Successful re-exposure to high-dose methotrexate after severely delayed methotrexate elimination and renal toxicity in children with acute lymphoblastic leukemia

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Abstract

High-dose methotrexate (HDMTX) is a cornerstone of contemporary treatment protocols for both pediatric and adult acute lymphoblastic leukemia (ALL); however, up to 4% of children and 15% of adults develop renal toxicity with severely delayed MTX elimination (DME). Evidence-based guidance on re-exposure after DME is lacking, and omission of further HDMTX may compromise anti-leukemic efficacy and potentially increase the risk of relapse. This study, conducted within the Ponte di Legno International Toxicity Working Group, aimed to evaluate the safety of HDMTX re-challenge in pediatric patients after DME. National investigators from 12 countries provided case-level data on initial DME events and subsequent HDMTX re-exposures via structured questionnaires. Data from 189 patients treated for ALL who experienced DME were analyzed, of whom 143 were subsequently re-exposed to HDMTX. Clinical toxicities after the initial DME included gastrointestinal complications (vomiting, diarrhea, mucositis), infections, and neurological events (encephalopathy, seizures, MTX stroke-like syndrome). Laboratory toxicities comprised cytopenias and hepatic abnormalities. Two patients transiently required dialysis. DME led to chemotherapy modifications in 73% of the patients. After re-exposure, toxicities were similar in spectrum, self-limited, and non-fatal. Twenty children (14%) developed recurrent DME, including three with two additional episodes. Recurrent DME

could neither be predicted by clinical, pharmacokinetic, or demographic variables, nor by uniform MTX dose reduction during re-exposure. In conclusion, re-exposure to HDMTX following DME is feasible and generally well tolerated, although the risk of recurrence is increased. Re-challenge should be considered once renal function has normalized, with careful monitoring and individualized dose adjustment.

Introduction

Epidemiology, definition, clinical manifestations, and risk factors

High-dose methotrexate (HDMTX, defined as ≥500 mg/m^2) constitutes a fundamental part of most contemporary pediatric acute lymphoblastic leukemia (ALL) treatment protocols, as it significantly reduces the risk of central nervous system (CNS) relapse and improves overall survival (OS).[1-3] Owing to its potent anti-leukemic activity, HDMTX has also been incorporated into adult treatment regimens.[4]

HDMTX courses are usually administered at 3-5 g/m^2 over 24 hours, accompanied by intensive hydration and urine alkalinization, followed by folinic acid (FA) rescue to mitigate toxicity. Nevertheless, despite optimal supportive care and careful monitoring, up to 4% of the children and as many as 15% of adults receiving HDMTX develop renal toxicity resulting in severely delayed MTX elimination (DME).[5-10]

MTX enters cells primarily by active transport via the reduced folate carrier receptor and undergoes polyglutamylation, promoting intracellular retention. At higher serum concentrations, MTX can also cross the cell membrane by passive diffusion. By inhibiting dihydrofolate reductase (DHFR), MTX disrupts the synthesis of methionine, thymidine, and purines. During HDMTX therapy, monitoring of serum creatinine and MTX levels is crucial. Prolonged exposure to MTX and its metabolites can result in acute renal injury with reduced MTX clearance, leading to sustained, elevated plasma MTX concentrations, and enhanced systemic toxicities that may result in irreversible damage.[6,11,12] The severity of methotrexate-related toxicity is highly schedule-dependent and directly proportional to the duration of exposure (AUC); however, MTX concentrations show wide inter- and intra-individual variations, even when using the same dose and duration.[6]

Proposed mechanisms of MTX-induced renal dysfunction include direct tubular toxicity due to prolonged exposure to MTX and reduced renal perfusion due to arteriolar vasoconstriction.[6,11]

Several risk factors for DME have been identified, including reduced renal function, acidic urine pH, inadequate hydration,[12] hypoalbuminemia,[13,14] and concomitant drugs or food interfering with MTX clearance.[5,12,15] Population pharmacokinetic modeling has demonstrated that, beyond those known risk factors, patients' characteristics (age, sex, body mass index [BMI], weight, and ethnicity) as well as polymorphisms in genes involved in MTX metabolism and transport, contribute to inter-individual variation in MTX pharmacokinetics.[16-19] Recently developed models that integrate early MTX and creatinine plasma measurements, alongside clinical and genetic variables, aim to personalize MTX dosing and reduce the risk of DME in children with ALL.[20-23]

The Ponte di Legno Toxicity Working Group (PTWG) defines severe DME as a rise in plasma creatinine >0.3 mg/dL or a 1.5-fold increase above baseline, with markedly elevated plasma MTX concentrations at key time points post-infusion: 36 hours >20 μmol/L, 42 hours >10 μmol/L, or 48 hours >5 μmol/L.[24] DME can be accompanied by gastrointestinal symptoms (vomiting, diarrhea), neurological complications, and other systemic toxicities; however, most patients remain initially asymptomatic and present with non-oliguric renal dysfunction characterized by an abrupt rise in serum creatinine during or shortly after MTX infusion, resulting in significantly elevated plasma MTX concentrations.[11,12,25,26] Early recognition of DME, followed by urgent intervention with plasma MTX-adapted FA rescue, and when indicated, glucarpidase administration, is therefore critical. Most ALL protocols include guidelines for prevention and management of DME, including criteria for glucarpidase use (*Online Supplementary Table S1*). However, the dilemma of HDMTX re-exposure after severe DME remains insufficiently addressed.

