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ORIGINAL ARTICLE

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High-dose methotrexate: on the relationship of methotrexate elimination time vs renal function and serum methotrexate levels in 1164 courses in 264 Swedish children with acute lymphoblastic leukaemia (ALL)

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Abstract *Purpose*: The objectives of the present study were to determine the relationship between methotrexate (MTX) elimination time and various aspects of renal function and to evaluate the prognostic value of elevated serum MTX and creatinine for delayed MTX elimination. *Patients and methods*: The majority of the 264 children were being treated for ALL. According to the NOPHO-92 protocol, 5 or 8 g MTX/m^2 was administered over 24 h. Serum creatinine was assessed daily. In 11 patients from one centre, renal function was studied in more detail using serum cystatin C, iohexol clearance, and urinary albumin, IgG and protein HC. *Results*: Increased serum creatinine correlated significantly with the elimination time of MTX, whereas no indications were found of tubular or barrier function damage. Of the 1164 courses, 44 had delayed elimination of MTX (≥120 h). Serum MTX >150 μ*M* at the end of infusion had a sensitivity of 0.27 and a specificity of 0.94 to predict delayed MTX elimination, and ≥50% increase in serum creatinine during the first treatment day (creatinine ratio) had a sensitivity of 0.32 and a specificity of 0.99. The corresponding risk ratios were 5 and 19 for MTX >150 μ*M* and creatinine ratio, respectively. In courses with a normal elimination time (<72 h), 99% of the courses had a rise in serum creatinine of less than 50%. *Conclusions*: Elevation of serum creatinine by more than 50% is a better predictor of delayed elimination than the level of serum MTX at the end of MTX infusion, especially if information on previous creatinine measurements is used to reduce the impact of an occasionally low serum creatinine value before the start of the MTX infusion.

Keywords MTX elimination and renal function · Creatinine · Glomerular filtration rate · Proximal tubule

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Introduction

Methotrexate (MTX) is used against a variety of disorders in oncology. As well as serum concentrations, the time of exposure is also of importance for both toxicity and antileukaemic activity [19, 20, 29]. The introduction of folinic acid rescue has enabled high doses of MTX to be administered with reduced toxicity. Serum MTX (S-MTX) levels are routinely used for the dosing of folinic acid, and the elimination of S-MTX is therefore assessed as a clinical routine. A factor complicating the use of high-dose MTX (HDMTX) is that the elimination of MTX can be profoundly delayed. MTX is mainly excreted by renal elimination but the exact mechanism is not known. Studies in monkeys have suggested that clearance is chiefly determined by renal tubular function at lower concentrations (0.1–3.7 μ*M*) and by glomerular filtration rate (GFR) at higher levels (13–70 μ*M*) [3]. The results of Abelson et al. in humans suggest that

HDMTX induces a transient decrease in GFR [1]. With a longer follow-up, GFR is not attenuated [17]. Although MTX clearance has been found to be significantly correlated with GFR, glomerular function is reported to explain only a small part of the variability in clearance of MTX [17].

A suggested mechanism for delayed elimination is precipitation of 7-OH-MTX in the renal tubule leading to renal dysfunction [13, 14, 23, 26]. To prevent tubular precipitation of 7-OH-MTX, most HDMTX protocols stipulate alkalization and standardized hydration to keep urinary pH and diuresis high. Previous studies [7, 8, 24] have indicated that the MTX concentrations achieved are related to the level of hydration. Apart from reduced S-MTX concentrations, toxicity is also reduced with more vigorous hydration and alkalization [5, 22]. A general feature of many HDMTX protocols is therefore to augment hydration if there is an indication that MTX elimination will be delayed.

In the NOPHO-92 (Nordic Society for Paediatric Haematology and Oncology) protocol, a S-MTX level of 3 μM at 36 h after the start of MTX infusion is a cut-off level for increasing hydration and alkalization. In the ALL-BFM-95 (Berlin-Frankfurt-Münster) protocol, it is suggested that the hydration and alkalization should be augmented as early as 24 h after the start of the infusion if the steady-state level of S-MTX is above 150 μM.

It is a well-known clinical observation that delayed elimination of MTX appears to be related to elevated serum creatinine (S-creatinine) [16]. S-creatinine is an indicator of GFR but a number of markers for renal function have been suggested to describe various aspects of renal function better, and in more detail. For example, urinary protein HC (α_1-microglobulin) is freely filtered in glomeruli and is normally reabsorbed in the renal tubules. Increased urinary levels of protein HC has been suggested as a sensitive and reliable indicator of tubular dysfunction [9]. Increased excretion of urinary albumin and immunoglobulin G indicates glomerular damage with an impaired barrier function. These parameters have previously been used to assess renal function after cisplatin therapy [15] and reference limits have been proposed for adults [28] and children [12]. Serum cystatin C has been claimed to be a better indicator of GFR than S-creatinine [9]. This has also been shown in children with and without renal dysfunction, and age-independent reference limits have been proposed [11]. Iohexol clearance is an established measure of GFR [2, 4, 18].

The present study was performed to evaluate the recommendations of the ALL-BFM-95 and NOPHO-92 protocols, and to examine the relationship between MTX elimination time and various aspects of renal function. If an early predictor of delayed MTX elimination could be applied, measures could be taken earlier (e.g. intensified alkalization and hydration) to reduce renal impairment and avoid the use of excessive folinic acid rescue which may adversely affect the efficacy of HDMTX.

Materials and methods

In Sweden 388 children were diagnosed with ALL from January 1992 to December 1997. Data regarding MTX dose, body surface area, S-creatinine and S-MTX concentrations in 1164 courses could be recovered for 264 patients treated during that period at ten paediatric oncology units in Sweden with 1 to 79 patients per unit (4–425 courses per unit). The mean patient age at the start of the first course was 6.5 years (range 0.7–18 years) and the male/female ratio was 151/113. Of the patients, 95% were treated according to the NOPHO-92 protocol. The remainder were treated according to the ALL BFM-90 protocol. Of the 264 patients, 91% had acute lymphoblastic leukaemia (ALL) and the rest had non-Hodgkin's lymphoma (NHL).

