

Identifying risk factors for high-dose methotrexate-induced toxicities in children with acute lymphoblastic leukemia

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Background: Whether monitoring of the methotrexate (MTX) concentrations after high-dose MTX (HD-MTX) infusion can predict toxicities is still controversial, especially when HD-MTX therapy is used in the treatment of children with acute lymphoblastic leukemia (ALL), which is different than the previous schedules. The relationship between patient characteristics and severe adverse events (AEs) has yet to be determined.

Objective: To analyze the relationship between the MTX concentration and toxicities and to identify the risk predictors from patient characteristics for severe AEs during HD-MTX therapy in children with ALL.

Methods: We conducted a retrospective study on children with ALL who were treated with 388 HD-MTX infusions. The chi-square test and univariate and logistic regression analyses were used to analyze the relationship between the MTX concentrations and toxicities and to identify predictors for severe AEs.

Results: Febrile neutropenia ($P=0.000$) and vomiting ($P=0.034$) were more likely to occur if the infusion had an MTX level ≥ 1 $\mu\text{mol/L}$ at 44 h, but other toxicities had no correlations with MTX concentration. Predictive factors for toxicities were as follows: higher risk stratification and higher values of albumin (ALB) for leucopenia, higher values of white blood cell count (WBC) for anemia, higher values of ALB and creatinine (Cr) for neutropenia, higher risk stratification and higher 44-h MTX concentration for febrile neutropenia, higher values of alanine transferase (ALT) for elevated ALT, higher values of ALT for elevated aspartate transferase (AST), and higher values of total bilirubin (TBil) for vomiting.

Conclusion: Routine monitoring of 44-h MTX concentrations is essential to identify patients at high risk of developing febrile neutropenia and vomiting. This study may provide a reference for clinicians to distinguish patients with a relatively high risk of severe AEs based on certain characteristics before HD-MTX infusion.

Keywords: high-dose methotrexate, methotrexate concentration, toxicities, patient characteristics, risk predictors, acute lymphoblastic leukemia

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Introduction

Acute lymphoblastic leukemia (ALL) is a relatively infrequent malignant hematopoietic neoplasm in children.¹ Methotrexate (MTX) is an effective drug for the treatment of ALL. Antineoplastic activity is enhanced when MTX is used at a high dose. High-dose MTX (HD-MTX), defined as MTX doses ≥ 1 g/m², is a useful therapy for central nervous system prophylaxis in the treatment of children with ALL.²⁻⁵ The administration of calcium folinate (CF) following HD-MTX treatment is essential to prevent severe myelosuppression and oral mucositis.^{6,7} However,

excessive CF rescue reduces both the toxicity and the desired antitumor effect of MTX.^{8,9} Therefore, the balance between efficacy and adverse events (AEs) is one of the major clinical challenges to achieve a high cure rate of the disease. Monitoring the serum concentrations of HD-MTX is a well-accepted method to identify patients who are at high risk for severe toxic effects of the drug¹⁰ and to prevent toxic effects by early administration of a modified dose of CF in patients with evidence of abnormal plasma MTX clearance.^{6,7}

The plasma concentration of MTX and the duration of a concentration above a certain threshold are important for the development of toxicity in the treatment of children with ALL.^{11–14} However, currently, HD-MTX therapy is administered with 24-h continuous infusion and as part of a multiagent anticancer chemotherapy, which is different from the schedules used when these studies were originally performed.^{6,15,16}

Since the purpose of cytotoxic therapy is to provide the best possible therapeutic index, an improved understanding of predictors for the occurrence of severe AEs is necessary. Recently, some newer studies concluded that the risks of oral mucositis and hematological toxicity are associated with serum MTX concentrations.^{3,17} However, another study indicated the opposite conclusions: there was no significant relationship between the serum MTX concentration and oral mucositis.¹⁸ Additionally, another study indicated that some patients sometimes encounter severe AEs during HD-MTX therapy, even if serum MTX concentrations comply with recommended values, and concluded that the plasma concentration of MTX is not a predictive value for clinical AEs during HD-MTX therapy.¹⁹ Therefore, whether monitoring of the serum concentrations after HD-MTX is suitable as the predictor of severe AE occurrence is still controversial, especially when HD-MTX therapy is used in the treatment of children with ALL today, which is different than the previous schedules. In addition, other relevant clinical prediction factors for AEs in HD-MTX therapy in children with ALL include patient characteristics such as age, gender, body surface area, subtype of leukemia, kidney function tests, complete blood counts and liver function tests that have yet to be clearly determined.

