British Journal of Rheumatology 1997;**36:**338–344

QUANTITATIVE LIVER FUNCTION IN PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH LOW-DOSE METHOTREXATE: A LONGITUDINAL STUDY

C. BEYELER, J. REICHEN,* S. R. THOMANN, B. H. LAUTERBURG* and N. J. GERBER

*Department of Rheumatology and *Department of Clinical Pharmacology, University of Berne, Switzerland*

SUMMARY

The objectives were to determine quantitative liver function prospectively in patients with rheumatoid arthritis (RA) treated with low-dose methotrexate (MTX), to search for risk factors for a loss of quantitative liver function and to assess the relationship between quantitative liver function and histological staging. A total of 117 patients with RA (ACR criteria, 85 women, mean age 59 yr) had measurements of galactose elimination capacity (GEC), aminopyrine breath test (ABT) and liver enzymes [aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (AP), γ-glutamyl transferase (GGT), bile acids, bilirubin, albumin] before treatment with weekly i.m. MTX injections and every year thereafter. In 16 patients, liver biopsies were performed. Before the introduction of MTX, mean GEC was 6.6 mg/min/kg [5th to 95th percentile (5–95 PC) 5.1–8.5; reference range 6.0–9.1] and mean ABT was 0.80%kg/mmol (5–95 PC 0.42–1.30; reference range 0.6–1.0). During treatment with MTX [mean weekly dose 11.8 mg (5–95 PC 5.4–20.2), mean observation period 3.8 yr (5–95 PC 0.4–6.9)], significant declines of GEC (-0.12 mg/min/kg per year, $t = 3.30$, $P < 0.002$) and ABT (-0.06%kg/mmol per year, $t = 4.81$, $P < 0.001$) were observed. Negative correlations were found between the annual change in GEC and GEC at baseline ($R_S = -0.40$, $P < 0.0001$), and the annual change in ABT and ABT at baseline ($R_S = -0.43$, $P < 0.0001$). No correlations were found between the annual change in GEC or ABT and weekly MTX dose, age or percentage of increased liver enzymes, and no effect of a history of alcohol consumption >30 g/week became evident. Two patients with Roenigk grade III had impaired quantitative liver function, while 14 patients with Roenigk grades I and II exhibited a high variability of GEC and ABT from normal to abnormal values. The continuous declines in GEC and ABT observed deserve attention in patients with prolonged treatment. Patients with a low GEC or ABT at baseline seem not to be at increased risk for a further loss of quantitative liver function. An impaired GEC or ABT does not necessarily concur with hepatic fibrosis on histological examination.

KEY WORDS: Rheumatoid arthritis, Methotrexate, Liver.

LOW-DOSE methotrexate (MTX) is increasingly used in the treatment of RA even in early stages of the disease because of its favourable risk/benefit ratio compared to other disease-modifying anti-rheumatic drugs [1–4]. Hepatic side-effects are a limiting factor in a minority of patients during the first several years of MTX treatment [5]. Elevation of liver enzymes, typically the transaminases, does not exceed 2–3 times normal values, is temporary and regresses spontaneously or during temporary discontinuation of MTX in most patients. The majority of liver biopsies show only minor abnormalities, including fatty change, nuclear variability, portal inflammation and spotty hepatocellular necrosis. Similar changes may be seen in patients who have never received MTX and may be confounded by concomitant drugs including non-steroidal anti-inflammatory drugs, unrecognized alcohol ingestion and infectious hepatitis. However, advanced hepatic fibrosis and cirrhosis have been described in 1.1% of 719 patients usually biopsied within the first several years of MTX treatment with a mean cumulative dose ranging from 1.3 to 3.0 g. Longitudinal studies showed a moderate progression in a subgroup of patients. Because reports of histological follow-up for long periods are limited, it is unclear whether these favourable results also hold for long-term treatment. Therefore, hepatotoxicity after prolonged administration remains a concern. The development of serious liver disease can remain unrecognized until the late clinical findings of hepatic decompensation, such as ascites, oesophageal varices, splenomegaly, hepatic encephalopathy or hyperbilirubinaemia, are present. The cumulative risk was estimated to be 1/1000 patients at 5 yr [6]. Monitoring of liver blood tests on a regular basis should identify patients at risk for clinically serious liver disease and serial liver biopsies in unselected patients are no longer thought to be required or justified [7].