At one centre (Lund), in a subpopulation of these patients (11 consecutive children; 58 courses of HDMTX; two to nine consecutive courses per patient; median 5) renal function was studied prospectively and in more detail. The study protocol in these 11 patients required that S-creatinine, S-cystatin C and iohexol clearance should be measured prior to each course. S-creatinine was thereafter followed daily during the course. Protein HC, immunoglobulin G, albumin and creatinine were analysed in spot-urine before the start and during the 2nd day of treatment. To adjust for different voiding volumes, the first three of these are expressed as ratios in relation to urinary creatinine [12]. The ratios in relation to urinary creatinine are abbreviated U-alb for albumin, U-IgG for immunoglobulin-G and U-HC for protein HC.

Iohexol clearance was measured during the initial phase of MTX infusion. All the other baseline values were sampled on the day of treatment before the start of MTX infusion. S-MTX was analysed using EMIT (enzyme multiplied immunoassay technique; Behring Diagnostics, Syva Business, San Jose, Calif.) on a Cobas Mira S analyser (Roche, Basel, Switzerland). Cystatin C was determined by an immunoassay on a Cobas Mira Plus Instrument (Roche). U-HC, IgG and albumin were analysed using immunoturbidimetry as previously described [27]. Serum and urinary creatinine (U-creatinine) were determined using a Kodak Ektachem 700 XR-C analyser using the enzyme creatinine amidinohydrolase. GFR was estimated in terms of iohexol clearance measuring the concentration at two time points during the elimination phase (for details, see reference 2). At some of the centres in the entire study group MTX was analysed using FPIA (fluorescence polarization immunoassay; Abbott Scandinavia, Stockholm, Sweden).

Depending on the risk group classification [10], the stipulated doses of MTX were 5 or 8 g/m^2 of which one-tenth was infused intravenously (i.v.) over the first hour and the remaining nine-tenths over the following 23 h. I.v. hydration using 5% glucose containing 42 mM $NaHCO_3$/l and 20 mM KCl/l was stipulated to 3000 ml/m^2 over 24 h and was increased to 4500 ml/m^2 over 24 h if S-MTX 36 h after the start of infusion was ≥3 μmol/l. Urinary pH (U-pH) was measured at every voiding. $NaHCO_3$ (20 mmol in the courses with 5 g MTX/m^2 and 2 mmol/kg in the courses with 8 g MTX/m^2) was administered i.v. if U-pH was < 7. Furosemide (0.5–1 mg/kg, maximum 20 mg) was required to be administered i.v. for diuresis < 100 ml/m^2 per h. S-MTX levels were monitored at 23 and 36 h after the start of infusion and thereafter every 6 h until the S-MTX level was below 0.2 μM. In the 5- and 8-g courses racemic folinic acid (N5-formyl-tetrahydrofolic acid) was administered i.v. 36 h after the start of infusion at the doses 15 and 50 mg/m^2, respectively. Folinic acid 15 mg/m^2 was also given 39 and 42 h (8-g courses) or 42 h (5-g courses) after the start of MTX infusion. The dose of folinic acid was increased if S-MTX was ≥1 μM at 42 h. Otherwise 15 mg/m^2 was thereafter given every 6 h until 6 h after S-MTX had reached a level < 0.2 μM. S-creatinine was required to be measured daily.

The study was approved by the Research Ethics Committee. Patients and/or parents and/or guardians gave their informed consent to participation in the study.

The Lund subpopulation

The baseline values for urinary parameters were categorized using the upper reference limits proposed by Hjorth et al. [12] as cut-off limits (U-alb 3.8 mg/mmol at 1 month to 1 year, 3.3 mg/mmol at 1–5 years, 2.7 mg/mmol at 6–10 years, 2.1 mg/mmol at 11–15 years; U-IgG 1.0 mg/mmol at 1 month to 15 years; and U-HC 0.8 mg/mmol at 1 month to 15 years). The elimination times in courses with levels above the upper reference limits were compared to those with levels below each limit. Defining the day of treatment start as day 0, the change until day 2 was categorized according to whether an increase or a decrease was seen (i.e. day 2 value−day 0 value, >0 or ≤ 0). Column statistics were performed calculating the mean elimination times with 95% confidence intervals (CI) and multiple linear regressions to assess the differences between means.

For serum parameters (iohexol clearance, S-cystatin C and S-creatinine), both multiple linear regression and correlation were performed.

The entire study group

S-MTX >150 μM sampled 23 h after the start of infusion (proposed by the BFM group) was one of the three discrimination criteria examined for its ability to predict delayed S-MTX elimination. The other two were creatinine ratio and mean pretreatment creatinine ratio. The creatinine ratio was obtained by dividing the S-creatinine measured 12–24 h after the start of MTX infusion by the value obtained before the start of the course. The mean pretreatment creatinine ratio was calculated when data from at least one previous course existed (946 courses in 238 patients). In this case, S-creatinine measured 12–24 h after the start of MTX infusion was divided by the mean pretreatment S-creatinine calculated from the present and all previous courses in each specific individual.

Sensitivity and specificity were calculated for the three different discrimination criteria. The predictive value of a positive and negative value, and risk ratios for the three different approaches were also computed. Linear regression of the correlation between the number of days with a creatinine ratio ≥1.5 and elimination time was performed.

Data analysis

Data collected were entered into a database application (Microsoft Access 97) especially designed for the purpose. Data analysis was performed using GraphPad Prism, version 3.0 (GraphPad Software, San Diego, Calif.). Permission to use the generated database was granted by the Data Inspection Board of Sweden.

Statistics

Elimination time was defined as the time in hours from the start of MTX infusion until the first measurement of a S-MTX concentration <0.2 μM. "Delayed" MTX elimination was defined as ≥120 h and "normal" elimination as <72 h from the start of MTX infusion until the first occasion a S-MTX <0.2 μM was reached. These definitions were arbitrary but based on clinical experience (<72 h meant that the patient did not have to spend the weekend in hospital) and were decided before the start of data analysis.

The two-tailed Student's *t*-test was used if not otherwise stated. A level of $P<0.05$ was regarded as statistically significant. Values given in the text denote mean ± SEM. No adjustment for multiple comparisons was performed.