Although MTX-induced renal toxicity is generally reversible, and several studies have reported successful resuming once renal function has normalized,[8,20,27,28] many clinicians remain hesitant to re-challenge patients due to concerns about recurrence. If re-exposure is considered, MTX doses are often empirically reduced.[20,28] However, evidence-based guidelines for the timing, dosing, and safety of re-exposure are lacking. Consequently, clinical decisions are often based on institutional practice or physician discretion. For some patients, this results in permanent discontinuation of HDMTX, which may compromise treatment efficacy and increase relapse risk (RR).

Purpose

We conducted a retrospective, multinational study to evaluate the safety and tolerability of HDMTX re-exposure in pediatric patients with ALL following an episode of DME. Pharmacokinetic, clinical, and toxicity data were collected from 12 pediatric ALL working groups to support the development of international, evidence-based recommendations for HDMTX re-administration.

Methods

Patients

Inclusion criteria

Children (≤18 years) with newly diagnosed ALL who developed severe DME following treatment with HDMTX. Only the most severe cases, meeting the PTWG criteria,[24] were included. Each case was independently reviewed twice to confirm eligibility.

Exclusion criteria

Children with pre-existing renal dysfunction prior to the HDMTX course, therapy with tyrosine kinase inhibitor, or insufficient data to apply the PTWG definition of DME.

Data capture and editing

Data on DME events were captured as serious adverse events (SAE) within the national pediatric ALL treatment protocols both prospectively and retrospectively. A detailed overview of the treatment protocols is provided in *Online Supplementary Table S2*. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki, and informed consent, approved by national ethics committees, was obtained for all participants before study registration. Primary national investigators completed detailed questionnaires (*Online Supplementary Table S3*) for each HDMTX course complicated by DME, documenting clinical, laboratory, and pharmacokinetic parameters, as well as comprehensive data on subsequent HDMTX courses.

Classification and data collected

DME events were classified as either first occurrences or subsequent events following HDMTX re-exposure. Data analysis comprised treatment details on MTX dosage, hydration, and concomitant chemotherapy, leukemia lineage, risk group, and treatment protocol. Laboratory data included baseline creatinine levels and serial MTX and creatinine values, blood counts, and biochemical profiles. Clinical toxicities (grade ≥3, per Common Terminology Criteria for Adverse Events [CTCAE]) during DME events and subsequent MTX courses were documented, for gastrointestinal, hepatic, CNS, and infectious complications. Finally, the impact on scheduled chemotherapy, resumption of treatment, and modifications was assessed.

Statistical analysis

Outcome events

We examined the relationship between potential explanatory variables and the occurrence of severe DME at the index MTX re-exposure, and correlates of clinical and laboratory variables measured at the time of the index exposure, and the occurrence of DME at the next MTX exposure. We particularly focused on MTX dose, MTX level at 48 hours, and time to MTX level <0.25 μmol/L.

Analysis

The effect of categorical variables on the outcome was tested using Fisher's exact test. The effect of continuous variables was tested using the Wilcoxon rank sum test. For all variables, we computed the area under the ROC curve (AUC) and the Goodman-Kruskal gamma. Analyses were done with missing values imputed (mode for categorical variables and median for continuous variables) and with the exclusion of patients with missing values. Variables with 20% or more missing values were excluded. As an additional analysis, we examined the effects of the explanatory variables using the generalized estimating equations (GEE) method, which accounts for possible dependence between multiple observations on the same patient. The GEE analysis was done in a logistic regression framework. We computed both raw *P* values and adjusted *P* values using the Benjamini-Hochberg adjustment for multiple testing. All analyses were univariate analyses examining one explanatory variable at a time. Multivariate analysis was not undertaken because of the small number of DME events.

Results

Study cohort

A total of 209 questionnaires were submitted from 12 countries. Twenty cases were excluded; ten did not meet the PTWG criteria for severe DME, and ten lacked essential data. The final cohort comprised 189 pediatric ALL patients, from 11 countries, treated between 1992 and 2022, who developed severe DME following HDMTX therapy (Figure 1; *Online Supplementary Figure S1*). Baseline patient characteristics are summarized in Table 1.

For the entire cohort, the OS rate was 84%, and the relapse rate (RR) was 17%. When stratified by treatment era, patients treated between 1995-2010 (N=100) had an OS of 83% and RR of 25%, whereas those treated between 2011-2022 (N=89) achieved an OS of 87% and RR of 8%.

First delayed methotrexate elimination events

More than half of the events (103 patients, 55%) occurred after the first HDMTX course, and an additional 15% occurred after the second of the four to eight scheduled HDMTX courses (Table 2). The remaining cases were distributed across later infusions, including some after the eighth course (*Online Supplementary Table S4*). Glucarpidase was administered to 51 patients (27%).

Markedly elevated MTX plasma concentrations were first detected at 24 hours (>150 μmol/L) in 34% of patients, at 36 hours (>20 μmol/L) in 16%, at 42 hours (>10 μmol/L) in 18%, and at 48 hours (>5 μmol/L) in 30%. Most patients (77%) exhibited pathological MTX levels at multiple time points, and nearly all (N=169, 89%) had severely elevated 48-hour MTX levels (>5 μmol/L) (Figure 2).