In this study, to answer these clinical issues, we performed a retrospective study to analyze the relationship between the serum MTX concentrations and toxicities (hematological toxicities, transient liver dysfunctions, vomiting and oral mucositis). In addition, this study also

aimed to identify predictors from variables extracted from patient characteristics for the occurrence of severe AEs during HD-MTX therapy in children with ALL.

Patients and methods

Patients

We conducted a retrospective noninterventional study on patients aged ≤ 14 years with ALL who were treated by HD-MTX and who had adequate medical records available for review between May 2015 and May 2018 at the Affiliated Hospital of Qingdao University, China. Children with newly diagnosed ALL who received HD-MTX treatment and had plasma MTX concentrations determined at 44 h after the start of treatment as a routine practice were enrolled in this study. This study was approved by the Institute Medical Ethics Committee of the Affiliated Hospital of Qingdao University and was conducted in accordance with the ethical principles of the Declaration of Helsinki. The requirement for informed consent was waived by the Institute Medical Ethics Committee because of the retrospective nature of this study, but patient confidentiality was protected.

HD-MTX administration

All of the included patients were treated on the 2015 Chinese children's leukemia cooperation group-ALL protocol (CCCG-ALL-2015), which was based on the St. Jude Children's Research Hospital Total XV Study. Low-severity children received 3 g/m² of MTX, whereas moderate- and high-severity children received a 5 g/m² dose. The first dose (1/10 of the full dose) was intravenously transfused within 0.5 h. The remainder of the full dose was infused during the following 23.5 h. Hydration was employed before HD-MTX administration and lasted for 4 days. Urinary alkalinization was performed before HD-MTX administration with 5% sodium bicarbonate and lasted for 3 days to ensure the urine pH was between 7 and 8.

CF rescuing regimen

At 42 h after MTX administration, the rescue was started every 6 h for 3 to 5 doses. For patients with absolute toxicity, the rescue was started 36 h after MTX administration. For patients with 44-h plasma MTX concentrations below 1.0 $\mu\text{mol/L}$, 15 mg/m² was given, whereas for those with 44-h plasma MTX concentrations above 1.0 $\mu\text{mol/L}$, the rescue regimen was given according to the 44-h

plasma MTX concentrations as previously reported.^{20,21} Briefly, the CF rescue dosage was increased 15 mg/m² every 6 h per $\mu\text{mol/L}$ increase in the 44-h plasma MTX concentration. If the 44-h plasma MTX concentration was higher than 5 $\mu\text{mol/L}$, the dosage of CF was calculated according to the following formula: [CF (mg) = body weight (kg) multiplied by 44-h plasma MTX concentration ($\mu\text{mol/L}$)]. The rescue continued until the plasma MTX concentration was $<0.1 \mu\text{mol/L}$. In addition, CF (3 mg/d) was distributed to the oral mucosa to prevent oral mucositis during chemotherapy after HD-MTX infusion.

Determination of plasma MTX concentration

Serum concentrations of MTX for ALL patients monitored at our hospital from May 2015 to May 2018 were acquired via the Viva-E[®] system (Siemens Healthcare, Forchheim, Germany) and were retrospectively collected. For patients with a 44-h plasma MTX concentration above 1.0 $\mu\text{mol/L}$, monitoring was continued every 24 h until the plasma MTX concentrations were $<0.1 \mu\text{mol/L}$.

Date collection

The following clinical data were collected by retrospective chart review before every infusion: age, gender, subtype of leukemia, and body surface area (BSA) calculated as reported.²² Patient characteristics, including complete blood counts, liver function tests and kidney function tests that included white blood cell count (WBC), hemoglobin (Hb), platelet (PLT) count, albumin (ALB), total bilirubin (TBil), alanine transferase (ALT) and creatinine (Cr) were also collected.

Toxicities

The toxicity (hematological toxicity, transient liver dysfunction, vomiting and oral mucositis) was assessed according to the National Cancer Institute Common Toxicity Criteria (NCI-CTCAE version 4.0). The hematological toxicity included the reduction in WBC, neutrophil count (NEUT), Hb and PLT count. Here, we also included febrile neutropenia as one of the hematological toxicities. Hepatic toxicity included increases in ALT, aspartate transferase (AST) and TBil.

