5). This study evaluates two non-invasive methods for detection of significant liver fibrosis in MTX-treated psoriasis patients.

The first test is a recently developed patented artificial intelligence algorithm (Fibrotest) that has been validated to detect fibrosis in hepatitis C patients (6). It is a biochemical fibrosis index that needs input values of five serum markers and is corrected for age and sex, leading to a composite value (between 0 and 1) to determine the presence of significant liver fibrosis (7, 8).

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As a second test, we measured liver elasticity, using one-dimensional transient elastography, the Fibroscan®. As liver stiffness roughly correlates with the degree of hepatic fibrosis, it can serve as a non-invasive test for fibrosis. This latter approach seems promising for the assessment of fibrosis and cirrhosis in patients with hepatitis C and a fine correlation has been established between significant fibrosis (F2 according to the Metavir histology grade) and elastography (Fibroscan®) outcome (7–10).

We compared the test characteristics of Fibrotest and Fibroscan® in a cohort of psoriasis patients treated with MTX and compared the results with the histology of the liver. Our ultimate aim was to identify effective test alternatives for a liver biopsy.

Patients and methods

Patients

Our study population was drawn from 60 psoriasis patients who were on MTX treatment at the end of 2005. Patients who underwent a percutaneous liver biopsy within 18 months of Fibrotest (Biopredictive, Paris, France) and Fibroscan® (Echosens, Paris, France), as part of their regular MTX monitoring procedure, were eligible for inclusion in this study. We contacted 34 patients and asked them to participate in the study. Twenty-four patients agreed to participate and gave their written informed consent. We collected information about the risk factors for liver damage such as excessive alcohol consumption, obesity, diabetes mellitus and chronic viral hepatitis using a structured interview.

Histological assessment

Liver biopsies for histological evaluation of MTX effects were obtained using a 1.6 mm diameter Menghini-type needle via the right intercostal approach with local lidocaine anaesthesia (Hepafix, Braun, Melsungen, Germany). The biopsy site was marked by ultrasound examination. There were no complications. Biopsy specimens were fixed in 4% formaldehyde and subsequently embedded in 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 biopsies were revised and analysed by two independent investigators (M. A. M. B and J. S.) and verified by an experienced pathologist (J. H. v. K.). Disagreements were resolved by consensus. All liver biopsy specimens were scored according to the Metavir histology score (11). Furthermore, the number of portal tracts were noted and all liver biopsies (previously taken liver biopsies included), as well as their results (Metavir score) were reported. A consistent result was defined as a Roenigk score that was stable over time or increased gradually. An inconsistent result was defined as a decrease of more than two grades or a random pattern with an increase and later a decrease with one grade on Roenigk classification.

Non-invasive measurements of fibrosis

Fibrotest

Blood samples were collected to analyse five serum markers included in the Fibrotest score. For this purpose, we measured the following biological parameters: γ-glutamyl-transpeptidase (γ-GT), total bilirubin level, α2-macroglobulin, apolipoprotein A1 and haptoglobin. In addition, we measured albumine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and bilirubine.

Measurements were performed immediately on fresh obtained samples using validated methods. The results were used as input for the Fibrotest. This is a patented artificial intelligence algorithm that generates a measure of liver fibrosis. It provides a numeric quantitative estimate of liver fibrosis ranging from 0.00 to 1.00. It is a continuous linear biochemical assessment of fibrosis stage, which corresponds with the stages F0–F4 of the Metavir scoring system. Based on the literature data, we chose a cut-off value of 0.31 to identify patients with significant fibrosis ($\geq$F2) (8, 12, 13). Fibrotest results were kindly provided by T. Poynard, University Paris VI, Paris, France (www.biopredictive.com).