The median time to reach a plasma MTX concentration

<0.25 μmol/L was 192 hours (range, 48-476 hours). Additional analysis, including only patients who did not receive glucarpidase (N=136), revealed similar results with a median time of 190 hours (range, 48-360 hours).

A significant negative correlation was found between the timing of DME onset and MTX clearance (*P*=0.0319): patients with DME detected at 24 hours, had a longer median time to MTX concentration <0.25 μmol/L (204 hours), compared with those whose DME occurred at 48 hours (170 hours) (*Online Supplementary Figure S3*).

Elevated serum creatinine was first observed 24 hours post-infusion, with a median relative increase of 1.58, and remained elevated at subsequent measurements. The peak increase occurred at a median of 54 hours, with a 2.51-fold rise. Renal function subsequently normalized, with creatinine returning to baseline after a median of 18 days (range, 2-120 days).

Toxicities following the first delayed methotrexate elimination event

Clinical toxicities were assessed in 100 patients (52%). Significant events (CTCAE grade ≥3) included gastrointestinal toxicities: vomiting, diarrhea, and mucositis in 33%; infections in 28%; and neurological complications: encephalopathy, seizures, and MTX stroke-like syndrome in 7%. Significant laboratory toxicities were assessed in 78 patients (41%) and included: cytopenia in 41% and hepatic abnormalities (elevated bilirubin and transaminases) in 16%. Two patients required transient dialysis (for 6 and 16 days); both had clinical and laboratory profiles similar to the rest of the cohort. Two patients died following persistent pancytopenia after the DME event and HDMTX-related myelotoxicity. One

Table 1. Study cohort N=189.

Characteristics	Values
Age, years, median (range)	10 (1-20.9)
Sex, N (%)	
Female	68 (36)
Male	121 (64)
Risk group, N (%)	
Standard risk	50 (26)
Medium risk	58 (31)
High risk	80 (42)
Not assessed	1
Treatment protocol, N (%)	
BFM	51 (27)
DCOG	29 (15)
NOPHO	109 (58)
MTX dose at 1st severe DME, gr/m^2, N (%)	
5	155 (82)
8	34 (18)
Re-exposed, N (%)	143 (76; 80 of scheduled)
Additional severe DME, N (%)	20 (14)
Last follow-up status, N (%)	
Alive	159 (84)
Relapse	32 (17)
Dead	28 (15)
Lost to follow-up	2 (1)

BFM: Berlin-Frankfurt-Münster; DCOG: Dutch Childhood Oncology Group; NOPHO: Nordic Society of Pediatric Hematology and Oncology; MTX: methotrexate; HDMTX: high-dose MTX; DME: delayed MTX elimination.