Statistics

All data were analyzed with SPSS statistical software (version 19.0; SPSS Inc., Chicago, Illinois, USA). Collected data were expressed as the mean \pm SD for

continuous variables with a Gaussian distribution, while the median (min, max) was used for continuous variables without Gaussian distribution. The distribution of categorical variables was presented as frequencies and percentages. The 44-h plasma MTX concentrations were expressed as the median (min, max), and the comparisons were calculated using the chi-square test or Fisher's exact test, as appropriate.

To identify potential predictors for toxicities, we used univariate analyses and multivariate binary logistic regression analyses. Univariate analyses were used to test the relationship between the patient characteristics and toxicities. In the univariate analysis, Pearson's chi-square test and, if necessary, Fisher's exact test were used to evaluate the correlation of toxicities with categorical variables, and the independent sample *t*-test (means, Gaussian populations) as well as the Mann-Whitney U-test (medians, non-Gaussian populations) were used to investigate the correlation of toxicities with continuous variables.

Patient variables with *P*-values less than 0.2 were entered stepwise into a multivariate binary logistic regression model.²³ The binary variables for these analyses were male =0 and female =1 for sex; B-cell ALL =0, T-cell ALL =1 for subtype of leukemia; low risk =0, intermediate and high risk =1 for degree of risk. All tests were two-sided, and *P*<0.05 was considered statistically significant.

Results

Patient characteristics

A total of 104 children (59 boys and 45 girls) with newly diagnosed ALL who received HD-MTX treatment (388 infusions) and had plasma MTX concentrations determined 44 h after the start of treatment as a routine practice were enrolled in this study. According to subtype of leukemia, 93 patients were diagnosed as B-cell ALL, and 11 patients were diagnosed as T-cell ALL. The mean patient age at the time of HD-MTX treatment was 5.4 ± 3.3 years (median: 4.5 years; range: 0.75–14.00 years). According to risk stratification, 56 patients were from the low-risk group, and 48 were from the intermediate- and high-risk groups. A total of 209 infusions of HD-MTX at 3 g/m² were administered, while a total of 179 infusions of HD-MTX at 5 g/m² were administered. The rest of the demographic and patient characteristics collected before every infusion are summarized in Table 1. All AEs categorized by differential grade are listed in Table 2. Anemia was the most

Table 1 Patient demographics and baseline characteristics

Characteristic N=104 (388 infusions)	Median (min, max)
Age (years)	4.50 (0.75, 14.00)
Weight (kg)	18.25 (8.00, 69.00)
Height (cm)	108.00 (75.00, 175.00)
BSA (m ²)	0.74 (0.41, 1.77)
WBC ($\times 10^9/L$)	2.98 (1.09, 20.64)
Hb (g/L)	102.00 (68.00, 137.00)
PLT ($\times 10^9/L$)	245.50 (67.00, 871.00)
ALB (g/L)	40.04 (26.00, 49.80)
TBil ($\mu\text{mol/L}$)	7.20 (2.10, 33.50)
ALT (U/L)	21.00 (3.90, 199.60)
Cr ($\mu\text{mol/L}$)	42.95 (10.00, 73.90)

Note: Descriptive data are presented as the median (min, max).

Abbreviations: WBC, white blood cell count; Hb, hemoglobin; PLT, platelet count; ALB, albumin; TBil, total bilirubin; ALT, alanine transferase; Cr, creatinine.

common mild drug-induced toxicity (AEs > grade 1) (77.06%), followed by leucopenia (76.55%) and neutropenia (62.63%) (Table 2). Neutropenia was the most common severe drug-induced toxicity (AEs \geq grade 3) (26.54%), followed by leucopenia (24.49%) and febrile neutropenia (10.83%) (Table 2).

