Use of *MTHFR C677T* polymorphism and plasma pharmacokinetics to predict methotrexate toxicity in patients with acute lymphoblastic leukemia

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Abstract

Background. Methotrexate (MTX) is a key component of acute lymphoblastic leukemia (ALL) therapy, but it is associated with serious toxicities in a considerable number of patients.

Objectives. The aim of the current study was to determine which variables were associated with MTX toxicity in children, adolescents and young adults with ALL.

Material and methods. In this prospective study, 35 patients with newly diagnosed ALL, treated according to the 58951 European Organization for Research and Treatment of Cancer – Children's Leukemia Group (EORTC-CLG) protocol, were prospectively enrolled. Toxicity data was collected objectively after each high-dose methotrexate (HD-MTX) course. The risk factors of MTX toxicity were determined using multiple linear regression analysis, with age, gender, immunophenotype, risk group, plasma MTX levels, plasma homocysteine (HCY) levels, and *MTHFR C677T* included as independent variables.

Results. Twenty-five (71.4%) patients experienced toxicity on at least 1 course of HD-MTX. In the univariate linear regression, the global toxicity score was associated with a significant rise in plasma HCY concentrations within 48 h after MTX administration ($\beta = 0.4$; $R^2 = 0.12$; $p = 0.02$). In the multiple regression model, the global toxicity score was significantly associated with a higher MTX plasma levels at 48 h ($\beta = 0.5$; $R^2 = 0.38$; $p = 0.001$) and CT 677 *MTHFR* genotype ($\beta = 0.3$; $R^2 = 0.38$; $p = 0.01$).

Conclusions. Routine monitoring of plasma MTX concentrations is essential to detect patients at a high risk of MTX toxicity. *MTHFR C677T* genotyping may be useful for predicting MTX toxicity.

Key words: methotrexate, acute lymphoblastic leukemia, *MTHFR C677T* polymorphism, toxicity

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer; its survival rate has improved, with 5-year event-free survival (EFS) rates of 70–80% and overall cure rates of 80%.[1,2] Such an improvement in the treatment outcome is largely due to the advances in chemotherapy. Methotrexate (MTX), an antifolate chemotherapeutic agent, plays an important role in the chemotherapy regimen for ALL and has significantly reduced the recurrence rate of ALL in children.[3]

Methotrexate is predominantly taken up into cells via the reduced folate carrier (RFC).[4] Inside the cell, MTX is converted to its active polyglutamate forms (methotrexate polyglutamates – MTXPGs).[5] Both MTX and MTXPGs inhibit dihydrofolate reductase, an enzyme that catalyzes the conversion of dihydrofolate to its active form tetrahydrofolate (THF), a substrate of thymidylate synthase (TS), to convert deoxyuridine monophosphate to deoxythymidine-5'-monophosphate, resulting in DNA synthesis.[6] Tetrahydrofolate deficiency leads to the depletion of intracellular folates, and thereby to decreased synthesis of both purines and pyrimidines, contributing to the inhibition of nucleic acid synthesis and favoring cell death.[7] Methotrexate polyglutamates can also interfere with methylenetetrahydrofolate reductase (*MTHFR*), which converts 5,10-methylene-THF to 10-methyl-THF, the major circulating form of folate that provides a methyl group for homocysteine (HCY) methylation to methionine, and channels the methyl group into DNA and protein methylation reactions.[6]

A high-dose methotrexate (HD-MTX) refers to infused MTX in doses of more than 1 g/m^2.[8] The use of HD-MTX has shown great benefit in the treatment of childhood ALL and the prevention of extramedullary leukemia, i.e., central nervous system (CNS) leukemia and testicular leukemia.[2] However, MTX is associated with various toxicities, including severe mucositis, myelosuppression, gastrointestinal toxicity, hepatic toxicity, neurotoxicity, and hematological toxicity, requiring a dose reduction and the interruption of chemotherapy, and subsequently an increased risk of relapse.[9] Methotrexate-related toxicity remains a common and often unpredictable clinical problem, because of a wide interindividual variation in pharmacokinetics and pharmacodynamics of this drug.[8]

The aim of the current study was, therefore, to determine factors associated with the high risk of MTX toxicity that could help to develop personalized therapies in children, adolescents and young adults with ALL.