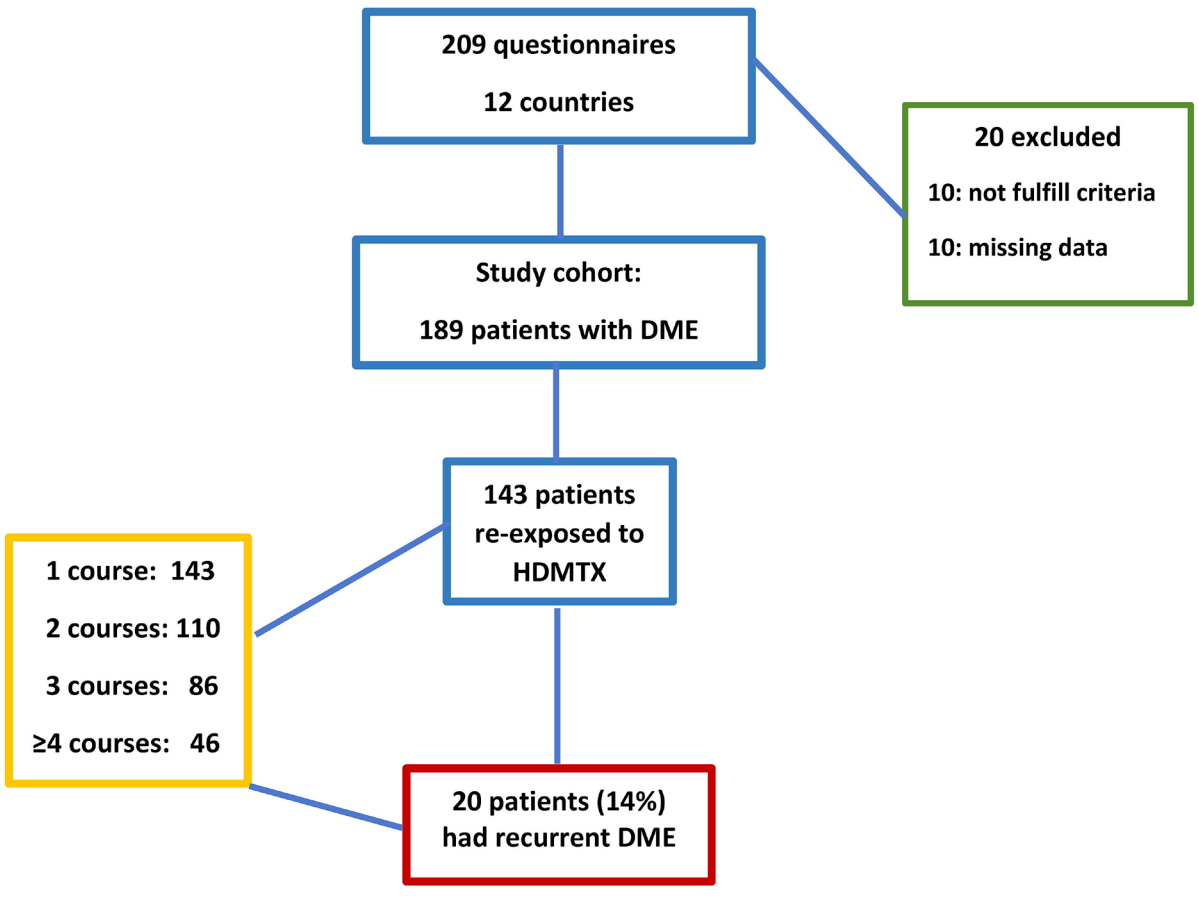


Figure 1. Study cohort. MTX: methotrexate; DME: delayed methotrexate elimination; HDMTX: high-dose MTX.

died after sepsis with multi-organ failure, and one after stem cell transplantation performed for unrecovered bone marrow aplasia (Table 3; *Online Supplementary Table S5*). All other laboratory abnormalities were transient .

Modifications of therapy

The impact of DME on subsequent chemotherapy was evaluated in 172 patients (91%). Treatment modifications occurred in 126 patients (73%), and included treatment delays in 105 (61%) and chemotherapy regimen adaptations in 66 (38%). Among these, 51 patients (30%) received a reduced MTX dose in subsequent courses, and 15 (9%) had the next MTX course omitted or permanently discontinued.

Re-exposure to high-dose methotrexate

Of the 189 patients who experienced DME, 143 (80% of those scheduled for additional HDMTX courses) continued treatment and received between 1-7 further HDMTX infusions (Table 3; *Online Supplementary Table S6*). The main reason for discontinuing HDMTX was physicians' concern regarding recurrent DME.

First methotrexate re-exposure

Most patients (81%) received >50% of the planned MTX dose, while 62% received the full dose. MTX dosing during re-exposures was determined at the treating physician's discretion and was not guided by algorithms or dose-adjustment models targeting specific plasma concentrations. Clinical toxicities were reported in 69 patients (48%) and included mucositis (N=3), neurological symptoms (N=3), and infectious complications (N=9). Laboratory toxicities were documented in 24 patients and included cytopenia (N=11) and elevated liver enzymes (N=2). Adverse events were less frequent than those observed after the first DME events, and all were transient and non-fatal.

Subsequent high-dose methotrexate re-exposures

Among re-exposed patients, 110 received two additional HDMTX courses, 86 received three, and 46 received more than three courses (Figure 1). During subsequent re-exposures, the proportion of patients receiving higher MTX doses gradually increased: the percentage of patients receiving >50% of the planned dose rose from 85% to 94%, and the percentage receiving the full dose rose from 65% to 88% across the second to fourth re-exposures (Table 4; *Online Supplementary Figure S4*). MTX doses administered during re-exposures were not influenced by the course number at which the initial DME occurred, or its severity, nor by pharmacokinetic or predictive modeling.

Table 2. The first delayed methotrexate elimination event.

Parameter	N (%)
MTX course number	
1st	103 (55)
2nd	29 (15)
>2nd	57 (30)
Concomitant IV chemotherapy	
Single MTX	152 (80)
Combined IV	37 (20)
Time of 1st pathological MTX level,* hours	
24	65 (34)
36	30 (16)
42	34 (18)
48	56 (30)
Time of any pathological MTX, hours	
24	65 (34)
36	69 (49)
42	106 (72)
48	169 (93)
Pre-hydration duration, hours	
4	111 (59)
≥12	56 (30)
NA	22 (11)

Parameter	Values
Time to plasma MTX <0.25, hours	
Median (IQR)	192 (160-234)
100-150, N (%)	35 (22)
150-200, N (%)	52 (32)
200-250, N (%)	47 (29)
>250, N (%)	27 (17)
Time of maximal creatinine, hours	
Median (IQR)	48 (36-72)
Mean (range)	58 (23-210)
24, N (%)	23 (15)
25-48, N (%)	74 (48)
49-72, N (%)	31 (20)
>72-120, N (%)	27 (17)
Time to baseline creatinine, days	
Median (IQR)	18 (10-30)
Mean (range)	23 (2-120)
≤10, N (%)	35 (26)
11-30, N (%)	78 (58)
>30, N (%)	21 (16)
Relative increase in creatinine∞, hours Median (IQR)	
0-24	1.58 (1.26-2.07)
0-36	2.49 (1.91-3)
0-42	2.2 (1.8-2.9)
0-48	2.43 (1.9-3.2)
0-54	2.51 (2-4)

*Pathological methotrexate (MTX) levels: 24 hours (h) >150 μmol/L, 36 h >20 μmol/L, 42 h >10 μmol/L, or 48 h >5 μmol/L. ∞The relative increase in creatinine level was measured from the time before starting intravenous (IV) high-dose MTX infusion to the highest creatinine level during this course. Creatinine units: mmol/L, MTX units: μmol/L; IQR: interquartile range; NA: not assessed.

Toxicities following re-exposure to high-dose methotrexate

Clinical and laboratory toxicities observed after HDMTX re-exposures were generally mild and self-limiting, with no treatment-related deaths. Reducing MTX dose during re-exposure did not reduce the rate or severity of these toxicities.

