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Creatinine clearance rate and serum creatinine concentration are related to delayed methotrexate elimination in children with lymphoblastic malignancies

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Methotrexate (MTX) is an effective treatment for childhood acute lymphoblastic leukemia (ALL) or Non-Hodgkin lymphoma (NHL); however, toxicity can arise with high doses MTX (HDMTX), especially in patients with delayed MTX elimination. Routine monitoring of plasma MTX concentrations is clinically important, but unfortunately is not always feasible. The aim of this study was to examine the relationship between MTX elimination and renal function to identify parameters that may be useful for predicting delayed MTX elimination in Chinese children with ALL and NHL. A total of 105 children with ALL and NHL were included in the study. Each patient received HDMTX (3 or 5 g/m^2) over 24 hours. Plasma MTX concentrations were measured at 24, 48, and 96 hours. Delayed elimination was indicated by plasma MTX concentrations ≥1.0 at 48 hours or ≥0.1 μmol/L at 96 hours. Creatinine clearance rate (CCr) and serum Cr concentrations were measured at 0, 24, and 48 hours. There were 39 patients (37.1%) with delayed MTX elimination. For patients with delayed MTX elimination, the 24 hour plasma MTX concentration was negatively correlated with the 24 hour CCr (P=0.019). The 48 hour plasma MTX concentration was positively correlated with 24 and 48 hour serum Cr concentrations (P=0.001 and P<0.001, respectively), and negatively correlated with the 24 and 48 CCr (both P<0.001). Both MTX concentrations and elimination time decreased with increasing CCr (P<0.05 and P<0.001, respectively). Receiver operating characteristic curves revealed that the best predictors of delayed MTX elimination were 24 hour CCr <100 mL/min (sensitivity: 67.6%; specificity: 72.1%) and 24 hour Cr concentration >36 mmol/L (sensitivity: 64.7%; specificity: 77.4%) (both P < 0.001). CCr and serum Cr concentration may be useful for monitoring plasma MTX concentrations in children receiving HDMTX for ALL and NHL and for predicting delayed MTX elimination.

Key words: acute lymphoblastic leukemia, child, creatinine clearance, kidney function, methotrexate, non-Hodgkin lymphoma, pharmacokinetics

Use of methotrexate (MTX) has led to significant improvements in the long-term survival of children with acute lymphoblastic leukemia (ALL) and is important for extramedullary leukemia prophylaxis in many effective ALL and lymphoma chemotherapeutic regimens [1-5]. Different from other antineoplastic agents, the cytotoxic effects of MTX can be ameliorated by folic acid (leucovorin). This allows for the administration of high dose (up to 33.6 g/m^2) intravenous MTX (HDMTX) [5, 6]. Despite the use of leucovorin, MTX-related toxicity remains an important issue, manifesting as myelosuppression, nephrotoxicity, hepatotoxicity, gastrointestinal mucositis, as well as both transient and persistent neurotoxicity [2, 7-9]. These toxic effects are most severe in patients with delayed elimination of MTX [9]. Therefore, being able to identify patients at risk of experiencing delayed elimination of MTX after HDMTX is of obvious clinical importance.

A number of population-based pharmacokinetic models of HDMTX infusion in patients with lymphoblastic malignancies have been described [10-12]. However, the clinical application of these models, particularly for patients with delayed elimination of MTX, is limited because of the considerable variation between patients in terms of MTX dosage, hydration and alkalization, renal function, coadministration of other anticancer agents, and pharmacogenomics [2, 7, 13]. Therefore, the standard practice for prospective identification of patients at high risk of developing toxicity following HDMTX is routine monitoring of plasma MTX concentrations. With such monitoring, early leucovorin treatment can be given to patients with abnormal plasma MTX clearance to reduce the associated toxicity. Unfortunately, there

are still many oncology institutions that do not have the capability to routinely monitor plasma MTX concentrations; hence, having a simple and convenient alternative means of screening patients to identify those at high risk of developing toxicity would be highly applicable.

