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Prospective Comparison of Transient Elastography, Liver Biochemistries, and APRI for the Assessment of Methotrexate Induced Liver Damage in Patients with Dermatologic or Rheumatic Diseases

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ABSTRACT

Background: Concerns about methotrexate-induced liver damage in patients with psoriasis and rheumatoid arthritis have led to the recommendation of serial liver biopsies to monitor the progression of liver injury. However, liver biopsies may be associated with life-threatening complications. There is a need for accurate noninvasive methods in assessing the degree of liver damage. The present study assessed the performance of transient elastography (Fibroscan®) for the prediction of liver fibrosis in patients with psoriasis or rheumatoid arthritis on methotrexate treatment, in comparison with liver biochemistries and the aspartate aminotransferase (AST) to platelets ratio index (APRI).

Methods: We prospectively enrolled outpatients who received cumulative doses of methotrexate more than 1500 mg and gave their written informed consent for liver biopsy examination between November 2007 and November 2008. For each patient, biochemical and hematologic determination, and liver stiffness measurement using Fibroscan followed by liver biopsy were performed on the same day. All liver biopsy specimens were analyzed independently by one experienced pathologist with blinded.

Results: Seventeen patients with psoriasis and one patient with rheumatoid arthritis were recruited. Patients had a mean age (± SEM) of 46.3 ± 2.8 years, 67% were female, and their mean body mass index was 22.9 ± 0.8 kg/m^2. The mean serum alanine aminotransferase (ALT), the mean AST/ALT ratio, and the APRI score were 23 ± 2 units/L, 1.10 ± 0.06, and 0.23 ± 0.03, respectively. The mean value of Fibroscan was 6.2 ± 0.5 kPa. According to the Roenigk classification, 6 biopsy specimens were classified as grade II, 7 as grade IIIa, 4 as grade IIIb, and 1 as grade IV. The areas under the receiver operating characteristic curve of Fibroscan, ALT, AST/ALT ratio, and APRI values for Roenigk grade ≥ IIIa were 0.97, 0.76, 0.71, and 0.61, and for Roenigk grade ≥ IIIb were 0.88, 0.52, 0.62, and 0.60, respectively. The optimal cutoff value of Fibroscan for the prediction of significant fibrosis (grade IIIa) was 5.2 kPa, which yielded a sensitivity of 83%, specificity of 100%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 75%. Similarly, the optimal cutoff value of Fibroscan for the prediction of advanced fibrosis (grade IIIb) was 7.6 kPa, which provided a sensitivity of 80%, specificity of 92%, PPV of 80%, and NPV of 92%.

Conclusion: Fibroscan is a rapid, noninvasive, and reproducible method for assessing methotrexate-induced liver damage, with better performance than liver biochemistries or APRI. Fibroscan help avoid the need for liver biopsy in most patients with dermatologic or rheumatic diseases on methotrexate therapy.

Key words : Fibroscan, transient elastography, liver fibrosis, methotrexate-induced liver damage

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INTRODUCTION

Methotrexate (MTX) was initially used as a cytotoxic chemotherapy for leukemia and other malignancies, and is a commonly prescribed systemic drug for the treatment of severe psoriasis. There is substantial evidence to suggest that MTX acts by inhibiting DNA synthesis, while possessing also potent anti-inflammatory effect on T-cell mediated immune responses by inhibiting proliferation or inducing apoptosis in activated T-cell as well as blocking abnormal rapid epidermal cell proliferation[1-3]. Subsequently, it has become a valuable second-line agent in the treatment of rheumatoid arthritis (RA)[3]. MTX has also been shown to be effective in the treatment, acting as a disease-modifying anti-rheumatic drug (DMARD)[3]. Low-dose treatment with MTX is also an effective therapy for psoriasis and RA.