Results

Urinary parameters in the Lund subpopulation

Of the 58 courses included, urine samples (U-alb, U-IgG, U-HC) were available from 38 courses for day 0, 36 courses for day 2, and 24 courses for both days 0 and 2. Baseline values for the urinary parameters were categorized as above or below the upper reference limits. The means and the 95% CIs of the MTX elimination times were calculated for the groups and are shown in Fig. 1. The corresponding calculations were performed with the urinary parameters measured on day 2 (data not shown). The differences between day 2 and baseline were categorized according to whether the value was increased or not. Interestingly, in the course with a higher day-2 U-HC the patient received diclofenac before and during the entire MTX course. The MTX elimination time in that course was 198 h and the U-HC increased from 1.3 to 10.8 mg/mmol but U-alb and U-IgG were essentially unaffected. Courses with U-alb above the upper reference limits before the start of the course had significantly ($P=0.02$) longer MTX elimination times than those below. However, if the course with diclofenac was excluded, it was not significant ($P=0.25$). There was no significant difference in MTX elimination time between courses with U-IgG and U-HC below or above the upper reference limits, whether or not the course with diclofenac was excluded.

Serum parameters in the Lund subpopulation

Iohexol clearance and S-creatinine on day 0 were measured in all 58 courses. S-cystatin C (sampled before the start of the course) and S-creatinine on day 2 were measured in 47 and 57 courses, respectively. In only one course was the iohexol clearance below the reference value (80 ml/min per 1.73 m^2) but the elimination time in that course was normal. All cystatin C values were below the upper reference limit [11] (1.33 mg/l).

Multiple linear regression revealed no significant correlation between the baseline values and the elimination time of MTX. If the course, discussed above, with concomitant diclofenac treatment was excluded, GFR was, however, significantly correlated with elimination time ($P=0.04$, $r^2=0.31$). S-creatinine on day 2 was also correlated significantly with elimination time ($P<0.0001$, $r^2=0.68$). Figure 2 illustrates the difference in the levels of S-creatinine between day 0 and day 2 which was significantly correlated with time of MTX elimination ($P<0.0001$, $r^2=0.63$) whether or not the diclofenac course was excluded. The results were essentially the same even if the interindividual differences were not taken into account (i.e. all courses were evaluated in a correlation analysis).

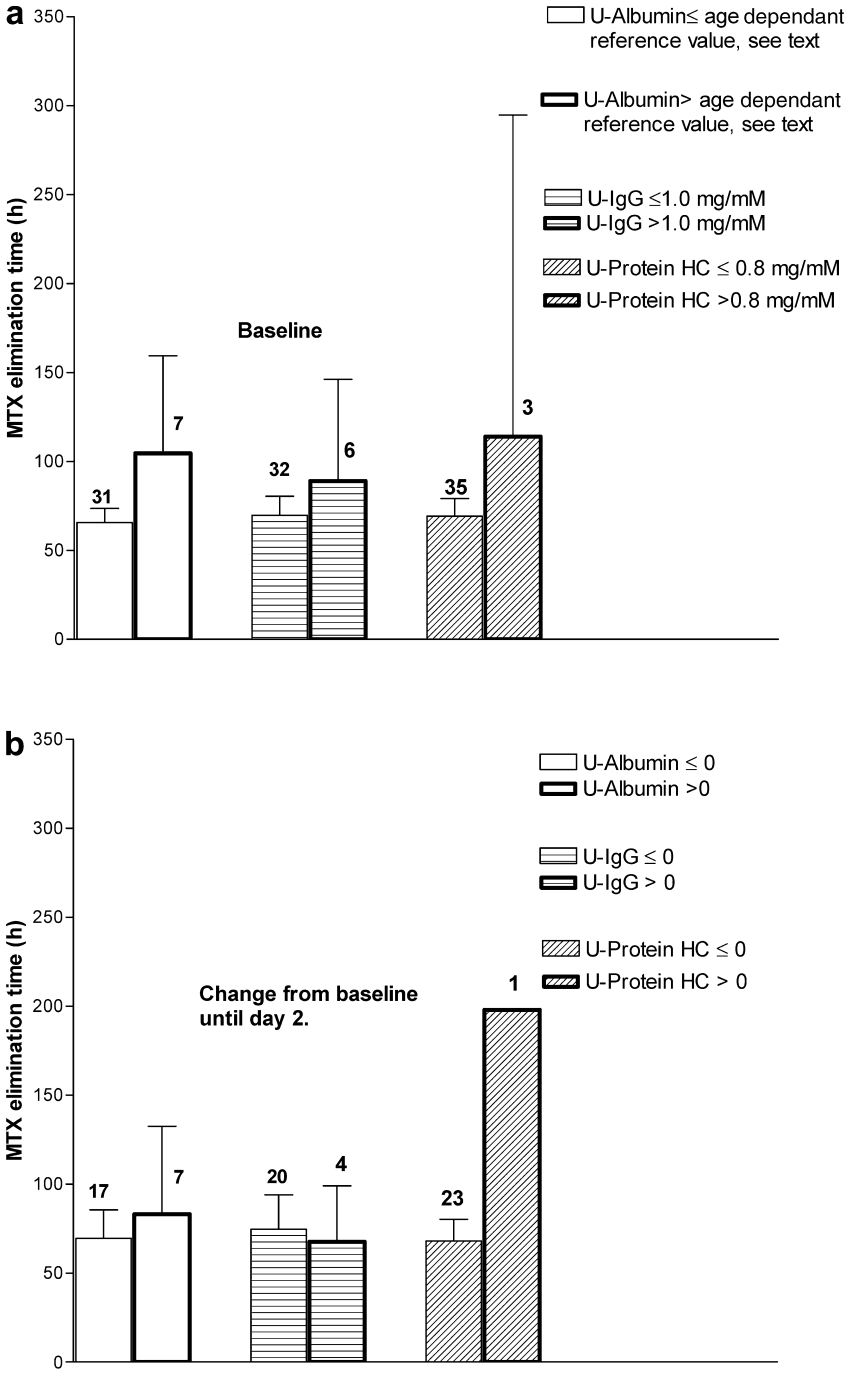

Fig. 1a, b Urinary parameters and elimination time of MTX in the Lund subpopulation. The number above each bar is the number of treatment courses in each group. The height of the columns represents the elimination times with 95% CI. **a** U-albumin, U-IgG and U-Protein HC before the start of each MTX course. Each parameter is divided into two groups according to whether the values are below or above the upper reference limit for that parameter. **b** Each parameter is divided into two groups according to whether the values were increased or not on day 2 of treatment compared to before the start of the course

The entire study group (1164 courses)

Although the stipulated doses were 5 and 8 g/m^2, the doses ranged between 0.96 and 10.8 g/m^2 with 42 courses <4 g/m^2, 995 courses ≥ 4 and <7 g/m^2, and 127 courses ≥ 7 g/m^2. The 127 courses with doses ≥ 7 g MTX/m^2 had significantly longer elimination times (77.9 ± 2.2 h) than the 1037 courses with doses <7 g MTX/m^2 (65.8 ± 0.7 h, $P < 0.0001$).