It is well known that renal clearance is the principal pathway of MTX elimination and that the elimination of MTX appears to be related to kidney function [14-16]. Indeed, Skarby et al previously reported that the increase in serum creatinine (Cr) concentration within the first 12 to 24 hours after the start of MTX infusion was a good predictor of delayed elimination of MTX in Swedish children with ALL [14]. The aim of this study was to further examine the relationship between MTX elimination and indicators of renal function to identify parameters that may be useful for predicting delayed elimination of MTX in Chinese children with ALL and non-Hodgkin's lymphoma (NHL). Different to Skarby et al [14], we generated receiver operating characteristic (ROC) curves to identify specific cut-off values for predicting delayed elimination of MTX.

Patients and methods

Patients. Patients with newly diagnosed ALL and NHL who were treated at the Children's Hospital of Zhejiang University School of Medicine between May 2009 to December 2009 were enrolled in this study. All patients were treated following the modified National Protocol of Childhood Leukemia in China 2006 (NPCLC-ALL2006). Patients with ALL were stratified to low, intermediate, and high risk groups for treatment according to the their clinical characteristics as previously described [17]. Patients with NHL were included as a low risk group (none of these patients had extramedually infiltration). All patients were in complete remission before receiving HDMTX.

This study was approved by the Medical Ethics Committee of Children's Hospital of Zhejiang University, School of Medicine. Written informed consent was obtained from the guardian of each patient.

Treatment. *Methotrexate administration.* HDMTX was administered after consolidation and during maintenance therapy. Before each HDMTX dose, patients were required to have a white blood cell count ≥ 3.0×10^9/L, an absolute neutrophil count > 1.0×10^9/L, and a platelet count ≥ 80×10^9/L, without any evidence of liver or kidney impairment, or manifestations of infection. The total MTX dose was 3 g/m^2 for patients in the low risk group (group A) and 5 g/m^2 for patients in the intermediate and high risk groups (group B). For each course of treatment, 1/6 of the total MTX dose (maximum: 0.5 g) was given intravenously during the first hour, while the remainder was administered evenly during the subsequent 23 hours.

Hydration and urinary alkalization. Urinary alkalinization was performed 3 days before HDMTX administration with 5% of sodium bicarbonate at a dosage of 60 mL/m^2/day. Heavy hydration and urinary alkalinization, intravenous hydration (2000-3000 mL/m^2/day for group A, 3000-4000 mL/m^2/day for group B) and urinary alkalinization (3-4 mL/kg/day 5% sodium bicarbonate for group A, 4-5 mL/kg/day 5% sodium bicarbonate for group B) were continued during and after MTX infusion until the plasma MTX concentration was < 0.1 μmol/L.

Leucovorin rescue. For patients without delayed elimination of MTX, leucovorin was administered 36 hours after the start of MTX administration as an intravenous bolus at a dose of 15 mg/m^2 every six hours (three to eight doses in total) until the plasma MTX concentration was < 0.1 μmol/L. For patients with delayed elimination of MTX (definitions ensue), leucovorin was administered according to the 48 and 96 hour MTX concentrations shown in Table 1.

Measurement of plasma methotrexate concentrations. Plasma MTX concentrations were measured 24, 48, and 96 hours after the start of treatment by fluorescence polarization immunoassay [18]. For patients with delayed elimination of MTX, plasma MTX concentrations were measured every 48 hours until the MTX concentration fell below 0.1 μmol/L.

Delayed elimination of MTX was indicated by plasma MTX concentrations ≥ 1.0 μmol/L at 48 hours or ≥ 0.1 μmol/L at 96 hours [9].

