

Hepatic fibrosis in rheumatoid arthritis patients treated with methotrexate: application of a new semi-quantitative scoring system

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Abstract

Objective. Evaluation of hepatic lesions in patients treated with methotrexate (MTX) generally used the Roenigk histological score. However, the sensitivity of the method for hepatic fibrosis assessment has been discussed. The semi-quantitative histological scoring system (SSS) offers a sensitive and specific evaluation of liver fibrosis. Both scores have been evaluated in liver biopsies of patients with rheumatoid arthritis.

Methods. Seventy-four liver biopsies were obtained in 57 rheumatoid arthritis patients before initiation of MTX (group 1, 38 cases), in cases of a persistently high level of transaminases during 1 yr of treatment (group 2, 10 cases) and after a MTX total dose of 2 g (group 3, 26 cases). Eleven biopsies of groups 1 and 3 originated from the same patient in 11 cases. Specimens were examined blindly by two anatomopathologists. The three groups were compared with an ANOVA. Sequential biopsies performed in 11 patients were compared with the Wilcoxon paired test.

Results. The Roenigk score and the SSS were significantly correlated ($P < 0.0001$). Only a mild fibrosis was found in 33.8% (25/74) of the biopsies with the Roenigk score. Liver fibrosis, graded as mild (48.6%), moderate (41.8%) or severe (4%), was demonstrated in 94.6% (70/74) of the biopsies with the SSS. The Roenigk score and the SSS of the three patient groups were not statistically significantly different. The scores did not progress in the 11 patients who had serial biopsies.

Conclusion. SSS is much more sensitive than the Roenigk score for the evaluation of hepatic fibrosis. However, SSS did not show progression of hepatic fibrosis in patients with rheumatoid arthritis treated with MTX.

KEY WORDS: Semi-quantitative scoring system of hepatic fibrosis, Methotrexate, Rheumatoid arthritis, Hepatic fibrosis.

Introduction

Methotrexate (MTX), a folic antagonist, is the most widely used slow-acting anti-rheumatic agent in the treatment of rheumatoid arthritis (RA), with the best efficacy/toxicity ratio [1]. The major concern of its long-term use is hepatotoxicity. Histological abnormalities in patients treated with MTX include non-specific histological features such as fatty change, focal liver cell necrosis, portal tract inflammation, nuclear pleomorphism, and specific lesions such as fibrosis with collagen accumulation, particularly in the perisinusoidal space [2]. Depending on the study, the reported overall risk of fibrosis ranges from 3 to 52% [2]. In RA patients

receiving MTX, even in long-term studies, only a mild fibrosis is reported and cirrhosis seems exceptional [3]. However, psoriatic patients treated with MTX may develop significant liver disease, including cirrhosis, which may not be predicted by liver enzymes [2]. These patients are monitored according to dermatologists' guidelines for MTX with routine liver biopsies [4].

In RA patients, MTX monitoring relies on ACR (American College of Rheumatology) recommendations [5] for liver biopsy, using Roenigk histological grading [6], and serum transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]. However, liver enzymes are not always correlated with histological data [7] and the Roenigk score was found to be insensitive for detection of small changes in the degree of fibrosis [8]. In France, a new semi-quantitative histological scoring system (SSS) is used by hepatologists to assess specifically hepatic fibrosis in alcoholic

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and viral hepatitis [9]. It analyses four sites of fibrosis: centrilobular vein (CLV), portal tract (PT), perisinusoidal space (PS), and width and number of septa (WS and NS) when present.

Both the Roenigk score and the SSS have been used for the evaluation of liver biopsies of patients with rheumatoid arthritis.

Patients and methods

Patients

Fifty-seven RA patients (51 women) accepted liver biopsies (LB) in the Lyon Sud Hospital between 1986 and 1990. They all had definite RA according to the ACR criteria. Mean age of patients was 65.8 ± 13.5 yr (range 29–90). Mean disease duration was 18.4 ± 8.8 yr (range 5–37).

Before entering the study, patients had received 2.2 ± 1.3 other disease-modifying anti-rheumatic drugs (DMARDs). Their daily dose of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids (milligrams prednisone equivalent) was recorded. Patients starting MTX after liver biopsy initially received a 7.5 mg oral weekly dose. They were asked not to drink any alcohol.