The most common side effects of MTX are gastrointestinal in nature, such as anorexia, nausea, stomatitis and diarrhea. More serious adverse effects include hepatotoxicity, bone marrow suppression, pulmonary hypersensitivity, opportunistic infections, anaphylactoid reactions, and possible neoplasms[1-3]. A meta-analysis involving 15 studies on psoriasis and on rheumatoid arthritis revealed that the cumulative dose of methotrexate for each gram taken correlated with a 7% chance of progression of liver histology[7]. Concerns about methotrexate-induced liver damage in such patients have led to the recommendation of serial liver biopsies to monitor the progression of liver injury, as

Table 1.

Recommendations for monitoring MTX-induced hepatotoxicity in psoriasis patients.[5,6]

Pretreatment	During therapy
Laboratory tests Obtain routine liver function tests, complete blood count, urinalysis, serum BUN and creatinine, creatinine clearance	*Laboratory tests* Obtain liver function tests at 2 months interval (1 week after last MTX dose). If significant and persistent abnormalities are seen, withhold MTX for 1-2 weeks and repeat tests. If significant abnormalities persist 2-3 months, consider liver biopsy.
Liver biopsy Recommended in patients with risk factors for liver disease (ie, past or current alcohol intake of ≥ 1-2 drinks per day, abnormal liver function tests, history of liver disease, history of intravenous drug use, family history of inheritable liver disease, diabetes, obesity, history of significant exposure to known hepatotoxic drugs).	*Liver biopsy* Recommended in patients with normal histology, physical examination and liver function tests, and no risk factors for liver disease. Liver biopsy is recommended after a cumulative MTX dose of approximately 1,500 mg. If biopsy is normal, repeat at subsequent 1,000-1,500 mg cumulative dose intervals.

Recommendations for monitoring hepatotoxicity in RA patients during MTX therapy.[4]

Pretreatment	During therapy
Laboratory tests Obtain routines liver function tests, hepatitis B and C serology, complete blood count, serum BUN and serum creatinine.	*Laboratory tests* Monitor serum AST, ALT and albumin at 1-2 month intervals. If persistent elevations are seen, change non-steroidal anti-inflammatory drugs, or reduce MTX dosage, or discontinue temporarily. If enzyme elevations persist despite these measures and other etiologies for the elevations have been excluded, consider liver biopsy.
Liver biopsy Recommended in patients with prior excessive alcohol consumption, persistently abnormal baseline AST values, or chronic hepatitis B or hepatitis C infection.	*Liver biopsy* Liver biopsy is recommended if 5/9 AST determinations within a given 12-month period (6/12 if tests are performed monthly) are above the upper limit of normal, or if there is a decline in serum albumin to less than normal levels in the setting of well-controlled RA.

evident in an established guideline to monitor MTX-induced liver injury in the treatment of psoriasis and RA (Table 1). However, guidelines for monitoring patients for MTX-induced hepatotoxicity during treatment differ considerably between the two diseases.

Liver biopsy may be associated with potential life-threatening complications, sampling errors, and intra- and inter-observer variability[8]. Non-invasive monitoring may be very helpful to detect and monitor these patients. Currently, the only accepted markers are AST/ALT and other liver function tests.

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. This study evaluates two non-invasive methods for detection of significant liver fibrosis in MTX-treated psoriasis patients.

The first test is the aspartate aminotransferase to platelet ratio index (APRI), that has been employed as a predictor of the presence or absence of significant fibrosis on liver biopsy of patients with chronic hepatitis C (HCV) patients[11,12]. The second test is Fibroscan, measures liver elasticity by means of one-dimensional transient elastography. Fibroscan is essentially an ultrasound transducer that generates vibrations, and the velocity of the waves passing through the liver is tracked by pulse-echo ultrasound and is correlated to tissue stiffness. This latter innovation appears promising for the assessment of fibrosis and cirrhotic changes. In patients with hepatitis C, a good correlation has been shown between significant fibrosis and elastography outcome[12-15].

The aim of the present study is to compare the diagnostic performance of non-invasive tests for the prediction of liver fibrosis in patients with psoriasis or rheumatoid arthritis, who have consumed methotrexate at the cumulative dosage of greater than 1,500 mg. We also wanted to determine the correlations between of cumulative doses of MTX and liver biochemistries, liver histology, and Fibroscan results.