Measurement of creatinine clearance rate and serum creatinine concentration. 24-hour urine samples were collected over the first 48 hours (0 to 24 and 24 to 48 hours) after the start of MTX treatment and treated with 6N HCl as preservative. A portion of the sample was used to measure the urinary Cr concentration. Within the last hour of urine sample collection, a venous blood sample was drawn for the measurement of serum Cr concentration. The following formula was used to calculate the endogenous Cr clearance rate (CCr):

CCr = urine Cr (mg/dL) × 24 hour urine volume (mL) × 1.73 m^2 / body surface area (m^2) × serum Cr (mg/dL) × 1440.

Statistical analysis. Data are presented as mean with standard deviation and were compared between groups (A vs B, normal vs delayed elimination of MTX) by Mann-Whitney U test. The correlations between patient characteristics and plasma MTX concentrations were determined by assessing Spearman's rank correlation. The serum Cr concentration and CCr cut-off values for predicting delayed MTX elimination were determined by constructing ROC curves. All statistical assessments were two-tailed and considered statistically sig-

Table 1. Leucovorin rescue according to plasma methotrexate concentration

MTX Concentration (μmol/L)	Leucovorin Regimen
< 0.1	15 mg/m^2, q6h×3
~1.0	15 mg/m^2, q6h × [3+ (C_{MTX}–24—0.4)/0.1]
1.0~2.0	30 mg/m^2, q6h×8
2.0~3.0	45 mg/m^2 , q6h×8
3.0~4.0	60 mg/m^2, q6h×8
4.0~5.0	75 mg/m^2, q6h×8
> 5.0	C_{MTX}–48 × weight (kg) mg, q6h×8, or half of that dose q3h×16

C, concentration; MTX, methotrexate; q6h, every 6 hours.

nificant at the 0.05 level. Data were analyzed using SPSS 15.0 statistical software (SPSS Inc, Chicago, IL).

Results

A total of 95 children with ALL and 10 children with NHL were included in the study. Of the 105 patients, 67 were boys and 38 were girls, with a median age of 6.2 years (range: 2.2 years to 15.0 years). Of the 105 patients, 59 were rated low risk (including the 10 patients with NHL), 27 were rated high risk, and 19 were rated intermediate risk.

The patients' demographic and clinical characteristics are presented (stratified by dose group and MTX elimination status) in Table 2. Body height, body weight, age, and body surface area (BSA) were all significantly higher in group B compared with group A (all $P < 0.001$). Serum Cr concentrations at 24 and 48 hours were significantly higher in group A compared with group B ($P = 0.040$ and $P = 0.037$, respectively). In contrast, the CCr at 24 and 48 hours was significantly higher in group B compared with group A ($P = 0.007$ and $P = 0.009$, respectively). Delayed elimination occurred in 39 of the 105 patients. There were no statistically significant differences in body height, body weight, age, or BSA between patients who had normal and delayed elimination of MTX. Serum Cr concentrations at 24 and 48 hours were significantly higher in the delayed elimination group compared with the normal elimination group ($P = 0.001$ and $P = 0.002$, respectively). In contrast, the CCr at 24 and 48 hours was significantly higher in the normal elimination group compared with the delayed elimination group ($P < 0.001$ and $P = 0.001$, respectively)

Table 3 summarizes the correlations between patients' demographic and clinical characteristics and plasma MTX

Table 2. Comparison of patients' characteristics by methotrexate (MTX) dose and by MTX elimination status