Fibroscan®

We performed the Fibroscan® measurements on the same day as the measurements of the biological parameters that are used for the Fibrotest. The Fibroscan® is an ultrasound transducer that generates vibrations that cause a slow elastic shear wave. The propagation and velocity of the wave in the liver are tracked by pulse-echo ultrasound and correlate to tissue stiffness. Measurements were performed on the right lobe of the liver, at the same target area for liver biopsy. The procedure was performed through the intercostal space while the patients were lying on their backs with their arms in maximal abduction behind their heads.

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Each patient underwent a series of 10 validated electrographic measures. The success rate was calculated as the number of validated measurements divided by the total number of measurements. The results were expressed in kilopascals (kPa) and according to Castera a cut-off value of 7.1 kPa was chosen to identify patients with significant fibrosis ($\geq$F2) (7). The median value was considered to be representative for the elasticity of the liver. Only procedures with 10 validated measurements and a success rate of at least 60% were considered to be reliable. Fibroscan® assessments were performed by an experienced physician (R. J. d. K.) who was strictly blinded to the histological outcome.

Cumulative MTX dose and the three tests

We next went on to test the effect of cumulative MTX usage on the presence of liver fibrosis as assessed by the three tests under study. The presence of liver fibrosis was defined as the presence of Metavir $\geq$F2 on a liver biopsy specimen.

Statistical analysis

Frequency tables were provided for both demographic and clinical information (cumulative MTX dose, alcohol consumption and the presence of diabetes mellitus). The diagnostic performance of the non-invasive methods for liver fibrosis was measured as sensitivity, specificity, positive predictive value, negative predictive value and accuracy for the presence and absence of significant fibrosis (>F2). The results of both tests were drawn in a scatterplot with regression lines of individual Metavir scores in relationship with the results of Fibrotest and Fibroscan®. Furthermore, we compared the two non-invasive tests using the non-parametric Spearman correlation test, both continuously and subdivided for Metavir score. Finally, the relation between the cumulative MTX dose and the results of the three tests was tested with the Wilcoxon's rank sum analysis. All statistical analyses were undertaken with SAS statistical software (SAS institute, Minneapolis, MN, USA), version 8.2.

Results

Demographics

We included 24 psoriasis patients (13 females, 11 males) with a mean age of 55 years (range 34–73). The reasons for performing a liver biopsy were as follows: all patients underwent a liver biopsy in keeping with the guideline after a median dosage of 1635 mg MTX (range 162–2354 mg). Only one patient had elevated liver enzymes more than twice the upper limit. Sixteen patients were biopsied before.

Table 1. Main demographic and clinical features of the studied population

	N = 24
Male gender (%)	11 (46)
Median age, years (range)	55 (34–73)
Median body mass index, kg/m^2 (range)	26 (20–38)
Diabetes mellitus (%)	4 (17)
Median cum dose MTX, mg (range)	3352 (314–20 235)
Median Fibroscan, kPa (range)	6.4 (3.3–18.4)
Median Fibrotest (range)	0.32 (0.06–0.93)

MTX, methotrexate.

Table 2. Three patients with inconsistent histology

Patients	Histology according to Roenigk score				
1	R1	R3a	R1	R3a	R2
2	R1	R3a	R1	R1	
3	R1	R1	R3a	R2	R1

The median body mass index was 26 kg/m^2 (range 20–38 kg/m^2) and 14 patients were considered to be overweight (defined as body mass index >25 kg/m^2). Ten patients consumed alcohol, while a single patient was an excessive consumer (>14 U/week) and four patients had diabetes mellitus. Patients received a median cumulative MTX dose of 3352 mg (314–20 235) during a median follow-up period of 346 weeks (111–2162). Four patients had a cumulative dose of more than 5000 g. Table 1 shows the main demographic and clinical features of the population studied.

Histology

In the population studied, the median biopsy length was 30 mm (range 10–60 mm). Five patients (21%) had a liver biopsy specimen shorter than 15 mm. The mean number of portal tracts was 22 (SD 9). Scoring with the Metavir classification resulted in five patients with a liver biopsy classified as F0, 13 as F1, 4 as F2, 1 as F3 and 1 as F4.