Patients and Methods

From November 2007 to November 2008, we performed a single institution prospective study in psoriasis and RA patients. Eligible patients were at least 18 years old and had been receiving MTX at cumulative doses of ≥ 1,500 mg. The cumulative dose of MTX was based on the established guidelines for monitoring hepatotoxicity in psoriasis. All enrolled patients gave their written informed consent.

Clinical data and complete medical history with past and current medications were recorded including: age, gender, body mass index (BMI), liver function tests in the past 12 months, co-morbid diseases, treatment with other medications, and other risk factors of liver disease. All patients had been treated with MTX for a long period of time before enrollment, and the treatment duration and cumulative doses of MTX were recorded. The laboratory data included serum albumin, AST, ALT, alkaline phosphatase (AP), cholesterol and complete blood count (CBC).

Exclusion criteria were (1) current alcoholic drinking > 40 gm/day, (2) other causes of liver disease, such as viral hepatitis, autoimmune liver disease, metabolic liver disease, drug-induced liver injury, and skeletal muscle or cardiac diseases.

All patients (41 psoriasis, 20 rheumatoid arthritis and 1 seronegative spondylo-arthropathy SNSA patient) gave informed consent and underwent blood testing, APRI score evaluation, and Fibroscan test. Fibroscan was obtained on the right lobe of liver, via an intercostal space, with the patient lying in dorsal decubitus and the right arm in maximal abduction behind the head. The tip of the transducer probe was covered with coupling gel and placed on the skin, between the ribs overlying the right hepatic lobe. When the target area was located, the operator pressed the probe button to start measurements at the depths of 25 mm and 65 mm. Ten validated measurements were taken on each case. The results were expressed in kilopascals (kPa). The median value was considered representative of the elastic modulus of the liver. The examination took at least 5 minutes. Only procedures with 10 validated measurements and a success rate of at least 60% were considered reliable. Fibroscan assessment was performed by an experienced medical technician who was strictly blinded to the clinical data.

Oral MTX was given on a weekly basis at doses ranging from 5 to 50 mg. starting at either the intended maintenance dose, or at lower doses with gradual increases over a few weeks toward the intended maintenance dose. Some patients also received oral folate supplementation. Cumulative MTX dose and duration of treatment were determined on the same day as Fibroscan examination. Laboratory data from blood

samples were obtained at the same laboratory and on the same day as Fibroscan examination. The APRI score was calculated from the equation:

$$\text{APRI score} = \frac{\text{AST of the sample} \times 100}{\text{AST of upper normal limit} \times \text{platelets count } (10^3/\text{mL}).}$$

Liver biopsy was performed after obtaining an informed consent, using a G16 Jamshidi Menghini soft tissue core biopsy needle. The biopsy specimen was placed in 10% neutral buffered formalin solution, processed and embedded in paraffin blocks. Five-micron-thick sections were microtomed and stained with hematoxylin-eosin and modified trichrome. The slides were reviewed and graded according to Roenigk's criteria for MTX-induced hepatotoxicity by an experienced pathologist who was blinded to MTX doses and results of non-invasive methods. (Table 2)

Results are expressed as mean ± SEM. Comparisons of quantitative data were made using the student t-test, or the non-parametric Mann-Whitney rank-sum test for data not exhibiting a normal distribution. Qualitative data were analyzed using Spearman rank correlation, and their probabilities (p) evaluated the association between liver stiffness and clinical or biochemical data. Comparison of diagnostic accuracy between non-invasive test and other parameters was determined by area under the receiver operative characteristic curve (AUROC) at optimal cut-off point. All statistical testing was done at the conventional 2-tailed α level of 0.05.