Material and methods

Patients and study design

From January 2013 to December 2014, 35 patients with newly diagnosed ALL were prospectively enrolled from the Hematology Department of Hedi Chaker University Hospital (Sfax, Tunisia). The diagnosis of ALL was based on morphologic, cytochemical and immunophenotypical criteria.

Patients were selected according to the following inclusion criteria: availability of clinical data, treatment according to the European Organization for Research and Treatment of Cancer – Children's Leukemia Group (EORTC-CLG) 58951 protocol, administration of at least 1 course of intravenous MTX chemotherapy, and no history of other active malignancies requiring a modification of chemotherapy regimen.[10]

Patients were stratified into 4 risk groups (low-risk – LR; average risk 1 – AR1; average risk 2 – AR2, and high-risk – HR) on the basis of their presenting clinical features, the biologic features of their leukemic cells, and their early response to remission-induction treatment.[10,11]

This study was conducted in accordance with the Helsinki Declaration and informed consent was given by all the persons participating in this study.

Treatment

All patients were treated according to the 58951 EORTC-CLG protocol, a Berlin–Frankfurt–Munster-like trial, with treatment phases including induction (IA), consolidation (IB/IB9), CNS prophylaxis without cranial irradiation, late intensification II, and maintenance.[11,12]

According to this protocol, the infusion of HD-MTX was given intravenously in each course at 5 g/m^2 body surface area (BSA) over a 4-hour period. Intravenous hydration and urinary alkalinization were performed 1 day before HD-MTX administration, and continued during and after MTX infusion. Leucovorin rescue (25 mg/m^2) was administered every 6 h, starting at 24 h after the initiation of HD-MTX infusion.

High-dose methotrexate infusions were administered during the interval therapy to all patients, in the induction phase to AR2/HR-ALL, in the consolidation phase to HR-ALL and in the R1-R2 Bloc to HR-ALL.

Plasma methotrexate and homocysteine levels determination

In each course of HD-MTX, blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes at the following times: at 24, 48 and 72 h from the start of intravenous MTX infusion. Plasma was recuperated by centrifugation at 3000 rpm for 10 min and was stored at –20°C for the determination of MTX and HCY levels.

Plasma levels of MTX and HCY were determined by a fluorescence polarization immunoassay. Plasma MTX levels were considered high if the concentration was above 10 µmol/L at 24 h, 1 µmol/L at 48 h or 0.1 µmol/L at 72 h.[13,14]

MTHFR C677T genotyping

The *MTHFR C677T* polymorphism was determined in 28 patients with ALL and 70 healthy subjects taken from the general population (35 males and 35 females, age range: 18–29 years). DNA was extracted from peripheral blood samples and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed for the molecular diagnosis of the *C677T MTHFR* polymorphism. The primers, lengths and restriction enzymes have been described previously.[15] The 677C → T base pair substitution creates a *Hinf1* restriction site. Then, CC genotype would be reflected by a single band of 265 bp, CT genotype by 3 bands of 265, 171, and 94 bp, and TT genotype by 2 bands of 171 and 94 bp.

Toxicity

Toxicity data obtained from questionnaires and case records were prospectively collected objectively after each HD-MTX course, in the period from the end of HD-MTX infusion to the next HD-MTX course or until 14 days after HD-MTX infusion.

The toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE, v. 5.0).[16]

According to the modified method of Radtke et al., the global toxicity score for each patient was calculated in our study by adding up the grading of all adverse events that occurred during courses of MTX.[17] This score integrates both frequency and severity of MTX toxicity. In the study, the higher grade of toxicity in each HD-MTX course was considered.

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics v. 20 software (Chicago, USA). Quantitative variables were expressed as means and standard error of the mean (SEM); they were compared using the t-test or the Wilcoxon-Mann-Whitney test according to the characteristics of the distribution. Qualitative variables were presented as a total number and proportion, and were compared using the χ^2 test or Fisher's exact test according to sample sizes.

To identify the risk factors of MTX toxicity, a multivariate linear regression model was constructed, using variables identified from the univariate analysis, which included age, gender, immunophenotype, risk group, MTX plasma levels, HCY plasma levels, and *MTHFR C677T*. A stepwise selection was used with probability value of $p < 0.2$ for entry and $p < 0.05$ for removal. For all comparisons, differences were considered statistically significant at $p < 0.05$.

