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*Original Article*

# Significance of liver biopsy for the evaluation of methotrexate-induced liver damage in patients with rheumatoid arthritis

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**Abstract:** It is well recognized that long-term administration of methotrexate (MTX) in patients with rheumatoid arthritis (RA) can induce liver fibrosis via a steatohepatitis-like inflammatory process. Several non-invasive tests have been investigated as alternatives to liver biopsy, which is, however, still recognized as a final diagnostic modality to detect the MTX-induced liver damage. To clarify whether there is a significant discrepancy between clinical estimations and pathologic findings of this hepatic condition, we performed a following comparative study. Four RA patients (4 women, age 67-80 yr) with MTX-induced liver damage were reviewed. The severity of hepatic damage estimated clinically was compared with histopathologic findings. Consequently, the liver biopsies showed the relatively earlier stages of and milder degrees of hepatic damages than the clinical estimations. The histopathologic findings were more reliable and useful than any other clinical examinations, to plan and modify the treatment strategies, especially in cases of liver damages with multiple etiologies besides MTX. These findings suggest that liver biopsy is an unavoidable examination to assess precisely MTX-induced liver damage. Non-invasive tests may be useful to monitor the hepatic condition of RA patients receiving MTX but do not constitute an acceptable alternative to liver biopsy.

**Keywords:** Methotrexate, rheumatoid arthritis, steatohepatitis, liver fibrosis, biopsy

## Introduction

Over five decades, administration of low-dose methotrexate (MTX) has been used as a therapy for inflammatory disorders such as psoriasis and rheumatoid arthritis (RA) [1, 2]. Despite its efficacy and relative safety, long-term MTX administration has the potential to cause diverse organ toxicities, including hepatotoxicity [3, 4]. Concomitant use of folic acid can reduce the possibility of this side effect but does not prevent it completely in every patient [3, 4]. Therefore, close follow-up during MTX therapy is necessary for the early detection of this side effect.

The typical MTX-induced liver damage is fibrosis associated with a steatohepatitis-like inflammatory process, which potentially can progress to cirrhosis [4-6]. Liver biopsy provides the most reliable information to diagnose and evaluate the liver damage [4-6]. However, to reduce the burden on patients, a non-invasive test has been sought as an alternative to biopsy for early detection of liver damage. Several recent clinical studies evaluated various non-invasive methods for estimating liver fibrosis [7-9]. However, these simple methods, which only estimate the degree of liver fibrosis, may not be sufficient to assess precisely the patients' liver conditions.

Because the underlying conditions vary among patients, the causal role and extent of responsibility of MTX for liver damage are not considered to be uniform in every patient. Some patients receiving MTX may have the other liver disorders. To differentiate straightforward MTX liver damage from such complex conditions, liver biopsy is still considered important and unavoidable [4, 5].

To clarify the significance of liver biopsy in determining MTX-induced liver damage, we

**Table 1.** Clinical backgrounds of the patients

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| Age (yr) | 80 | 78 | 67 | 78 |
| Sex | Female | Female | Female | Female |
| Dose of MTX (mg/week) | 4 | 8 | 6 | 4 |
| Concomitant drugs | – | Folic acid | Steroids Infliximab | Folic acid |
| Clinical diagnosis | Cirrhosis | Cirrhosis | Liver dysfunction | Liver dysfunction |
| Child-Pugh class | B | A | – | – |
| Body mass index (kg/m$^2$) | 21.2 | 18.6 | 25.5 | 23.8 |
| Metabolic disorders | – | Dyslipidemia | Dyslipidemia | – |

MTX, methotrexate.

reviewed four cases that we experienced recently (**Table 1**).