RESULTS

Demographic and clinical data

Demographical and clinical characteristics of 62 patients and their underlying diseases are summarized in Table 3. Most patients were female (69%). The mean age was 47.5 ± 1.6 years, the mean BMI was 22.8 ± 0.6 kg/m^2, the mean cumulative dose of methotrexate was 2,685 ± 158 mg, the mean treatment duration was 71 ± 5 months, the mean APRI score was 0.25 ± 0.02 and the mean Fibroscan of all patient was 5.76 ± 0.28 kPa, respectively. The mean serum alanine aminotransferase (ALT), the mean AST/ALT ratio, and the mean APRI score were 23 ± 2 units/L, 1.10 ± 0.06, and 0.23 ± 0.03, respectively. There were 41 psoriasis patients (26 females, mean age 47.1 ± 2.1 years, mean BMI 23.0 ± 0.7 kg/m^2, mean cumulative dose 2,569 ± 202 mg, mean duration of treatment 64 ± 6 months), and 21 RA patients (17 females, mean age 48.3 ± 2.7 years, mean BMI 22.4 ± 1.1 kg/m^2, mean cumulative dose 2,911 ± 251 mg, mean duration of treatment 83 ± 9 months). There were no differences between psoriasis and RA patients with regard to demographic, clinical and laboratory data.

Correlation between Fibroscan and others parameters

Correlation between Fibroscan and other predictive parameters of MTX induced liver damage are shown in Table 4. Serum AST, ALT and APRI were correlated to liver stiffness measurement (r = 0.32, 0.32 and 0.30; p = 0.01, 0.01 and 0.04, respectively). Only liver histology showed excellent relationship to Fibroscan results. (r = 0.74; p = 0.0004)

Comparative performance of liver biopsy and non-invasive tests

Liver biopsy was obtained in 18 patients (psoriasis 17, RA 1 patient). The median length of biopsy was 23 mm (range 13-38 mm) and the median number of portal tracts was 14 (range 6-27). Based on the Roenigk classification, grades II, IIIa, IIIb and IV were present in 6, 7, 4 and 1 specimen respectively.

Table 2. Roenigk's criteria for MTX induced hepatotoxicity.

Roenigk's grade	Fatty change	Necroinflammation	Fibrosis
Grade I	none or mild	with or without mild portal inflammation	none
Grade II	moderate or severe	moderate or severe portal inflammation	none
Grade IIIa	with or without	with or without	mild (fibrosis extend to acini)
Grade IIIb	with or without	with or without	moderate or severe
Grade IV	with or without	with or without	cirrhosis

Table 3. Demographic and laboratory characteristics of 62 patients.

Characteristics	All patient (n = 62)	Psoriasis (n = 41)	Rheumatoid arthritis (n = 21)	*p*-value
Age (year)	47.5 ± 1.6	47.1 ± 2.1	48.3 ± 2.7	0.8
Sex; female (%)	45 (69%)	26 (63%)	17 (81%)	0.3
BMI (kg/m^2)	22.8 ± 0.6	23.0 ± 0.7	22.4 ± 1.1	0.5
AST (IU/L)	24 ± 1	24 ± 1	23 ± 2	0.4
ALT (IU/L)	22 ± 2	23 ± 2	21 ± 3	0.1
AST/ALT ratio	1.24 ± 0.06	1.18 ± 0.06	1.35 ± 0.09	0.2
ALP (IU/mL)	75 ± 3	73 ± 3	79 ± 5	0.4
Albumin (g/dL)	4.08 ± 0.05	4.07 ± 0.07	4.11 ± 0.05	0.8
Platelets (× 10^3/mL)	322 ± 18	322 ± 26	322 ± 23	0.8
APRI score	0.25 ± 0.02	0.25 ± 0.02	0.26 ± 0.04	0.8
Methotrexate				
Cumulative dose (mg)	2,685 ± 158	2,569 ± 202	2,911 ± 251	0.07
Duration (months)	71 ± 5	64 ± 6	83 ± 9	0.08
Liver stiffness (kPa)	5.76 ± 0.28	5.86 ± 0.35	5.55 ± 0.46	0.5

Table 4. Correlations between liver stiffness and other predictive parameters.

