Original Article

# Factors influencing delayed high-dose methotrexate excretion and its correlation with adverse reactions after treatment in children with malignant hematological tumors

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**Background:** High-dose methotrexate (HDMTX) is crucial in treating pediatric malignant hematological tumors. However, its use is often complicated by delayed excretion and associated adverse reactions, which can significantly affect treatment outcomes and patient safety. Identifying risk factors is essential for safer, more effective therapy. This study aimed to investigate the influencing factors for delayed excretion and their correlation with adverse reactions in children with malignant hematological tumors after receiving HDMTX chemotherapy.

**Methods:** From April to October 2021, the clinical information of children who had undergone HDMTX chemotherapy and had their blood tested for drug concentration was gathered by the Department of Hematology and Oncology at Shanghai Children's Medical Center. Via univariate and multivariate logistic regression, the factors affecting the delayed excretion of HDMTX were examined, and the relationship between delayed excretion and unfavorable effects in children was determined.

**Results:** This study included 99 patients comprising 199 courses of HDMTX. The occurrence rate of HDMTX delayed excretion was 20.1%. Age ≥9 years and a 24-hour methotrexate (MTX) concentration of 64 μmol/L were independent risk factors for delayed MTX excretion according to multivariate logistic regression analysis ($P<0.05$). Negative side effects, such as fever, infection, mucositis, gastrointestinal response, and decreased platelet count in children with delayed excretion were statistically significant when compared to those of children with normal excretion. White blood cell reduction, hemoglobin levels below 65 g/L, MTX excretion delay, and concomitant etoposide treatment were all independent risk factors for infection in children.

**Conclusions:** To estimate the risk of delayed MTX excretion during HDMTX therapy, patient laboratory data should be scrutinized, especially for patients ≥9 years or those with a 24-hour MTX concentration of greater than 64 μmol/L.

**Keywords:** Leukemia; lymphoma; high-dose methotrexate (HDMTX); delayed excretion; adverse reactions

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## Introduction

Methotrexate (MTX), a common anticancer medication, works by inhibiting dihydrofolate reductase to prevent dihydrofolate from being converted to physiologically active tetrahydrofolate, thus obviating DNA biosynthesis. Clinical terminology refers to an MTX dose of 500 mg/m$^2$ as high-dose MTX (HDMTX). This dosage is frequently used to treat malignant hematologic neoplastic disorders such osteosarcoma, non-Hodgkin lymphoma, and acute lymphoblastic leukemia, among others (1). Unless the toxic effects of MTX are terminated by repeated calcium folinate (CF) injections over a sustained period of 2–3 days, vigorous rehydration and urinary alkalinization before and after therapy are also required. Infusion of HDMTX alone for 4–36 hours may be fatal. Even with the aforementioned precautions, a small percentage of patients nevertheless experience delayed MTX excretion, which leads to severe negative effects, such as acute liver and kidney injury, bone marrow suppression, mucositis, gastrointestinal problems, fever, and infection (2). Blood concentrations of >1 μmol/L at 48 hours and >0.1 μmol/L at 72 hours following injection are considered to indicate delayed MTX excretion (3).

According to research, the medication dose, body surface area dose, and patient age are the main variables that affect the delayed excretion of HDMTX in adults (4). Additionally, while receiving HDMTX treatment, patients may concurrently receive proton pump inhibitors (5,6), nonsteroidal anti-inflammatory drugs (NSAIDs) (7), and penicillin medication (8), which may alter how well MTX is cleared from the body. The Chinese Guidelines for the evidence-based use of HDMTX recommends clinical monitoring of blood concentrations and adjustment of the administered dose of the patient's next cycle of chemotherapy based on the patient's situation, including results of therapeutic efficacy, drug tolerance, and therapeutic drug monitoring (9). Further clinical research is required to identify the variables affecting children's blood levels because of their distinct physiology and high growth rates, which can result in different pharmacokinetics from adults. By preventing and controlling disease, preventive medicine seeks to improve patients' overall health. For better malignant tumor prevention, treatment, and progression control, it is especially crucial to increase public health awareness and popularize knowledge concerning malignant tumors (10).

