Two Dose Adjustment Programs in High-dose Methotrexate Treatment for Pediatric Acute Lymphoblastic Leukemia

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Abbreviations	
MTX	methotrexate
ALL	acute lymphoblastic leukemia
CCCG-ALL-2015	chemotherapy protocol under Chinese Children's Cancer Group for pediatric acute lymphoblastic leukemia (2015)
HDMTX	high-dose methotrexate
LR	low-risk
IR/HR	intermediate/high risk
BSA	body surface area
C_{44h}	methotrexate concentration at 44h after infusion
C_{68h}	methotrexate concentration at 68h after infusion
C_{16h}	methotrexate concentration at 16h after infusion
RFS	Relapse-free survival
CCR	creatinine clearance rate
ANC	absolute neutrophil count
sCr	serum creatinine

Abstract

Background: Methotrexate is safely administered to most patients but can also cause severe toxicities. It is necessary to individualize methotrexate dose to maintain sufficient exposure while minimizing toxicities.

Procedure: We enrolled 1174 cycles of high-dose methotrexate chemotherapy from 294 patients treated following the CCCG-ALL-2015 protocol and explored risk factors of toxicities, methotrexate clearance delay and relapse. We compared those who received a fixed-dose reduction (Program 1) with those who were dose-adjusted by added methotrexate concentration test at 16h (Program 2) after methotrexate clearance delay existed the last cycle.

Results: Female, IR/HR group, BSA<0.69m^2 and C_{44h}≥1.0 μ mol/L were risk factors of toxicities(P<0.05). Significant covariates on methotrexate clearance delay were age >6years, male and IR/HR group (P<0.01). Male, IR/HR and C_{68h}≥0.2μmol/L group patients were at higher risk of relapse(P<0.05). No significant association was observed between methotrexate dose and relapse-free survival. 405 cycles from 168 patients were dose-adjusted by Program 1 and 118 cycles from 43 patients by Program 2. Patients who used Program 2 had a higher actual methotrexate infusion dose and infusion rate and was better in keeping C_{44h} in our target value (P<0.001). Abnormal serum potassium was more frequently in patients using Program2 (P<0.001), and prolonged myelosuppression was more commonly seen in IR/HR patients with Program2(P=0.003).

Conclusions: No significant correlation between methotrexate dose or C_{44h} and relapse-free survival time was found. Patients who were dose-adjusted by Program 2 received a higher therapeutic dose and better controlled the methotrexate concentration to our target range.

1 | Introduction

Methotrexate (MTX), an antimetabolite that interferes with folic acid metabolism, is a critical component of the successful treatment of many hematologic, solid and central nervous system tumors since its first clinical trial in 1953 with mini-dosage.[1] In recent chemotherapy regimens for acute lymphoblastic leukemia (ALL) conducted by major cooperative study groups, high-dose methotrexate (HDMTX) at a range of 1-5g/m2 was indispensable in consolidation treatment. Intensive chemotherapy can prove the overall prognosis of cancer to some extent, but decreased organ function accompanied.[1-4]

HDMTX is safely administered to most patients, but it can also cause severe toxicities, including acute kidney injury, neurotoxicity and hepatotoxicity.[3,5] It is hard to predict toxicity as large interindividual and intraindividual variability of MTX exists.[6] Aggressive monitoring and prompt intervention, as well as dose adjustment, can generally promote methotrexate excretion, prevent toxicity and allow patients to receive subsequent therapy.[3] The most widely adopted standard of HDMTX personalization after severe HDMTX-related toxicities or delayed MTX clearance has occurred was a fixed dose-reduction in 20–25% off or complete omission of subsequent HDMTX cycles. [7] In recent decades, researchers developed various pharmacokinetic models use different parameters, which showed a certain degree of practicality with constraints. [8-11]It is still one of the major clinical challenges to keep the balance between efficacy and side effects.