Characteristic	Overall	MTX Dose[a]		*P* Value	MTX Elimination[b]		*P* Value
	N = 105	Group A N = 58	Group B N = 47		Normal N = 66	Delayed N = 39	
Body height (cm)	119.3 ± 20.4	109.6 ± 14.3	132.2 ± 20.4	<0.001	120.2 ± 20.7	118 ± 20.4	0.672
Body weight (kg)	24.9 ± 10.5	20.2 ± 6.8	31.1 ± 11.3	<0.001	25.4 ± 10.7	24.2 ± 10.3	0.653
Age (years)	8.1 ± 6.0	5.8 ± 2.6	10.0 ± 3.7	<0.001	7.8 ± 3.8	8.7 ± 8.6	0.471
BSA (m^2)	0.9 ± 0.3	0.8 ± 0.2	1.1 ± 0.3	<0.001	0.9 ± 0.3	0.9 ± 0.3	0.333
Creatinine[c] (mmol/L), 0h	31.4 ± 8.9	30.3 ± 8.7	32.7 ± 9.1	0.199	31.6 ± 9.3	31.1 ± 8.3	0.769
Creatinine[c] (mmol/L), 24h	35.9 ± 14.8	38.5 ± 17.7	32.3 ± 8.9	0.040	31.2 ± 7.9	44.4 ± 20.1	0.001
Creatinine[c] (mmol/L), 48h	36.3 ± 18.3	39.4 ± 22.8	32.4 ± 8.2	0.037	30.9 ± 8.3	46.3 ± 26.1	0.002
CCr (mL/min), 0h	150.7 ± 62.4	150.5± 70.7	150.9 ± 50.4	0.978	149.3 ± 60.6	153.1 ± 66.3	0.788
CCr (mL/min), 24h	117.1 ± 44.7	107.5 ± 41.5	129.7 ± 41.0	0.007	132 ± 40.4	90.5 ± 39.8	<0.001
CCr (mL/min), 48h	117.4 ± 42.6	106.7 ± 42.6	131.3 ± 42.6	0.009	127.9 ± 38.1	98 ± 43.9	0.001

Data are presented as mean ± standard deviation.

BSA, body surface area; CCr, creatinine clearance rate; MTX, methotrexate

[a] Group A = 3 g/m^2 MTX, group B = 5 g/m^2 MTX.

[b] Delayed elimination of MTX was indicated by plasma MTX concentrations ≥ 1.0 μmol/L at 48 hours or ≥ 0.1 μmol/L at 96 hours.

[c] Serum creatinine concentration.

Table 3. Correlations between patients' characteristics and plasma MTX concentrations

Characteristic	MTX 24h				MTX 48h			
	Normal		Delayed[a]		Normal		Delayed[a]	
	ρ	*P*	ρ	*P*	ρ	*P*	ρ	*P*
Body height (cm)	0.496	0.002	0.300	0.146	-0.099	0.555	-0.090	0.669
Body weight (kg)	0.420	0.008	0.292	0.157	-0.115	0.485	0.015	0.942
Age (years)	0.546	<0.001	0.142	0.390	-0.049	0.695	-0.190	0.248
BSA (m^2)	0.497	<0.001	0.263	0.106	-0.099	0.427	-0.076	0.644
Creatinine[a] (mmol/L), 0h	0.279	0.030	0.184	0.290	-0.074	0.573	0.014	0.934
Creatinine[a] (mmol/L), 24h	0.043	0.739	0.234	0.184	0.100	0.441	0.564	0.001
Creatinine[a] (mmol/L), 48h	0.348	0.005	0.219	0.206	0.230	0.068	0.811	<0.001
CCr (mL/min), 0h	-0.068	0.619	-0.128	0.484	-0.176	0.194	0.021	0.908
CCr (mL/min), 24h	0.141	0.278	-0.401	0.019	-0.126	0.335	-0.659	<0.001
CCr (mL/min), 48h	-0.116	0.362	-0.279	0.104	-0.135	0.290	-0.802	<0.001

BSA, body surface area; CCr, creatinine clearance rate; MTX, methotrexate.