This study thus aimed to offer a theoretical foundation for the clinical prevention of delayed HDMTX excretion in children by retrospectively analyzing the influencing factors of delayed MTX excretion and the occurrence of adverse events in pediatric patients. We further sought to identify the risk factors during HDMTX treatment in children and to recommend preventive medical approaches to lower the likelihood of adverse reactions related to excretion delay by examining the relationship between excretion delay and adverse responses in children. This study's findings may bear significant clinical ramifications for preventing HDMTX excretion delay and its associated side effects. We present this article in accordance with the STROBE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-23-615/rc).

### Highlight box

**Key findings**

- To estimate the risk of delayed methotrexate (MTX) excretion during high-dose MTX (HDMTX) therapy, patient laboratory data should be scrutinized, especially for patients older than 9 years old or with a 24-hour MTX concentration of greater than 64 μmol/L.

**What is known and what is new?**

- There are numerous types adverse reactions associated with HDMTX treatment for hematological malignancies in children. In the course of chemotherapy, the MTX concentration should be monitored over time, and the subsequent treatment plan of children should be adjusted according to the blood concentration.
- Age ≥9 years and an MTX concentration ≥64 μmol/L at the 24 hours were independent risk factors for delayed MTX excretion, while delayed MTX excretion, reduced leukocyte count, hemoglobin level <65 g/L, and concurrent use of etoposide were independent risk factors for infection in children. Additionally, compared to those with normal excretion, children with delayed excretion were more likely to experience negative side effects, such as fever, infection, mucositis, gastrointestinal problems, and decreased platelet count.

**What is the implication, and what should change now?**

- Patient laboratory data should be monitored, especially in patients ≥ 9 years old or with a 24-hour MTX concentration of greater than 64 μmol/L.

## Methods

### *Ethics*

This retrospective single-center clinical study was conducted at Shanghai Children's Medical Center, Shanghai, China. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This research was approved by the Institutional Review Board

of Shanghai Children's Medical Center Affiliated with Shanghai Jiao Tong University School of Medicine (No. SCMCIRB-K2023177-1) and individual consent for this retrospective analysis was waived.

### *Participants*

Children who underwent HDMTX chemotherapy in the Department of Hematology and Oncology of Shanghai Children's Medical Center from April 2021 to October 2021 were selected, and their blood drug concentration was measured. All the children were free of infection, and their blood, liver, and kidney function met the standard of chemotherapy when they were treated with HDMTX. Children who received HDMTX via a 24-hour drip and had their CF stabilized at less than 0.3 μmol/L (Shanghai Children's Medical Center's lower limit of measurement) met the inclusion criteria. Children whose MTX blood content was not brought down to 0.3 μmol/L and those whose MTX concentration data during HDMTX treatment were lacking were excluded.

### *Research methods*

#### Treatment regimen

HDMTX (2–5 g/m$^2$) was dissolved in 0.9% sodium chloride injection or 5% dextrose injection; patients first received a loading dose of 1/10 of the total amount, which was rapidly infused intravenously over 0.5 hours, and the remaining dose was infused intravenously at an even rate over 23.5 hours. After 42 hours had passed since the first administration of MTX, CF was administered at a dose of 15 mg/m$^2$ q6h until the MTX concentration was <0.3 μmol/L (11). At least 12 hours before the delivery of HDMTX, monitoring of hydration, urine alkalinization, and maintenance of urine pH in the range of 7.0–8.0 was initiated. The patient's individual chemotherapy regimen was used to determine the appropriate relief treatment if delayed MTX excretion occurred according to the Chinese Children's Cancer Group study mature B non-Hodgkin lymphoma study plan 2015 (CCCG-BNHL-2015) and the Chinese Children's Cancer Group study acute lymphoblastic leukemia study plan 2020 (CCCG-ALL-2020). In this study, MTX 44th hour blood-drug concentration $C_{44h}$ >1.0 μmol/L and $C_{68h}$ >0.3 μmol/L were used as the criteria for delayed excretion.