Little is known about quantitative liver function in patients with RA and the effect of MTX. Galactose elimination capacity (GEC) and aminopyrine breath test (ABT) have been shown to be efficacious in identifying the severity of disease in patients with chronic hepatitis and cirrhosis [8]. These procedures might be helpful in detecting early stages of MTX-induced liver disease. We therefore investigated GEC and ABT longitudinally in a cohort of patients with RA with the aims: (1) to examine changes in quantitative liver function over time; (2) to search for risk factors for a loss of quantitative liver function; and (3) to assess the relationship between quantitative liver function and histological staging.

Submitted 19 April 1996; revised version accepted 6 September 1996.

Correspondence to: Christine Beyeler, Department of Rheumatology, University Hospital, CH-3010 Berne, Switzerland.

PATIENTS AND METHODS

Patients

Between August 1984 and February 1991, 117 patients with RA were recruited. All patients fulfilled the modified ACR criteria [9] and had disease activity sufficient to warrant the introduction of low-dose MTX. Patients were excluded from the study according to the following criteria: active liver disease defined by aspartate amino transferase (AST) or alanine amino transferase (ALT) greater than two times the upper limit of the reference range; carriers of hepatitis B surface antigen and patients testing positive for human immunodeficiency virus; patients with a history of alcohol consumption >80 g/day in males or >40 g/day in females; renal failure defined by a serum creatinine >1.1 times the upper limit of the reference range; active bone marrow disease defined by leucopenia $<3000/mm^3$ or thrombopenia $<100\,000/mm^3$; pregnant women and patients of child-bearing age not practising effective contraception. Informed consent was obtained from all patients at the beginning of the study.

Methotrexate treatment

Weekly i.m. MTX injections, usually between 10 and 15 mg, were started. Every single dose applied was documented on a patient control sheet and the cumulative dose calculated. Regular clinical follow-ups were performed, usually every 3 months or in the case of intermittent deterioration. These included a history of alcohol consumption and concomitant drug treatment and appropriate safety investigations [full haematology, AST, ALT, alkaline phosphatase (AP), creatinine and erythrocyte folate concentrations]. Weekly MTX doses were repeatedly adjusted according to disease activity, minor side-effects, potential interactions with concomitant drug treatment and renal function. Reasons for stopping MTX were carefully documented. In the case of incomplete follow-up, special attention was paid to the verification of non-compliance due to different reasons in order not to miss any adverse effects of MTX. If MTX had to be stopped for more than 1 month for any reason, results obtained after the interruption were not included in the evaluations. Non-steroidal anti-inflammatory drugs, low-dose oral corticosteroids and other drug treatments were continued as necessary. Folic acid was substituted in the case of decreased erythrocyte folate levels.

Quantitative liver function tests

Before the introduction of MTX, and every year thereafter, an extensive hepatological assessment was performed. A physical examination was carried out looking for clinical signs of hepatic disease and blood samples were drawn after an overnight fast. Serum was analysed for AST, ALT, AP, γ-glutamyl transferase (GGT), bile acids, bilirubin and albumin according to standard procedures. GEC was measured following Tygstrup's procedure [10]. Briefly, disappearance from plasma was calculated following zero-order kinetics after injection of 0.5 g/kg body weight of galactose. The ABT was performed according to Pauwel's procedure [11]. Briefly, the radioactivity of 2 mmol of exspiratory CO_2 was measured 30 min after i.v. injection of a tracer dose of 1.6 μCi dimethyl [^{14}C]aminopyrine. Both GEC and ABT have been found to be superior to conventional liver tests in predicting histological severity and survival of patients with chronic liver disease [8]. Liver biopsies were carried out in 16 patients with a decrease in quantitative liver function, increase of both aminotransferases or total MTX dose >3 g before adoption of the ACR guidelines [5]. Liver histology was evaluated by a pathologist blinded for all clinical and laboratory parameters using the Roenigk classification system [12]. Data collection was stopped in June 1994.