The correlation between plasma MTX concentration at 44 h and toxicities

The correlation between plasma MTX concentration and toxicities induced by MTX, including hematological toxicity, transient liver dysfunction, vomiting and oral mucositis, was statistically analyzed to determine which toxicity was associated with 44-h plasma MTX concentration. In general, AEs \geq grade 3 were collected for analysis, but vomiting and oral mucositis \geq grade 2 were used for

analysis because the number of vomiting incidents \geq grade 3 occurred in only 4 infusions, and oral mucositis \geq grade 3 was observed in only 16 infusions. In this study, we excluded reduced PLT count from the analysis of the hematological toxicity and reduction in TBil from the analysis of hepatic toxicity because a reduction in PLT count \geq grade 3 was observed in only one infusion, and a reduction in TBil \geq grade 3 also occurred in only one infusion. The 44-h plasma MTX concentrations in all 388 infusions are described in Figure 1. As described in Figure 1, most infusions had an MTX level $<1 \mu\text{mol/L}$; only 37 infusions had an MTX level $\geq 1 \mu\text{mol/L}$ at 44 h. The correlation between 44-h plasma MTX concentrations and MTX-related toxicities is shown in Table 3. As shown in Table 3, febrile neutropenia \geq grade 3 ($P=0.000$) and vomiting \geq grade 2 ($P=0.034$) were more likely to occur if the infusion had an MTX level $\geq 1 \mu\text{mol/L}$ at 44 h, but other hematological toxicities, including leucopenia, neutropenia and anemia, had no correlations with MTX levels. Hepatic toxicity and oral mucositis had no correlations with MTX levels.

Univariate and multivariate analysis of predictive factors for MTX-related toxicities in HD-MTX regimens

Univariate analyses were used to test the relationship between the patient characteristics and toxicities. The selected patient characteristics were age, gender, BSA, subtype of leukemia, WBC, Hb, PLT, ALB, TBil and Cr. In general, AEs \geq grade 3 were collected for analysis, but vomiting and oral mucositis \geq grade 2 were collected for the same reason in the analysis of the

Table 2 Number of courses per grade of adverse events (n=388)

Adverse events	Grade n (%)				
	0	1	2	3	4
Leucopenia	91 (23.45)	88 (22.68)	114 (29.38)	85 (21.91)	10 (2.58)
Anemia	89 (22.94)	136 (35.05)	141 (36.34)	22 (5.67)	0 (0)
Neutropenia	145 (37.37)	60 (15.46)	80 (20.62)	75 (19.32)	28 (7.22)
Febrile neutropenia	346 (89.18)	0 (0)	0 (0)	40 (10.31)	2 (0.52)
Depressed PLT	375 (96.65)	11 (2.84)	1 (0.26)	1 (0.26)	0 (0)
Elevated ALT	193 (49.74)	133 (34.28)	33 (8.51)	29 (7.47)	0 (0)
Elevated AST	191 (49.23)	155 (39.95)	15 (3.87)	27 (6.96)	0 (0)
Elevated TBil	379 (97.68)	5 (1.29)	3 (0.77)	1 (0.26)	0 (0)
Vomiting	348 (89.69)	11 (2.84)	25 (6.44)	3 (0.77)	1 (0.26)
Oral mucositis	303 (78.09)	43 (11.08)	26 (6.70)	14 (3.61)	2 (0.52)

Note: Data are presented as numbers (%).

Abbreviations: PLT, platelet count; ALT, alanine transferase; AST, aspartate transferase; TBil, total bilirubin.

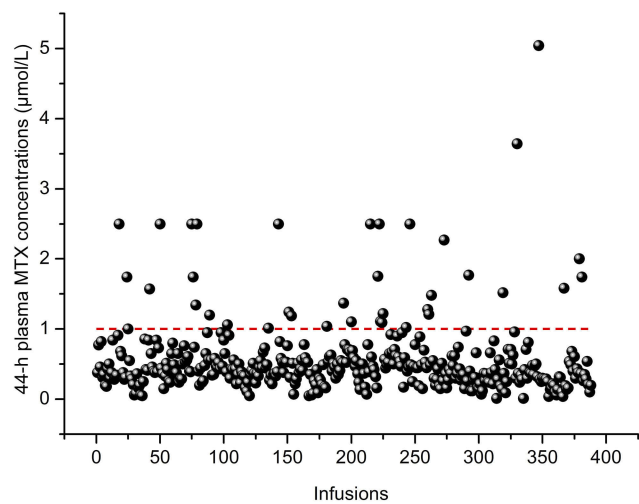


Figure 1 The 44-h plasma MTX concentrations in all 388 infusions.
Abbreviation: MTX, methotrexate.

