The effect of the plasma methotrexate concentration during high-dose methotrexate therapy on prognosis of childhood acute lymphoblastic leukemia

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Abstract

Aim: High-dose methotrexate (HD-MTX) therapy is commonly used in acute lymphoblastic leukemia (ALL) which need drug monitoring. This study was to analyze the influence factors and prognostic role of the plasma MTX concentration in ALL. Methods: 1435 HD-MTX courses of 246 childhood ALL were enrolled. MTX doses were 3 g/m2 for low-risk (LR) group and 5 g/m2 for intermediate or high-risk groups. The target 24-hours (24h) MTX concentrations were set at 33 μmol/L for LR and 65 μmol/L for non LR group. Results: The median 24h MTX concentrations were 42.0 μmol/L for LR and 70.0 μmol/L for non LR group. Only SLCO1B1 genotype in the LR group and age in the non LR group was associated with the 24h MTX concentrations. The survival results were comparable between patients with or without courses failed to reach the target 24h MTX concentration. MTX excretion delay was observed in 211/1435 (14.7%) courses of 125 patients, which more commonly caused MTX-induced toxicities. All the 6 CNS relapses occurred in patients with MTX excretion delay, while in those without did not have any type of CNS relapse (6/119 vs. 0/121, P=0.015). Patients with more than 20% of MTX excretion delay courses had significant worse survival (83.8%±5.0% vs. 93.7%±1.8% for EFS, P=0.014, and 88.8%±4.3% vs. 97.2%±1.1% for OS, P=0.010). Conclusions: Achieving the target 24h MTX concentration during HD-MTX therapy is not the useful indicator to predict the survival of ALL patients. MTX excretion delay is an independent factor for predicting the CNS relapses and worse prognosis.

Title Page

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Running title: Methotrexate concentration and prognosis

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Keywords: methotrexate, pharmacokinetics, pharmacogenomics, childhood, survival, acute lymphoblastic leukemia

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What is already known about this subject:

High-dose methotrexate is commonly used in acute lymphoblastic leukemia. The target 24-hours methotrexate concentrations were set at 33 μmol/L for 3 g/m^2 dose group and 65 μmol/L for 5 g/m^2 dose group. The relationship between target 24-hours methotrexate concentration and methotrexate excretion delay with survival have not been fully confirmed.

What this study adds:

Achieving the target 24-hours methotrexate concentration during high-dose methotrexate therapy is not the useful indicator to predict the survival of acute lymphoblastic leukemia patients. Patients with more than 20% of methotrexate excretion delay courses had significant worse prognosis. More serious consideration should be taking into account on the leucovorin rescue.

Abstract:

Aim: High-dose methotrexate (HD-MTX) therapy is commonly used in acute lymphoblastic leukemia (ALL) which need drug monitoring. This study was to analyze the influence factors and prognostic role of the plasma MTX concentration in ALL.

Methods: 1435 HD-MTX courses of 246 childhood ALL were enrolled. MTX doses were 3 g/m^2 for low-risk (LR) group and 5 g/m^2 for intermediate or high-risk groups. The target 24-hours (24h) MTX concentrations were set at 33 μmol/L for LR and 65 μmol/L for non LR group.**Results:** The median 24h MTX concentrations were 42.0 μmol/L for LR and 70.0 μmol/L for non LR group. Only SLCO1B1 genotype in the LR group and age in the non LR group was associated with the 24h MTX concentrations. The survival results were comparable between patients with or without courses failed to reach the target 24h MTX concentration. MTX excretion delay was observed in 211/1435 (14.7%) courses of 125 patients, which more commonly caused MTX-induced toxicities. All the 6 CNS relapses occurred in patients with MTX excretion delay, while in those without did not have any type of CNS relapse (6/119 vs. 0/121, P=0.015). Patients with more than 20% of MTX excretion delay courses had significant worse survival (83.8%±5.0% vs. 93.7%±1.8% for EFS, P=0.014, and 88.8%±4.3% vs. 97.2%±1.1% for OS, P=0.010).

Conclusions: Achieving the target 24h MTX concentration during HD-MTX therapy is not the useful indicator to predict the survival of ALL patients. MTX excretion delay is an independent factor for predicting the CNS relapses and worse prognosis.

