The value of dynamic hepatic scintigraphy and serum aminoterminal propeptide of type III procollagen for early detection of methotrexate-induced hepatic damage in psoriasis patients

R.J. van DOOREN-GREEBE,* A.L.A.KUIJPERS,* W.C.A.M.BUIJS,† P.H.M.KNIEST, †
F.H.M.CORSTENS, † F.M.NAGENGAST, † Th.DE BOO,§ J.L.WILLEMS, || P.DULLER*‡
AND P.C.M.VAN DE KERKHOF*†§

*Department of Dermatology, †Department of Nuclear Medicine, §Department of Gastroenterology and Hepatology,
†Department of Medical Statistics, ||Department of Clinical Chemistry and ††Department of Medical Psychology,
University Hospital Nijmegen, PO Box 9101, 6500 HN Nijmegen, the Netherlands
Accepted for publication 1 June 1995

Summary

Oral methotrexate (MTX) is a highly effective drug for the treatment of severe psoriasis. A limitation of this treatment is its potential hepatotoxicity. In the present prospective study the value of dynamic hepatic scintigraphy (DHS) and serum aminoterminal propeptide of type III procollagen (PIIINP) were investigated as screening methods for early detection of MTX-induced hepatic damage. These relatively non-invasive procedures were compared with the liver biopsy classification, until now the gold standard to assess MTX-induced liver damage. Twenty-five MTX patients were studied. The mean cumulative MTX dose was 3.9 g (range 0.2–11.1 g). Twenty-one patients had a normal liver histology (grade I), three patients had steatosis (grade II), and one patient mild fibrosis (grade IIIa). Seven additional patients with non-MTX related hepatic cirrhosis were included as disease controls.

DHS showed a clear-cut separation between the portal contribution of the MTX patients with grade I liver histology, and that of all other patients. A portal contribution larger than 52% was associated with a >95% chance of normal liver histology. If this cut-off value had been used to postpone the liver biopsy, this would have resulted in at least a 55% reduction in the number of biopsies in patients with a normal liver histology. DHS appeared to be very promising as a screening test to differentiate between the presence or absence of MTX-induced hepatic damage, but appeared not suitable to grade the severity of hepatic damage. Although a global relationship was demonstrated between serum PIIINP concentration and hepatic damage, single measurements in individual patients were not reliable. The combination of PIIINP measurements with DHS had only a limited additional value above DHS alone. The present study indicates that DHS has great promise for the detection of early MTX-induced hepatic damage. Pending further studies, regular liver biopsies remain mandatory for the safe prolonged use of MTX in psoriasis patients.

Oral methotrexate (MTX) is a highly effective drug for the treatment of severe psoriasis.1–4 MTX treatment, however, is associated with potential hepatotoxicity.1 Conventional serum liver function tests are not reliable as indicators of hepatic damage. According to international guidelines, a liver biopsy has to be performed in the initial phase of treatment and subsequently after each cumulative dose of 1.5 g.2,3,5 At present, histological evaluation of liver biopsies is the gold standard to assess MTX-induced liver damage. However, serious complications, e.g. haemorrhage, pneumothorax, and sepsis, may occur incidentally. The reported morbidity figures range from 0.02 to 10%,6,7 and the mortality rate is 0.01–0.1%.7,8 Liver biopsies are relatively expensive and uncomfortable for patients. Magnetic resonance imaging,9 ultrasound scanning,10,11 and static scintigraphy of the liver,12 have been reported as not being sufficiently reliable.

Recently, McHenry et al.11 reported promising results on dynamic hepatic scintigraphy (DHS), performed
according to the methodology of Ferguson et al. In short, the uptake of \( {\text{\textsuperscript{99m}}\text{Tc}} \) sulphur colloid in the spleen is proportional to the arterial uptake by the liver. By subtracting this arterial contribution from the total liver uptake, the portal contribution to the total liver blood flow can be computed. The low-pressure portal vein system is more sensitive for changes in the structure of the liver parenchyma than the high-pressure arterial system, and the portal contribution decreases in cases of fibrosis or cirrhosis.

Another interesting screening test is the measurement of the serum level of aminoterminal propeptide of type III procollagen (PIIINP). Hepatic fibrosis is characterized by increased synthesis and the deposition of collagen, predominantly type III. Collagen molecules are derived from procollagen by cleaving the terminal peptides, which are subsequently released into the circulation. Measurement of PIIINP, with a radioimmunoassay, may be suitable to detect and monitor liver damage. This test is not organ-specific, as increased PIIINP levels have also been demonstrated in systemic sclerosis, localized scleroderma, and rheumatoid arthritis.