correlation between plasma MTX concentration and toxicities. Patient variables with *P*-values less than 0.2

were entered stepwise into a multivariate binary logistic regression model to identify the predictive factors for occurrence of AEs (\geq grade 3) before every HD-MTX regimen (Tables S1–S8). The predictive factors for the occurrence of hematological toxicity (\geq grade 3) before every HD-MTX regimen in the HD-MTX therapy of children with ALL are shown in Table 4. The correlation between patient characteristics before every HD-MTX regimen and hepatic toxicity (\geq grade 3) in the HD-MTX therapy of children with ALL is shown in Table 5. The predictive factors for the occurrence of vomiting and oral mucositis (\geq grade 2) before every HD-MTX regimen in the HD-MTX therapy of children with ALL are shown in Table 6.

As shown in Table 4, the predictive factors for the occurrence of hematological toxicity were as follows: higher risk stratification [odds ratio (OR) = 2.013, confidence interval (CI) = 1.162–3.486; *P* = 0.013] for leucopenia, higher values of ALB [OR = 1.084, CI = 1.003–1.171; *P* = 0.041] for leucopenia,

Table 3 Relationship between 44 hr plasma MTX concentration and MTX-related toxicities

Toxicities	44 hr plasma MTX concentration ($\mu\text{mol/L}$)		P-value	
	<1 (n=351, %)	\geq 1 (n=37, %)		
Hematological toxicities (grade \geq3)	• Leucopenia Yes, n=95 No, n=293	88 (25.1) 263 (74.9)	7 (18.9) 30 (81.1)	0.408
	• Neutropenia Yes, n=103 No, n=285	96 (27.4) 255 (72.6)	7 (18.9) 30 (81.1)	0.269
	• Anemia Yes, n=22 No, n=366	20 (5.7) 331 (94.3)	2 (5.4) 35 (94.6)	1.000
	• Febrile neutropenia Yes, n=42 No, n=346	29 (8.3) 322 (91.7)	13 (35.1) 24 (64.9)	0.000*
Hepatic toxicities (grade \geq3)	• Elevated ALT Yes, n=29 No, n=359	28 (8.0) 323 (92.0)	1 (2.7) 36 (97.3)	0.339
	• Elevated AST Yes, n=27 No, n=361	26 (7.4) 325 (92.6)	1 (2.7) 36 (97.3)	0.495
Vomiting (grade \geq2)	Yes, n=29 No, n=359	23 (26.2) 328 (93.4)	6 (2.8) 31 (83.8)	0.034*
Oral mucositis (grade \geq2)	Yes, n=42 No, n=346	35 (10.0) 316 (90.0)	7 (18.9) 30 (81.1)	0.096

Notes: Descriptive data are presented as the median (min, max). **P* < 0.05.

Abbreviations: ALT, alanine transferase; AST, aspartate transferase.

Table 4 Results of logistic regression analysis for MTX-related hematological toxicities (leucopenia, anemia, neutropenia and febrile neutropenia)