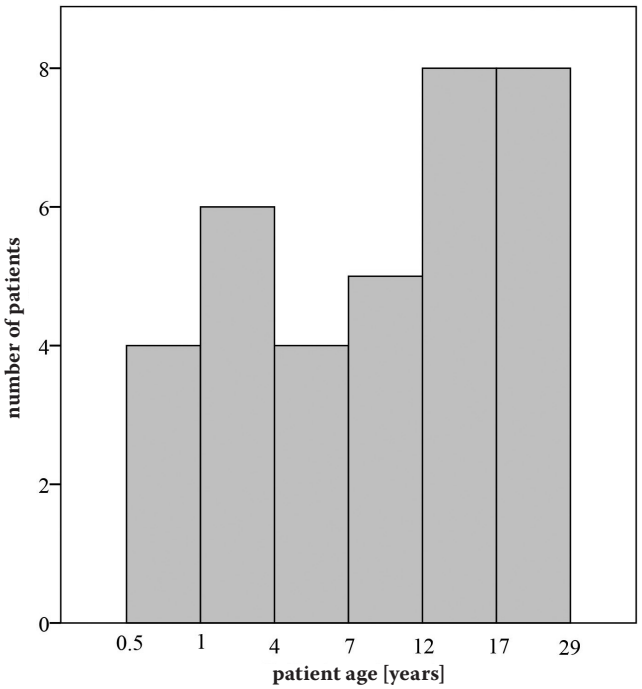

Fig. 1. Age distribution of patients

Results

Patient characteristics and plasma levels of methotrexate and homocysteine

A total of 35 patients (21 males and 14 females) with newly diagnosed ALL treated with the 58951 EORTC-CLG protocol were enrolled in this study. The mean patient age at diagnosis was 11.7 ±9 years (median: 10.1 years; range: 0.5–29 years) (Fig. 1). Patients' characteristics were shown in Table 1.

According to risk stratification, 12 patients were from AR1 group, 13 from AR2 group and 10 from HR group. A total of 173 courses of HD-MTX at 5 g/m^2 were administered.

In the study, 168 plasma MTX levels and 125 plasma HCY levels were analyzed. The results of these analyses are presented in Table 1.

Methotrexate levels ranged from 0.9 to 13 µmol/L at 24 h, from 0.02 to 6.7 µmol/L at 48 h and from 0.01 to 0.9 µmol/L at 72 h. Homocysteine levels ranged from 8 to 19 µmol/L at 24 h, from 5 to 15.5 µmol/L at 48 h and from 0.13 to 17 µmol/L at 72 h.

High-dose methotrexate-related toxicity

Of 35 patients, 25 (71.4%) experienced toxicity on at least 1 course of HD-MTX. The mean of global toxicity score for these patients was 5.1 ±2.1 (range: 1–12). A total of 59 cases of MTX-related toxicity were observed among 173 courses of HD-MTX (on average, 2 cases of toxicity per patient; range: 1–9) (Table 1).

Table 1. Characteristics of patients, their clinical condition and toxicity experienced

Characteristics of patients	Total number of patients (n = 35)	
Age at diagnosis [years], mean ±SEM (min–max) <10 years, n (%) ≥10 years, n (%)	11.7 ±1.6 (0.5–29) 17 (48.6) 18 (51.4)	
Gender male, n (%) female, n (%)	 21 (60) 14 (40)	
Immunophenotype B-ALL, n (%) T-ALL, n (%)	 23 (65.7) 12 (34.3)	
Weight [kg], mean ±SEM (min–max)	41.5 ±4 (11–60)	
Height [cm], mean ±SEM (min–max)	138 ±5.5 (80–183)	
BSA [m^2], mean ±SEM (min–max)	1.2 ±0.1 (0.5–2)	
Risk group AR1, n (%) AR2, n (%) HR, n (%)	 12 (34.3) 13 (37.1) 10 (28.6)	
MTX-related toxicity	Total number of patients (n = 35)*	Total number of HD-MTX courses (n = 173)
Hepatotoxicity, n (%) grade 2 grade 3 grade 4	13 (37.1) 7 4 2	17 (9.8) 9 6 2
Gastrointestinal toxicity, n (%) grade 1 grade 2 grade 3	10 (28.6) 1 3 7	17 (9.8) 1 9 7
Mucositis, n (%) grade 3 grade 4	6 (17.1) 3 4	8 (4.6) 3 5
Neurotoxicity, n (%) grade 1 grade 3 grade 4	3 (8.6) 1 2 2	5 (2.9) 1 2 2
Skin toxicity, n (%) grade 1 grade 2 grade 3	6 (17.1) 1 1 4	6 (3.4) 1 1 4
Hematotoxicity, n (%) grade 1 grade 4	2 (2.7) 1 1	2 (1.1) 1 1
Renal toxicity, n (%) grade 2	1 (2.8) 1	1 (0.6) 1
Phlebitis, n (%)	2 (2.7)	3 (1.7)
Plasma MTX levels [µM]	Total number (n = 168)	
24 h (n = 66), mean ±SEM, 48 h (n = 54), mean ±SEM 72 h (n = 48), mean ±SEM	5.6 ±0.6 1.3 ±0.3 0.1 ±0.02	
Plasma HCY levels [µM]	Total number (n = 125)	
24 h (n = 49), mean ±SEM 48 h (n = 48), mean ±SEM 72 h (n = 28), mean ±SEM	12.2 ±30.6 10 ±0.5 8 ±0.8	