At the time of liver biopsy, patients were evaluated clinically and biologically. Recorded clinical variables, morning stiffness and Lee and Ritchie indexes, were determined by the same physician. Laboratory assessment included: erythrocyte sedimentation rate (ESR) (Westergren method), C-reactive protein, complete blood count, rheumatoid factor (Latex nephelometric method), levels of AST (upper range 45 IU/l), ALT (upper range: 65 IU/l) and albumin (range: 39–46 mg/l) (Table 1).

Liver biopsies

A total of 74 liver biopsies (LB) were performed and divided into three groups: (1) before initiation of MTX (38 cases); (2) because of 'hepatotoxicity', defined as a persistent high level of transaminases (>twice the normal level) during 1 yr of treatment (10 cases); and (3) after a MTX total dose of 2 g (26 cases). Eleven biopsies of groups 1 and 3 originated from the same patients.

LB were performed by a gastroenterologist using a thin bore Menghini-type needle by a transthoracic approach. No side effects occurred and good quality samples were regularly obtained. Samples (>10 mm long) were fixed in Bouin's solution and embedded in paraffin-wax. Four micrometre-thick sections were stained in picosirius red solution.

Liver biopsy assessment

Two semi-quantitative scoring systems were performed blindly on the specimens by two liver pathologists. When their Roenigk scores differed, the pathologists conferred and then gave the mean of both measures. The inter-observer reproducibility for the SSS is not stated here because it has been determined in chronic viral hepatitis

by the same liver pathologists [9]. The inter-observer correlation was 0.756 (Kendall's t B) for the SSS and ranged from 0.735 to 0.884 for the different items (CLV, PS, PT, WS, NS).

The Roenigk scale. The Roenigk scale is divided into five grades measuring fibrosis and other histological lesions [6]. Grade I represents normal findings or mild fatty changes; grade II includes severe spotty hepatocellular necrosis; grade IIIA is a mild portal fibrosis, with or without fibrotic septa extending into the lobule; grade IIIB represents piecemeal necrosis or moderate-to-severe septal fibrosis with portal-to-portal, or portal triad-to-central vein bridging; grade IV is true cirrhosis with loss of normal architecture, fibrosis and nodular regeneration.

The semi-quantitative scoring system. Chevalier *et al.*'s SSS [9] was designed for the staging and follow-up of hepatic fibrosis in patients with chronic liver diseases. It is specifically focused on hepatic fibrosis which is graded in four main sites: CLV (graded 0–2), PT (graded 0–3), PS or Disse's space (graded 0–2), together with WS and NS when present (WS: graded 1–5 and NS: graded 0–3). SSS is given by $CLV + PS + PT + 2(WS \times NS)$, ranging from 0 to 35 (Fig. 1). For each item, the selected value corresponds to the most representative lesion of the biopsy sample. SSS is normal from 0 to 1, reflects mild fibrosis between 2 and 4, moderate fibrosis between 5 and 10, pre-cirrhosis from 11 to 15, and cirrhosis when >15.

Statistical methods

Results were expressed as the mean \pm s.d. Comparison between the Roenigk score and SSS was made with the Spearman test of correlation. Comparison between the different groups of liver biopsies was made with an ANOVA. The Wilcoxon test for paired groups was used to compare the sequential biopsies taken in 11 patients.

Results

Comparison of the two scores

The Roenigk score and the SSS were found to be significantly correlated ($P < 0.0001$, Spearman test of correlation) but the coefficient of correlation was only 0.54. The Roenigk score demonstrated fibrosis (grade >II) in 33.8% (25/74) of the biopsies. Fibrosis was regularly mild (IIIA). Grades IIIB and IV were not seen. Liver fibrosis was found in 94.6% (70/74) of the biopsies by the SSS. The grade of fibrosis was mild in 48.6% (36/74), moderate in 41.9% (31/74) and severe in 4% (3/74) of the biopsies. No cirrhosis was observed. The PS and PT were the most commonly altered areas: 1.35 ± 0.63 (0–2) for PS and 1.16 ± 0.65 (0–2) for PT.