#### Detection method for blood-drug concentration

The serum MTX concentration was assessed using a Viva-E drug concentration analyzer (Siemens Healthineers, Munic, Germany) with enzyme-expanded immunoassay 20 hours after the end of the MTX infusion and then every 24 hours continuously until the blood-drug concentration was <0.3 μmol/L.

#### Adverse reactions

During chemotherapy and 3 weeks after it had ended, data on the biochemical indicators of blood routine (white blood cell count, neutrophil count, hemoglobin, platelet count), liver and kidney function, and the adverse responses in the children receiving the chemotherapy were gathered. The Common Terminology Criteria for Adverse Events (CTCEA) version 5.0 were used to evaluate adverse events (12). Fever was defined in this study as a child's axillary temperature exceeding 37 ℃, oral temperature exceeding 37.3 ℃, and rectal temperature exceeding 37.6 ℃; infection was defined as the occurrence of fever following chemotherapy or the emergence of obvious clinical symptoms, such as a cough or runny nose, or the presence of clearly visible evidence of pneumonia or liver abscess on imaging; a neutrophil count <0.5×10$^9$/L was defined as grade 4 myelosuppression; hepatic function impairment injury was defined as aspartate transaminase (AST) or alanine aminotransferase (ALT) enzyme values three times higher than the upper limit of normal; and acute kidney injury was defined as a rise in creatinine level of more than 50% the baseline value.

#### Combination medication

Drugs that could potentially be associated with delayed MTX excretion, such as chemotherapy drugs, antiemetics, and other medications, but not topical treatments, were coadministered with MTX before and after the child underwent HDMTX treatment.

### *Statistical methods*

The proportion of missing nonlaboratory was less than 1%. Missing observations were excluded from the analysis. SPSS 27.0 (IBM Corp., Armonk, New York, USA) was used for the statistical analysis of data. The demographic data of children with normal distribution and laboratory indicators are expressed as the mean ± standard deviation (SD). The incidence of adverse reactions was tested by Chi-squared test, and logistic regression was used for the analysis of the delayed excretion of HDMTX and the single and multivariate factors leading to infections in the children. At a P value <0.05, a difference was deemed statistically

significant.

## Results

### *Delayed excretion in children*

In this study, a total of 99 children who underwent HDMTX chemotherapy and who undergone blood concentration tests were included. The average age was 6.76 (range, 1.08–15.17) years. There were 34 girls (34.3%) and 65 boys (65.7%) enrolled in the study. The most common diagnosis was acute lymphoblastic leukemia (53.8% of patients), followed by lymphoma (42.2%). Of the 199 sessions of HDMTX chemotherapy, 40 involved MTX delayed excretion, with an incidence of 20.1%.

### *One-way analysis of delayed HDMTX excretion*

In terms of gender and concurrent drug administration, one-way logistic regression analysis revealed no statistically significant differences between children in the non-delayed excretion group and the delayed excretion group (P>0.05), but there were statistically significant differences in age, body surface area, MTX concentration at 24 hours, and MTX dosage with delayed excretion of HDMTX (P<0.05). In the 199 sessions of HDMTX chemotherapy, age ≥9 years old, body surface area ≥1 $m^2$, body surface area dose ≥4 g/$m^2$, and MTX concentration ≥64 μmol/L at 24 hours were associated with a higher incidence of MTX excretion delay (*Table 1*).

### *Multivariate analysis of HDMTX excretion delay*

Factors that were statistically significant in the univariate analysis were included in the multivariate logistic regression analysis using the forward stepwise method, and the results showed that age ≥9 years and MTX concentration ≥64 μmol/L at 24 hours were independent risk factors for delayed MTX excretion (P<0.05) (*Table 2*).