CCCG-ALL-2015 is a prospective, randomized, multicenter study under the Chinese Children's Cancer Group for ALL. In this protocol, MTX was used at the dose of $3g/m^2$ in low-risk (LR) patients and $5g/m^2$ in intermediate/high-risk (IR/HR) every other week for four cycles in consolidation phase. In the original treatment regimen, MTX dose was adjusted by creatinine clearance rate (CCR) at the first exposure to HDMTX and then reduced by 20% off in subsequent cycle if methotrexate concentration at 44h (C_{44h}) was over 1.0μmol/L. In clinical practice, high MTX concentration at 44h was not always occurred in the first cycle and C_{44h} below our target was found in a large proportion of courses who received dose-reduced treatment. Therefore, the protocol was modified according to the research of St. Jude Total Therapy Study XV from the end of 2018.[8] In patients whose C_{44h} exceeded 1.0μmol /L or higher in the last cycle, CCR needs to be reassessed and a methotrexate concentration detection point at 16h (C_{16h}) was added, along with better monitored organ function. Whether to stop MTX infusion in advance, increasing the hydration rate, or receiving earlier leucovorin rescue depended on the C_{16h} value.

The new dose adjustment program requires extremely close monitoring of MTX concentration and organ function, which means more clinical resources consumed and experienced doctors are needed. Therefore, the targets of this follow-up study were to evaluate whether this change of dose adjustment program led to increased efficacy in maintaining sufficient exposure to MTX while minimizing toxicities than the traditional one.

2 | Materials and methods

Patients and methods

Our study was a sub-project of CCCG-ALL-2015. A total of 1172 cycles of HD-MTX from 294 patients were enrolled and analyzed between Aug 2015 to Sep 2020 in the Union Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology. The institutional research ethics committee approved the study, and informed consent from the patients' parents or guardians was obtained before enrollment for chemotherapy.

Data collection

Patients' demographic data, immunophenotype, risk stratification, chemotherapy details, pharmacokinetics and follow-up data were retrieved from recorded before. Additional data were collected retrospectively from the electronic medical record, including comorbidities and days of chemotherapy delay. Myelosuppression was shown as days absolute neutrophil count (ANC)<1.0*10^9/L. Toxicities were reported as per the National Cancer Institute Common Toxicity Criteria (CTCAE version 5.0).[12]

Treatment

ALL patients received upfront window therapy with dexamethasone and remission induction chemotherapy before consolidation treatment. Consolidation treatment con-

sists of HDMTX every other week for four courses and daily oral mercaptopurine at 25mg/m^2 at bedtime, along with an age-adjusted dose of triple intrathecal therapy on the day of HDMTX. MTX dose was 3g/m^2 in LR patients and 5g/m^2 in IR/HR. Patients received prehydration at 100ml/m^2/h for over 12h before HDMTX and hydration at 3000ml/m^2/d with 5% sodium bicarbonate at 5ml/kg for three days to maintain urine PH between 7-8. HDMTX was given a 10 % loading dose over 0.5 h, with the remaining 90 % administered over 23.5 h.

Leucovorin rescue was initiated at 42h from the beginning of the HDMTX infusion at a basic dose of 15mg/m^2 every 6h. Leucovorin dose was elevated in patients with methotrexate clearance delay (defined as C_{44h} >1.0μmol/L) and was continued until the plasma concentration was <0.2μmol /L.

In the original treatment protocol (we defined as Program1), MTX dose was adjusted by normalized creatinine clearance rate (CCR) at the first use of HDMTX. 80% ,70%, 50% and 40% of initial dose was used when CCR was in 70-85ml/min, 55-70ml/min, 40-55ml/min and 20-40ml/min, respectively. This dose reduced by 20% off in the next cycle if methotrexate concentration at 44h (C_{44h}) was over 1.0μmol /L and increased 20% when C_{44h} was below 0.5μmol/L (no more than 3g/m^2 in LR patients and 5g/m^2 in IR/HR).