[a] Delayed elimination of MTX was indicated by plasma MTX concentrations ≥ 1.0 μmol/L at 48 hours or ≥ 0.1 μmol/L at 96 hours.

concentrations at 24 and 48 hours. For patients with normal elimination of MTX, there were significant positive correlations between body height, body weight, age, BSA, and serum Cr at 0 and 48 hours and plasma MTX concentrations at 24 hours (all $P < 0.05$). There were no corresponding correlations at 48 hours. For patients with delayed elimination of MTX, there was a significant negative correlation between the CCr at 24 hours and the plasma MTX concentration at 24 hours ($P = 0.019$). For these patients, there were also significant positive correlations between serum Cr concentrations at 24 and 48 hours and plasma MTX concentrations at 48 hours ($P = 0.001$ and $P < 0.001$, respectively), and significant negative correlations between the CCr at 24 and 48 hours and the plasma MTX concentration at 48 hours (both $P < 0.001$).

Table 4 shows the plasma MTX concentrations at 24 and 48 hours and MTX elimination time by CCr. Plasma MTX concentrations at 24 and 48 hours decreased with increasing CCr at both 24 and 48 hours (all $P < 0.05$, except for the plasma MTX concentration at 24 hours by CCr at 24 hours). Similarly, MTX elimination time decreased with increasing CCr at both 24 and 48 hours (both $P < 0.001$).

Table 5 summarizes serum Cr concentration and CCr cut-offs for predicting delayed MTX elimination. The ROC curves generated revealed that the best predictors of delayed elimination of MTX were a CCr < 100 mL/min at 24 hours (sensitivity = 67.6%, specificity = 72.1%) and a serum Cr concentration > 36 mmol/L at 24 hours (sensitivity = 64.7%, specificity = 77.4%) (both $P < 0.001$).

Discussion

In this study, we examined the relationship between MTX elimination and measures of renal function in an effort to identify parameters that may be useful for predicting delayed elimination of MTX in Chinese children with ALL and NHL. Of note, by generating ROC curves, we found that low CCr and high serum Cr concentration at 24 hours were significant predictors of delayed MTX elimination. Assessment of these measures of renal function may be useful for identifying patients with delayed MTX elimination in certain clinical settings where plasma MTX monitoring is not possible.

We found that a 24 hour CCr < 100 mL/min was a significant predictor of delayed MTX elimination after HDMTX. Several other studies with much lower MTX doses (1 g, 0.9 to 3.7 g, and 1 to 2 g [3, 8, 19], respectively) found no correlations between plasma MTX concentrations and CCr. Further, the plasma MTX concentrations at 24 and 48 hours in these studies were much lower than in our study eg, the maximum 48 hour MTX concentration in the study of Joannon et al was 1.07 μmol/L [19]. Hence, the patients in these studies may have experienced a lesser degree of renal function

Table 4. 24 and 48 hour plasma methotrexate (MTX) concentrations and MTX elimination time by creatinine clearance rate

	C_{MTX}–24 h (μmol/L)		C_{MTX}–48h (μmol/L)		MTX Elimination Time[a] (hours)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
CCr 24 h						
< 70 mL/min	80.04 ± 31.94	74	6.42 ± 3.56	6	245.33 ± 74.73	216
70~100 mL/min	60.37 ± 22.75	59	1.83 ± 3.03	1	143.2 ± 64.42	120
> 100 mL/min	56.43 ± 20.12	56	0.59 ± 0.58	0	110.67 ± 30.72	96
P value	0.162		<0.001		<0.001	
CCr 48 h						
< 70 mL/min	73.56 ± 27.04	69	6.07 ± 4.73	5	221.54 ± 82.65	216
70~100 mL/min	58.64 ± 23.88	59	1.42 ± 1.48	0	144 ± 64.4	96
> 100 mL/min	57.15 ± 21.25	53	0.6 ± 0.59	0	111.24 ± 32.06	96
P value	0.019		<0.001		<0.001	

C, concentration; CCr, creatinine clearance rate; MTX, methotrexate; SD, standard deviation.
[a] Time to C_{MTX} < 0.1 μmol/L.