AST, ALT and daily dose of prednisone were not correlated with any of the two scores.

Comparison of the three groups of patients

Patient characteristics. The 38 patients of group 1 (pre-treatment group) were significantly ($P < 0.05$) older and had more active RA than patients of the other

TABLE 1. Clinical and biological data of the three groups of patients

	Liver biopsies before MTX (n = 38)	Liver biopsies for hepatotoxicity (n = 10)	Liver biopsies after 2 g of MTX (n = 26)
Age (yr)	70.39 ± 10.44 (46–90)	66.20 ± 12.25 (45–84)	60 ± 13.91 (29–84)
Sex (men/women)	4M/34W	2M/8W	5M/21W
RA duration (yr)	18.78 ± 7.93 (6–37)	20.60 ± 10.05 (6–37)	15.19 ± 8.08 (5–37)
No. of positive rheumatoid factors	35/38	9/10	20/26
No. of DMARDs	2.27 ± 1.37 (0–7)	1.89 ± 1.27 (0–3)	2.46 ± 1.39 (0–7)
Morning stiffness (min)	72.06 ± 69.38 (0–240)	56.67 ± 58.95 (0–180)	31.40 ± 39.46 (0–180)
Lee index	10.15 ± 7.08 (0–27)	7.83 ± 7.86 (1–21)	6.37 ± 4.77 (0–16)
Ritchie index	16.94 ± 8.11 (1–32)	14 ± 12.17 (1–35)	7.28 ± 5.56 (1–21)
ESR (min/1st hour)	60.20 ± 28.84 (3–100)	21.40 ± 12.38 (6–40)	25.46 ± 20.07 (4–84)
CRP (mg/l)	55.85 ± 60.20 (4–258)	29.89 ± 25.13 (2–62)	13.72 ± 17.87 (2–84)
ASAT (UI/l)	11.89 ± 3.78 (6–20)	40.78 ± 46.73 (25–63)	17.19 ± 6 (9–36)
ALAT (UI/l)	12.67 ± 8.85 (5–48)	49.89 ± 26.30 (30–9)	20.5 ± 10.05 (8–45)
Albuminaemia (g/l)	36.41 ± 4.46 (24–45)	35.29 ± 7.87 (21–44)	42.25 ± 6.19 (33–61)

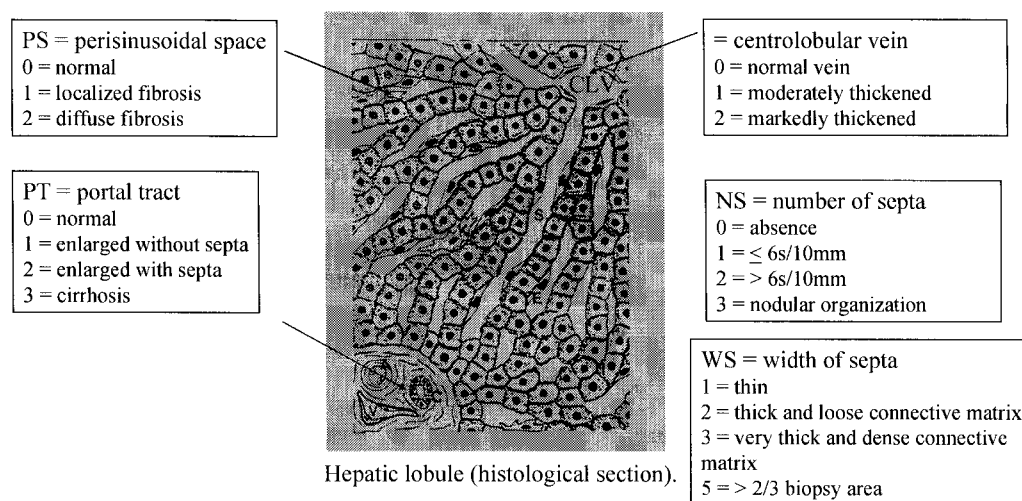


FIG. 1. The semi-quantitative scoring system of hepatic fibrosis. SSS = CLV + PS + PT + 2 (NS × WS) [9].

groups (Table 1). They differed significantly ($P < 0.05$) from other groups by a longer duration of morning stiffness, a higher Lee and Ritchie index, a higher ESR and a higher CRP level.