Recurrent delayed methotrexate elimination events after re-exposure

Across 387 re-exposures in 143 patients, 23 recurrent DME episodes occurred in 20 patients (14%). All events were clinically manageable, and most patients were able to continue HDMTX therapy without further complications. However, the cumulative incidence (CI) of recurrent DME increased with each successive course (Figure 3).

Recurrent DME events occurred after the first re-exposure in eight patients (6%), after the second in six (5%), after the third in seven (8%), and after the fourth in two (4%) (Table 3). Similar to initial DME events, 70% of recurrent cases were characterized by pathologically elevated MTX plasma concentrations at 24 hours (*Online Supplementary Table S6*). Three patients experienced two recurrent DME episodes, occurring after the first through fourth re-exposures, at both full and reduced MTX doses (*Online Supplementary Table S7*). All three subsequently continued HDMTX therapy without further complications.

Risk factor for additional delayed methotrexate elimination events

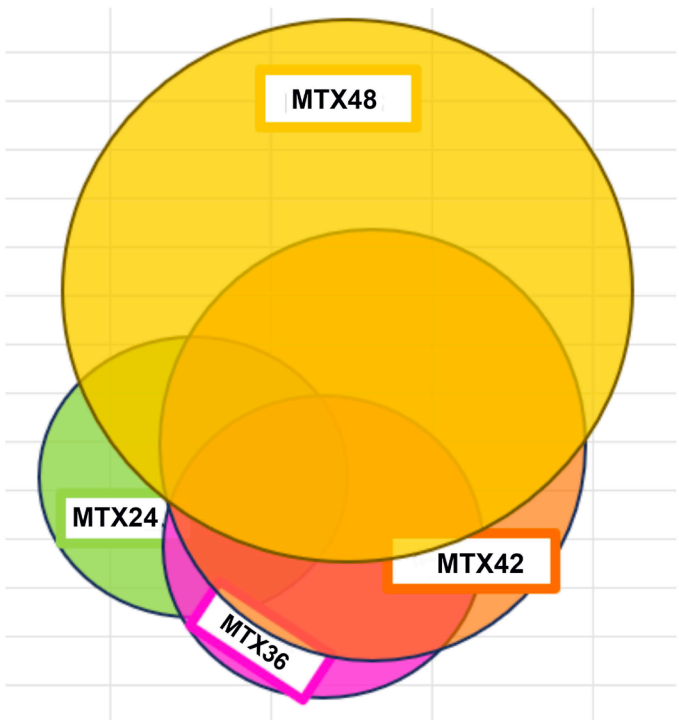
Multiple potential predictors of recurrent DME after re-exposure were evaluated. Variables related to the initial DME event included MTX course number, plasma MTX concentrations at defined time points, time to achieve MTX levels <0.25 µmol/L, relative increase in serum creatinine, time to creatinine normalization, glucarpidase use, and modifications to subsequent therapy. Re-exposure-related factors were also assessed, including MTX dose and course number, as well as plasma MTX and creatinine levels at multiple time points. In addition, demographic and treatment-related characteristics such as treatment protocol, sex, age, and risk group were analyzed (Table 4). None of these factors emerged as a statistically significant predictor of recurrent DME following re-exposure (*Online Supplementary Figures S5-7*). Specifically, neither MTX dose reduction during subsequent courses nor delays in re-exposure reduced the risk of additional DME events. Overall, among all subsequent HDMTX courses, recurrent DME events occurred in 9.6% of those receiving <3 g/m^2 MTX (5/52 courses) and in 5.4% of those receiving >3 g/m^2 MTX (18/333 courses) (*Online Supplementary Figure S8*).

Discussion

Severe DME is one of the most serious acute toxicities associated with HDMTX therapy. In this large international cohort, we evaluated 189 children with ALL who developed DME and assessed the feasibility and safety of subsequent HDMTX re-exposure.

The first severe delayed methotrexate elimination events

Consistent with previous reports identifying the first HDMTX infusion as carrying the highest risk for DME,[8] more than half of the DME events in our cohort occurred



	24 hours	36 hours	42 hours	48 hours
First pathological plasma MTX levels*	65 (34%)	30 (16%)	34 (18%)	56 (30%)
Persistence of pathological plasma MTX levels	39 (21%)			
	46 (24%)			
	42 (22%)			
		63 (33%)		
		61 (32%)		
			96 (51%)	
Pathological plasma MTX levels all cohort	65 (34%)	69 (36%)	106 (56%)	169 (89%)

* First pathological MTX plasma levels:

MTX24 > 150 µmol/L; MTX36 > 20 µmol/L; MTX42 > 10 µmol/L; MTX48 > 5 µmol/L

Figure 2. Time points of the first and subsequent pathological plasma methotrexate levels. Pathological plasma methotrexate (MTX) levels persisted across multiple time points following MTX infusion. Pathological MTX levels at 24 hours are shown in green, at 36 hours in pink, at 42 hours in orange, and at 48 hours in yellow. The majority of patients (89%) exhibited pathological MTX levels 48 hours after MTX infusion.

after the initial MTX course. Possible explanations include stricter adherence to preventive measures in later courses, such as maintaining urine alkalinization and adequate hydration,[12] correcting hypoalbuminemia,[13] and avoiding drug or food interactions,[6,12,15] especially if there were some degrees of clinical or laboratory toxicities during the previous course. Another potential mechanism may be the persistence of an expanded folate pool from prior HDMTX courses, which could displace MTX from DHFR, resulting in reduced intracellular MTX levels and providing partial protection during subsequent infusions.[29]

Most patients (89%) exhibited pathological plasma MTX concentrations at 48 hours (MTX48), reaffirming this as the most sensitive time point for identifying DME. However, elevated MTX levels were already evident at 24 hours in 34% of patients and at 36 hours in 16%. When considering both early time points together, nearly half of all DME cases could have been detected before 48 hours. This underscores the importance of early and close monitoring, while MTX sampling at 36 and/or 42 hours may enable timely recognition and intervention.