Calculations

The mean weekly MTX dose was estimated by dividing the cumulative dose at the time point of the last quantitative liver function testing by the duration of treatment. In every patient, the proportions of results outside the reference ranges were calculated for all laboratory parameters analysed. A change in quantitative liver function over time was assessed by the calculation of slopes for each individual. In the 17 patients with two measurements only, the slope of the fitted line was calculated. In the 87 patients with three or more values, linear regression analyses were performed. Each individual graph was carefully studied for deviation from linearity (sudden change, curvature). In six out of 87 patients (6.9%) only, a rapid fall of GEC and ABT during the first year of MTX treatment was followed by stable values. In all other patients, use of the linear model was judged appropriate for GEC and ABT. The mean coefficients of variation of the slopes calculated and the percentage of patients with coefficients of variation <0.2 as an indicator of good predictive power of the linear regressions were as follows: GEC: 0.075, 100%; ABT: 0.180, 64.7%. Further statistical analyses included calculations of the mean, S.D., 5th and 95th percentile (5–95 PC), one group t-test and Spearman's rank correlation (R_s). The significance level was set at $P = 0.05$ with two-sided testing.

RESULTS

The 117 patients with RA included into the study had a mean disease duration of 9.9 yr (S.D. 9.6) and 99 (85%) patients were rheumatoid factor positive. Mean age was 59 yr (S.D. 12.7), 85 were women and 32 were men. Mean weight was 64 kg (S.D. 12.0) and mean serum creatinine at entry to the study was 79 μmol/l (S.D. 15.9) (reference range 44–115). Serum creatinine was 109.6% of the baseline value (S.D. 18.9) at the end of the study. Twelve (10%) patients gave a history of alcohol consumption >30 g/week [mean 110 g/week (S.D. 60.9)] during MTX treatment.

TABLE I
Liver blood tests in 117 patients with rheumatoid arthritis before and during treatment with low-dose methotrexate (MTX)

			Before MTX treatment		During MTX treatment
Test	Unit	Reference range	Mean value (5th–95th percentile)	Percentage of values outside reference range	Mean percentage of values outside reference range (5th–95th percentile)
Aspartate amino transferase (AST)	U/l	11–36	15.5 (6–29)	0.8	7.6 (0–40)
Alanine amino transferase (ALT)	U/l	10–41	18.0 (5–43)	5.1	10.8 (0–50)
Alkaline phosphatase (AP)	U/l	36–120	98.3 (50–188)	17.9	18.3 (0–100)
γ-Glutamyl transferase (GGT)	U/l	8–45	50.8 (10–161)	33.0	23.1 (0–100)
Bile acids (fasting)	μmol/l	0–6	2.3 (1–6)	3.4	2.4 (0–20)
Bilirubin	μmol/l	3–26	7.6 (4–14)	0	0.8 (0–0)
Albumin	g/l	32–52	31.6 (23–41)	41.6	18.2 (0–100)

Liver blood tests before treatment with low-dose MTX are summarized in Table I. Mean GEC at baseline was 6.6 mg/min/kg (5–95 PC 5.1–8.5; 29.1% of the tests outside the reference range of 6.0–9.1). Mean ABT at baseline was 0.80%kg/mmol (5–95 PC 0.42–1.30; 25.2% of the tests outside the reference range of 0.6–1.0).Until the end of the study in June 1994, the mean observation period was 3.8 yr (5–95 PC 0.4–6.9) (Fig. 1) and the mean weekly MTX dose was 11.8 mg (5–95 PC 5.4–20.2). Five patients (4.3%) were lost to follow-up and in a total of 50 patients (42.7%) MTX was stopped. The reason was remission of RA in 14 patients (12.0%), inefficacy of MTX in eight patients (6.8%), disorders unrelated to RA or MTX in 12 patients (10.3%) and adverse effects of MTX in 16 patients (13.7%): gastrointestinal intolerance ($n = 9$), acute pneumonitis ($n = 1$), pancytopenia ($n = 1$), progression of rheumatoid nodules ($n = 1$), cutaneous vasculitis ($n = 1$), post-injection pruritus ($n = 1$) and development of liver disease ($n = 2$). A 62-yr-old woman had a steady deterioration of quantitative liver function over 5 yr (cumulative dose 2.2 g). GEC decreased from 6.3 to 3.6 mg/min/kg and ABT from 1.1 to 0.39%kg/mmol, respectively. All liver enzymes remained within the reference range with the exception of GGT, and hepatitis B and C serology was negative. Liver biopsy showed Roenigk grade I and II after 3 and 5 yr, respectively. One year after stopping MTX,