From the end of 2018, MTX dose was adjusted following the revised protocol (we defined as Program 2). If patients' C_{44h}>1.0μmol /L in the last cycle, reassessing CCR before new exposure to MTX and adding a methotrexate concentration detection point

at 16h (C_{16h}), along with better monitoring renal function. Subsequent treatment depended on C_{16h} to a target 24-hour steady-state MTX level of 35μmol/L in the LR group and 65μmol/L in the IR/HR group. If C_{16h} <100μmol /L and serum creatinine (sCr) was normal but rose more than 26μmol/L, increase hydration speed to 150 ml/m^2 and add MTX concentration and renal function test at 24h. Leucovorin rescue (30mg/m^2) advanced to 36h when C_{24h} and sCr kept elevating. When the renal function was abnormal, stop MTX infusion and reassess renal function and MTX concentration at 24h to determine whether advanced leucovorin rescue to 36h or dialysis is needed. If C_{16h} was in 100-149.9μmol/L, stop MTX infusion at 20h and increase hydration speed to 175ml/h/m^2. When C_{16h} was over 150μmol/L, stop MTX infusion at 18h and increase hydration speed to 200ml/h/m^2. Then retest MTX concentration at 24h and decide whether leucovorin rescue need to advance to 36h or dialysis is needed.

Statistical analysis

Categorical data were summarized by counts and percentages, while continuous data were described with median and inter-quartile range (IQR) or range. Association between categorical data was evaluated by χ^2test, and a nonparametric test was used in continuous data. Logistics regression analysis was used to detect the risk factors of methotrexate clearance delay at 44h and toxicities. To analyze predictors of relapse-free survival (RFS), the Cox proportional hazard regression model was used and the

survival curves were drawn by the Kaplan–Meier method added with the Log-rank test or Tarone-Ware test (when the survival curve crossed). $P< 0.05$ was considered statistically significant. The analysis was performed using SPSS 25.0 software.

3 | Results

Participants

The 294 patients receiving 1172 cycles of targeted HDMTX chemotherapy were as follows: 175 male (59.5%), 119 female (40.5%); 135 LR (45.9%), 159 IR/HR (54.1%); 268 B-ALL (91.2%), 26 T-ALL (8.8%); median age 4.8 years (range: 0.4-14).

As the methotrexate dose was 3g/m2 in the LR group and 5 g/m2 in the IR/HR risk group, we divided patients into two groups. The baseline patient demographics, treatment data and laboratory values of the two groups were listed in TABLE 1. The median actual MTX dose was 3g/m^2(IQR:2.4-3) in LR group and 4 g/m^2 (IQR:3.5-5) in IR/HR group and the median MTX infusion rate was 1.0 (IQR:0.8-1.0) and 0.8 (IQR:0.7-1.0), respectively. The median leucovorin dose was 75mg/m^2 (IQR:75-120) in LR patients and 75 mg/m^2 (IQR:75-255) in IR/HR.

Pharmacokinetic data

The median C_{44h} in the LR group was 0.47μmol/L (IQR:0.34-0.74) and 0.67μmol/L (IQR:0.44-1.6) in the IR/HR group. At 68h, they were 0.14μmol/L (IQR:0.1-0.22) and

0.2μmol/L (0.11-0.39), respectively. C_{68h} had a significant linear correlation with C_{44h}, and the Pearson correlation coefficient is 0.948($P<0.001$).18% (n=97) cycles in the LR group found a clearance delay at 44h and 27.3% (n=147) at 68h (TABLE 1). This was significantly lower than in the IR/HR group, which were 33.1% and 45.6% ($P < 0.001$). Furthermore, 4 cases in LR and 35 in IR/HR group got excessive MTX concentration over 10μmol/L at 44h. A proportion of 54.8% cycles in the LR group and 33%cycles in the IR/HR group were below our target (0.5-1.0μmol/L).

Logistic regression analysis was used to detect the risk factors of methotrexate clearance delay. Before that, univariate logistic regression was done to identify variables showing significant or nearly significant effects, which were included in a multivariate logistic regression model ($P<0.25$ was used as inclusion criteria of variables). Seven predictors were included in the multivariate model, but only age, sex, risk group and the number of cycles showed significant differences. Age> 6years, male and IR/HR patients had a higher risk of MTX clearance delay ($P_{age}=0.002$, OR: 1.583; $P_{sex}=0.003$, OR=1.545; $P_{risk}<0.001$, OR: 1.950). No significant difference between C1 and C2 was found, while patients were at a lower risk of MTX clearance delay when receiving the third or the fourth cycle of HDMTX. ($P_{C2}=0.15$, $P_{C3\text{-}C4}<0.001$; $OR_{C2}=0.771$, $OR_{C3}=0.498$, $OR_{C4}=0.338$).