Variable	β	SE	P	OR	CI of OR	
					Lower 95%	Upper 95%
Leucopenia (grade ≥ 3)						
Subtype of tumor	-0.064	0.399	0.872	0.938	0.429	2.050
Degree of risk	0.700	0.280	0.013*	2.013	1.162	3.486
WBC ($\times 10^9/L$)	-0.497	0.130	0.000*	0.608	0.471	0.786
PLT ($\times 10^9/L$)	-0.004	0.001	0.003*	0.996	0.994	0.999
ALB (g/L)	0.081	0.040	0.041*	1.084	1.003	1.171
TBil ($\mu\text{mol/L}$)	-0.002	0.030	0.952	0.998	0.941	1.059
ALT (U/L)	0.004	0.004	0.262	1.004	0.997	1.012
Cr ($\mu\text{mol/L}$)	0.008	0.009	0.401	1.008	0.990	1.027
Anemia (grade ≥ 3)						
WBC ($\times 10^9/L$)	0.224	0.101	0.026*	1.252	1.027	1.525
Hb (g/L)	-0.156	0.030	0.000*	0.855	0.807	0.907
PLT ($\times 10^9/L$)	-0.004	0.002	0.084	0.996	0.991	1.001
ALB (g/L)	0.108	0.089	0.224	1.114	0.936	1.327
Neutropenia (grade ≥ 3)						
Gender	-0.499	0.265	0.060	0.607	0.361	1.022
BSA	-1.742	1.752	0.320	0.175	0.006	5.433
Age	-0.005	0.135	0.968	0.995	0.763	1.296
WBC ($\times 10^9/L$)	-0.586	0.133	0.000*	0.556	0.428	0.723
PLT ($\times 10^9/L$)	-0.002	0.001	0.091	0.998	0.996	1.000
ALB (g/L)	0.096	0.039	0.015*	1.101	1.019	1.189
Cr ($\mu\text{mol/L}$)	0.021	0.010	0.038*	1.021	1.001	1.042
Febrile neutropenia (grade ≥ 3)						
BSA	0.995	2.502	0.691	2.705	0.020	364.542
Age	-0.304	0.205	0.137	0.738	0.494	1.102
Degree of risk	0.867	0.382	0.023*	2.379	1.124	5.034
WBC ($\times 10^9/L$)	0.134	0.075	0.076	1.143	0.986	1.325
ALT (U/L)	0.009	0.005	0.087	1.009	0.999	1.019
Cr ($\mu\text{mol/L}$)	0.030	0.017	0.075	1.030	0.997	1.065
C _{MTX44h} ($\mu\text{mol/L}$)	1.025	0.279	0.000*	2.786	1.613	4.813

Note: * $P < 0.05$.

Abbreviations: OR, odds ratio; CI, confidence interval; WBC, white blood cell count; Hb, hemoglobin; PLT, platelet count; ALB, albumin; TBil, total bilirubin; ALT, alanine transferase; Cr, creatinine; C_{MTX44h}, 44 hr plasma MTX concentration.

higher values of WBC [OR =1.252, CI =1.027–1.525; $P=0.026$] for anemia, higher values of ALB [OR =1.101, CI =1.019–1.189; $P=0.015$] for neutropenia, higher values of Cr [OR =1.021, CI =1.001–1.042; $P=0.038$] for neutropenia, higher risk stratification [OR =2.379, CI =1.124–5.034; $P=0.026$] for febrile neutropenia, and higher 44-h plasma MTX concentration [OR =2.786, CI =1.613–4.813; $P=0.000$] for febrile neutropenia. Protective factors for the occurrence of hematological toxicity were higher values of WBC [OR =0.608, CI =0.471–0.786; $P=0.000$] for leucopenia, higher PLT count [OR =0.996, CI =0.994–0.999; $P=0.003$] for leucopenia, higher Hb count [OR =0.855, CI

=0.807–0.907; $P=0.000$] for anemia, and higher values of WBC [OR =0.556, CI =0.428–0.723] for neutropenia.

As shown in Table 5, predictive factors for the occurrence of hepatic toxicity were higher values of ALT [OR =1.018, CI =1.009–1.027; $P=0.000$] for elevated ALT and higher values of ALT [OR =1.013, CI =1.003–1.022; $P=0.008$] for elevated AST.

As shown in Table 6, this analysis revealed only one predictive factor for the occurrence of vomiting: higher values of TBil [OR =1.077, CI =1.004–1.157; $P=0.040$]; there was no predictive factor for the occurrence of oral mucositis.

Table 5 Results of logistic regression analysis for MTX-induced transient liver dysfunction toxicities (elevated ALT and AST)

Variable	β	SE	P	OR	CI of OR	
					Lower 95%	Upper 95%
Elevated ALT (grade ≥ 3)						
Gender	-0.632	0.431	0.143	0.531	0.228	1.237
Subtype of tumor	-1.618	1.083	0.135	0.198	0.024	1.657
ALT (U/L)	0.018	0.005	0.000*	1.018	1.009	1.027
Elevated AST (grade ≥ 3)						
BSA	0.117	2.801	0.967	1.124	0.005	272.494
Age	-0.098	0.226	0.665	0.907	0.582	1.413
Hb (g/L)	-0.024	0.016	0.142	0.976	0.945	1.008
ALB (g/L)	-0.027	0.064	0.670	0.973	0.858	1.104
TBil ($\mu\text{mol/L}$)	-0.096	0.067	0.152	0.908	0.796	1.036
ALT (U/L)	0.013	0.005	0.008*	1.013	1.003	1.022

Note: * $P < 0.05$.