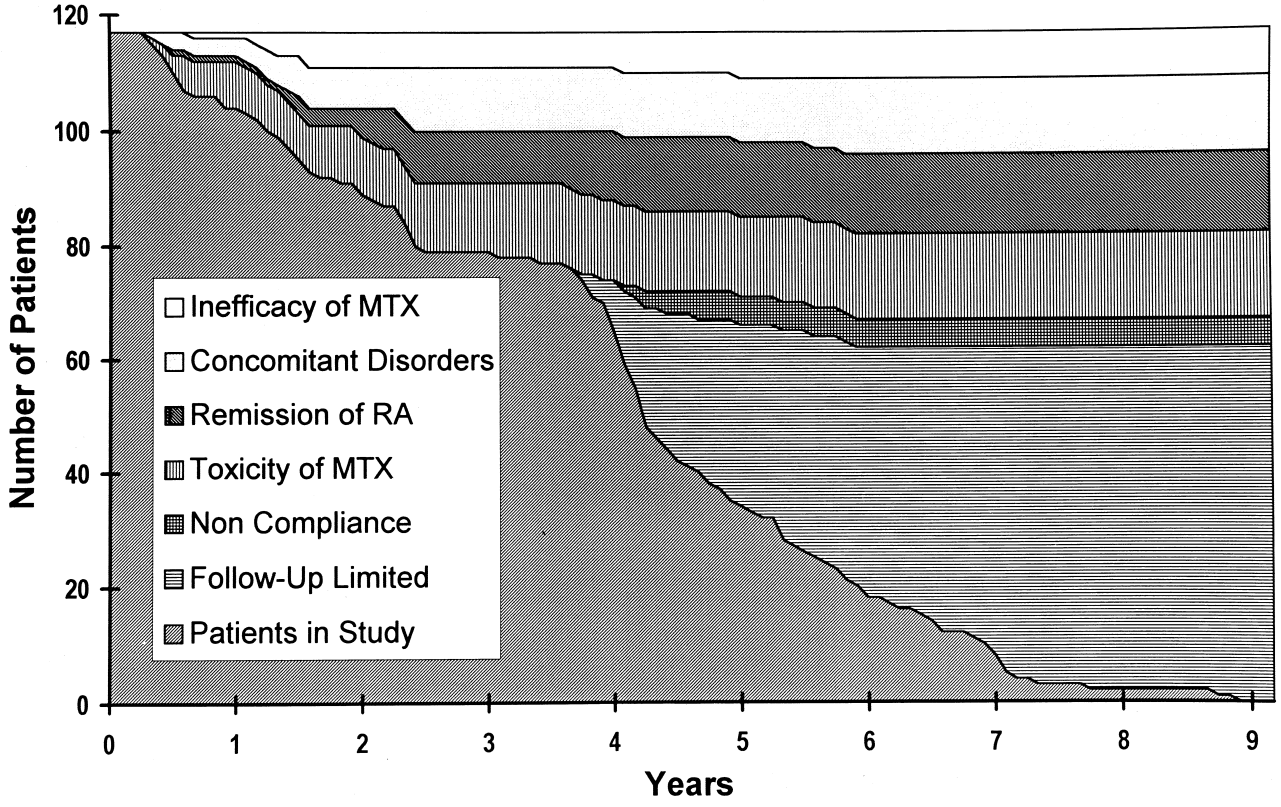

FIG. 1.—Life table graph of the number of patients with RA with quantitative liver function tests and withdrawal of methotrexate for different reasons.