Serum creatinine elevation, commonly the first indicator of DME, was observed in all patients, 24 hours post-infusion, peaking at a mean of 58 hours. This corresponds with sustained intracellular MTX accumulation and resultant tubular injury. Importantly, nephrotoxicity was always reversible, although recovery could be prolonged; MTX concentrations <0.25 µmol/L were occasionally achieved only after 20 days, and full renal recovery could take up to 4 months. A statistically significant negative correlation was observed between the timing of DME onset and MTX clearance, with the slowest decline in serum MTX concentrations among patients whose DME developed within 24 hours after infusion. This may suggest that early-onset DME events are the most severe and nephrotoxic.

Toxicities following the first DME events were consistent with prior studies, including gastrointestinal, infections, and neurological complications in up to 30% of patients, as well as cytopenia and hepatotoxicity in up to 40%. Notably, DME events prompted modifications to subsequent chemotherapy in 73% of patients, primarily treatment delays (61%) and MTX dose reductions (38%).

As our cohort included patients treated under multiple protocols spanning three decades, and since most (80%)

Table 3. High-dose methotrexate courses with the first delayed methotrexate elimination event and re-exposures to high dose methotrexate.

Parameter	First DME N=189	1st re-exposure N=143	2nd re-exposure N=110	3rd re-exposure N=86	4th re-exposure N=46
MTX dose, N (%)					
100%*	189 (100)	88 (62)	71 (65)	76 (88)	38 (83)
69-90%	-	28 (19)	22 (20)	5 (6)	5 (11)
≤ 50%	-	27 (19)	17 (15)	5 (6)	3 (6)
Time to MTX <0.25, hours					
Mean (range)	198 (48-476)	67.7 (42-249)	64 (42-192)	NA	NA
Time to baseline creatinine, days					
Mean (range)	23 (3-120)	3.8 (0-24)	-	NA	NA
Clinical Toxicities CTCAE grade ≥3, N (%)				NA	NA
GI	33/100 (33)	0	0	-	-
Mucositis	13/100 (13)	3/69 (4)	0	-	-
CNS	7/100 (7)	3/69 (4)	1/50 (2)	-	-
Infectious	28/100 (28)	9/69 (13)	8/50 (16)	-	-
Laboratory toxicities,N (%)				NA	NA
Hematological	32/78 (41)	11/24 (46)	10/35 (29)	-	-
Liver	9/78 (12)	2/24 (8)	2/35 (6)	-	-
DME, N (%)**	189 (100)	8 (6)	6 (5)	7 (8)	2 (4)
MTX % dose, N					
100	189	8	4	5	-
80	-	-	-	-	1
>50	-	-	2	-	-
50	-	-	-	2	1
Further re-exposure to MTX, N (%)	143 (76)	5 (62)	3 (50)	3 (43)	2 (100)
Death, N	2	0	0	0	0

*5 g/m^2 (155 patients; 82%), 8 g/m^2 (34 patients; 18%). **Data on additional delayed methotrexate elimination (DME) after re-exposure were provided for 132 patients (92%). Creatinine units: mmol/L; methotrexate (MTX) units: µmol/L; HDMTX: high-dose MTX; CTCAE: Common Terminology Criteria for Adverse Events; GI: gastrointestinal; CNS: central nervous system.

continue HDMTX therapy following DME, the potential long-term detrimental consequences of delaying or omitting MTX courses could not be directly assessed. Nonetheless, OS and RR in this cohort were comparable to those reported in the literature for children with ALL, suggesting that appropriate management and re-exposure strategies may mitigate the potential impact of DME on treatment outcomes.

Re-exposures to high-dose methotrexate

A total of 143 children were re-exposed to HDMTX following a prior DME event, and their subsequent courses were analyzed in detail. Clinical and laboratory toxicities during re-exposure were similar in type but less frequent than those observed after the initial DME episode; all were generally mild to moderate, self-limited, and without any treatment-related deaths.

The main reason for discontinuing HDMTX was the physician's concern about recurrence. Indeed, 20 patients (14%) experienced additional DME events, with three patients having two recurrences. The confidence interval (CI) of recurrent DME increased with each re-exposure and reached 20% after the fourth re-exposure. This rate is considerably higher than that reported in the general pediatric ALL population (up to 4%), suggesting that a prior DME confers a markedly elevated risk of recurrence upon re-exposure. Beyond the known acute toxicities of DME, each event may further delay or compromise subsequent chemotherapy, as was demonstrated in our cohort.