Sixteen patients had more than one liver biopsy. Thirteen patients (81%) had consistent pathological scoring of their liver biopsies over the years. In only three patients the histology results varied between biopsies (Table 2).

In patient 3, the time period between the first two biopsies was 6 months. Treatment with MTX was discontinued then, but was restarted 14 years later. Histology of the follow-up biopsy 18 months later

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Table 3. Diagnostic value of fibrotest and fibroscan in detecting patients with clinically significant fibrosis (METAVIR fibrosis score F2 or greater)

	Fibrotest ≥F2	Fibroscan ≥F2
Optimal cut-off	0.31	7.1 kPa
Sensitivity (%)	83	50
Specificity (%)	61	88
Accuracy (%)	67	70
Positive predictive value (%)	42	33
Negative predictive value (%)	92	86

resulted in an R1. In patient 2, the time period between the second and third biopsy was half a year. The last two biopsies were consistent and the time period between them was 3 years. In patient 1, time periods between biopsies were 3 years, 8 months, 4.5 years and 10 months. The median length of the consistent biopsies was 25 mm (8–85 mm) vs a median length of 24.5 mm (9–45 mm) of the inconsistent biopsies.

Fibrotest

Fibrotest values ($n=24$) ranged between 0.06 and 0.93 (median value 0.32). Table 3 shows the diagnostic test properties for the Fibrotest with respect to detection of significant liver fibrosis (Metavir fibrosis score ≥F2). Figure 1 shows the agreement in Metavir score between the fibrotest and histology. The results show that Fibrotest correctly identifies those patients with significant fibrosis in 83% of the cases, indicating good screening properties (Metavir fibrosis score ≥F2, Fibrotest < 0.31).

Fibroscan®

The mean number of measurements per patient was 12.1 ± 2 (range: 10–18) to obtain 10 evaluable values. The success rate of liver elastography measurements was 85 ± 12%. The total procedure failed in four (17%) patients because of the presence of obesity. These patients had a body mass index (BMI) of 35.4, 32.4, 38.2 and 32.7 kg/m^2, Metavir histology scores F0, F1, F3 and F2 and Metavir Fibrotest scores F0, F3, F3 and F4.

Liver stiffness measurements ($n=20$) ranged between 3.3 and 18.4 kPa (median value 6.4 kPa). Table 3 shows the diagnostic test properties for the Fibroscan® in our study population with respect to detection of significant liver fibrosis (Metavir fibrosis score ≥F2). The test detected 88% of the patients without significant fibrosis (Metavir fibrosis score < F2) in this population, indicating good diagnostic properties. Figure 1 shows the agreement in Metavir score between the fibrotest fibroscan and histology.

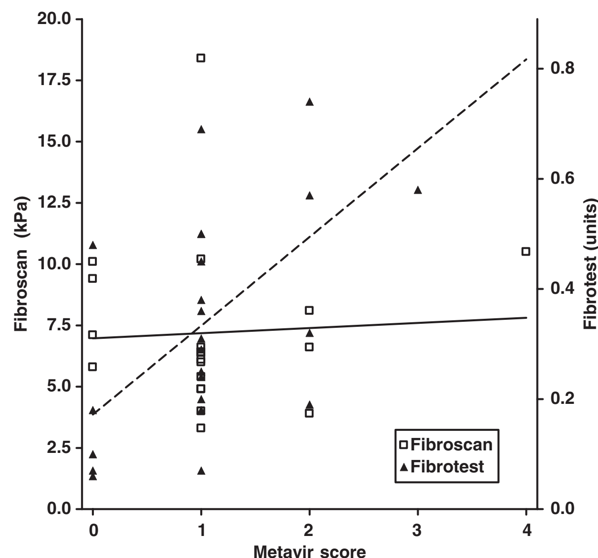

Fig. 1. Scatterplot representing all observations and regression lines for both Fibrotest (dashed line) and Fibroscan® (regular line).