## Case reports

*Case 1*

An 80-year-old woman, who had received MTX (4 mg/week) for 20 years as therapy for RA, complained of swelling of her lower extremities. Computed tomography (CT) revealed abundant ascites and atrophy of the right lobe of the liver, which suggested liver cirrhosis (**Figure 1A**). She was a non-drinker, and did not suffer from metabolic syndrome. Both viral hepatitis and autoimmune liver disorders were ruled out serologically. Laboratory test results showed elevated serum levels of liver fibrosis markers (hyaluronic acid and type IV collagen) and thrombocytopenia, probably associated with cirrhosis (**Table 2**). The clinical diagnosis was cirrhosis (Child-Pugh class B) due to long-term MTX administration. Liver biopsy was performed to evaluate her liver damage and MTX administration was immediately halted. Although the ascites disappeared, she died of advanced RA-related pulmonary fibrosis.

*Case 2*

A 78-year-old woman, who had received MTX (8 mg/week) and folic acid (5 mg/week) for 3 years as therapy for RA, complained of abdominal fullness. The abdominal CT findings suggested liver cirrhosis (**Figure 1B**). Laboratory tests revealed elevated serum levels of aspartate aminotransferase (AST) and markers of fibrosis (**Table 2**). Both viral hepatitis and autoimmune liver disorders were ruled out. She also had dyslipidemia, which was being controlled well by pravastatin. The clinical diagnosis was cirrhosis (Child-Pugh class A) due to MTX administration. Liver biopsy was performed and MTX therapy was halted. Subsequently, the ascites disappeared but the low-grade AST abnormality persisted.

*Case 3*

A 67-year-old obese woman (non-drinker, **Table 1**), who had received MTX (6 mg/week) and steroids for 20 years as therapy for RA, was found to have abnormally elevated serum levels of AST and alanine aminotransferase (ALT) (**Table 2**) and hypercholesterolemia (274 mg/dL). Abdominal CT findings suggested fatty liver (**Figure 1C**). Liver biopsy was performed to clarify whether the liver dysfunction was induced by MTX. Folic acid was instituted but the serum aminotransferases did not reduce to normal levels. Later, the patient suffered from a life-threatening infection and MTX therapy was halted. After recovery, AST and ALT reduced and were maintained at normal levels.

*Case 4*

A 78-year-old woman, who had received MTX (4 mg/week) and folic acid (5 mg/week) for more than a decade as therapy for RA, showed abnormal liver function test results (**Table 2**). She was an occasional drinker but did not suffer from viral hepatitis or metabolic syndrome. Clinically, autoimmune hepatitis could not be ruled out because of a high-titer of antinuclear antibody (× 160) but cessation of MTX therapy resulted in improvement of the liver function.

## Materials and methods

Liver biopsy samples obtained from the four patients were fixed in buffered formalin and embedded in paraffin blocks. Three-μm-thick sections were cut from the paraffin blocks and stained with hematoxylin-eosin and Azan-Mallory. The stained sections were observed under a light microscope and the histological pattern and severity of liver damage were