Abbreviations: OR, odds ratio; CI, confidence interval; Hb, hemoglobin; ALB, albumin; TBil, total bilirubin; ALT, alanine transferase.

Table 6 Results of logistic regression analysis for MTX-induced toxicities (vomiting and oral mucositis)

Variable	β	SE	P	OR	CI of OR	
					Lower 95%	Upper 95%
Vomiting (grade ≥ 2)						
Age	0.055	0.056	0.320	1.057	0.948	1.179
WBC ($\times 10^9/\text{L}$)	0.137	0.077	0.078	1.146	0.985	1.334
TBil ($\mu\text{mol/L}$)	0.074	0.036	0.040*	1.077	1.004	1.157
Oral mucositis (grade ≥ 2)						
Subtype of tumor	-1.736	1.028	0.091	0.176	0.023	1.321
WBC ($\times 10^9/\text{L}$)	0.067	0.086	0.438	1.069	0.903	1.266
Hb (g/L)	-0.023	0.012	0.053	0.977	0.955	1.000

Note: * $P < 0.05$.

Abbreviations: OR, odds ratio; CI, confidence interval; WBC, white blood cell count; Hb, hemoglobin; TBil, total bilirubin.

Discussion

Currently, ALL is nearly curable.¹⁷ Intravenous HD-MTX is a useful component in the treatment of children with ALL.²⁴ However, MTX was recently associated with severe AEs in a considerable number of patients despite CF rescue, hydration and urinary alkalinization.²⁵ In this study, reversible myelosuppression was the most common side effect, which was consistent with a previous study.²⁶ Neutropenia was the most common severe drug-induced side effect (\geq grade 3) (26.54%), followed by leucopenia (24.49%) and febrile neutropenia (10.83%). No life-threatening AEs were observed. It is worth noting that the occurrence of severe oral mucositis was rare (4.13%) in our study, which was different from previous studies.^{18,27} Our present finding is most likely a result of the fact that CF (3 mg/d) was also distributed to the oral

mucosa to prevent oral mucositis during chemotherapy after HD-MTX infusion, except for intravenous injection.

The plasma concentration of MTX is important for the development of toxicity in the treatment of children with ALL.¹¹⁻¹⁴ However, today, HD-MTX therapy for the treatment of children with ALL is administered with 24-h continuous infusion, CF rescue, hydration and urinary alkalinization and as part of a multiagent anticancer chemotherapy that is different from the previous therapies used when these studies were performed.^{6,15,16} Evaluating the association between the plasma concentration of MTX and the toxicities induced by MTX in the HD-MTX therapy of children with ALL is important. In this study, febrile neutropenia \geq grade 3 ($P=0.000$) and vomiting \geq grade 2 ($P=0.034$) were more likely to occur if the infusion had an MTX level $\geq 1 \mu\text{mol/L}$ at 44 h,

meaning that MTX level ≥ 1 $\mu\text{mol/L}$ at 44 h was the threshold of MTX concentration, which was related to febrile neutropenia \geq grade 3 and vomiting \geq grade 2. The results indicate that monitoring the plasma concentration of MTX at 44 h is important for adjusting the dose of CF to decrease the MTX-related toxicities in the original treatment protocol of HD-MTX in all children with ALL. Interestingly, a recent study of HD-MTX treatment in children with ALL showed relationships between polymorphisms in folate-related genes and toxicities.²⁸ However, routine monitoring of plasma concentration of MTX still has an important role in adjusting the dose of CF to decrease the MTX-related toxicities in practice before individual prediction of MTX-related toxicities based on pretreatment genotyping reach a practical use.^{26,29} In addition, we concluded that other toxicities, such as hepatic toxicity, oral mucositis, leucopenia, neutropenia and anemia, had no correlations with MTX levels at 44 h. This is in accordance with the findings that there was no significant relationship between the serum MTX concentration and clinical AEs during HD-MTX therapy.^{18,19} However, some studies have demonstrated that the risk of oral mucositis and hematological toxicity is associated with serum MTX concentrations.^{3,17} Nevertheless, remarkably, those previous studies still simultaneously employ 7-h continuous infusion and 24-h continuous infusion¹⁷ and took in only 28 cases of children with ALL and osteosarcoma.³ Therefore, a standardized treatment protocol and a homogenous diagnosis for all children with ALL ensure that the results of our study are credible. We concluded that a high 44-h plasma MTX concentration was associated with febrile neutropenia and vomiting, although it was not associated with mucositis and neutropenia. A review summarized that, in most cases, reversible myelosuppression, mucositis and hepatotoxicity can be prevented by leucovorin rescue.²⁶ The mechanism of our present finding is likely a result of the fact that children with high 44-h plasma MTX concentrations were adequately managed by escalating the dose of leucovorin rescue to decrease toxicities such as myelosuppression, mucositis and hepatotoxicity, but not to decrease toxicities such as mucositis and neutropenia.