Table 4. Relationship cum MTX dose vs test

	Median cum dose MTX, mg (range)		
	Metavir < F2	Metavir ≥F2	*P**
Histology	3879 (314–20 235)	2791 (1445–3882)	0.45
Fibroscan	4396 (314–18 502)	4467 (1400–15 173)	0.40
Fibrotest	2166 (314–6000)	4026 (1445–20 235)	0.10

*Tested with the Wilcoxon's rank-sum test.
MTX, methotrexate.

Relationship cumulative MTX dose results of three tests

There was no effect of cumulative MTX dosing on the presence of liver fibrosis. Patients with significant liver fibrosis (Metavir fibrosis score ≥F2) did not have a higher cumulative MTX dose than patients without significant liver fibrosis as assessed by any of the three tests (Table 4).

Discordance between fibrotest and fibroscan®

In nine patients, Fibroscan® and Fibrotest resulted in different Metavir scores with a discordance of two stages. In four of them, the total Fibroscan® procedure failed because of the presence of obesity. In the remaining five, biopsy length was significantly shorter

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compared with the biopsy length of the remaining patients. There was no significant difference in BMI between those patients. Other potential confounders for failure of Fibrotest like Gilbert, haemolysis and acute inflammation were ruled out.

Discussion

Our aim was to evaluate the accuracy and feasibility of two non-invasive methods, Fibrotest and Fibroscan®, in the detection of significant MTX-induced liver fibrosis, using liver biopsy as the gold standard, in a cohort of psoriasis patients. Specifically, in our population we found that the Fibroscan® is a good diagnostic test for *excluding* significant liver fibrosis ($\geq$F2 on the Metavir score) but less equipped in *detecting* significant fibrosis ($\geq$F2) in this population. On the other hand, we found that the fibrotest is a good screening test in detecting significant fibrosis. This suggests that a complementary use of both Fibrotest and Fibroscan® is beneficial in establishing the grade of liver fibrosis in MTX-induced liver fibrosis in psoriasis patients and might be instrumental in reducing the need for liver biopsies.

The Fibroscan® results of this study accord with those collected from the literature. It had a comparable accuracy in excluding significant liver fibrosis in a sample of patients with hepatitis C, but also in a series of patients with Crohn's disease on MTX treatment (7, 8, 10, 14, 15).

Our sample contained only a few patients with F3 or F4 liver fibrosis. Thus, we mainly based our conclusion on patients with a Metavir histology grade F0–F2. However, in another study fibroscan® was prospectively investigated in a large cohort of patients with chronic liver disease of various aetiologies including 144 (20%) of patients with F3/F4 fibrosis. There diagnostic performances for $\geq$F2 were comparable to our results, sensitivity 50% vs 64% and specificity 85% vs 88% (9).

Because continuous long-term use of MTX is associated with hepatotoxicity, frequent evaluation of liver function tests and periodic liver biopsy are recommended during therapy. Specifically, dermatologic guidelines call for a liver biopsy at every 1500 mg cumulative dose (2). Conventional liver enzyme tests (ALT or g-GT) correlate poorly with histological changes (3). Therefore, liver biopsy is considered as the gold standard method in the assessment of histological changes. A recent study by our group about liver injury in long-term MTX treatment in 125 patients with psoriasis with 278 liver biopsies found that 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. Furthermore, in this study, ASAT, ALAT and AF serum concentrations were not elevated beyond the normal range for any of the Roenigk grades (16).