S-MTX at the end of infusion (C_{23})

Had a S-MTX level of >150 μM at C_{23} been used as the cut-off level, as proposed by the BFM group, 12 courses in 12 patients who developed delayed elimination would have been identified and given increased alkalized hydration at that stage (Fig. 3). However, 32 courses in 26 patients who developed delayed elimination would not have received increased hydration based on the C_{23} MTX

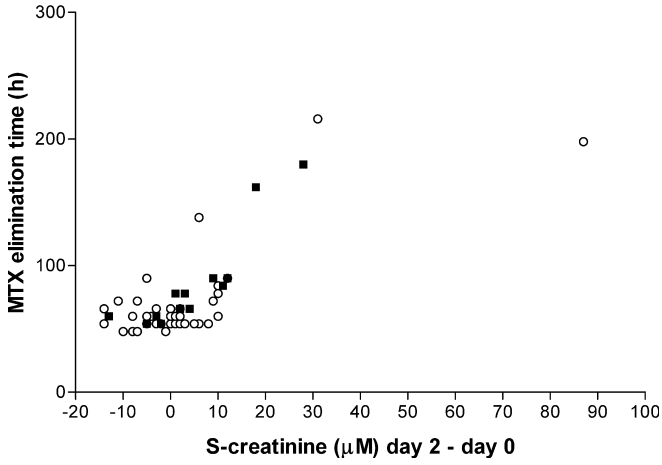

Fig. 2 Change in S-creatinine from day 0 (baseline) to day 2 of treatment in relation to the elimination time of MTX in the Lund subpopulation (○ treatment courses with 5 g MTX/m^2, $n = 43$; ■ treatment courses with 8 g MTX/m^2, $n = 14$)

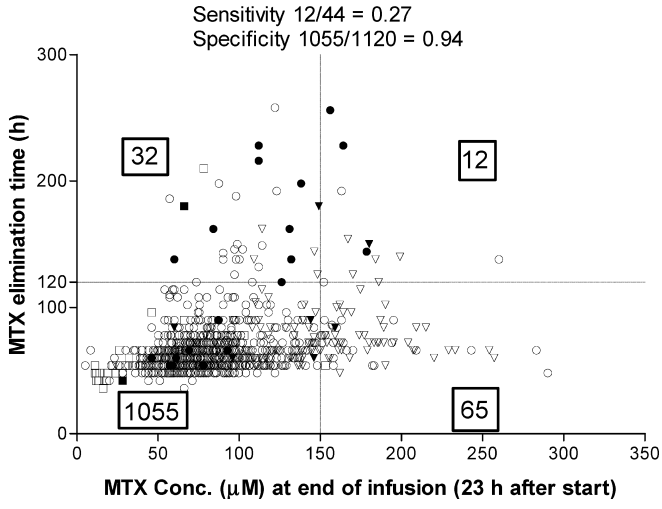

Fig. 3 MTX concentration at the end of infusion (23 h after the start of infusion) and elimination time in 1164 courses administered to 264 patients. The numbers indicate the number of courses in each region of the Figure (■ □ < 4 g MTX/m^2, ● ○ ≥4 g and < 7 g MTX/m^2, ▼ ▽ ≥7 g MTX/m^2; □ ○ ▽ creatinine ratio day 1 < 1.5, ■ ● ▼ creatinine ratio day 1 ≥1.5)

level. Furthermore, 65 courses in 43 patients with elimination times below 120 h would have been given increased hydration. Thus, the sensitivity and the specificity using this cut-off level were 0.27 and 0.94, respectively. The predictive value of a positive test was 0.16 (12/77) and the predictive value for a negative test was 0.97 (1055/1087). The risk ratio was 5.3 [(12/77)/(32/1085)].

Of the 1164 courses, data on MTX concentration 36 h after the start of infusion (C_{36}) were available for 1155. Of these courses, 76 had S-MTX at C_{23} > 150 μ*M*, and of these 76 courses, 11 had delayed elimination and 44 had C_{36} values ≥3 μ*M*. All 11 courses with S-MTX at C_{23} > 150 μ*M* and delayed elimination had C_{36} values ≥3 μ*M*.

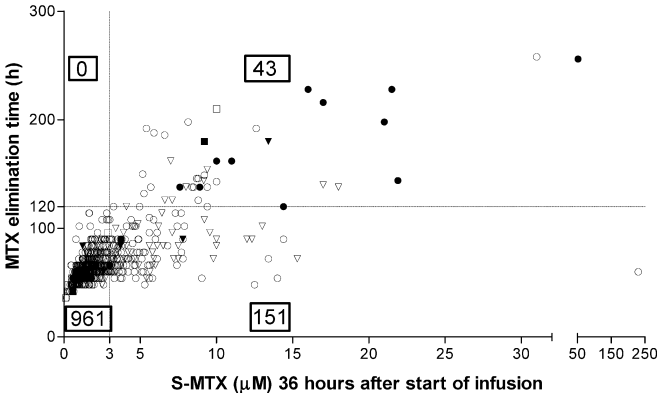

Fig. 4 MTX concentrations 36 h after the start of infusion (12 h after the end of infusion) and MTX elimination time in 1155 courses administered to 264 patients. The numbers indicate the number of courses in each region of the Figure (■ □ < 4 g MTX/m^2, ● ○ ≥4 g and < 7 g MTX/m^2, ▼ ▽ ≥7 g MTX/m^2; □ ○ ▽ creatinine ratio day 1 < 1.5, ■ ● ▼ creatinine ratio day 1 ≥1.5)