In the present study the value of both DHS and serum PIIINP, alone and in combination, for the detection of MTX-induced hepatic damage was prospectively investigated. The liver biopsy results were used as a gold standard. A small group of patients with non-MTX-related hepatic cirrhosis was included as disease controls.

Patients and methods

Patients

In the period September 1993 to October 1994, all MTX patients who were scheduled to undergo a liver biopsy were asked to participate in the study. All patients were treated according to the Weinstein schedule (maximum weekly dose 15 mg/day). All investigations in individual patients were completed within a period of 10 weeks. DHS was performed \( \geq 2 \) weeks after the liver biopsy. Details on age and gender of the patients, indication for MTX treatment, duration of treatment, cumulative dose, and the presence or absence of arthritis, were obtained from the medical records. Several patients from the Department of Gastroenterology, with non-MTX-related liver cirrhosis, were included as disease controls. The study was approved by the hospital’s Medical Ethical Committee and all patients gave written informed consent.

Liver biopsies in methotrexate patients

Liver biopsies were performed, according to the Menghini technique, at the Department of Gastroenterology. The biopsies were prepared and classified, according to the guidelines of Roenigk et al., by one pathologist: liver biopsy classification (LBC) grade I, no abnormalities or mild steatosis, nuclear variability or portal inflammation; LBC grade II, moderate or severe steatosis, nuclear variability portal inflammation or necrosis; LBC grade IIIA, mild fibrosis; LBC grade IIIB, moderate to severe fibrosis; LBC grade IV, cirrhosis.

Dynamic hepatic scintigraphy

After overnight fasting, the patient was positioned supine on the couch of the dual-headed gamma camera, connected to an ICON computer (MultiSPECT II, Siemens, Hofmann Estates, IL, U.S.A.). The camera was equipped with \(' Low Energy High Resolution\) collimators. The liver and spleen were positioned in the centre of detectors measuring 53 \( \times \) 39 cm. Immediately after a rapid injection of 111 MBq \( {\text{\textsuperscript{99m}}\text{Tc}} \) sulphur colloid, with a particle size of about 30 nm, dynamic planar gamma camera images were recorded for 160 frames of 0·5 s each, in a 64 \( \times \) 64 matrix. Analysis was performed according to the described methods. Regions of interest were drawn around the liver, on an anterior sum-image, and around the spleen, on a posterior sum-image. Time activity curves for both organs were generated and smoothed. The ratio of liver to spleen activity, for the first 80 s post-injection, was also generated. On the basis of this curve the onset of portal input was estimated as the moment when the ratio curve started to rise, followed by a plateau. The value of the ratio just before the curve started to rise was used to normalize the liver to the spleen curve over the complete duration of the study. The spleen curve was subtracted from the normalized liver curve, resulting in the portal curve. The portal contribution at 25, 30, 35 and 40 s post-injection, was calculated as the ratio between the activity in the portal curve and the normalized liver curve, and expressed as a percentage of the total liver curve. All studies were analysed blinded by three observers (the first three authors). The results of W.C.A.M.B., physicist at the Department of Nuclear Medicine, were used for further analysis. The interobserver variability was evaluated. The radioactive dose was 1·1 mSv/scan (the background radiation on earth is 2 mSv/year).
**Serum aminoterminal propeptide of type III procollagen**

Sera were collected and stored at $-20^\circ$C until analysis. PIIINP concentrations were measured with a radio-immunoassay based upon the human propeptide (FARMOS Diagnostica, Espoo, Finland). The reference range for adults (based on healthy Finnish blood donors), as provided by the manufacturer, is $1.7-4.2$ μg/l.

**Patient questionnaire**

After completion of the liver biopsy and DHS, a questionnaire was sent to all MTX patients. A subjective judgement of mild, medium, or strong discomfort scored 1–3 points, and a judgement of absent, minimum, medium, or strong fear scored 0–3 points. The patients were asked which procedure they preferred.

**Statistical methods**

In order to distinguish patients with a LBC grade I from the other patients, linear discriminant analysis was used. With this method, posterior probabilities for a grade I LBC can be computed, given the values of one or more discriminating variables and given the prior probability (i.e. the probability without any further knowledge). As possible discriminating variables, the portal contribution (DHS), and the PIIINP were used. A minimal prior probability of 66%, for a grade I LBC, was used; this percentage originates from an earlier study. When the corresponding posterior probability, given the cut-off value of a discriminating variable (e.g. the portal contribution) exceeds 95%, this means that, at most, 5% of the patients with such a high portal contribution have a higher LBC than grade I. The inter-observer variability of the DHS was evaluated by recomputing the posterior probabilities for a grade I LBC, using the values of the portal contribution as they were measured by the two other observers (R.J.v.D.G. and A.L.A.K.).