BSA – body surface area; AR – average risk; HR – high risk; MTX – methotrexate; HCY – homocysteine; HD-MTX – high-dose methotrexate; SEM – standard error of the mean; * the upper grade of toxicity in each HD-MTX course was considered.

Hepatic and gastrointestinal toxicities were the most frequently observed toxicities, accounting for 57.6% of the total number of observed toxicities. Methotrexate-induced hepatotoxicity was expressed by elevated aspartate aminotransferase/alanine aminotransferase (ASAT/ALAT). There were 55 cases of toxicity (93.2%) with grade 2 or greater, and only 4 cases of toxicity with grade 1 (6.8%) (Table 1).

Plasma methotrexate levels and methotrexate-related toxicity

The univariate linear regression revealed that the global toxicity score was significantly associated with plasma levels of MTX at 24 h ($\beta = 0.64$; $R^2 = 0.41$; $p = 0.001$), 48 h ($\beta = 0.43$; $R^2 = 0.19$; $p = 0.01$) and 72 h ($\beta = 0.37$; $R^2 = 0.14$; $p = 0.03$) (Table 2).

Methotrexate plasma levels ≥10 µmol/L at 24 h were significantly associated with higher HCY plasma levels at 24 and 48 h after the initiation of MTX infusion (Table 3).

Table 2. Risk factors for MTX toxicity (univariate linear regression)

Patients	p-value (Fisher's exact test, global)	β	β0	R^2 (%)
Age at diagnosis [years]	0.45	0.13	3.1	1.7
Gender (M vs F)	0.78	0.04	3.1	0.2
Immunophenotype (T vs B)	0.67	–0.07	4.5	0.6
Weight	0.37	0.15	2.7	2.4
Height	0.4	0.1	1.5	2.1
BSA	0.35	0.16	2.3	2.6
Risk group (AR1, AR2, HR)	0.14	0.2	0.5	0.6
Plasma levels of MTX 24 h 48 h 72 h	 10^{-3} 0.01 0.03	 0.64 0.43 0.37	 0.9 2.4 2.5	 41.1 19.1 14.3
Plasma levels of HCY 24 h 48 h 72 h	 0.14 0.02 0.18	 0.28 0.4 –0.31	 2.4 2.1 1.3	 4.5 12.8 4.8
MTHFR C677T (*CT* vs *CC*)	0.02	0.4	1.8	19

BSA – body surface area; MTX – methotrexate; HCY – homocysteine; M – males; F – females; AR – average risk; HR – high risk; β – regression coefficient; β0 – intercept coefficient.

As shown in Table 3, the global toxicity score was significantly correlated with MTX levels >10 µmol/L at 24 h, 1 µmol/L at 48 h and 0.1 µmol/L at 72 h. Gastrointestinal and skin toxicity were significantly associated with high MTX plasma levels 48 and 72 h after MTX infusion (Table 3).