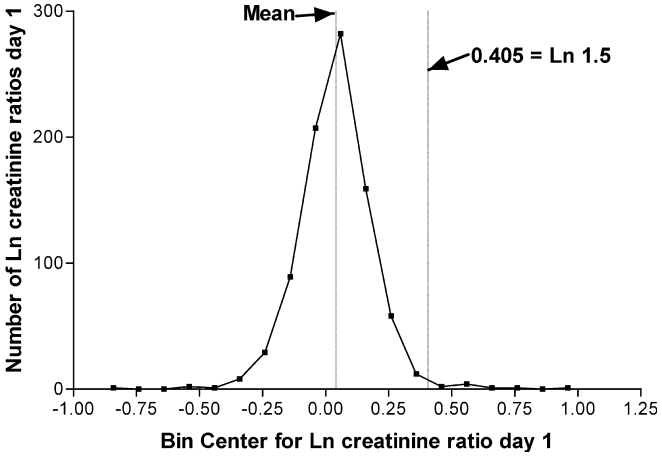

Fig. 5 Frequency distribution of ln creatinine ratios on day 1 in all 857 courses in 222 patients with an elimination time for MTX < 72 h. In the text, this elimination time is referred to as "normal". *Bins* are the intervals into which the ln ratios are grouped with a point representing the centre

S-MTX 36 h after the start of infusion (C_{36})

Figure 4 demonstrates that all those developing delayed elimination had ≥3 μ*M* MTX at C_{36}. Of the 205 courses in 131 children with MTX C_{36} ≥3 μ*M*, 43 in 36 patients had delayed elimination. Thus, delayed elimination did not develop in 162 courses in 107 patients despite MTX C_{36} ≥3 μ*M*.

S-creatinine in courses with a normal elimination time (< 72 h)

The frequency distribution of ln creatinine ratios in 857 courses with a "normal" elimination time, defined as < 72 h to reach < 0.2 μ*M* MTX, is illustrated in Fig. 5.

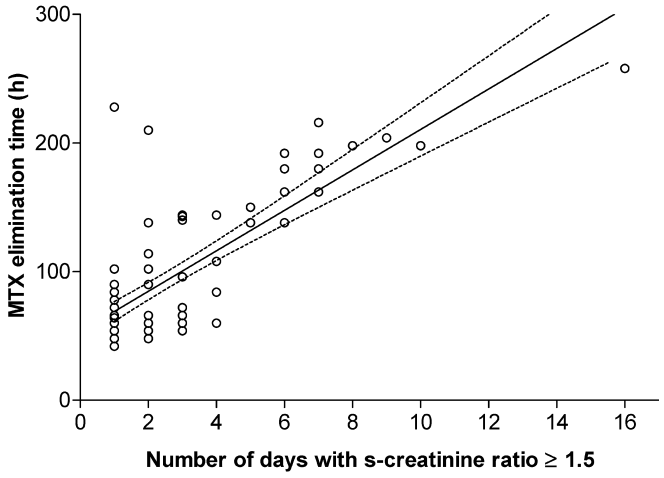

Fig. 6 Number of days with S-creatinine ratio (S-creatinine measured after the start of MTX infusion divided by that before the start of the course) ≥1.5 and MTX elimination time in 94 courses in 73 patients with an S-creatinine ratio ≥1.5 at any time during the course (only courses in which the number of days for MTX elimination corresponds to the number of days of S-creatinine measurement are included)

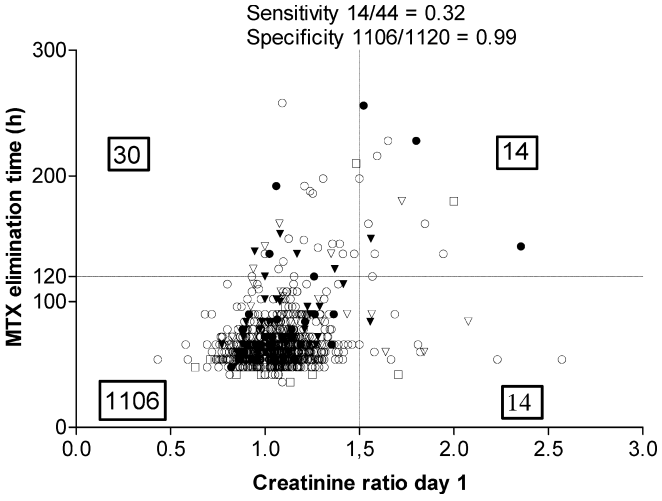

Fig. 7 S-creatinine ratios day 1 (S-creatinine measured 12–24 h after the start of MTX infusion divided by that before the start of the course) and MTX elimination time in 1164 courses administered to 264 patients. The numbers indicate the number of courses in each region of the Figure (■ □ <4 g MTX/m^2, ● ○ ≥4 g and <7 g MTX/m^2, ▼ ▽ ≥7 g MTX/m^2; □ ○ ▽ MTX C_{23} ≤150 μ*M*, ■ ● ▼ MTX C_{23} >150 μ*M*)

The mean ln creatinine ratio was 0.041, indicating a significant elevation of day 1 S-creatinine ($P<0.0001$; Wilcoxon signed rank's test) during these courses. The standard deviation was 0.144. Considering that mean ± 2 standard deviations shall comprise 95% of the ln creatinine ratios (if they had a Gaussian distribution), the data suggest that 97.5% of "normal" creatinine ratios during HDMTX treatment would be expected to be below 1.39 ($=e^{(0.041+2\times0.144)}$). Although the material was not normally distributed, 98.1% of the creatinine ratios were found to be <1.39, and 98.8% were <1.5 (ten courses in ten patients had a creatinine ratio ≥1.5 and an elimination time <72 h). A creatinine ratio ≥1.5 ($=e^{0.405}$) was therefore regarded as a reasonable approximation of a pathological value.

Number of days with creatinine ratio ≥1.5

In Fig. 6 all courses with S-creatinine measured daily during and after the course are illustrated. Of these courses, 94 (73 patients) had S-creatinine ratios ≥1.5. Plotting the number of days with S-creatinine ratios ≥1.5 against elimination time yielded a linear regression line significantly different from zero ($P<0.0001$) with $r^2=0.61$.