**Results**

**Patients**

Twenty-five MTX patients (12 males and 13 females) were included in the study. Twenty-four patients had severe psoriasis and one had Reiter's disease. The mean age was 52 years (range 31–75 years). The mean cumulative MTX dose was 3.9 g (range 0.2–11.1 g), and the mean duration of treatment was 90 months (range 4.2–288 months). Six of the 25 patients also had psoriatic arthritis. In seven patients, three males and four females, with non-MTX-related hepatic cirrhosis, DHS and/or PIIINP determinations were performed. Their mean age was 53 years (range 43–75 years). In four patients, cirrhosis was induced by alcohol abuse and in three by autoimmune hepatitis.

**Liver biopsies in methotrexate**

Twenty-one patients (84%) had LBC grade I, three (12%) had grade II, and one (4%) grade IIIA. No complications occurred during or after liver biopsies.

---

Dynamic hepatic scintigraphy

DHS was performed in 22 of the 25 MTX patients. The portal contribution computed at 35 s post-injection had the highest discriminating power and was used for further analysis. The mean portal contribution at 35 s was 50.4% [standard deviation (SD) 13.1]. In the 18 patients with LBC grade I, the mean portal contribution was 54.8% (SD 9.5). The three patients with LBC grade II had portal contributions of 20.4%, 28.9%, and 38.0%, and the patient with grade III LBC had a portal contribution of 37.0%. Figure 1 shows the portal contribution of all patients in relation to their LBC (grades I-III). There was a clear separation between the portal contribution of the patients with a normal liver histology (grade I) and all other patients. The values of the patients with grade II and III LBC were in the same range. The portal contributions of five cirrhosis patients are shown in a separate column of Figure 1 (grade IV). Their mean portal contribution (31%), was in the same range as that of the MTX patients with grade II-III LBC.

Serum-aminoterminal propeptide of type III procollagen

The mean PIIINP, in all 25 MTX patients, was 3.8 µg/l (SD 1.3). In the patients with LBC grade I, the mean PIIINP was 3.6 µg/l (SD 1.0). The three patients with LBC grade II had PIIINP values of 3.2, 3.0, and 7.0 µg/l, and the patient with LBC grade III had a PIIINP value of...
6.2 µg/l. Figure 2 shows the PIIINP values of all patients in relation to their LBC (grades I–III). Although the mean PIIINP values in patients with grade II–III LBC were significantly higher than those with grade I LBC, isolated values did not discriminate between individuals with and without hepatic damage. The six patients with psoriatic arthritis all had a LBC grade I, and their mean PIIINP was 3.7 µg/l. The PIIINP values of five cirrhosis patients are shown in a separate column (grade IV). Their mean PIIINP (8.0 µg/l) was significantly higher than that of the MTX patients, with an overlap for the individual results. If the MTX and the cirrhosis patients are combined, three of the nine patients (33%) with abnormal histology, had normal PIIINP values.

Discriminant analysis (DHS and PIIINP) for methotrexate patients

The cut-off value of the portal contribution (DHS), corresponding to a >95% posterior probability to a LBC grade I, was 52% (Fig. 3). Of the 18 patients with a normal liver histology, 10 (55%) had posterior probabilities >95%, while none of the patients with abnormal histology met this criterion (Fig. 3). Subsequently, it was investigated whether the addition of PIIINP as second discriminating variable could enhance the posterior probabilities. This appeared to be the case as, with this, 14 (78%) of the 18 patients with normal liver histology had posterior probabilities >95%, compared to none of those with abnormal histology (Fig. 3). The variable PIIINP, on its own, did not produce a useful cut-off point. The cirrhosis patients are included in Figure 3 to show that their position is clearly left of the cut-off limit. In the discriminant analysis, however, only the MTX patients have been included.

To evaluate inter-observer variability of the DHS, after performance of discriminant analysis, it was computed whether the two other observers would have reached other conclusions about the percentage of patients with a >95% chance of LBC grade I. It is important that none of the observers produced false negative results (computed posterior probability > 95% and abnormal liver histology). The percentage of false-positive results (computed posterior probability ≤ 95% and normal liver histology) differed between the three observers (W.C.A.M.B., 45%; A.L.A.K., 22%; and R.J.v.D.G., 17%). If the PIIINP is added as a second discriminating variable, only the percentage false positive results for observer W.C.A.M.B. changed, namely from 45 to 22%. If the decision was made to postpone the liver biopsy, in the instance of a portal contribution >52%, the number of biopsies in patients with a normal liver would have been reduced from 55 to 83%, depending on the observer.