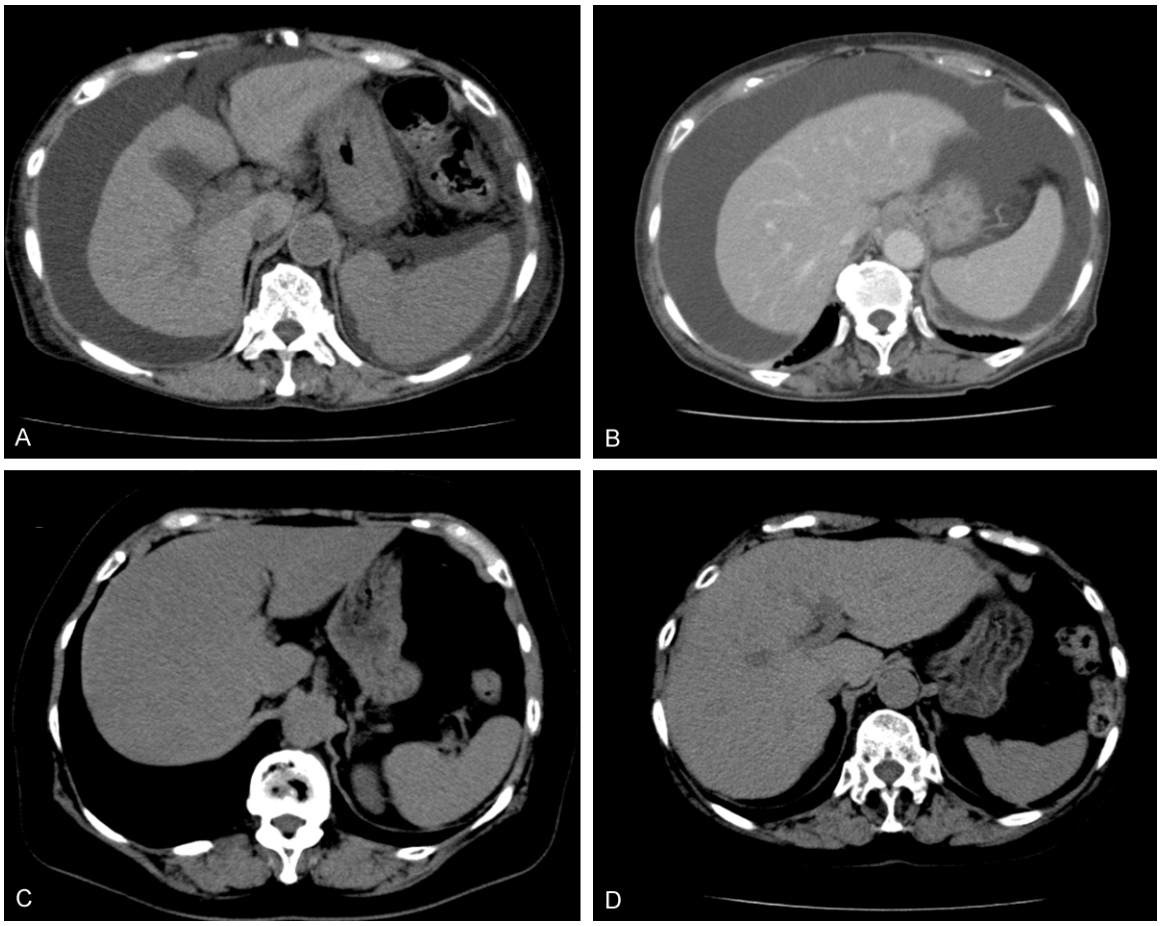


**Figure 1.** Computed tomography findings of the four cases. A. Case 1. Atrophy of liver, splenomegaly, and ascites suggest cirrhosis. B. Case 2. Findings almost identical with Case 1 suggest cirrhosis. C. Case 3. The low-density of liver indicates fatty change. D. Case 4. No significant change is seen in the liver.

**Table 2.** Laboratory test results of the patients at the time of liver biopsy*

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| AST (IU) | 30 | 59 | 110 | 120 |
| ALT (IU) | 13 | 29 | 119 | 136 |
| GGT (IU) | 16 | 14 | 32 | 129 |
| Albumin (g/dL) | 2.9 | 3.0 | 4.1 | 4.0 |
| Total Bilirubin (mg/dL) | 1.6 | 1.0 | 0.7 | 0.7 |
| Platelet (count/mm$^3$) | 5.4 | 13.6 | 13.2 | 17.0 |
| PT-INR | 1.76 | 1.26 | 1.24 | 1.09 |
| Hyaluronic acid (ng/ml) | 670 | 242 | 494 | – |
| Type IV collagen (ng/ml) | 211 | 399 | 317 | – |

*Abnormal values are underlined. AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: Gamma glutamyl transpeptidase; PT-INR: International normalized ratio.

assessed. The histopathologic findings were compared with the clinical data, including radiologic findings, in each case. Informed consent was obtained from each patient and the hospital ethics committee approved the study protocol.