S-creatinine ratio on day 1 (creatinine ratio)

Figure 7 demonstrates the relationship between creatinine ratio and elimination time. If a cut-off level ratio of ≥1.5 was used, 14 of 44 courses in which delayed elimination (≥120 h) developed could be identified as early as during the first 24 h. However, 14 courses with an elimination time <120 h also had a creatinine ratio ≥1.5 and 1106 of 1120 courses with an elimination time <120 h had a creatinine ratio <1.5. Thus, the sensitivity of this cut-off level was 0.32 and the specificity 0.99. The predictive value of a positive test was 0.50 and the predictive value for a negative test was 0.98. The risk ratio was 19 [(14/28)/(30/1136)].

Interestingly, 14 courses in 14 patients with an elimination time <120 h and a creatinine ratio ≥1.5 had significantly ($P<0.0001$) lower S-creatinine values before the start (25.86 ± 3.34 μ*M*) than did those 1106 courses in 257 patients with an elimination time <120 h and a creatinine ratio <1.5 (40.73 ± 0.34 μ*M*). Patient age at the start of the first HDMTX course was available for 13 of 14 patients in the former and 243 of 257 in the latter group. The patient age did not differ significantly ($P=0.07$; 4.12 ± 0.64 years, $n=13$ vs 6.26 ± 0.27 years, $n=243$). Creatinine values before the start in courses with an elimination time ≥120 h ($n=44$) were, however, not significantly different ($P=0.14$) from those in the group with an elimination time <120 h and a creatinine ratio <1.5 ($n=1106$). Creatinine values before the start in the 14 courses with a creatinine ratio ≥1.5 and an elimination time <120 h were significantly ($P=0.002$) lower than in the other 61 courses in the same 14 patients in whom the S-creatinine value before the start of the course was available (25.86 ± 3.34 μ*M*, $n=14$ vs 34.08 ± 0.97 μ*M*, n = 61), but the number of preceding courses did not differ between the groups ($P=0.10$; 3.92 ± 0.39, $n=61$ vs 2.43 ± 0.67, $n=14$). Of the 1155 courses for which data on MTX concentration 36 h after the start of infusion (C_{36}) were available, 27 courses had a creatinine ratio ≥1.5, and of these 27 courses, 13 had delayed elimination and 17 had C_{36} values ≥3 μ*M*. All 13

courses with delayed elimination had C_{36} values ≥3 μ*M*. Thus, four courses had a creatinine ratio ≥1.5 and a C_{36} ≥3 μ*M* without delayed elimination.

Mean pretreatment S-creatinine ratio

Ten courses in ten patients with a mean pretreatment creatinine ratio ≥1.5 had elimination times ≥120 h, six courses in six patients with a mean pretreatment creatinine ratio ≥1.5 had elimination times < 120 h, 18 courses in 15 patients with mean pretreatment creatinine ratio < 1.5 had elimination times ≥120 h and 912 courses in 207 patients with mean pretreatment day-1 creatinine ratio < 1.5 had elimination times < 120 h. The sensitivity and specificity using this approach were 0.36 and 0.98, respectively (Fig. 8). The predictive value of a positive test was 0.63 and the predictive value for a negative test was 0.98. The risk ratio was 32 [(10/16)/(18/930)].

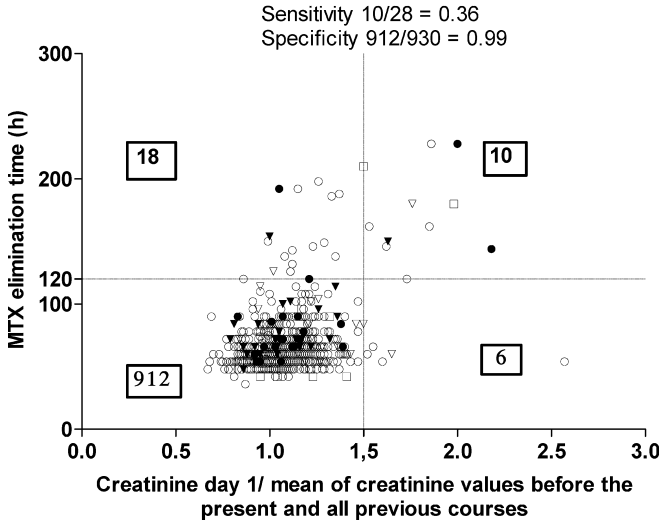

Fig. 8 Creatinine day 1/mean creatinine value before the present and all previous courses for 946 courses administered to 238 patients (only courses with at least one previous course are included). The numbers indicate the number of courses in each region of the Figure (■ □ < 4 g MTX/m^2, ● ○ ≥4 g and < 7 g MTX/m^2, ▼ ▽ ≥7 g MTX/m^2; □ ○ ▽ MTX C_{23} ≤150 μ*M*, ■ ● ▼ MTX C_{23} > 150 μ*M*)

Of the 946 courses, data on MTX concentration 36 h after the start of infusion (C_{36}) were available for 937. Of these, 15 courses had a mean pretreatment creatinine ratio ≥1.5, and of these 15 courses, only 9 with delayed elimination had C_{36} values ≥3 μ*M*. Thus, no courses had a mean pretreatment creatinine ratio ≥1.5 and C_{36} ≥3 μ*M* without delayed elimination.

Combined prognostic value of MTX C_{23} and mean pretreatment creatinine ratio

In Table 1 the prognostic values of both MTX C_{23} and mean pretreatment creatinine ratio are summarized. If both a mean pretreatment creatinine ratio ≥1.5 and MTX C_{23} > 150 is demanded for the test to be positive and the rest are regarded negative, the sensitivity is 3/28 = 0.11, the specificity is 918/918 = 1.00, and the predictive value for a positive test is 3/3 = 1.00 and for a negative test is 918/943 = 0.97. If both a mean pretreatment creatinine ratio < 1.5 and S-MTX ≤150 is demanded for the test to be negative and the rest are regarded positive, the sensitivity is 13/28 = 0.48, the specificity is 867/918 = 0.94, and the predictive value for a positive test is 13/64 = 0.20 and for a negative test is 867/882 = 0.98.

Discussion

The results of the present study suggest that HDMTX induces significant elevations in S-creatinine. Furthermore, the increase in creatinine and the number of days with a creatinine ratio ≥1.5 are closely related to the elimination time of MTX. These findings indicate that the elimination time of MTX during HDMTX is mainly related to glomerular impairment. None of the markers for renal function measured before the start of HDMTX was correlated with time of MTX elimination. Neither were any of the urinary parameters measured on the 2nd day of treatment related to MTX elimination time. Furthermore, U-alb, U-IgG and U-HC were not elevated on the 2nd day of treatment as compared to day 0.