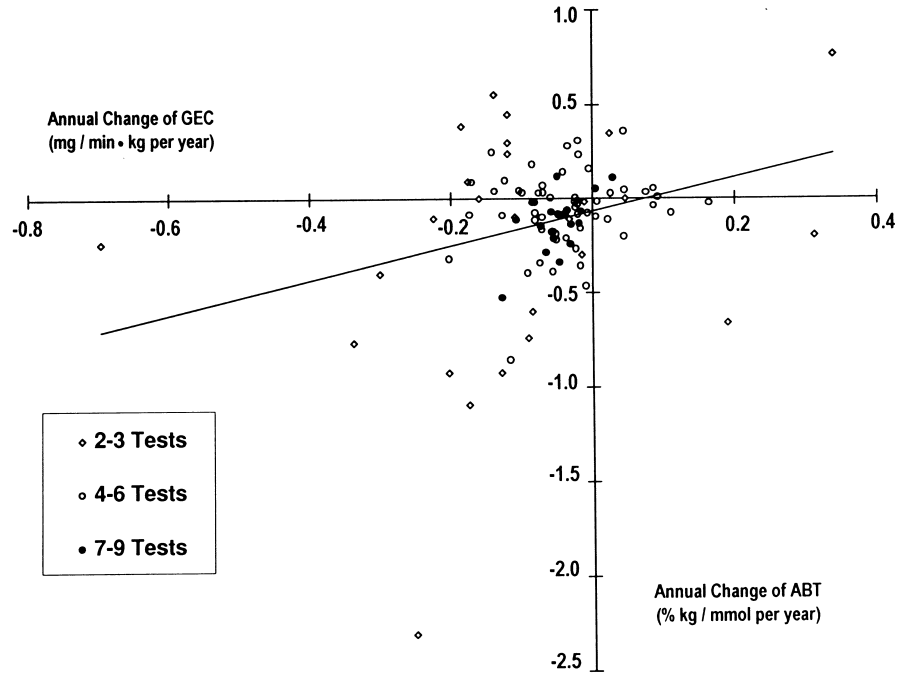

FIG. 2.—Plot of the annual change in galactose elimination capacity (GEC) *vs* the annual change in aminopyrine breath test (ABT). Patients with small numbers of tests showed a high variability of the annual changes calculated. However, mean annual changes remained significantly different from nought in all subgroups except one. GEC: 2–3 tests: -0.24 mg/min/kg, $t = 1.96$, n.s.; 4–6 tests: -0.60 mg/min/kg, $t = 2.17$, $P < 0.05$; 7–9 tests: -0.12 mg/min/kg, $t = 3.72$, $P < 0.002$. ABT: 2–3 tests: -0.094%kg/mmol, $t = 3.18$, $P < 0.01$; 4–6 tests: -0.035%kg/mmol, $t = 3.81$, $P < 0.001$; 7–9 tests: -0.042%kg/mmol, $t = 4.07$, $P < 0.002$.

quantitative liver function had recovered with a GEC of 6.3 mg/min/kg and an ABT of 0.65%kg/mmol. Half a year after the reintroduction of MTX, GEC was 5.5 mg/min/kg and ABT 0.68%kg/mmol. A 47-yr-old woman exhibited a continuous decline of quantitative liver function over 2.5 yr (cumulative dose 1.1 g). GEC decreased from 7.0 to 4.8 mg/min/kg and ABT from 0.91 to 0.32%kg/mmol, respectively. All liver blood tests increased to levels above the reference range (AST 200 U/l, ALT 120 U/l, AP 190 U/l, GGT 125 U/l, bile acids 17.5 μmol/l) and remained unchanged 3 months after stopping MTX. Hepatitis B and C serology, anti-nuclear factor and anti-mitochondrial antibodies were negative. Liver histology revealed signs of chronic hepatitis with plasma cell infiltration, piece meal necrosis and incomplete septal fibrosis without nodular formation (Roenigk grade III B) 12 and 30 months after the introduction of MTX. A diagnosis of chronic active autoimmune hepatitis was made and successful treatment with oral corticosteroids was established with normalization of all liver enzymes.

The percentage of liver blood tests outside the reference range during MTX treatment is summarized in Table I. A significant decline of quantitative liver function was observed. The mean annual change in GEC was -0.12 mg/min/kg per year (5–95 PC -0.76 to 0.35, $t = 3.30$, $P < 0.002$). The mean annual change in ABT was -0.057%kg/mmol per year (5–95 PC -0.203 to 0.092, $t = 4.81$, $P < 0.001$). The annual change in GEC was only weakly correlated with the annual change in ABT ($R_S = 0.18$, $P = 0.067$) (Fig. 2), in analogy to a weak correlation between GEC and ABT at baseline ($R_S = 0.20$, $P = 0.033$). A strong negative correlation was found between the annual change in GEC and GEC at baseline ($R_S = -0.40$, $P < 0.0001$) which was maintained after analysis of the proportional annual change in GEC in relation to GEC at baseline ($R_S = -0.36$, $P < 0.0002$). There was also a strong negative correlation between the annual change in ABT and ABT at baseline ($R_S = -0.43$, $P < 0.0001$) which became weaker with analysis of the proportional annual change in ABT in relation to ABT at baseline ($R_S = -0.24$, $P = 0.015$). Therefore, patients with a low GEC or ABT at baseline seemed not to be at increased risk for further declines during MTX therapy. No effect of alcohol consumption, the presence of rheumatoid factor and gender became evident. In addition, no correlations were found between the annual change in GEC or ABT and age, mean weekly MTX dose or percentage of liver blood tests, including albumin, outside the reference range.