Toxicities

No one died because of serious adverse events. As a representative indicator of myelosuppression, ANC < 1.0*10^9/L over 7 days was presented in 18.9%(n=222) cycles. There were 64 (5.5%) cycles acquired a delayed start of subsequent HDMTX therapy for toxicity recovery, 3.6% in 1-7days and 1.9% over 7 days. None experienced complete omission of subsequent HDMTX cycles. Hypokalemia and hyperkalemia were found in 148(12.6%) and 13 (1.1%) cycles, respectively.

A total of 535 times of toxicities graded 1/2 and 168 graded 3/4 were collected (here we recorded all the toxicity occurred as each cycle could have more than one kind of toxicity). A majority of higher-grade cycles (Grade 3 and 4) were infection, which might be related to neutropenia to some extent. Ten cycles (0.9%) had Grade3/4 nephrotoxicity, of which three cases (all in the first cycle of HD-MTX treatment) required dialysis due to exorbitant MTX concentration. Two cycles postponed the next HDMTX treatment until three continuation therapy courses and the MTX dose was halved. Gastrointestinal toxicity (e.g., nausea, vomiting, hemorrhage, stomachache, gastroenteritis), mucositis, hepatotoxicity and neurotoxicity that Graded 3/4 were recorded in 11 (0.9%), 14(1.2%),59 (5%) and 9(0.8%) cycles, respectively. The difference of serum potassium ($P<0.001$), mucositis ($P=0.025<0.05$), hepatotoxicity ($P<0.001$) between LR and IR/HR group was statistically significant.

Logistic regression analysis was also used to detect the risk factors of toxicity in Grade3/4 (TABLE 2). Here we enrolled the highest grade when two or more toxicities occurred simultaneously in one cycle. Male were at a lower risk of toxicity than fe-

male (P=0.026, OR=0.704) while patients in IR/HR group (P=0.016, OR=1.491) were at a higher risk. There was a growing risk of toxicities when C_{44h} was more than 1.0μmol/L (P=0.022,OR:1.578) compared to that was below 0.5μmol/L, especially when it was over 10μmol/L(P<0.001, OR:6.437). Besides, a decreased risk was found in C4 compared to C1 (P=0.004, OR:0.511).

Survival analysis

By Aug 2020, the median follow-up time was 27.9 months (range:4-69); 247 patients survived without incidents, 27 patients relapsed, 8 patients lost to follow-up, 3 patients gave up treatment because of serious adverse events, 6 patients transferred and 3 patients died.

To analyze RFS, predictors with a statistically significant difference in the univariate analysis were added in the Cox proportional hazard regression model after the test of proportional hazards assumption by Cox time-dependent covariant analysis (here we defined each cycle as a case). Seven variables were included in the model, but only sex, risk group and C_{68h} showed statistically significant differences (TABLE 3). Male, IR/HR group and C_{68h} ≥0.2μmol/L were risk factors of relapse (P<0.05, OR_{sex}:2.669, OR_{risk}:2.734, OR_{C68h}:1.561). No significant correlation between C_{44h}, MTX infusion dose or body surface area (BSA) and RFS was found. The survival curves drawn by the Kaplan–Meier method added with the Log-rank test (or Tarone-Ware test when the survival curve crossed) were shown in Fig. 1.

Comparison between two dose adjustment programs

In all the 1172 cycles we enrolled, 405 cycles from 168 patients were dose-adjusted by Program 1, which was based on C_{44h} of the last cycle, and 118 cycles from 43 patients were adjusted by Program 2, which also relied on reassessed serum creatinine clearance and added test of C_{16h}. χ^2test showed that the patients' risk group in these two programs was statistically significant(P=0.003). As different doses of MTX were used between two risk groups (3g/m2 in LR group and 5g/m2 in IR/HR group), and toxicity, as well as MTX clearance, were associated with risk group which described earlier in our study, we started this part in groups.