## Results

Generally the patients' liver tissues showed steatohepatitis-like inflammatory injury, which differed slightly from typical alcoholic and nonalcoholic steatohepatitis (**Figure 2**). Macrovesicular steatosis was rather mild but, in contrast, ballooning degeneration was prominent. Inflammation and fibrosis were accentuated in the portal areas and lobular inflammation was faint. Perivenular fibrosis was essentially absent. These findings were identical with the known histopathological features of MTX-induced liver damage [5, 6]. However, the findings varied, depending on the patients' clinical backgrounds.

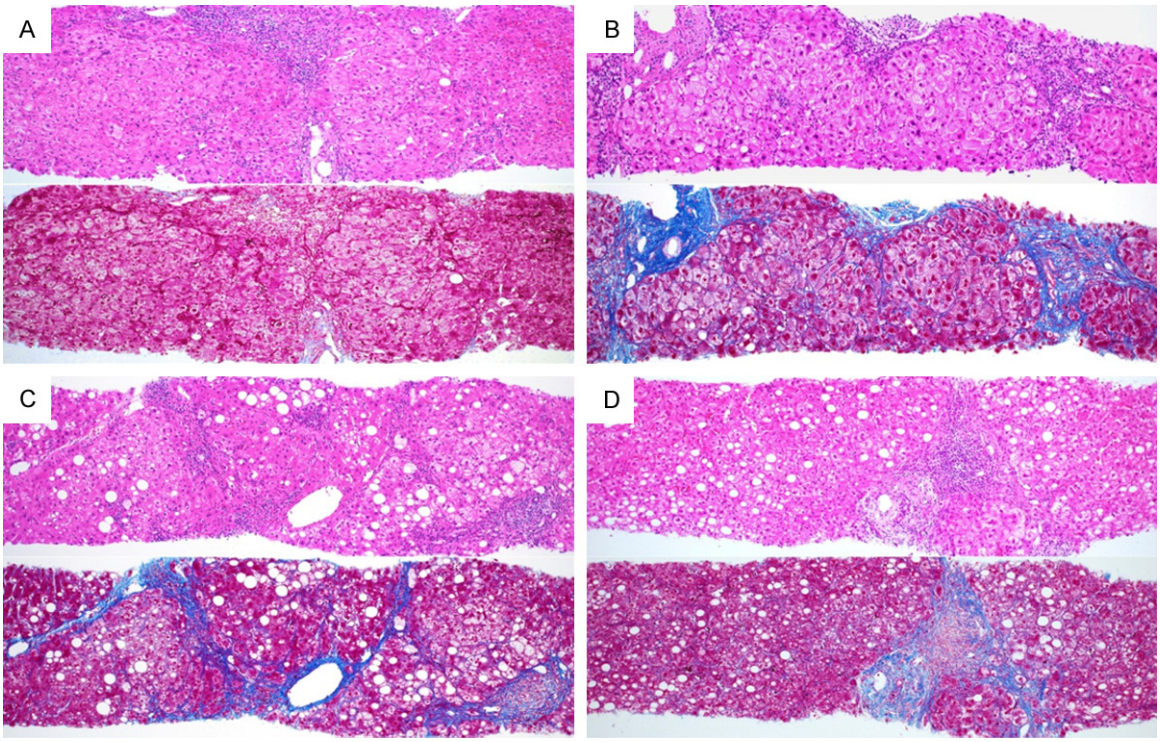


**Figure 2.** Histological findings of liver biopsies of the four cases. A. Case 1. Faint steatosis, hepatocyte ballooning, portal inflammation, and bridging fibrosis (portal-to-portal) are seen, but regenerative nodules of cirrhosis are absent. B. Case 2. Findings are almost identical with Case 1. C. Case 3. Marked macrosteatosis, hepatocyte ballooning, portal and lobular inflammation, and perivenular fibrosis with bridging are seen. The findings are similar to alcoholic and nonalcoholic steatohepatitis. D. Case 4. Steatosis, hepatocyte ballooning, and bridging fibrosis are seen, but interface hepatitis is absent. These findings are not consistent with autoimmune hepatitis. (Upper rows, hematoxylin-eosin; lower rows, Azan-Mallory stain; original magnification, ×20).