The results of 18 liver biopsies in 16 patients and their corresponding quantitative liver function tests are presented in Table II. In 11 patients, a liver biopsy was performed because of a pronounced fall in GEC or ABT (-1.8 mg/min/kg and -0.39%kg/mmol, respectively). In two of them, liver biopsies had to be repeated because of a further decline of quantitative liver function. In an additional five patients, liver biopsies were performed because of increases in AST and ALT or after a total MTX dose >3 g. No relationships were revealed between Roenigk grading

TABLE II
Liver biopsy findings and quantitative liver function tests in 16 patients with rheumatoid arthritis. Reasons for liver biopsy: a = drop of quantitative liver function; b = increase of both aminotransaminases; c = cumulative methotrexate dose >3 g before adoption of the ACR guidelines [5]

				Galactose elimination capacity (mg/min/kg)		Aminopyrine breath test (%kg/mmol)			
Number	Age (yr)	Sex	Roenigk grade	Value at time of liver biopsy	Change to value before methotrexate treatment	Value at time of liver biopsy	Change to value before methotrexate treatment	Cumulative methotrexate dose at time of liver biopsy (mg)	Reasons for liver biopsy
1	57	f	I	7.7	− 0.6	1.28	− 0.05	3210	c
2	50	f	I	6.3	− 2.6	0.41	− 0.64	2100	a
3	29	f	I	6.2	− 1.1	0.44	+ 0.02	4350	c
4	63	m	I	6.2	− 0.3	–	–	3130	c
5	51	m	I	5.6	− 0.6	0.47	− 0.07	3130	c
6	62	f	I	4.9	− 1.4	0.44	− 0.66	1350	a
7	68	f	I	4.7	− 1.5	0.36	− 0.25	490	a
8	68	f	I	4.6	− 1.3	0.33	− 0.47	460	a
9	51	f	I	4.6	− 1.7	–	–	2680	a
10	57	f	I	4.1	− 1.6	0.70	− 0.23	500	a
11	61	f	I	3.8	− 2.8	0.23	− 0.69	2320	a
12	50	f	II	7.7	− 0.3	–	–	930	b
13	62	f	II	4.9	− 1.8	0.40	− 0.21	1140	a
14	56	f	II	4.8	− 2.8	0.89	+ 0.01	640	a, b
6*	64	f	II	3.6	− 2.7	0.37	− 0.73	2200	a
15	67	m	III A	5.0	− 0.6	0.53	− 0.14	1540	a, b
16	49	f	III B	5.7	− 1.3	0.34	− 0.57	580	a, b
16*	50	f	III B	4.8	− 2.2	0.32	− 0.59	1050	a, b

* = follow-up biopsy.

and quantitative liver function or total MTX dose, respectively.

DISCUSSION

This is the first longitudinal study demonstrating a continuous decline of quantitative liver function assessed by GEC and ABT in patients with RA treated with low-dose weekly MTX. The mean annual losses of GEC (0.12 mg/min/kg per year) and ABT (0.06%kg/mmol per year) were higher than the decreases expected due to ageing, estimated in healthy subjects to be 0.03 mg/min/kg per year and 0.003%kg/mmol per year, respectively [13]. Both tests showed a decline of quantitative liver function, although they are only weakly related to each other and measure activities of different enzyme systems. ABT, quantifying microsomal liver function, is influenced by a variety of xenobiotics, which can either induce or inhibit the drug-metabolizing enzyme system. In contrast, GEC, assessing cytosolic liver function, does not appear to be markedly influenced by genetic and environmental factors. This could, at least in part, explain the higher variability of the individual time course of ABT than GEC, reflected by higher coefficients of variation of the slopes calculated by regression analyses.

The present study failed to identify risk factors for a loss of quantitative liver function during MTX treatment. No effect of gender became evident and no correlations were found between annual change in GEC or ABT and age or mean weekly MTX dose. This is in agreement with histological findings, where the incidence of increased fibrosis was the same in male and female patients [7, 14]. Progression of MTX-associated liver abnormalities was also not related to age, nor was advanced age a feature of MTX-associated cirrhosis in case reports of patients with RA [15]. However, a retrospective case–control study found higher age at first use of MTX to be a predictor of serious liver disease [6]. In an electron microscopic study, weekly MTX dosage was also not different in patients with progression of fibrosis compared to those without [14]. No associations were found between the annual change in GEC or ABT and the percentage of liver blood tests outside the reference range. This finding on quantitative liver function contrasts with a report in the literature that histological deterioration, as observed in hepatic tissue obtained from annual liver biopsies, was positively correlated with the number of elevations of AST, when monitoring was performed every 4–6 weeks [7]. However, the prediction of histological abnormality may be limited to serial as opposed to isolated aminotransferase abnormalities [16, 17]. Finally, a low GEC or ABT at baseline seemed not to be risk factors for further declines during MTX therapy in view of negative correlations between annual change in quantitative liver function and baseline value for both tests. These findings on function are in agreement with those on histology, where changes on pre-treatment liver biopsy did not appear to be predictors of subsequent fibrosis or cirrhosis [16].