Plasma homocysteine levels and methotrexate-related toxicity

The univariate linear regression revealed a positive correlation between global toxicity score and plasma HCY

Table 3. Correlation between folate pathway and MTX toxicity

Plasma HCY and MTX toxicity	Plasma MTX						*MTHFR*	
	at 24 h (n = 31)		at 48 h (n = 32)		at 72 h (n = 30)			
	<10 μM (n = 25)	≥10 μM (n = 6)	<1 μM (n = 18)	≥1 μM (n = 14)	<0.1 μM (n = 19)	≥0.1 μM (n = 11)	CC (n = 22)	CT (n = 6)
Plasma HCY at 24 h [μM], mean ±SEM	11.6 ±0.6	15 ±1.8*	12.2 ±0.8	12.3 ±1.3	12 ±0.7	13 ±1.3	12 ±0.7	13.3±2
Plasma HCY at 48 h [μM], mean ±SEM	8.8 ±0.5	11.6 ±0.9*	9.2 ±0.6	10 ±0.7	9.1 ±0.6	10 ±0.8	8.2 ±0.5	10 ±0.9
Plasma HCY at 72 h [μM], mean ±SEM	8.5 ±0.8	6.3 ±4	9.6 ±1	7 ±1.1	8.7 ±1	8.2 ±1.7	8.1 ±0.9	5.9 ±3
Global toxicity, mean ±SEM	2.5 ±0.5	7.5 ±1.6*	1.6 ±0.5	6.3 ±0.7*	1.5 ±0.4	6.7 ±0.8*	2.5 ±0.6	6.1 ±1.7*
Gastrointestinal toxicity (n)	8	3	4	9†	4	7†	8	3
Mucositis (n)	2	2	1	4	1	5†	2	1
Nausea/vomiting/diarrhea (n)	4	3	2	7†	3	4	4	3
Abdominal pain (n)	3	1	2	3	1	3	4	1
Liver toxicity (n)	6	5	6	6	6	6	5	4
Renal toxicity (n)	0	1	0	1	0	1	0	1
Neurotoxicity (n)	2	2	1	3	1	1	1	2
Seizure (n)	1	0	0	1	0	0	1	0
Somnolence (n)	1	0	1	0	1	0	0	1
Paralysis (n)	0	1	0	1	0	1	–	–
Agitation (n)	1	0	0	1	0	0	1	0
Anxiety/mood disorder (n)	0	1	0	1	0	0	0	1
Skin toxicity (n)	4	3	1	6†	2	5†	3	2
Hematotoxicity (n)	2	0	2	0	1	1	2	0
Thrombocytopenia (n)	1	0	1	0	1	0	1	0
Hemorrhage (n)	1	0	1	0	0	1	1	0
Phlebitis (n)	1	1	1	1	1	1	1	1

MTX – methotrexate; HCY – homocysteine; SEM – standard error of the mean; * statistically significant differences between groups estimated using Mann-Whitney U test; † statistically significant differences between groups estimated using Fisher's exact test.