The basic demographic information and treatment data of the two groups were listed in TABLE 4. Patients had a higher actual MTX infusion dose and infusion rate after dosage adjustment according to Program 2(P<0.001), while higher leucovorin rescue level is seen only in IR/HR group(P=0.023). IR/HR patients who adjusted MTX dose by Program 2 were more likely to experience prolonged myelosuppression(P=0.003). Abnormal serum potassium, especially hypokalemia, was more frequent in patients who received dose adjustment by Program2, either in LR or in IR/HR patients. The comparison on C_{44h} and C_{68h} between these groups was showed in Fig. 2. As depicted in Figs. 2A, patients in IR/HR group using Program2 for dose adjustment were more likely to have C_{44h}>0.5μmol/L(P=0.013), although no statistically significant difference was found in the part that defined as MTX clearance delay

(P=0.087). C_{68h} showed no significant difference between these two programs, neither in LR nor in IR/HR group (Figs. 2B). In Figs. 2C, we found that C_{44h} <0.5μmol/L showed an increasing trend when the number of treatment cycles increased after dose adjustment by Program 1, while patients used Program 2 had such increasing ratio in our target value (0.5-1.0μmol/L), both along with a decreased number of MTX clearance delay.

In patients who were dose-adjusted by Program 2, the median C_{16h} was 77μmol/L (range:31.1-196.5). C_{16h} >100μmol/L and 150μmol/L were recorded in 18.6% and 3.4% cycles, respectively. In those cycles who had taken full dose of MTX and whose C_{16h} below 100μmol/L, only 28.1% cycles experienced MTX clearance delay again and even quite a few cases' (30.2%) C_{44h} below 0.5μmol/L.

Moreover, in patients who used either Program 1 or Program 2, their MTX dose in the first cycle was only adjusted by creatinine clearance rate. Although CCR was normal or MTX dose reduced in some courses, a significant proportion of cases experienced MTX clearance delay (35.5%) and severe adverse events (24.7% in Grade 3/4). All the three cases that need dialysis because of exorbitant C_{44h} were occurred in the first cycle, of which two were with normal CCR and one with low CCR and had received a 20% off of MTX dose.

4 | Discussion

Methotrexate is a critical therapeutic agent of the successful treatment of many pediatric and adult tumors. Despite safely administered in most patients with HDMTX, it can cause severe toxicities, treatment delays or even death. It is essential to adjust MTX dose to an effective but safe rate.[1,5] Researchers have attempted to find better ways to individualize HDMTX in recent years but the methods accessed have many constraints (e.g., requiring complete and complex pharmacokinetic parameters, previous exposure of MTX or normal renal function), which was not available most of the time in practice.[8-11]

Here in the CCCG-ALL-2015 chemotherapy protocol, two programs of dose adjustment in HDMTX treatment were provided. The initial aim of our project was to investigate whether the modified dosage adjustment program is more efficiently and safe compared to the traditional one. As all the patients enrolled in our study received chemotherapy follow the same risk-directed regime, the patients were highly homogeneous, making the comparison results more convincing.

Only 168 of 1174 cycles recorded grade 3 or 4 toxic events attributable to MTX treatment. Although three cases underwent dialysis because of excessive MTX concentration, none experienced complete omission of subsequent HDMTX cycles or death. This toxicity rate is lower or in concordance with previous studies, mainly because of the use of clinical guidelines developed at St.Jude for monitoring HDMTX treatment and dose adjustment.[7,8,13,14]

The predisposing factors of MTX clearance delay (age over 6years, male, IR/HR group) and toxicities (female, IR/HR, BSA<0.69m^2 and 44h MTX concentration) demonstrated in our study was roughly consistent with previous studies. [7,15-18] It is presented in our study that as the number of HDMTX cycles increased, lower risk of MTX clearance delay and toxicities occurred, which was the same as Kawakatsu et al.'s research. However, Panetta et al. considered that toxicity was not associated with the cycle of HDMTX therapy.[11,15]This finding may be largely attributed to our dosage adjustment, either with Program 1 or with Program 2. Neither MTX clearance nor toxicities were found directly correlated to MTX infusion dose in this study, which may be because of the large interindividual and intraindividual variability of HDMTX.[3,6]