AST and ALT were higher in the third group than in the first one ($P = 0.0002$ and $P = 0.001$, respectively), but their mean values remained within the normal range. Albuminaemia was significantly ($P = 0.03$) lower in group 1 than in group 3.

The number of previous DMARDs and NSAIDs, cortisone intake, and the rheumatoid factor level were similar in the three groups.

Histological scores The Roenigk mean score was 1.8 ± 0.9 , 2.3 ± 0.8 and 1.6 ± 0.9 in groups 1, 2 and 3,

respectively. No statistically significant difference between the three groups was found. A mild fibrosis (grade IIIA) was observed in 39.5% (15/38), 40% (4/10) and 23.1% (6/26) of patients in groups 1, 2 and 3, respectively (Fig. 2).

Mean SSS was 5.1 ± 3.2 , 4.2 ± 1.6 and 4.2 ± 2.2 in groups 1, 2 and 3, respectively. No statistically significant difference between groups was found. A mild fibrosis (SSS from 2 to 4) was seen in 50% (19/38), 40% (4/10) and 50% (13/26) of patients in groups 1, 2 and 3 respectively. A moderate fibrosis (5–10) was seen in 36.8% (14/38), 60% (6/10) and 42.3% (11/26) of patients in groups 1, 2 and 3, respectively. Severe fibrosis (11–15) was only found in three patients of group 1 (Fig. 2).

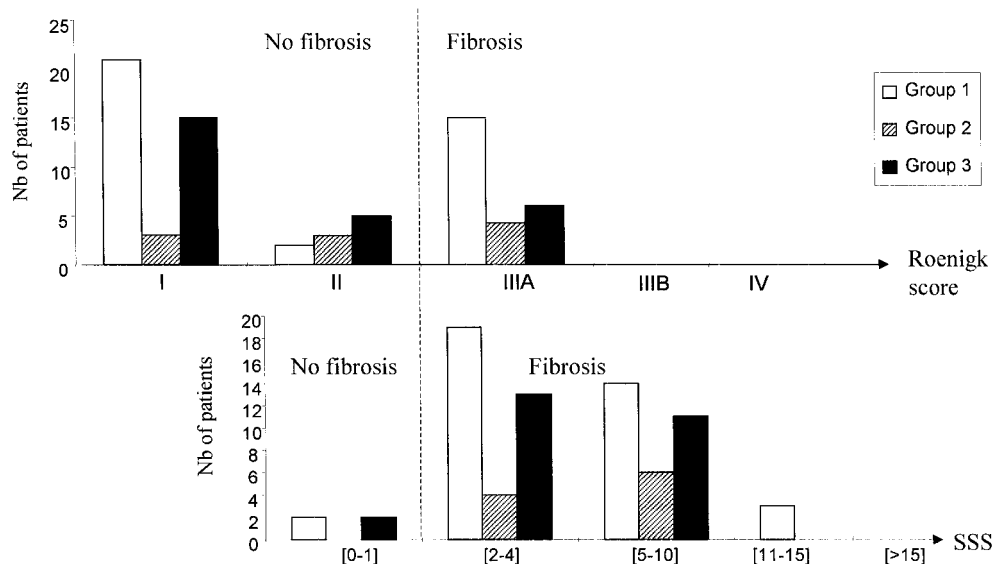


FIG. 2. (Top) Distribution of the Roenigk score in three groups of RA patients. (Bottom) Distribution of the semi-quantitative scoring system in the three groups of RA patients.

The width of septa (WS) was slightly improved ($0.1 < P < 0.2$) after 2 g of MTX using the ANOVA: 0.81 ± 1.08 before MTX vs 0.36 ± 0.57 after 2 g of MTX. The PS and PT were the most commonly altered areas: 1.35 ± 0.63 (0–2) for PS and 1.16 ± 0.65 (0–2) for PT before MTX was not significantly different from 1.24 ± 0.66 (PS) and 1.04 ± 0.61 (PT) after 2 g. There was no significant difference between the three groups for CLV and NS.