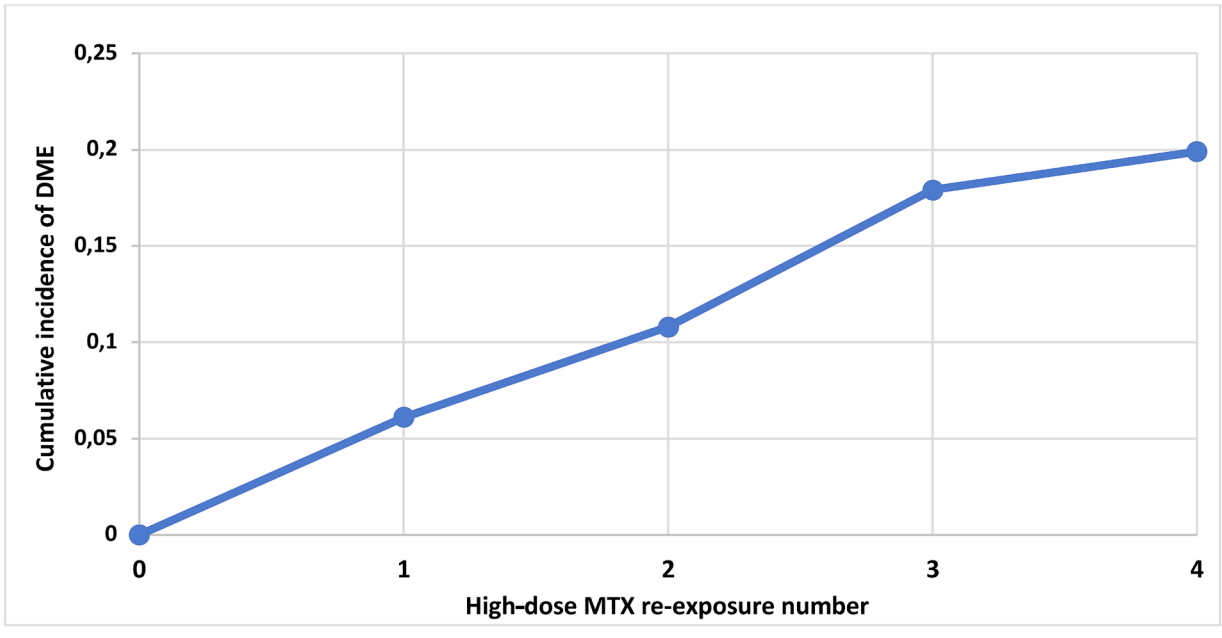
Notably, in 70% of recurrent DME events, pathological MTX levels were detected first after 24 hours (>150 μmol/L), underscoring the critical importance of this time point for early detection and intervention.

Risk factors for additional delayed methotrexate elimination events

To identify predictors of DME recurrence after re-exposure, we evaluated multiple variables related to the first DME event, subsequent re-exposures, and patient characteristics. However, none of these factors emerged as a statistically significant predictor of DME recurrence.

Reducing the MTX dose or delaying the next MTX course did not mitigate the risk of recurrence; in fact, 9.6% of courses at doses <3 gr/m^2 were complicated by recurrent DME, compared to 5.4% of courses at doses >3 gr/m^2. Similarly, none of the laboratory or pharmacokinetic parameters from the initial DME event, nor the patients' or ALL characteristics, reliably predicted recurrence.

Christensen *et al.*[28] reported successful resumption of HD-MTX following DME by adjusting the MTX dose to target a steady-state plasma concentration of 65 μM, based on the clearance from the previous course. Likewise, Foster *et al.*[20] demonstrated that recurrence of DME might be prevented



		Additional DME	Cumulative incidence
*Number of children in the 1st re-exposure	131	8	6.10%
Number of children in the 2nd re-exposure (and no DME at the 1st re-exposure)	99	5	10.80%
Number of children in the 3rd re-exposure (and no DME at the 1st and 2nd re-exposure)	76	6	17.90%
Number of children in the 4th re-exposure (and no DME at the 1st, 2nd, and 3rd re-exposure)	40	1	20.10%

Figure 3. The cumulative incidence of additional severe delayed methotrexate elimination after re-exposure to high-dose methotrexate. The cumulative incidence of additional delayed methotrexate elimination (DME) increases with each additional exposure; *143 patients received 1st re-exposure, 12 were excluded from analysis due to missing full DME data. MTX: methotrexate.

by individualizing 24-hour HDMTX infusion rates according to real-time MTX concentrations measured 2 and 6-8 hours after infusion initiation. Implementing such adaptive pharmacokinetic algorithms could enhance the safety and efficacy of future HDMTX re-exposures.

Fifty-one patients (27%) received glucarpidase during their first DME event. As glucarpidase became more available only after 2008 and was approved in 2012, 65% of patients treated thereafter (N=78) received it, indicating adherence of most pediatric ALL protocols to the international consensus guidelines for glucarpidase use in DME.[11] While glucarpidase effectively reduced plasma MTX concentrations and mitigated acute toxicities, it did not reduce the likelihood of recurrent DME upon re-exposure in our study. Theoretically, genetic variants of MTX metabolism and clearance may increase susceptibility to DME recurrence. Polymorphisms in MTX transport genes (*SLCO1B1, SLC19A1* and *SLCO1A2*), and ATP-binding cassette transporters (*ABCB1, ABCG2, ABCC2* and *ABCC4*), have been associated with DME. Polymorphisms in polyglutamation pathway genes

Table 4. Univariate analysis: risk factors for additional delayed methotrexate elimination upon re-exposure.