No significant correlation between C_{44h} and RFS was found in this study, whereas Evans et al. considered that longer average systemic exposure to methotrexate improved the outcome of children with B-lineage leukemia.[19] Likewise, there was no significant relationship between MTX dose and RFS. This probably because our follow-up time was not long enough, or although reduced, the MTX dose was still in a valid range and C_{44h} was above the value associated with an increased risk of relapse.

It was shown in our research that C_{68h} ≥0.2μmol/L contributed to a higher risk of relapse. Similar results have not been found yet in other studies. As C_{68h} ≥0.2μmol/L was more common in the IR/HR groups and C_{68h} was related to C_{44h} and leucovorin rescue dose, it may not be a direct risk factor to relapse-free survival.

The same as what we observed in practice, the data in this study demonstrated that although a fixed-dose reduction reduced MTX clearance delay and toxicities successfully, it might lead to an increased probability of lower concentrations. This ensured the safety of patients receiving HDMTX chemotherapy but at the cost of reducing the potential efficacy of the treatment. By contrast, patients followed Program2 acquired a higher MTX infusion rate and better controlled the concentration in our target range, without increasing toxicities obviously. Although no evidence of MTX dose or concentration was found associated with prognosis in our study, this cannot be neglected. As Evans et al. considered that steady-state concentration at 24h under 16μmol/L was a risk factor for recurrence.[19] However, despite the fact that fixed-dose reductions do not allow a patient to receive the benefits of a maximally tolerated dose of HDMTX, it is safe, efficient, simple and universally feasible in institutions with a scarcity of resources.

It is worth noting that abnormal serum potassium, especially hypokalemia, was more frequent in patients who were dose-adjusted by Program2. This might be mainly due to the accelerated rate of hydration after exorbitant MTX concentration occurred. Therefore, appropriately added electrolyte content to the hydration liquid is essential, so are electrolyte testing and electrocardiograph monitoring.

In patients who were dose-adjusted by Program 2 and took full dose over 24h, C_{16h} <100μmol/L that need no additional processing accounted for 78%. Among these patients, only 28.1% of cases experienced MTX clearance delay at 44h, and even quite a

few cases' (30.2%) C_{44h} were below 0.5μmol/L. This suggests that MTX's clearance rate in vivo is not directly related to prior exposure to MTX. People who experienced clearance delay may acquire low concentration when retaking HDMTX treatment, and high concentration may also occur in patients with low concentration before. [18]

A great proportion of patients experienced MTX clearance delay and severe adverse event in the first treatment with HDMTX (35.5% and 24.7% aforementioned) under normal CCR conditions. It is still unpredictable despite many researches in recent years focused on risk factors for eliminating delays, such as renal dysfunction, gene polymorphism, and concomitant medications. [5,20-22] These patients at higher risk can avoid severe adverse events through dose reduction, but even small doses can still be toxic. Assiduous monitoring of plasma concentrations still plays an important role in preventing MTX toxicities in practice. Therefore, we suggest that the C_{16h} test should be added in every exposure to HDMTX and then take appropriate measures according to the results, no matter CCR is normal or not.

Conflict of interest

All authors declare no conflicts of interest.

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Legends

Figure 1 Correlation between sex, risk group, C_{44h} and C_{68h} with relapse-free survival(**A**). sex with relapse-free survival (**B**). risk group with relapse-free survival (**C**). C_{44h} with relapse-free survival (**D**). C_{68h} with relapse-free survival

Figure 2 Comparison on MTX concentration at 44h and 68h between patients using Program1 and Program2 for MTX dose adjustment (**A**). MTX concentration at 44h after dose adjustment (**B**). MTX concentration at 68h after dose adjustment (**C**). MTX concentration at 44h in different treatment cycles after dose adjustment.