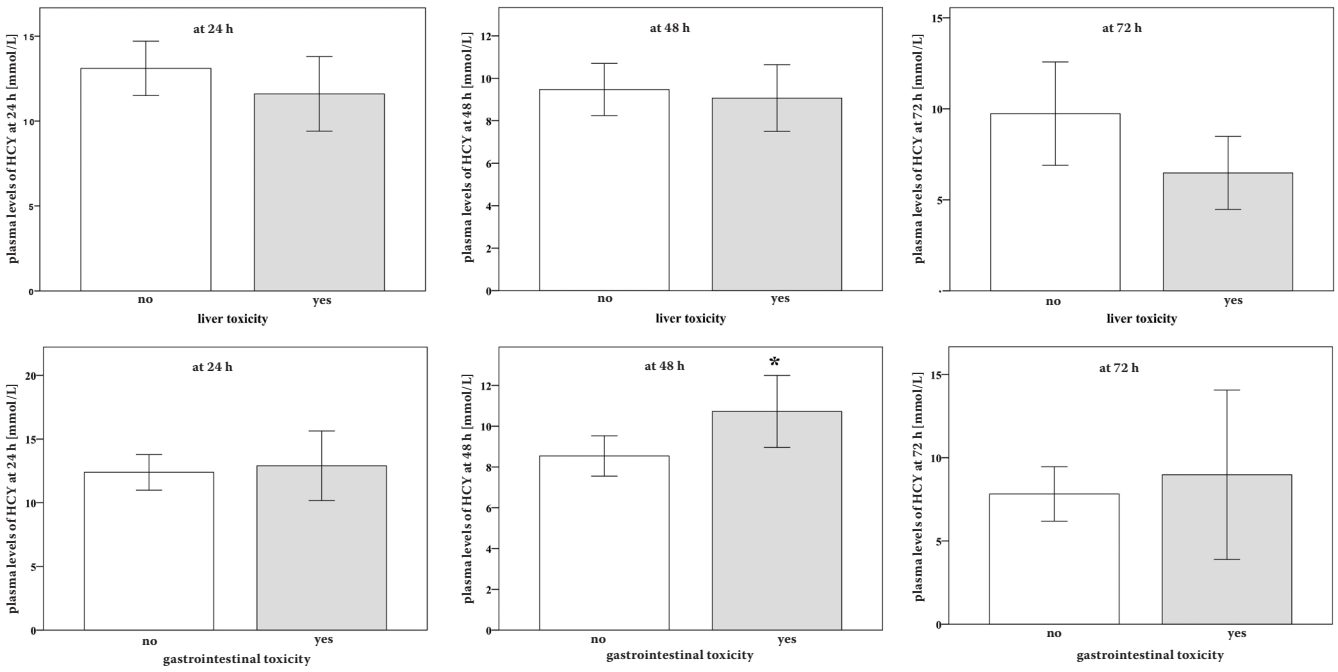

Fig. 2. Association of liver toxicity and gastrointestinal toxicity with plasma homocysteine levels

Each column represents mean with standard deviation (±SD); HCY – homocysteine; * p = 0.02.

levels within 48 h after MTX administration (β = 0.4; R^2 = 0.12; p = 0.02) (Table 2). Figure 2 shows that HCY plasma levels at 48 h were significantly higher in patients with gastrointestinal toxicity (p = 0.02).

Gene polymorphisms and methotrexate-related toxicity

The *MTHFR C677T* polymorphism was in Hardy-Weinberg equilibrium. *MTHFR 677C>T* was of the wild type (CC) in 22 patients (78.6%) and 70 controls (100%), and heterozygous (CT) in 6 (21.4%).

The influence of this polymorphism on MTX toxicity was analyzed in 28 patients with ALL included in this study. The mean global toxicity score was significantly higher in *MTHFR 677 CT* genotype than in wild genotype (p = 0.02) (Tables 2,3).

There was no significant association between *MTHFR 677 C>T* polymorphism and plasma MTX levels.

Risk factors for methotrexate toxicity

In a multiple regression model, the global toxicity score was significantly associated with 2 variables: higher MTX plasma levels at 48 h (≥1µmol/L) (β = 0.5; R^2 = 0.38; p = 0.001) and CT 677 *MTHFR* genotype (β = 0.3; R^2 = 0.38; p = 0.01).

Discussion

Intravenous HD-MTX is a key component in the therapy of ALL.[3] However, despite leucovorin rescue with hydration and urinary alkalinization, MTX is associated with serious toxicities in a considerable number of patients.[15] This could lead to the interruption of treatment, which may increase relapse risk.

This study identified several clinical variables that influence MTX toxicity in patients with ALL treated according to the EORTC-CLG 58951 protocol. We used the global toxicity score that integrates both severity and frequency of MTX toxicity during 173 HD-MTX courses.

One of the major limitations of the current study was the small sample size; studies with a greater number of patients would be necessary to confirm our results. However, a homogenous diagnosis, a standardized treatment protocol followed by all patients, objective and well-recorded toxicity data make the results credible.

High-dose methotrexate was associated with toxicities in the majority of patients included in this study (71.1%). Consistent with previous studies, we found that the most common side effects following HD-MTX therapy were hepato-, skin and gastrointestinal toxicity, particularly the oral mucositis.[18]

This toxicity is unpredictable because of large inter-patient variability in the pharmacokinetics and pharmacodynamics of this drug, even with the same treatment protocol.[5,7,18] The mechanism of MTX-induced toxicity could be mainly explained by an inhibition of normal cells and tissue adjacent to the target abnormal cells.[8]

In this study, acute MTX-induced hepatotoxicity was expressed by elevated ASAT/ALAT and was observed in 37.1% of patients. The pathophysiology of this side effect remains unclear. Holmboe et al. suggested that 7-OH-MTX, a main metabolite of MTX, was involved in the development of HD-MTX hepatic toxicity in patients with osteosarcoma treated with HD-MTX.[19]