Parameters	r	*p*-value
Age	0.78	0.6
BMI	0.12	0.4
AST	0.32	0.01
ALT	0.32	0.01
AST/ALT ratio	0.12	0.3
ALP	0.45	0.8
Albumin	0.17	0.2
Globulin	0.07	0.6
APRI score	0.30	0.04
Cumulative dose of MTX	0.17	0.2
Duration of MTX used	0.14	0.3
Liver histology: Roenigk grade	0.74	0.0004

Comparison was made between patients who underwent liver biopsy (n = 18) and did not perform liver biopsy (n = 44). In the biopsy group, the mean age was 46 ± 2.8 years, the mean BMI was 22.9 ± 0.8 kg/m^2, the mean cumulative dose was 2,669 ± 364 mg, the mean duration of treatment was 68 ± 8 months and mean Fibroscan was 6.2 ± 0.5 kPa. In the non-biopsy group, the mean age was 48 ± 2.0 years, the mean BMI was 22.8 ± 0.8 kg/m^2, the mean cumulative dose was 2,691 ± 169 mg, the mean duration of treatment was 72 ± 6 months and the mean Fibroscan was 5.58 ± 0.34 kPa. Relevant data are displayed in Table 5, no statistical significance between the two groups are detected.

According to psoriasis treatment guidelines, Roenigk classification grade IIIa was recommended for liver biopsy within 6 month, and discontinuation of MTX was recommend for Roenigk classification grade IIIb. The diagnostic role of Fibroscan and other serums for detection of MTX induced liver damage is shown in Table 6 and Table 7. Fibroscan was associated with the highest AUROC, as compared to liver histology in both Roenigk Grade IIIa and at least grade IIIb groups.

At a cut-off point of 5.2 kPa, Roenigk grade IIIa carried a 100% positive predictive value and 95% negative predictive value. Only 1 case (12.5%) was misclassified. At a cut-off point of 7.6 kPa, Fibroscan predicted the presence of Roenigk grade IIIb with 80% sensitivity and 92% specificity. Comparisons with other parameters are shown in Table 6 and Table 7.

DISCUSSION

MTX is a recommended treatment for steroid sparing and maintenance. However, its use is limited by toxicity. The correlation between cumulative MTX dose and hepatotoxicity, however, has not been observed by all investigators. In these groups of patients with RA and psoriasis, little to no relationship was found among the cumulative MTX dose, liver function tests, APRI score and Fibroscan results. Liver biopsy remains the gold standard for the detection

Table 5. Comparison between the liver biopsy and the non-biopsy group.

Characteristics	Biopsy group (n = 18)	Non-biopsy (n = 44)	*p*-value
Age (year)	46 ± 2.8	48 ± 2.0	0.8
Sex; female (%)	12 (67%)	31 (71%)	0.8
BMI (kg/m^2)	22.9 ± 0.8	22.8 ± 0.8	0.7
AST (IU/L)	23 ± 2	24 ± 1	0.9
ALT (IU/L)	23 ± 2	22 ± 2	0.4
AST/ALT ratio	1.09 ± 0.06	1.29 ± 0.07	0.2
ALP (IU/mL)	74 ± 5	75 ± 3	0.9
Albumin (g/dL)	4.06 ± 0.1	4.09 ± 0.04	0.7
Platelets (× 10^3/L)	339 ± 32	315 ± 23	0.4
APRI score	0.23 ± 0.03	0.26 ± 0.02	0.5
Methotrexate			
Cumulative dose (mg)	2,669 ± 364	2,691 ± 169	0.9
Duration (months)	68 ± 8	72 ± 6	0.9
Liver stiffness (kPa)	6.2 ± 0.5	5.58 ± 0.34	0.2

Table 6. Comparative performance of non-invasive tests for detecting Roenigk Grade IIIa.