While Cases 1 and 2 were clinically diagnosed as cirrhosis, mainly by the CT findings (**Figure 1A** and **1B**), histological examination revealed that the livers had bridging fibrosis but not cirrhosis (**Figure 1A** and **1B**). The fibrous bands formed portal-to-portal linkage and perivenular fibrosis was absent. Dense mononuclear cell infiltration was seen in the portal tracts. Necroinflammatory foci were also seen in the lobular parenchyma but were rare. Compared to the degree of steatosis, ballooning degeneration was prominent. In these cases, MTX was considered the sole and primary cause of the liver damage.

In Case 3, the patient was obese and had received steroids in addition to MTX. The histological findings were consistent with those of MTX-induced liver injury (**Figure 2C**). However, marked macrosteatosis and the presence of perivenular fibrosis, extending to the adjacent central veins, suggested overlapping of true, nonalcoholic (i.e., metabolic syndrome-associated) steatohepatitis. Hard exhaustion after the crucial infectious/inflammatory event might have contributed partially to the improvement of liver function after the cessation of MTX.

The biopsy specimen of Case 4 showed typical histological patterns known as MTX-induced liver injury, including steatosis, dense accumulation of inflammatory cells in the portal areas, and fibrosis extending from the portal areas (**Figure 2D**). The pathologic diagnosis based on these histological findings allowed physicians to rule out autoimmune hepatitis.

## Discussion

The results of this study demonstrated that liver biopsy was superior to the other clinical examinations in assessing MTX-induced liver damage. Importantly, the severity of liver damage determined by the biopsies tended to be milder than expected from the clinical assessment. Especially in Cases 1 and 2, both of

which were clinically diagnosed as cirrhosis, the pathologic reports of non-cirrhosis provided valuable information that allowed positive modifications of the treatment strategies.

Cessation of MTX is essential to avoid further liver damage but means the loss of a good therapeutic option against RA. Therefore, physicians must make the decision on the basis of a correct diagnosis. Surprisingly, a recent study reported that MTX-specific histopathologic changes were quite rare in the livers of RA patients with liver dysfunction [10]. This indicates that the differentiation of MTX-induced liver damage from the other liver disorders is a pivotal matter. Liver biopsy is currently the best diagnostic tool for this differentiation.

As was shown in Case 4, it may be difficult to differentiate clinically between MTX-induced liver damage and autoimmune hepatitis in patients with RA. Also, as in Case 3, if liver tissues show steatohepatitis-like findings that do not match a typical MTX-induced damage pattern, it is necessary to consider the potential contribution of pathogenic factors other than MTX, e.g., the effects of metabolic disorders, steroid administration, etc. On the other hand, for minimizing the burdens of medical examinations, it is necessary to replace liver biopsy by non-invasive tests [7-9, 11-14]. Without doubt, non-invasive methods of assessment of liver fibrosis are valuable to monitor the liver condition and detect liver fibrosis early in patients treated with MTX because many aspects of MTX-induced liver damage progress as subclinical disorders. However, since these non-invasive tests can only evaluate the severity of liver fibrosis and cannot provide information about the etiology [11-14], liver biopsy cannot be excluded from the management protocols of patients with RA treated with MTX.

The application of a drug-induced stimulation test (DLST) for the diagnosis of MTX-induced liver damage is controversial. A previous case report suggested its merit in the diagnosis of MTX-induced hepatitis [15]. On the other hand, DLST is not recognized as helpful in the diagnosis of MTX-induced pneumonitis because it frequently yields positive results against MTX in RA patients treated with long-term MTX administration [16]. Probably this caveat is appropriate in MTX-induced liver damage too.

From all points of view, there are no laboratory tests superior to liver biopsy for the detection of MTX-induced liver damage. Finally we caution that non-invasive tests cannot yet take the place of liver biopsy.

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### Disclosure of conflict of interest

None.

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