### *Effect of HDMTX excretion delay on adverse reactions*

During 199 HDMTX chemotherapy sessions, 43.7% of the children experienced fever (within the 3-week observation window afterward), 46.7% infection, 53.3% myelosuppression, 26.6% mucositis, 19.1% gastrointestinal problems, 4.0% acute kidney injury, and 15.0% abnormal liver function as side effects. Children with excretion delay were significantly more likely than children with normal excretion to experience fever, infection, mucositis, gastrointestinal symptoms (vomiting, nausea, abdominal discomfort), or reduced platelet count. The probability of other types of adverse events was not significantly impacted by delayed MTX excretion (*Table 3*).

### *Univariate analysis of the effect of HDMTX excretion delay on infection*

Univariate analysis revealed that delayed excretion of MTX, drug dosage, body surface area dosage, MTX concentration at the 24 and 44 hours, granulocyte deficiency, leukocyte decrease, hemoglobin decrease, platelet decrease, and the combination of medications including prednisone, isocyclophosphamide, etoposide, vinblastine, mercaptopurine, and rituximab, pembrolizumab were among the factors associated with the emergence of infection in children following the administration of HDMTX (P<0.05) (*Table 4*).

### *Multivariate analysis of the effect of delayed HDMTX excretion on infection*

The results of the multivariate logistic regression analysis in the forward stepwise regression analysis revealed that delayed MTX excretion, decreased leukocyte count, a hemoglobin level <65 g/L, and etoposide intake were independent risk factors for postchemotherapy infection in children (P<0.05) (*Table 5*). These factors were also found to be statistically significant in the univariate analysis.

## Discussion

Despite HDMTX being used to treat a variety of malignant tumors for more than 50 years, its delayed excretion is still not completely understood. MTX-related toxicities are more common in courses with MTX excretion delay (13). In this retrospective, single-center study, an analysis of 199 HDMTX sessions revealed a 20.1% incidence of delayed MTX excretion. Age ≥9 years and MTX concentration ≥64 μmol/L at 24 hours were independent risk factors for delayed MTX excretion, while delayed MTX excretion, reduced leukocyte count, a hemoglobin level <65 g/L, and concurrent use of etoposide were independent risk factors for infection. Additionally, compared to children with normal excretion, children with delayed excretion were more likely to experience negative side effects of fever, infection, mucositis, gastrointestinal problems, and