The decline of quantitative liver function observed would be, at least in part, compatible with the evidence

of progression of fibrosis on electron microscopy in a subgroup of patients receiving MTX [14]. However, no clear-cut correlation between quantitative liver function and liver histology grading was evident from our results. Nevertheless, it is noteworthy that all patients with Roenigk grade III had an impaired quantitative liver function, while patients with Roenigk grades I and II exhibited a high variability of GEC and ABT from normal to abnormal values. It is unlikely that these disparities between functional and histological alterations are due to sampling errors only. GEC and ABT are efficacious in identifying the severity of disease in patients with hepatitis and cirrhosis [8]. However, in agreement with our results, oral and i.v. ABT seemed to be ineffective in identifying early MTX-induced liver disease in patients with RA or psoriasis [15, 18–20]. Similarly, antipyrine clearance was negatively correlated with liver biopsy scores for fatty changes, but not for fibrosis, liver cell necrosis and inflammatory infiltration of the portal tracts [21].

Part of the deterioration of quantitative liver function during MTX treatment might be reversible and not related to irreversible structural changes. One case reported in the present study illustrates the possibility of recovery of GEC and ABT after stopping MTX. This observation is in agreement with a previous report of four patients who showed a return to normal values of oral ABT 6–24 months after MTX withdrawal [18]. A possible explanation might be the reduction of the regenerative potential of hepatic cells by MTX. Alternatively, MTX might inhibit various enzyme systems, resulting in slowed metabolism of the test compounds used.

In the present study, no associations between quantitative liver function and disease activity were examined in view of the continuous fluctuation of the latter. A subclinical hepatobiliary involvement is known in active RA [16, 22–24]. This is supported by high proportions of increased alkaline phosphatase and GGT, enzymes known to be correlated with disease activity [25]. In addition, interactions with concomitant drug treatments, including non-steroidal anti-inflammatory drugs, remain unexplored in the present study because of the changing compounds and dosages in response to the changing disease activity.

In summary, the continuous decline of quantitative liver function at a higher rate than that due to the normal process of ageing deserves attention in patients treated with low-dose MTX. To what extent a hepatobiliary involvement of RA or MTX-unrelated adverse drug reactions play a role in the accelerated declines of GEC and ABT remains open. No risk factors for a marked loss of quantitative liver function, such as mean weekly MTX dose, age, gender or a low quantitative liver function at baseline, could be established. A loss of GEC and ABT points to a decline in hepatic function, but is not helpful in detecting early stages of MTX-induced liver disease and identifying the subgroup of patients with RA who will progress to advanced hepatic fibrosis and cirrhosis.

Acknowledgements

We are grateful to Prof. Arthur Zimmermann for reviewing the pathology specimens. We thank Dr Rolf Gerny, Dr Franz Koelliker, Prof. Ruedi Preisig, Dr Eberhard Renner, and all colleagues from the Departments of Rheumatology and Clinical Pharmacology for their help with the clinical data collection, and Dr Christoph Minder for statistical advice. We also thank D. Messer and M. Keiser for secretarial assistance. We acknowledge financial support from the Swiss Federal Health Office Research Fund, Lederle (Switzerland) and private funds.