A S-creatinine ratio ≥1.5 within the first 12–24 h after the start of MTX infusion may be regarded as a reasonably good approximation of a pathological value. It

Table 1 Number of courses with and without delayed MTX elimination (≥120 h or < 120 h from the start of MTX infusion until the first S-MTX < 0.2 μ*M*) in relation to MTX C_{23} (S-MTX 23 h after the start of infusion) and the mean pretreatment creatinine ratio (S-creatinine value on day 1divided by the mean S-creatinine value before the present and all previous courses)

	MTX elimination time	
	≥120 h	< 120 h
Number of courses with mean pretreatment creatinine ratio ≥1.5 and MTX C_{23} > 150 μM	3	0
Number of courses with mean pretreatment creatinine ratio ≥1.5 and MTX C_{23} ≤150 μM	7	6
Number of courses with mean pretreatment creatinine ratio < 1.5 and MTX C_{23} > 150 μM	3	45
Number of courses with mean pre-treatment creatinine ratio < 1.5 and MTX C_{23}≤150 μM	15	867

is a better predictor of delayed elimination than the level of MTX sampled just before the end of infusion (C_{23}), especially if information on previous creatinine measurements is used to reduce the impact of an occasionally low S-creatinine value before the start of MTX infusion.

The present findings suggest that none of the renal markers measured before the start of MTX infusion can be used to predict the MTX elimination time before the start of HDMTX, at least in those patients with relatively normal renal function. However, MTX elimination time was correlated significantly ($P = 0.04$) with pretreatment GFR, if the course with diclofenac was excluded. The r^2 value (0.31) was modest, suggesting that the clinical relevance of GFR for predicting MTX elimination time is limited: only 31% of the variability in MTX elimination time could be explained by the pretreatment GFR. In the study by Murry et al. pretreatment GFR was shown to vary fivefold in patients treated with MTX, and the mean GFR was 131 ml/min per 1.73 m^2 [17], which is similar to the results of the present study (sixfold variation in GFR with a mean of 155 ml/min per 1.73 m^2). Corresponding well with the present results, they found a significant correlation between GFR and MTX clearance, with only 37% of the variance in MTX clearance being explained by GFR. In good agreement with these results, Rees et al. have suggested that routine estimation of GFR does not contribute to the clinical management of HDMTX [21].

The increase in S-creatinine from baseline to day 2 indicates that glomerular impairment develops during HDMTX treatment, but barrier and tubular functions remain unaffected, as suggested by the lack of elevation in U-HC, U-alb and U-IgG. The close correlation between elevation in S-creatinine, but not U-HC, and MTX elimination time suggests that the elimination time of MTX is closely dependent upon glomerular but not tubular function. Thus, the suggestion of Murry et al. [17] that tubular function might explain some of the variance in MTX clearance is not supported by the present results.

The present results correlate well with those of Lawrenz-Wolf et al. indicating that delayed elimination is often associated with a rise in S-creatinine [16]. The results of Abelson et al. [1] suggest a transient 43% decrease in GFR in nontoxic courses when measured 24–40 h after a 6-h infusion of HDMTX. In the present study, S-creatinine was only elevated by 4% in courses with a "normal" elimination time. Furthermore, although GFR was not measured during treatment, consecutive courses (2–8 weeks between courses) did not indicate any trend towards a time-dependent deterioration in GFR, suggesting that the glomerular impairment was transient.

As previously pointed out [1], secondary elevation of S-creatinine induced by obstruction of the renal tubule cannot be excluded. In our study only one patient had higher levels of U-HC on day 2 compared to baseline, whereas Johnsson et al. found an increase in eight of ten patients treated with cisplatinum, indicating that MTX is less toxic to the tubules than cisplatinum [15]. However, tubular damage cannot be excluded since others, using other markers for tubular function, have obtained results indicating such damage [6]. In the study by Deray et al. [6], 4 g/m^2 MTX administered over 4 h to adults resulted in a mean increase in S-creatinine of 69% on day 6. Urinary β_2-microglobulin and urinary *N*-acetyl glucosaminidase, both (like U-HC) indicators of tubular damage, were elevated sevenfold and threefold, respectively. However, no individual concentration data on MTX were given so that closer comparison with the present study was not possible.

In both the NOPHO-92 and BFM ALL 95 protocols, S-MTX 36 h after the start is used as a predictor of delayed elimination requiring increased alkalized hydration if the level is ≥3 μ*M*. The results (Fig. 4) demonstrate that all courses with delayed elimination actually had a S-MTX ≥3 μ*M* 36 h after the start. Thus, even if alkalized hydration is not increased during the first 24 h, all those developing delayed elimination will have an augmented alkalized hydration after 36 h. As elimination time is dependent on the amount of alkalized hydration [5, 7, 8, 22, 24, 25], it is not an independent variable and it is not possible to determine whether the elimination in the 151 courses with an elimination time < 120 h and S-MTX ≥3 μ*M* at 36 h would have been delayed had alkalized hydration not been increased.

It is a well-known clinical observation that increased S-creatinine during treatment with HDMTX is often associated with delayed elimination [16]. Although the NOPHO-92 and other protocols stipulate analyses of S-creatinine during treatment with HDMTX, the degree of increase in S-creatinine that may be regarded as augmented has not been defined and its predictive role for delayed elimination has not been fully elucidated. In the present study, 99% of S-creatinine ratios on day 1 in courses with a normal elimination time were < 1.5. A creatinine ratio ≥1.5 on day 1 may therefore be regarded as a reasonable approximation of a pathological elevation in S-creatinine. The close correlation between the number of days with a creatinine ratio ≥1.5 and the days of elimination indicates a close relationship between elevated S-creatinine and MTX elimination time. This is further supported by the close correlation between the creatinine increase on day 2 and MTX elimination time.