Oral mucositis was the most severe MTX-related toxicity observed in this study. Considerable effort has been expended to identify the etiopathophysiology of this side effect.[20,21] Pico et al. reported that MTX may be secreted in the saliva, leading to increased direct mucotoxicity.[22]

In the present study, we analyzed the relation between MTX pharmacokinetics and MTX-related toxicities during HD-MTX courses. In multiple linear regression analysis, we found that plasma MTX levels at 48 h were significantly correlated with the global toxicity score. Currently, few studies among patients with ALL have reported that the plasma levels of MTX may influence the risk of MTX toxicity.[23–25]

In the current study, acute MTX-induced hepatotoxicity, which was the most common side effect, was not associated with plasma MTX levels. This can be explained by the fact that the small number of children in this study could influence its power to detect a significant association.

We found that the risk of oral mucositis was significantly associated with high MTX plasma levels 72 h after drug infusion (Table 3). This finding is consistent with those of Cheng, who revealed that 64% of children with oral mucositis had plasma MTX levels above the defined upper limit of the expected profile at 66 h.[25] It is rather remarkable to find a higher frequency of nausea/vomiting episodes in patients with high MTX plasma levels at 48 h. Although the exact mechanism is unclear, it was reported that nausea/vomiting can lead to dehydration, causing decreased glomerular filtration rates, and thus limited renal clearance of MTX.[24]

It was also reported that MTX-related toxicity might be explained through the disruption of folate homeostasis.[26] In this study, we determined the plasma HCY levels, since it was considered a sensitive marker of deficient folate homeostasis.[27]

We found that elevated levels of HCY were associated with higher MTX plasma levels at 24 h, which is consistent with the previous study.[28] This can be explained by the interference of MTX with the metabolism of HCY by reducing the level of 5-methyl-THF, which serves as the donor of the methyl group for the methylation of HCY to methionine. As a result, the levels of HCY increase, whereas the levels of methionine decrease.[29]

Moreover, we found that global toxicity score, particularly gastrointestinal toxicity, was associated with

a significant rise in plasma HCY levels within 48 h after MTX administration. Although a strong association between blood levels of HCY and the risk of the development of CNS disorders has been shown, there are few studies reporting such an association with gastrointestinal toxicity.[30–32] Hyperhomocysteinemia may induce cell damage through a number of complex mechanisms, including interference with the methylation process and disturbance of oxidative stress balance.[33–35]

Therefore, an increased level of HCY might be considered a sensitive marker of MTX toxicity.

The *MTHFR* gene is located at the end of the short arm of chromosome 1 (1p36.3), and the encoded protein, *MTHFR*, is a key enzyme in folate metabolism.[36,37] The C677T single nucleotide polymorphism (SNP) is the most studied polymorphism in the *MTHFR* gene and results in an alanine-to-valine substitution at codon 222. Its variant alleles cause a substantial reduction of the *MTHFR* enzyme activity in vitro compared with the wild type allele.[38] People with a heterozygous *MTHFR 677 CT* genotype have 60% enzyme activity compared with those with the wild-type allele.[39] In the present study, we investigated whether there exists an influence of *MTHFR C677T* polymorphism on MTX-related toxicity. We found a significantly increased risk of MTX-related toxicity in patients with 677CT genotype compared with the wild genotype 677CC. This result was consistent with previous studies.[40,41] A meta-analysis of studies concerning the toxicity of low-dose MTX (10–15 mg/week) in rheumatoid arthritis suggested that C677T polymorphism was significantly associated with increased toxicity.[40] Another meta-analysis in ALL patients, including 21 articles published before September 2010, supported this association and suggested that the 677T allele serves as a toxicity predictor during treatment with MTX.[41]

In conclusion, the results of our study suggest that routine monitoring of plasma MTX levels during 48–72 h is essential to detect patients at a high risk of developing toxicity and to adjust leucovorin rescue and hydration. Moreover, we suggest that *MTHFR C677T* genotyping may be useful for predicting MTX toxicity. Future studies with large sample sizes should be undertaken to verify current findings, which may provide further biomarkers of treatment efficacy and toxicity in patients with ALL.

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