**Table 1** Univariate analysis of delayed methotrexate excretion

| Variant | Excretion delay group (n=159) | No excretion delay group (n=40) | OR | 95% CI for OR | P value |
|---|---|---|---|---|---|
| Sex (male *vs.* female) | | | 1.38 | 0.65–2.91 | 0.403 |
| Male | 100 (62.9) | 28 (70.0) | | | |
| Female | 59 (37.1) | 12 (30.0) | | | |
| Age (years) | 6.30±3.35 | 8.59±4.10 | 1.19 | 1.08–1.32 | <0.001 |
| ≥9 *vs.* <9 | | | 6.19 | 2.94–13.04 | <0.001 |
| Height (cm) | 117±23.5 | 133±27.9 | 1.03 | 1.01–1.04 | <0.001 |
| Weight (kg) | 24±12.2 | 32.8±16.1 | 1.05 | 1.02–1.07 | <0.001 |
| Body surface area ($m^2$) | 0.875±0.30 | 1.09±0.378 | 6.7 | 2.34–19.19 | <0.001 |
| ≥1 *vs.* <1 | | | 4.8 | 2.31–9.97 | <0.001 |
| Cr (μmol/L) | 23.96±9.635 | 29.64±10.469 | 1.055 | 1.018–1.094 | 0.004 |
| BUN (mmol/L) | 3.989±1.3779 | 3.436±1.7141 | 0.755 | 0.544–1.048 | 0.093 |
| MTX dose (g) | 3.69±1.67 | 4.7±1.90 | 1.37 | 1.13–1.67 | 0.002 |
| MTX body surface area dose ($g/m^2$) | 4.15±0.964 | 4.3±0.883 | 1.19 | 0.81–1.74 | 0.37 |
| ≥4 *vs.* <4 | | | 2.38 | 1.06–5.34 | 0.035 |
| Type of disease (lymphoma *vs.* acute lymphoblastic leukemia) | | | 0.81 | 0.4–1.65 | 0.569 |
| Acute lymphoblastic leukemia | 83 (52.2) | 24 (60.0) | | | |
| Lymphoma | 68 (42.8) | 16 (40.0) | | | |
| Medulloblastoma | 4 (2.5) | 0 | | | |
| Others | 4 (2.5) | 0 | | | |
| $C_{MTX24h}$ (μmol/L) | 63.9±37.4 [10.4–275] | 78±36.3 [8.6–178] | 1.01 | 1–1.02 | 0.041 |
| ≥64 *vs.* <64 | | | 4.29 | 2.03–9.09 | <0.001 |
| Combination of drugs | | | | | |
| Isocyclophosphamide | 59 (37.1) | 14 (35.0) | 0.91 | 0.44–1.88 | 0.805 |
| Cyclophosphamide | 8 (5.0) | 3 (7.5) | 1.53 | 0.39–6.05 | 0.544 |
| Etoposide | 52 (32.7) | 14 (35.0) | 1.11 | 0.53–2.3 | 0.783 |
| Vincristine | 55 (34.6) | 12 (30.0) | 0.81 | 0.38–1.72 | 0.583 |
| Vincristine | 10 (6.3) | 3 (7.5) | 1.21 | 0.32–4.61 | 0.782 |
| Thiopurine | 79 (49.7) | 16 (40.0) | 0.68 | 0.33–1.37 | 0.275 |
| Rituximab | 26 (16.4) | 6 (15.0) | 0.9 | 0.34–2.37 | 0.835 |
| Pegaspargase | 12 (7.5) | 3 (7.5) | 0.99 | 0.27–3.7 | 0.992 |
| Ondansetron | 50 (31.4) | 17 (42.5) | 1.61 | 0.79–3.28 | 0.188 |
| Granisetron | 74 (46.5) | 14 (35.0) | 0.62 | 0.3–1.27 | 0.191 |
| Palonosetron | 40 (25.2) | 12 (30.0) | 1.28 | 0.59–2.74 | 0.534 |
| Omeprazole | 7 (4.4) | 5 (12.5) | 3.1 | 0.93–10.35 | 0.066 |

Data are presented as n (%), mean ± standard deviation, or [range]. OR, odds ratio; CI, confidence interval; Cr, creatinine; BUN, blood urea nitrogen; MTX, methotrexate; $C_{MTX24h}$, MTX 24th hour blood-drug concentration.

**Table 2** Multivariate analysis of delayed methotrexate excretion

| Factors | OR | 95% CI | P value |
|---|---|---|---|
| Age ≥9 years | 4.725 | 2.053–10.873 | <0.001 |
| $C_{MTX24h}$ ≥64 μmol/L | 3.236 | 1.387–7.547 | 0.007 |

OR, odds ratio; CI, confidence interval; $C_{MTX24h}$, MTX 24th hour blood-drug concentration.

**Table 3** Effect of delayed methotrexate excretion on the incidence of various types of adverse reactions

| Event | Non-delayed excretion, n (%) | Delayed excretion, n (%) | Total, n (%) | P value |
|---|---|---|---|---|
| Fever | 61 (38.4) | 26 (65.0) | 87 (43.7) | 0.003 |
| Infections | 67 (42.1) | 26 (65.0) | 93 (46.7) | 0.011 |
| Granulocyte deficiency | 82 (51.6) | 24 (60.0) | 106 (53.3) | 0.341 |
| Platelet count <$50\times10^9$/L | 48 (30.2) | 19 (47.5) | 67 (33.7) | 0.041 |
| Hemoglobin count <65 g/L | 37 (23.3) | 14 (35.0) | 51 (25.6) | 0.132 |
| Mucositis | 34 (21.4) | 19 (47.5) | 53 (26.6) | 0.001 |
| Gastrointestinal reactions | 21 (13.2) | 17 (42.5) | 38 (19.1) | <0.001 |
| Vomiting | 9 (5.7) | 7 (17.5) | 16 (8.0) | 0.019 |
| Nausea | 6 (3.8) | 7 (17.5) | 13 (6.5) | 0.004 |
| Abdominal pain | 12 (7.5) | 12 (30.0) | 24 (12.1) | <0.001 |
| Diarrhea | 2 (1.3) | 1 (2.5) | 3 (1.5) | 0.572 |
| Elevated transaminase level (3× upper limit) | 23 (14.5) | 7 (17.5) | 30 (15.1) | 0.606 |
| Renal impairment | 16 (10.1) | 6 (15.0) | 22 (11.1) | 0.349 |

decreased platelet count.