The Roenigk score and the SSS did not vary significantly in the 11 patients with paired biopsies. In this latter group, the Roenigk mean score was 1 ± 0 and 1.12 ± 0.64 , and mean SSS was 5.1 ± 3.2 and 4.5 ± 2.6 before and after MTX, respectively.

Discussion

The SSS of Chevalier *et al.* [9] was designed for the staging and follow-up of hepatic fibrosis in patients with chronic liver diseases. SSS was also made to tackle the heterogeneity in the distribution of fibrosis in the same biopsy specimen. The high intra- and inter-observer reproducibility of SSS has been demonstrated in viral and alcoholic hepatitis [9]. SSS was also found to correlate well with the surface density of collagen measured by an image analysis system (morphometry) which is the gold standard assay of hepatic fibrosis [9].

SSS was found to be much more sensitive than the Roenigk score for the assessment of hepatic fibrosis in RA patients ($\chi^2 = 64$, $P < 0.005$). The Roenigk score demonstrated a mild fibrosis in 33.8% of the specimens. By contrast, fibrosis was demonstrated by SSS in 94.5% of the same specimens and was graded as mild, moderate and even severe. The frequency of fibrosis was lower with the Roenigk score than with the SSS for two reasons. The Roenigk score is a composite five grade score measuring fatty infiltration, inflammation, nec-

rosis, cirrhosis and only portal fibrosis while the SSS quantifies fibrosis at different localizations and ranges largely from 0 to 35. The relative sensitivity to change of the two scores has not been evaluated since they did not differ in the three groups of patients. Nevertheless, SSS might be recommended for the evaluation of hepatic fibrosis in RA patients treated with MTX.

Hepatic histological alterations were frequently observed in patients who were biopsied before MTX initiation. The findings were similar to previously reported observations [10, 11]. Such lesions are generally attributed to RA itself and also to the intake of NSAIDs, analgesics, corticosteroids and DMARDs, which may induce fatty change, inflammation, necrosis or fibrosis [12]. The alterations were only graded IIIA with the Roenigk score. Hepatic fibrosis was routinely detected by SSS in such specimens but was rarely graded as severe, in agreement with the study of Ruderman *et al.* [11].

Hepatic fibrosis is the main concern of long-term treatment with MTX. The main target of MTX is the space of Disse [2]. Fibrogenesis in PS is frequently attributed to other drugs such as vitamin A, azathioprine or mercaptopurine [13]. Fibrosis is considered as a slower process that may be initiated by recurrent flares of necrosis, or may occur independently, in relation to another stimulant [7]. Neither the Roenigk score nor the SSS demonstrated any progression of hepatic fibrosis in patients having received 2 g of MTX, or showing abnormal levels of transaminases. Such stability has been reported and attributed to a tapering of NSAIDs, steroids and alcohol that often accompanies MTX therapy [12]. However, such a reduction in other drug intake was not observed in this small group. The currently used sensitive method for fibrosis confirms the apparent lack of hepatotoxicity of long-term administration of MTX in RA patients, the estimated risk of

cirrhosis being 0.1% after 5 yr of treatment [1]. However, we had only 11 follow-up biopsies and we have not performed liver biopsies after cumulative doses larger than 2 g.

According to the ACR recommendations [5], a pre-treatment liver biopsy should be considered for patients with a risk factor for liver disease (excessive alcohol consumption, chronic viral hepatitis B or C, abnormal baseline AST values). Post-treatment liver biopsies must be performed only when a patient develops persistent biological abnormalities: increase in AST levels or decrease in albumin, below the normal range, in at least six assays over a 12-month period with monthly monitoring. Liver enzymes or albuminaemia were unrelated to the Roenigk score or the SSS in the present study, confirming the current opinion that there is no reliable marker of MTX hepatotoxicity [8]. Liver biopsy has a potential morbidity and mortality [5] and its cost-effectiveness in MTX-treated RA patients is debatable [15]. Other methods for detection of MTX hepatotoxicity would be helpful but remain to be evaluated [8, 16].

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