Cut-off value	AUROC	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Fibroscan ≥5.2 kPa	0.97	83% (55-95)	100% (61-100)	100% (72-100)	75% (41-93)
AST ≥25 U/L	0.69	58% (32-81)	100% (61-100)	100% (65-100)	55% (28-79)
ALT ≥17 U/L	0.76	83% (55-95)	67% (30-90)	83% (55-95)	67% (30-90)
AST/ALT ≥1.0	0.61	58% (32-81)	20% (4-62)	64% (35-85)	17% (3-56)
APRI ≥0.26	0.61	42% (19-68)	83% (44-97)	83% (45-97)	42% (19-68)
Albumin ≥3.5 g/dL	0.60	17% (5-45)	83% (44-97)	67% (21-94)	33% (15-58)

Table 7. Comparative performance of non-invasive tests for detecting Roenigk Grade ≥IIIb.

Cut-off value	AUROC	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Fibroscan ≥7.6 kPa	0.88	80% (38-96)	92% (67-99)	80% (38-96)	92% (67-99)
AST ≥30 U/L	0.59	40% (12-77)	92% (67-99)	67% (21-94)	80% (55-93)
ALT ≥14 U/L	0.52	40% (12-77)	77% (50-92)	40% (12-77)	77% (50-92)
AST/ALT ≥1.0	0.61	80% (38-96)	42% (19-68)	36% (15-65)	83% (44-97)
APRI ≥0.37	0.60	20% (4-62)	92% (67-99)	50% (10-91)	75% (51-90)
Abumin ≥3.5 g/dL	0.52	20% (4-62)	85% (58-96)	33% (6-79)	73% (48-89)

of patients who may have MTX-related hepatotoxicity.[1-3,5-7] Normal liver function tests have been observed in most patients. In both psoriasis patients and RA patients, cumulative doses and liver function tests are unreliable predictors of MTX-related hepatic injury. Liver biopsy can detect significant hepatic injury, and the prevalence of abnormal histological findings is greater after cumulative doses exceeding 1,500 mg.[6,7] The prevalence of hepatotoxicity with MTX therapy is low,[7,9] although slight elevations of aminotransferases have been noted in significant fibrosis (Roenigk grade IIIb) in both RA patients and

psoriasis patients.

The optimal cutoff value of Fibroscan for the prediction of significant fibrosis (grade IIIa) was 5.2 kPa, which provided a sensitivity of 83%, specificity of 100%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 75%. Similarly, the optimal cutoff value of Fibroscan for the prediction of advanced fibrosis (grade IIIb) was 7.6 kPa, which provided a sensitivity of 80%, specificity of 92%, PPV of 80%, and NPV of 92%.The results of the present prospective study comparing non-invasive parameters to liver biopsy indicate that Fibroscan is the most accurate method for detection of MTX induced liver damage in patients on MTX therapy.

We also noted that 80% of patients with Fibroscan <5.2 kPa had Roenigk grade II on liver biopsy. All of patients with Fibroscan 5.2-7.5 kPa had liver biopsy showing Roenigk grade IIIa. Interestingly, all patients with liver biopsy showing Roenigk grade IIIa and higher had Fibroscan of ≥7.6 kPa (data not shown). These results suggest that Fibroscan can predict the degree of fibrosis in patients on MTX. Therefore, liver biopsy may be reserved only for patients with intermediate values of Fibroscan between 5.2-7.5 kPa.

The main limitation in our study is related to the small sample size, which may limit the precision of the estimated effects. Also sampling error may occur with the liver biopy taking in some of our cases with small biopsy specimens. However, the average biopsy length in our study compares favorably with those in other studies[10]. Our overall results suggest that Fibroscan appears useful in detecting and / or good in excluding significant MTX-induced liver damage (Roenigk grade ≥IIIb).

In conclusion, significant liver fibrosis is rare in psoriasis and RA patients treated with high doses of MTX, and is not accurately reflected by cumulative dose of MTX, duration of MTX treatment, liver function tests and APRI score. The Fibroscan® is a more accurate, more rapid, noninvasive, and reproducible method for assessing liver fibrosis compared to APRI, liver function tests and MTX cumulative dose. Fibroscan is useful as a fibrosis screening test in selected patient population. Fibroscan helps to avoid liver biopsy in most patients with dermatologic or rheumatic diseases on MTX therapy.

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