A number of retrospective investigations have been conducted on the factors influencing the delayed excretion of HDMTX, and the prevalence of delayed HDMTX excretion reported in these studies ranges from 7.8% to 19.8% (14-16). Among the 199 chemotherapy sessions in our study, even when the conventional treatments of CF rescue (performed 42 hours after MTX initiation), hydration, and alkalinization (performed 12 hours before MTX initiation) were administered, the incidence of delayed MTX excretion was 20.1%. This outcome was comparable to that of the study by Xu *et al.* (14) (19.8%), with the exception that the CF rescue in their study began 6 hours earlier than it did in ours—which may be the reason for its somewhat lower frequency of delayed excretion—and at 36 hours following MTX administration. In contrast, the study by Li *et al.* reported an incidence of delayed MTX excretion of 13.67% (15), which may be related to the fact that hydration was advanced by 1 day (3 L/m$^2$ of hydration) and continued for 4 days, with 5% sodium bicarbonate (5 mL/kg) used to alkalinize the urine on the day of chemotherapy for 3 days. In the study by Xu *et al.*, 12.1% of children who were administered MTX experienced excretion delay. This finding may be related to the fact that CF rescue was initiated 36 hours after MTX medication administration, urine was alkalinized 3 days prior to medication administration, and the urine was heavily hydrated and alkalinized for 3 days following MTX's use (16). A recent small-sample study conducted outside of China found that the incidence of delayed MTX excretion was only 7.8%, which may be due to the study's sample size and high hydration levels (>3 L/m$^2$) (17). According to the Chinese guidelines for the evidence-based use of HDMTX, intravenous hydration (2.5–3 L/m$^2$ for ≥72 hours) should begin 12 hours or earlier before HDMTX infusion, and the MTX concentration in blood should be tested at least once at 24, 48, and 72 hours after the start of titration until the MTX blood concentration is ≤0.1–0.2 μmol/L.

**Table 4** Univariate analysis of methotrexate-induced infections in children

| Variant | No infection group (n=106) | Infection group (n=93) | OR | 95% CI for OR | P |
|---|---|---|---|---|---|
| MTX delayed excretion | 14 (13.2) | 26 (28.0) | 2.55 | 1.24–5.25 | 0.011 |
| MTX dose (g) | 3.63±1.82 | 4.18±1.65 | 1.2 | 1.02–1.41 | 0.029 |
| MTX body surface area dose (g/m$^2$) | 3.86±0.989 | 4.54±0.753 | 2.3 | 1.65–3.21 | <0.001 |
| ≥4 *vs.* <4 | 50 (47.2) | 75 (80.6) | 4.67 | 2.46–8.85 | <0.001 |
| $C_{MTX24h}$ (μmol/L) | 59.7±35.5 | 74.8±38.4 | 1.01 | 1–1.02 | 0.006 |
| ≥64 *vs.* <64 | 35 (33.0) | 49 (52.7) | 2.26 | 1.27–4.01 | 0.005 |
| $C_{MTX44h}$ (μmol/L) | 0.426±0.882 | 0.955±1.68 | 1.52 | 1.08–2.14 | 0.015 |
| Granulocyte deficiency | 36 (34.0) | 70 (75.3) | 5.92 | 3.19–10.99 | <0.001 |
| White blood cell count | 1.99±1.23 | 0.853±0.898 | 0.32 | 0.22–0.46 | <0.001 |
| Hemoglobin content | 89.5±14.9 | 72.6±21.2 | 0.95 | 0.93–0.96 | <0.001 |
| Hemoglobin count <65 g/L | 6 (5.7) | 45 (48.4) | 15.62 | 6.23–39.16 | <0.001 |
| Decreased platelet count | 153±83.8 | 73.7±83 | 0.99 | 0.98–0.99 | <0.001 |
| Platelet count <50×10$^9$/L | 16 (15.1) | 51 (54.8) | 6.83 | 3.49–13.35 | <0.001 |
| Combination of drugs | | | | | |
| Prednisone | 20 (18.9) | 48 (51.6) | 4.59 | 2.43–8.65 | <0.001 |
| Isocyclophosphamide | 21 (19.8) | 52 (55.9) | 5.13 | 2.74–9.63 | <0.001 |
| Etoposide | 18 (17.0) | 48 (51.6) | 5.21 | 2.72–9.99 | <0.001 |
| Vincristine | 18 (17.0) | 49 (52.7) | 5.44 | 2.84–10.43 | <0.001 |
| Thiopurine | 70 (66.0) | 25 (26.9) | 0.19 | 0.1–0.35 | <0.001 |
| Rituximab | 7 (6.6) | 25 (26.9) | 5.2 | 2.13–12.7 | <0.001 |
| Pegaspargase | 3 (2.8) | 12 (12.9) | 5.09 | 1.39–18.63 | 0.014 |