References

1. Bannwarth B, Labat L, Moride Y, Schnaeverbeke T. Methotrexate in rheumatoid arthritis. Drugs 1994;47:25–50.
2. Schnabel A, Gross WL. Low-dose methotrexate in rheumatoid diseases—efficacy, side effects and risk factors for side effects. Semin Arthritis Rheum 1994;23:310–27.
3. Goodman TA, Polisson RP. Methotrexate: adverse reactions and major toxicities. Rheum Dis Clin North Am 1994;20:513–28.
4. Fries JF, Williams CA, Ramey D, Bloch DA. The relative toxicity of disease-modifying antirheumatic drugs. Arthritis Rheum 1993;36:297–306.
5. Kremer JM, Alarcon GS, Lightfoot RW, Willkens RF, Furst DE, Williams HJ *et al*. Methotrexate for rheumatoid arthritis. Suggested guidelines for monitoring liver toxicity. Arthritis Rheum 1994;37:316–28.
6. Walker AM, Funch D, Dreyer NA, Tolman KG, Kremer JM, Alarcon GS *et al*. Determinants of serious liver disease among patients receiving low-dose methotrexate for rheumatoid arthritis. Arthritis Rheum 1993;36:329–35.
7. Kremer JM, Lee RG, Tolman KG. Liver histology in rheumatoid arthritis patients receiving long-term methotrexate therapy: a prospective study with baseline and sequential biopsy samples. Arthritis Rheum 1989;32:121–7.
8. Reichen J. Assessment of hepatic function with xenobiotics. Semin Liver Dis 1995;15:189–201.
9. Arnett FC, Edworthy SM, Bloch DA. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
10. Tygstrup N. Determination of the hepatic galactose elimination capacity after a single i. v. injection in man. Acta Physiol Scand 1963;58:162–72.
11. Pauwels S, Geubel AP, Dive C, Beckers C. Breath $^{14}CO_2$ after intravenous administration of ^{14}C aminopyrine in liver diseases. Dig Dis Sci 1982;27:49–56.
12. Roenigk HH, Auerbach R, Maibach HI, Weinstein GD. Methotrexate guidelines revised. J Am Acad Dermatol 1982;6:145–55.
13. Schnegg M, Lauterburg BH. Quantitative liver function in the elderly assessed by galactose elimination capacity, aminopyrine demethylation and caffeine clearance. J Hepatol 1986;3:164–71.
14. Bjorkman DJ, Hammond EH, Lee RG, Clegg DO, Tolman KG. Hepatic ultrastructure after methotrexate therapy for rheumatoid arthritis. Arthritis Rheum 1988;31:1465–72.
15. Bjorkman DJ, Boschert M, Tolman KG, Clegg DO,

Ward JR. The effect of long-term methotrexate therapy on hepatic fibrosis in rheumatoid arthritis. Arthritis Rheum 1993;36:1697–701.
16. Brick JE, Moreland LW, Al-Kawas F, Chang WWL, Layne RD, Di Bartolomeo AG. Prospective analysis of liver biopsies before and after methotrexate therapy in rheumatoid arthritis. Semin Arthritis Rheum 1989; 19:31–44.
17. Tolman KG, Clegg DO, Lee RG, Ward JR. Methotrexate and the liver. J Rheumatol 1985;12(Suppl): 29–34.
18. Williams CN, McCauley D, Malatjalin DA, Turnbull GK, Ross JB. The aminopyrine breath test, an inadequate early indicator of methotrexate-induced liver disease in patients with psoriasis. Clin Invest Med 1987;10:54–8.
19. Ytterberg SR, Knodell RG, Mahowald ML. Use of the aminopyrine breath test to monitor hepatotoxicity in the treatment of rheumatoid arthritis with methotrexate. Contemp Orthoped 1988;15:59–65.
20. Arias JM, Morton KA, Albro JE, Patch GG, Valdivia S, Greenberg HE *et al*. Comparison of methods for identifying early methotrexate-induced hepatotoxicity in patients with rheumatoid arthritis. J Nuclear Med 1993;34:1905–9.
21. Paramsothy J, Strange R, Sharif H, Collins M, Shaw P, Lawrence CM. The use of antipyrine clearance to measure liver damage in psoriatic patients receiving methotrexate. Br J Dermatol 1988;119:761–5.
22. Weinblatt ME, Tesser JRP, Gilliam JH. The liver in rheumatic diseases. Semin Arthritis Rheum 1982;11:399–405.
23. Mill PR, Sturrock RB. Clinical associations between arthritis and liver disease. Ann Rheum Dis 1982;41:295–307.
24. Fernandes L, Sullivan S, McFarlane IG, Wojcicka BM, Warnes TW, Eddleston ALWF *et al*. Studies on the frequency and pathogenesis of liver involement in rheumatoid arthritis. Ann Rheum Dis 1979;38:501–6.
25. Beyeler C, Banks R, Thompson D, Forbes MA, Cooper EH, Bird HA. Bone alkaline phosphatase in rheumatoid arthritis. J Rheumatol 1996;23:241–4.