In the ALL-BFM-95 protocol it is suggested that alkalized hydration should be augmented if the level of MTX sampled just before the end of infusion (C_{23}) is higher than 150 μ*M*. The results of the present study suggest that the S-creatinine ratio measured during the first 12–24 h after the start of MTX infusion is a better predictor of delayed elimination than the MTX C_{23}, especially if information on previous creatinine measurements is used to reduce the impact of an occasionally low creatinine value before the start of MTX infusion. Using a mean pretreatment creatinine ratio of ≥1.5 as a predictor of delayed elimination, as compared

to MTX C_{23} >150 μM yielded a higher sensitivity (0.36 vs 0.27), specificity (0.99 vs 0.94), and predictive values for a positive (0.63 vs 0.16) and a negative test (0.98 vs 0.97). Risk ratios were 32 vs 5. This indicates that a patient is 32 times more likely to have a delayed elimination when the mean pretreatment creatinine ratio is ≥1.5 than when the mean pretreatment creatinine ratio is <1.5, whereas a patient is only five times more likely to develop delayed elimination when the S-MTX is >150 μ*M* at C_{23} than when it is ≤150 μ*M*.

Although the probability of detecting delayed elimination (sensitivity) did not differ greatly (36% vs 27%), the probability that the elimination will become delayed is considerably higher if the mean pretreatment creatinine ratio is ≥1.5 than if MTX C_{23} is >150 μ*M* (63% vs 16%; predictive value of a positive test). This indicates that increased alkalized hydration would have been used in nine times as many (65/1120 = 5.8% vs 6/918 = 0.65%) courses with a "normal" elimination time (i.e. <120 h) had MTX C_{23} >150 μ*M* been used as a predictor of delayed elimination, instead of a mean pretreatment creatinine ratio ≥1.5. Furthermore, delayed elimination developed in 50% more courses with MTX C_{23} ≤150 μ*M* (3%) as compared to those with a mean pretreatment creatinine ratio were <1.5 (2%).

In several patients delayed elimination developed in the first course. Clinically there is normally ample knowledge of a patient's S-creatinine values before the start of the first course of MTX. It is therefore mostly possible to divide the S-creatinine value on day 1 of the first MTX course by the mean of a number of previous creatinine values normal for that patient in order to reduce the impact of an occasionally low creatinine value before the start of MTX infusion. Still, if these values are missing, dividing the S-creatinine value on day 1 with a single creatinine value taken before the start of the course renders a higher sensitivity (0.32 vs 0.27), a higher specificity (0.99 vs 0.94) and a higher predictive value of a positive (0.50 vs 0.16) and a negative (0.98 vs 0.97) test and a higher risk ratio (19 vs 5), as compared to the use of MTX C_{23} >150 μ*M*. Several of the creatinine ratios ≥1.5 in courses with an elimination time <120 h were due to an occasionally low S-creatinine value before the start as these were significantly lower than the corresponding values in the other courses in the same patients. In courses with a normal elimination time neither patient age nor the number of preceding courses differed significantly between those with normal and high (≥1.5) creatinine ratios.

Combining the predictive ability of the mean pretreatment creatinine ratio with that of MTX C_{23} suggests that the predictive value of a positive test can be enhanced to 1.00, although the number is small (three courses) if both mean pretreatment creatinine ratio ≥1.5 and MTX C_{23} >150 μ*M* are demanded for the test to be regarded as positive. Even if that means that all courses with these characteristics have a delayed elimination, only 11% of the courses with delayed elimination will be identified. On the other hand if either a mean pretreatment creatinine ratio ≥1.5 and/or MTX C_{23} >150 μ*M* are demanded for the test to be regarded as positive, 46% of the courses with a delayed elimination will be identified within 24 h of the start of MTX infusion. Thereby the predictive value of a positive test is reduced to 0.20, indicating that increased alkalized hydration will be used in four courses with a normal elimination time for each course with delayed elimination.

In the study by Lawrenz-Wolf et al. [16], HDMTX courses with S-MTX >5 μ*M* 42 h after the start of MTX infusion were analysed. A steady-state level of S-MTX >150 μ*M* was encountered in 11/14 patients (79%) given 5 g MTX/m^2 per 24 h and an increase in S-creatinine greater than 50% was found in 14/16 patients (88%) after 24 h. They suggested that the S-creatinine level 24 hours after the start of MTX infusion was highly predictive of the subsequent course of events. However, comparisons with "normal" HDMTX courses were not performed. In contrast to the present study, their results imply that impaired elimination of MTX occurs only once in each patient. In their study the numbers of patients and courses were also considerably smaller than in the present study.

In the study by Relling et al. [22], factors associated with S-MTX concentrations >1 μ*M* 42 h after the start of infusion (high-risk MTX concentrations) were examined in a multivariate analysis. Their results suggest that 47% of courses with high-risk MTX concentrations are associated with an increased AUC of MTX, low urine pH and emesis. However, renal toxicity was not evaluated in the study, and furthermore the number of courses associated with these three predictive factors without high-risk MTX concentrations was not stated. Their results and those of Christensen et al. [5] also indicate that S-MTX and toxicity are reduced by more vigorous alkalized hydration. In other studies, augmented alkalized hydration has also been shown to reduce S-MTX concentrations [7, 8, 24]. In the study by Sand and Jacobsen [25] the issues of hydration and U-pH were explored separately. Their results show that increased U-pH but not hydration leads to a significant elevation in renal clearance of MTX.

Summing up the predictive role of a combination of mean pretreatment creatinine ratio and MTX C_{23}, the latter do not appear to add anything further than the use of mean pretreatment creatinine ratio alone. Using mean pretreatment creatinine ratio identified 10 of 28 courses with delayed elimination (but also 6 courses that did not develop delayed elimination). Using also MTX C_{23} >150 μ*M* identified another 3 courses with delayed elimination, but also another 45 courses that did not develop delayed elimination. Although late in the course, all of the courses with mean pretreatment creatinine ratio ≥1.5 and MTX C_{36} >3 μ*M* developed delayed elimination, whereas none of the courses with a mean pretreatment creatinine ratio ≥1.5 and MTX C_{36} ≤ 3 μ*M* did.

In conclusion, of the parameters examined in the present study, S-creatinine appeared best for predicting

delayed MTX elimination, especially if information on previous creatinine measurements were used to reduce the impact of an occasionally low S-creatinine value before the start of MTX infusion. Thus, applying a mean pretreatment creatinine ratio of ≥1.5 as an early predictor of delayed MTX elimination, alkalization and hydration could be intensified earlier to reduce renal impairment and avoid the use of excessive folinic acid rescue which may adversely affect the efficacy of HDMTX.

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