Data are presented as n (%) or mean ± standard deviation. OR, odds ratio; CI, confidence interval; MTX, methotrexate; $C_{MTX24h}$, MTX 24th hour blood-drug concentration.

**Table 5** Multivariate analysis of methotrexate-induced infection in children

| Factor | OR | 95% CI | P value |
|---|---|---|---|
| Delayed excretion of methotrexate | 2.685 | 1.118–6.450 | 0.027 |
| Decreased white blood cell count | 0.499 | 0.334–0.745 | <0.001 |
| Hemoglobin level <65 g/L | 6.171 | 2.262–16.829 | <0.001 |
| Etoposide | 2.522 | 1.126–5.649 | 0.025 |

OR, odds ratio; CI, confidence interval.

The monitoring interval should be shortened and the monitoring frequency should be increased in cases of delayed excretion, acute renal damage, or other major adverse effects, but there is no specific requirement for the increase in frequency, and the concentration test can be increased according to the actual clinical situation. For the 24-hour continuous titration program, it is advised that the first dose of CF be given for relief 36–44 hours after the start of the titration, and when major adverse effects occur, the timing of the initial dose of relief should be altered according to the

clinical situation and on an individual basis (9).

In this investigation, the effects of patient variables on the delayed excretion of HDMTX, such as the age of the child and body surface area, were independent risk factors for delayed MTX excretion, which is consistent with Li *et al.*'s findings (18). According to a study, younger children have a higher MTX clearance than do older children, indicating that MTX clearance declines with age (19). In our study, logistic regression analysis indicated that advanced age was a risk factor for delayed MTX clearance, with children aged ≥9 years having a larger chance of delayed clearance. Early management may lower the frequency of delayed MTX excretion and make HDMTX therapy safer and more effective. As a result, older children should drink more fluids and be more stringently monitored in terms of therapeutic agents.

We discovered a strong correlation between the MTX concentration at the 24 hours and the delayed excretion of MTX in children. The MTX concentration at the 24 hours should, according to the Chinese guidelines for evidence-based use of HDMXT, be between 16 and 40 μmol/L (9). In children, MTX with a $C_{24h}$ >16 μmol/L can improve clinical efficacy, whereas MTX with a $C_{24h}$ >40 μmol/L may raise the risk of drug toxicity (3). Therefore, the guidelines suggest 16 and 40 μmol/L as the lower and upper limits of MTX concentration at the 24 hours in children with acute lymphoblastic leukemia, respectively, according to efficacy and safety concerns. Our multivariate analysis showed that a 24-hour MTX concentration >64 μmol/L was associated with delayed excretion. A study has been conducted on reducing the incidence of high 24-hour MTX concentrations via the detection of MTX concentrations at 2, 6, and 8 hours into the HDMTX chemotherapy course, which may help to adjust the drug dose in time (20). No delay in HDMTX excretion caused by the medication combination was encountered in this investigation (P>0.05). According to other research, concurrent proton pump inhibitors and HDMTX increase the blood concentration of MTX, which in turn increases the likelihood of delayed excretion (5). In our study, proton pump inhibitor utilization was also higher in children in the delayed excretion group (12.5% *vs.* 4.4%), but there was no statistically significant difference (P=0.066), which may be due to the rarity of pediatric proton pump inhibitor combination use, and thus this potential correlation needs to be confirmed by a clinical study with a larger sample size.