Categorical variable	Values	Total	DME post re-exposure, N (%)	Fisher's exact test raw *P* value	AUC	GAM
Sex	F M	132 231	11 (8.33) 12 (5.19)	0.266 -	0.561 -	0.624 -
Current re-exposure N	0** 1 2 3	130 105 82 46	8 (6.15) 6 (5.71) 7 (8.54) 2 (4.35)	0.820 - - -	0.557 - - -	0.579 - - -
Treatment protocol	NOPHO DCOG BFM	270 55 38	20 (7.41) 2 (3.64) 1 (2.63)	0.508 - -	0.568 - -	0.700 - -
Risk group	SR MR HR	136 143 80	7 (5.15) 10 (6.99) 6 (7.50)	0.729 - -	0.544 - -	0.567 - -
Glucarpidase	No Yes	274 86	15 (5.47) 8 (9.30)	0.211 -	0.560 -	0.641 -
Modification of therapy	No Yes	219 144	15 (6.85) 8 (5.56)	0.667 -	0.526 -	0.556 -
Continuous variable	**Total#**	**DME post-re-exposure Median (range)**		**Wilcoxson test raw *P* value**	**AUC without imputation**	**GAM without imputation**
		No	**Yes**			
Age	363	6.5 (1-20.9)	8 (2-9.3)	0.873	0.510	0.510
MTX course number*	363	1 (1-8)	1 (1-7)	0.179	0.574	0.640
MTX level 24*	324	134 (10-623)	145 (42-300)	0.451	0.547	0.548
Maximal increase in creatinine*	310	2.608 (0.774-8.897)	3.045 (1.745-5.644)	0.164	0.591	0.592
Time of maximal Creatinine, hours*	308	48 (23-210)	48 (24-180)	0.687	0.474	0.470
Maximal creatinine*	314	90 (24-333)	118 (46-333)	0.269	0.572	0.573
N of re-exposures	363	4 (1-9)	3 (1-9)	0.399	0.551	0.564
MTX dose, gr/m^2, current exposure	362	5 (0.5-8)	5 (2.5-8)	0.510	0.510	0.518
MTX48 level	340	0.8 (0.1-79)	1.88 (0.2-28.14)	0.356	0.559	0.559
Time to MTX <0.25	307	66 (42-476)	84 (48-258)	0.549	0.539	0.541

**Current re-exposure number =0 signifies the exposure of the initial delayed methotrexate elimination (DME) event. *At the exposure of the initial DME event. #No not available (NA) values. Creatinine units: mmol/L; methotrexate (MTX) units: μmol/L. M: male; F: female; NOPHO: Nordic Society of Pediatric Hematology and Oncology; DCOG: Dutch Childhood Oncology Group; BFM: Berlin-Frankfurt-Münster; MR: medium risk; SR: standard risk; HR: high risk; AUC: area under the curve; GAM: generalized additive model.

(*FPGS* and *GGH*), MTX target genes (*MTHFR* and *ARID5B*), and metabolism enzymes (*GSTP1*) have also been linked to elevated MTX concentrations and may predispose patients to DME recurrence.[30-32] However, none of these genes has been specifically validated as a predictor of DME recurrence following HDMTX re-exposure. Moreover, the applicability of these polymorphisms remains uncertain, particularly regarding whether interventions such as MTX dose reduction and stringent hydration and alkalization protocols could mitigate their effects. Future genome-wide association studies may elucidate genetic determinants of susceptibility to DME recurrence.

Limitations of the study

While data regarding DME occurrence were available for the entire cohort, information regarding other toxicities was incomplete. Furthermore, risk factors for the first DME could not be evaluated, as data were collected only for patients who developed DME, nor could we compare with the prevalence of other toxicities among patients without DME. Lastly, the extended study period of more than three decades, stemming from the rarity of DME, inevitably led to heterogeneity in treatment protocols and supportive care. Nevertheless, this prolonged observation period enabled the establishment of the largest international cohort of children with DME, providing the most comprehensive analysis currently available.

In conclusion, re-exposure to HDMTX after a severe DME event was achievable, did not result in unexpected toxicities, and adverse events were less frequent than those observed during prior HDMTX courses. However, 14% of re-exposed children experienced recurrent DME events. Upfront MTX dose reduction during re-exposure did not decrease the recurrence rate.

Given the central role of HDMTX in ALL therapy, we propose the following recommendations: (i) timing: re-exposure to HDMTX is feasible but should be undertaken only after full recovery of renal function; (ii) monitoring: as re-exposure carries an increased risk of recurrent DME, early and close monitoring of plasma MTX and creatinine levels, alongside strict control of modifiable risk factors, is essential; (iii) dose adjustment: since uniform MTX dose reduction may not reduce recurrence, individualized dosing strategies should be implemented. Further studies validating the effectiveness of algorithms for MTX dose adjustment based on early plasma concentrations,[20] targeting a plasma concentration of 65 μM,[28] or employing pharmacokinetic modeling to predict the elimination profile,[23] may better support safe and effective re-exposure

Disclosures

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Contribution

KS, SBB, and TSM were responsible for the study concept and methodology. DZ, JH, KS, SBB, and TSM performed the data analysis and interpretation. SBB and TSM wrote the original draft of the manuscript. SBB, NAC, EB, JH, GK, MM, AM, NO, SS, FS, GEV, IMvdS, SW, EZ, and TSM contributed to the collection of patients' data. All authors critically reviewed and edited the manuscript and approved the final version for submission.

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Data-sharing statement

The data supporting the findings of this international and multicenter study are not publicly available due to ethical and legal restrictions. De-identified data may be shared upon reasonable request to the corresponding author after completion of required data-use agreements.

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