A liver biopsy has some important limitations. It is an invasive procedure and carries several known risk factors such as pain, localized bleeding and less often pneumothorax, haemothorax, bile peritonitis, haemobilia, and inadvertent puncture of the kidney or intestine (17, 18). The complication risk of a liver biopsy is approximately 1–2%, with a 0.01–0.1% risk of mortality (4). Furthermore, it causes anxiety, which is an issue, as most psoriasis patients on the MTX require repeated liver biopsy during the course of their treatment. Another limitation of the liver biopsy is the possible sampling error, an intra- and inter- pathologist inconsistency in observations and that there are discontinuous and hence semi-quantitative histological scoring systems (2, 3, 7, 12, 15, 17, 19, 20). In three patients, histology results varied considerably between biopsies (inconsistent). In one patient, this might have been caused by the improvement of liver tissue during a long period in which the patient had not been treated with MTX, which favours the hypothesis that MTX-induced injury is reversible and in another patient the last two biopsies were consistent. To conclude, only in one patient histology results might have reflected a sampling error of liver biopsy.

There is an urgent need for alternative, non-invasive and reliable methods of monitoring and detecting MTX-induced liver injury in psoriasis patients. Fibrotest has been studied in patients with hepatitis B and C, alcoholic liver disease and in patients with non-alcoholic fatty liver disease (21–27). Previous studies found that non-invasive assessment of liver stiffness by use of the Fibroscan® is a reliable test to detect significant fibrosis or cirrhosis (8–10, 14, 15). The positive predictive value for detecting significant fibrosis is only 76%, but the combined use of Fibroscan® and Fibrotest does allow the evaluation of hepatitis C liver fibrosis (7, 28). In one study the stepwise combination of non-invasive markers of liver fibrosis improved the diagnostic performance in chronic hepatitis C patients. The need for liver biopsy in that study was reduced by 50–70% (28).

The main advantage of Fibroscan® compared with the Fibrotest is that it provides a direct quantative physical parameter. In obese patients, the fatty thoracic belt attenuates both elastic waves and ultrasound, rendering it difficult or even impossible (15). In our study, 12 patients suffered from obesity and in three of

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them the Fibroscan® failed. Psoriasis patients tend to be more obese compared with the normal population, with a prevalence of 34% in psoriasis patients vs 18% in the normal population (29), which might make this approach less successful in detecting MTX-induced liver injury in psoriatic patients.

In the search for a non-invasive method to monitor and detect MTX–induced liver injury, Amino terminal peptide of type III procollagen (PIIINP) was studied before (4, 30). PIIINP may serve as an alternative for routinely performed liver biopsies when measured levels at regular intervals are consistently normal in otherwise healthy adults. This is because PIIINP is not an organ-specific protein and may be raised in children and various pathological states associated with development of fibrosis including inflammatory arthritis, scleroderma, hyperthyroidism, scar formation following burns and myocardial infarction (4, 30). The advantage that Fibrotest offers over PIIINP is that one cross-sectional measurement is sufficient to judge whether liver fibrosis is present.

A limitation of our study is the relatively small study population, which may limit the precision of the effect estimates. Furthermore, as mentioned above, a liver biopsy, although considered as the gold standard, can have sampling variability problems. This might have been the case in our study because some patients had small liver biopsies. However, in our study liver biopsy length compares favourably with that of some other studies (9, 31) and only one patient had a liver biopsy with less than 10 portal tracts.

The pattern of results indeed supports the notion that the diagnostic test properties of Fibroscan® and screening properties of Fibrotest should be evaluated in a prospective manner. Specifically, we would like to propose a prospective controlled trial that evaluates the combined use of non-invasive markers such as the Fibrotest, Fibrotest and PIIINP in the detection of MTX-associated fibrosis (7, 28).

Although it should be kept in mind that this is a small pilot study, based on our results we conclude that the Fibrotest seems to be good in *detecting and* the Fibroscan® seems to be good in *excluding* significant MTX-induced liver fibrosis ($F \geq 2$) in patients with psoriasis treated with MTX. This suggests that the combined use of Fibrotest and Fibroscan® may be beneficial in establishing the grade of liver fibrosis in MTX-induced liver fibrosis in psoriasis patients.

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