During the course of 199 HDMTX chemotherapy sessions examined in our study, the children experienced a range of systemic adverse events, including mucositis, myelosuppression, infection, gastrointestinal problems, hepatic impairment, and renal impairment. In comparison to the non-delayed group, the delayed group had a higher incidence of adverse responses. Children with delayed excretion were significantly more likely to experience fever, infection, mucositis, gastrointestinal symptoms, and platelet count decrease than were children with normal excretion. It has been reported that the occurrence of negative effects and MTX blood concentration are closely connected. Neutropenia, hemoglobin, and thrombocytopenia are frequent clinical signs of hematopoietic poisoning. Myelosuppression in children was discovered to be the most common adverse reaction in this study, which is consistent with other research (21). Infection frequency is increased by granulocyte shortage, and mucosal injury with MTX increases the likelihood of oral infections and perianal abscesses. According to Jiang *et al.*'s findings, children who experience delayed MTX excretion are more likely to experience gastrointestinal side effects, such as vomiting, nausea, and abdominal discomfort (P<0.001) (22). Children should maintain good oral hygiene, and if grade III or IV oral ulcers develop, oral hypothermia can be administered based on standard care. The frequency of oral mucositis and ulcers in the delayed excretion group (47.5%) was higher than that in the normal excretion group (21.4%), and the difference was statistically significant (P=0.001).

Infection is a common and serious postchemotherapy adverse effect that can be fatal. The results of this study showed that in addition to the common infection susceptibility factors, such as delayed MTX excretion, leukopenia, and hemoglobin <65 g/L, the combination of other antitumor drugs including etoposide, cyclophosphamide, vincristine, and rituximab is a risk factor for infection in children. Etoposide and HDMTX are frequently used in our facility's treatment of young patients with CCCG-BNHL-2015 (MTX 5 g/m$^2$ d1 + etoposide 100 mg/m$^2$ d3–5 + vincristine 3 mg/m$^2$ d1 + isocyclophosphamide 1,200 mg/m$^2$ d1–5 + prednisone 60 mg/m$^2$ d1–7). Children with lymphomas on this regimen have a higher risk of infection because the chemotherapy intensity is higher than that in consolidation therapy for children with CCCG-ALL-2020 (MTX 3–5 g/m$^2$ + mercaptopurine 25 mg/m$^2$/day).

### *Limitations*

This is a retrospective study and the number of cases was limited. There is still uncertainty as to whether combined

use of certain drugs causes delayed excretion. In addition, this study is a single-center study, and the results have some limitations. Therefore, a multi-center prospective study can be conducted in the future, and a relatively balanced control group study can be set up.

## Conclusions

In conclusion, there are numerous types adverse reactions associated with HDMTX treatment for hematological malignancies in children. Over the course of chemotherapy, MTX concentration should be monitored over time, and the subsequent treatment plan for children should be adjusted according to the blood concentration so as to effectively reduce the occurrence of adverse reactions and effectively control infection. In addition, it is necessary to strengthen nursing support for children during HDMTX treatment, and actively managing adverse reactions such as vomiting and diarrhea can also substantially contribute to the prevention of delayed MTX excretion.

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## Footnote

*Reporting Checklist:* The authors have completed the STROBE reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-23-615/rc

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*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-23-615/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Shanghai Children's Medical Center Affiliated with Shanghai Jiao Tong University School of Medicine (No. SCMCIRB-K2023177-1) and individual consent for this retrospective analysis was waived.



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