Since the purpose of cytotoxic therapy is to provide the best possible therapeutic index, an improved understanding of the predictors of severe AEs is necessary. Although some reports have described the relationship between the effectiveness and the dosage of MTX used in the HD-MTX infusion,³⁰ as well as patient

characteristics and plasma MTX concentration,²⁰ this is a well-designed study that evaluated the relationship between patient characteristics and severe AEs induced by MTX. Univariate analyses were used to test the relationship between patient characteristics and toxicities. The selected patient characteristics were age, gender, BSA, subtype of leukemia, WBC, Hb, PLT, ALB, TBil and Cr. Patient characteristics with *P*-values less than 0.2 were entered stepwise into a multivariate binary logistic regression model to identify the predictive factors for the occurrence of severe AEs before every HD-MTX regimen. MTX dosage was not included in the multivariate binary logistic regression because it was not an independent predictor (the dosage was adjusted based on BSA and risk stratification of the patients). The model is feasible, as these characteristics are routinely assessed before every HD-MTX infusion in our center. Thus, the model may help identify patients with a high risk of severe AEs in HD-MTX infusions and improve early prevention. We found that the predictive factors for the occurrence of hematological toxicity were as follows: higher risk stratification was a significant predictor for leucopenia and febrile neutropenia, higher values of ALB were a significant predictor for leucopenia and neutropenia, higher values of WBC were a significant predictor for anemia, higher values of Cr were a significant predictor for neutropenia, and higher 44-h plasma MTX concentration was a significant predictor for febrile neutropenia. Since higher 44-h plasma MTX concentration and higher risk stratification were identified as significant predictors for the occurrence of febrile neutropenia, monitoring for signs of febrile neutropenia should be prioritized, especially when patients with higher risk stratification have a higher 44-h plasma MTX concentration. Higher values of ALB and Cr were significant predictors of neutropenia, which may provide some reference for clinicians to distinguish those patients with a relatively high risk of neutropenia, especially when patients had higher values of ALB and Cr at the same time before HD-MTX infusion.

This analysis also revealed predictive factors for the occurrence of hepatic toxicity: higher values of ALT were a significant predictor for elevated ALT and elevated AST, which clearly indicated that mild abnormal liver function before HD-MTX infusion is the predictive factor for the occurrence of hepatic toxicity. This analysis revealed that higher values of TBil were the only predictive factor for the occurrence of vomiting. The results may help identify

patients with mild abnormal liver function before HD-MTX infusion who easily suffer from hepatic toxicity and vomiting after HD-MTX infusions.

Several noteworthy limitations should be mentioned. First, this study employed a retrospective design with a limited sample size and the possibility of having other unknown factors. Second, our findings are limited to our center, as the study was based on a single center rather than multiple centers. Third, due to the deficiency of the test of renal function after HD-MTX infusion, we were not able to assess the risk factors for the occurrence of renal toxicity (\geq grade 3), which were analyzed in many other studies.^{2,31,32} Despite these limitations, this study still provided a reference for clinicians to improve the understanding of the predictors for the occurrence of severe AEs and early prevention.

Conclusion

The results of our study suggest that routine monitoring of plasma MTX levels at 44 h after HD-MTX infusion is essential to identify patients at a high risk of developing febrile neutropenia and vomiting. The study developed and validated a feasible risk model to test the predictive factors for the occurrence of severe AEs before every HD-MTX infusion. The study provides a reference for clinicians to distinguish those patients with a relatively high risk of severe AEs before HD-MTX infusion.

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Disclosure

The authors report no conflicts of interest in this work.

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