Patient questionnaire

The questionnaire was returned by 17 of the 25 MTX patients (68%). The total discomfort score was 28 points for the liver biopsy and 20 points for the DHS. The total fear score was 10 points for the liver biopsy and six points for the DHS. Fifteen out of the 17 patients (88%) preferred the DHS.

Discussion

In the present study a clear-cut separation between the portal contribution of MTX patients with a LBC grade I and all other patients could be demonstrated. DHS appeared not to be suitable for the determination of the severity of hepatic damage. The patients with steatosis (grade II) had a much lower mean portal contribution than the patients with grade I LBC (-29.1 vs. 54.8%). This suggests that a grade II LBC indeed reflects early MTX-induced hepatic damage. McHenry et al.13 performed 87 paired DHS and liver biopsies in 63 psoriatic MTX patients, and reported a high predictive value of normal DHS for fibrosis grade 0–1 (98.5%), and a low predictive value of an abnormal DHS for fibrosis of grade 2 or worse (25%). McLaren et al.25 reported a significant reduction in the portal contribution in 41 non-MTX-related cirrhosis patients, compared to 33 healthy controls.

By means of discriminant analysis, it has been demonstrated, in our study, that a portal contribution of >52%, in a MTX patient, was associated with a >95% chance of a LBC grade I. If this cut-off value of 52% had been used to postpone the liver biopsy, this would have reduced the number of biopsies, by at least 55%, in the patients with a normal liver histology. None of the three observers produced any false negative results.

Zachariae et al.15,16 report on PIIINP levels in 170 patients with psoriasis. Patients with liver fibrosis or cirrhosis, and patients with psoriatic arthritis, had significantly elevated mean PIIINP levels. The authors claimed that the number of liver biopsies performed on MTX patients might be reduced to a minimum, in patients with normal PIIINP levels. Like Kanzler and Gorsulowsky,26 we cannot confirm these conclusions.
as a considerable number (i.e. six to eight of 24, 25–33%) of patients without arthritis and with fibrosis, had normal values of PIIINP. Furthermore, four of 18 (22%) of patients with both arthritis and liver fibrosis had normal PIIINP values. Ristell et al.\textsuperscript{18} reported elevated PIIINP level in eight of nine patients with fibrosis or cirrhosis, and concluded that the number of biopsies could be significantly reduced in patients with normal PIIINP values, despite the fact that six of 15 patients (40%) with PIIINP values in the normal range had fibrosis or cirrhosis. Mitchell et al.\textsuperscript{17} reported on a controlled study with 51 MTX-treated psoriatics, and concluded that isolated PIIINP measurements cannot discriminate between individuals with and without significant liver pathology. The conclusion from the present study is in accordance with that of Mitchell et al.\textsuperscript{17} there was a global relation between PIIINP values and hepatic damage, but one-off readings appeared to be unreliable for the screening of individual patients. Patients with psoriatic arthritis had PIIINP levels in the same range as the other patients. Probably, there is scope for the use of PIIINP values for the regular monitoring of individual patients. The additional value of PIIINP measurements to DHS in individual patients, was very limited in the present study, and could be demonstrated only in one of the three observers (W.C.A.M.B.). The subjective judgement of both procedures by the MTX patients was clearly in favour of DHS.

The present study indicates that DHS, a relatively non-invasive procedure, is very promising for the detection of early MTX-induced hepatic damage. This approach may lead to a considerable reduction of the number of liver biopsies, in the near future. Pending further studies, performing liver biopsies according to the international guidelines,\textsuperscript{7} remains the gold standard for the safe and prolonged use of MTX in the treatment of psoriasis.

Acknowledgments

We would like to thank Dr J.M.J.I. Salemans, M.I. Verhulst and B.J.M. Witteman (Department of Gastroenterology) for performing liver biopsies, Professor Dr U.J.G.M. van Haelst (Department of Pathology) for histopathological evaluation of the liver biopsies, A.P.W. Meeuws (Department of Nuclear Medicine) for performance of the DHS, G.A.S. van de Wiel (Department of Clinical Chemistry), for determination of the PIIINP values, and the dermatologists, P. van Andel, Dr D. de Hoop, W. J.A. deKort (Ede) and I.H. Go (Tilburg), for referring patients for this study.

References

21 Frei A, Zimmerman A, Wiegand K. The N-terminal propeptide