Table 5. Serum creatinine concentration and creatinine clearance ratio for predicting delayed methotrexate elimination

Factor	Cut-off	Sensitivity	Specificity	AUC	OR (95% CI)	*P* Value
Creatinine (mmol/L), 24 h	>36	0.647	0.774	0.770	6.3 (2.5-15.8)	<0.001
Creatinine (mmol/L), 48 h	>36	0.600	0.719	0.737	3.8 (1.6-9.1)	<0.001
CCr (mL/min), 24 h	<100	0.676	0.721	0.759	5.4 (2.2-13.5)	<0.001
CCr (mL/min), 48 h	<100	0.571	0.781	0.678	4.8 (1.9-11.6)	0.008

AUC, area under the curve; CCr, creatinine clearance rate; CI, confidence interval; OR, odds ratio.

impairment than those in our study [20]. As the kidney has a powerful compensatory capacity [21], slight impairment would be likely to have minimal influence on the clearance of MTX. The dose-response effect of CCr variation on MTX elimination may not be obvious in patients with slightly impaired renal function, perhaps explaining the lack of any relationship between CCr and plasma MTX concentrations in the aforementioned studies.

Stratification of our CCr results by time (24 hours or 48 hours) provides relevant information concerning appropriate monitoring of plasma MTX concentrations. For example, if the CCr at 24 hours is < 70 kL/min, the patient should be carefully monitored for at least 216 hours to ensure elimination (ie, MTX concentration <0.1 μmol/L). Of course, further analysis, involving a much larger group of patients, is needed to establish a validative rule for clinical practice.

In addition to CCr, we found that a 24 hour serum Cr concentration > 36 mmol/L was a significant predictor of delayed MTX elimination after HDMTX. Our finding broadly agrees with that reported by Skarby et al [14], who also found that serum Cr concentration was a predictor of delayed elimination. Specifically, Skarby et al [14] found that an increase from baseline in serum Cr concentration by > 50% after the start of treatment was a significant predictor of delayed MTX elimination; however, our means of prediction does not require baseline assessment of serum Cr concentrations. Nevertheless, we also found that an increase from baseline in serum Cr concentration by > 50% 24 hours after the start of treatment was a predictor of delayed MTX elimination; in our study population, using serum Cr concentration at 24 hours > 50% above Cr baseline, the sensitivity is 41.9%, and the specificity is 94.8%; albeit with much lower sensitivity but higher specificity than our criteria. Although baseline serum Cr concentrations are typically available, there may be instances in which such results are not available; our means of predicting delayed MTX elimination would be readily applicable in such instances.

Interestingly, we found that in patients without delayed elimination, age was positively correlated with plasma MTX concentrations at 24 hours; however, there was no significant correlation between age and plasma MTX concentrations at 48 hours (Table 3). Age is one of the criteria for risk assessment. Specifically patients who were > 10 years old were grouped in the intermediate risk group and received 5g/m^2 MTX (group B). This may explain the aforementioned positive correlation. Further, because the patients in group B received higher doses of hydration and alkalization, the elimination rate was likely faster, reducing the correlation between age and plasma MTX concentration at 48 hours. Our findings demonstrate that proper hydration and alkalization, combined with appropriate monitoring, allows for safe and effective MTX therapy.

Our study is limited by the relatively small number of patients included. Hence, our findings require replication in a study involving a larger cohort of patients.

In summary, we have investigated the relationship between renal function and MTX elimination delay. Our findings suggest that a 24 hour CCr < 100 mL/min and 24 hour serum Cr > 36 mmol/L may be useful predictors of delayed MTX elimination following HDMTX in children with ALL. Clearly our findings warrant confirmation and further exploration. However, if these indicators of renal function prove to be reliable predictors of delayed MTX elimination, clinical measurement may allow for early adjustments in alkalization, hydration, and/or leucovorin dosage so that toxicity of MTX can be reduced/minimized. As already noted, such predictors would be very useful in settings where measurement of plasma MTX concentrations is not possible.

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