Introduction

Due to the success of risk stratification-based combination chemotherapy, 5-year event-free survival (EFS) rate of children with acute lymphoblastic leukemia (ALL) now exceeds 80%. Methotrexate (MTX) is an important component of all treatment protocols for ALL. High-dose MTX (HD-MTX) with leucovorin rescue is commonly used. Some patients may experience MTX excretion delay, which may cause significant toxicity, such as mucositis, nephrotoxicity, hepatotoxicity, myelosuppression and neurotoxicity and require treatment suspension. Therefore, therapeutic drug plasma level monitoring is essential during the HD-MTX treatment[1]. However, there is great interindividual pharmacokinetics variability in MTX which leads to high

variation in the efficacy and toxicity. In order to identify the factors related to the interindividual variability during MTX administration, many studies have been investigated.

MTX polyglutamates (MTXPGs) is a major determinant of both efficacy and toxicity of MTX in vivo. The accumulation of MTXPGs varies widely among different subtypes of ALL. The patients with T-cell ALL (T-ALL) and B-precursor ALL (B-ALL) with TCF3-PBX1 or ETV6-RUNX1 fusion genes have lower levels of MTXPGs, while those with hyperdiploidy and Philadelphia chromosome (Ph)-like subtypes have higher MTXPGs levels [2]. Gains of chromosomes 10, 18 [3] and chromosomes 4, 21[4] were reported to be associated with increased MTXPGs accumulation. MTX metabolism pathway includes several enzymes and transporters, such as methylenetetrahydrofolate reductase (MTHFR), folylpolyglutamate synthetase (FPGS), solute carrier (SLC) transporters, ATP-binding cassette (ABC) efflux transporters, etc[5]. Several pharmacogenetic studies have investigated the role of single nucleotide polymorphisms (SNPs) in relation to the vast variability of MTXPGs accumulation and toxicity of MTX, but the conclusions remain controversial[6-13].

Lopez et al. [2] have shown that the concentrations of MTXPGs are related to MTX efficacy and toxicity, after the intracellular MTXPGs were measured in bone marrow leukemia cells, but this approach is challenging because too few leukemia cells are usually available in bone marrow when patients receive the HD-MTX courses. Therefore, the plasma concentrations of MTX are monitored in our protocols. However, the MTX concentrations vary widely among patients and even within a patient on different cycles. Trevino et al.[14] from St Jude Children's Research Hospital suggested that target MTX concentrations at 24 hours when HD-MTX was given for ALL children might have impact on prognosis, but the ultimate outcome of this approach has not been elucidated. Due to the fact that the clinical determinants of MTX peak concentration at 24h after administration of HD-MTX and its relationship with survival have not been fully confirmed yet, we launched an investigation on this issue from October 2017 to March 2020 in our hospital: we set the target 24h MTX concentrations of 33 μmol/L for LR ALL and 65 μmol/L for both IR and HR ALL following the study by the St Jude Children's Research Hospital[14].

In the study, 1435 HD-MTX courses performed on 246 patients were evaluated. The aims of the study were as follows: Firstly, to identify the influence factors of 24h MTX concentration and MTX excretion delay; Secondly, to investigate the effect of achieving the target 24h MTX concentration or MTX excretion delay on the prognosis in Chinese ALL children.

Methods

2.1 Patients

Between October 2017 and March 2020, a total of 246 newly diagnosed ALL patients, median age of 3.8 years (range: 0.2 years to 16.3 years) with pharmacogenomics of MTX who received HD-MTX treatment in the Children's Hospital, Zhejiang University School of Medicine were enrolled in this study. The protocol was approved by the Medical Ethics Committee of the hospital.

Immunophenotyping at diagnosis and the minimal residual disease (MRD) in the bone marrow (BM) during the therapy were performed by flow cytometry (FACS canto II with FACSDiva software, Becton Dickinson, San Jose, CA, USA) using a standard panel of antibodies, as previously described[15]. The BM-MRD was determined at the following time points (TP): TP1, on day 15 of remission induction; TP2, on day 33, at the end of remission induction; TP3, on week 10 after the consolidation therapy.

Genetic screening

All patients were screened for genetic abnormalities at diagnosis. The cytogenetic abnormalities were detected by karyotyping and fluorescence in situ hybridization. The gene rearrangement was tested by polymerase chain reaction (PCR).

Pharmacogenomics based multi-gene detection, which included 52 genes (Supplementary Table S1), was performed by next-generation sequencing at Acornmed Biotechnology Co. Ltd (Tianjin, China). Multiplex libraries were sequenced using NovaSeq instrument (Illumina). Alignment of the trimmed reads was per-

formed using Burrows-Wheeler alignment (BWA, version 0.7.12). PCR duplicates were marked using the MarkDuplicates tool from Picard. BaseRecalibrator from Genome Analysis Toolkit (GATK; version 3.8) was applied for realignment and recalibration of the BWA data. Variant calling was performed in Mutect2. ANNOVAR software was used to annotate all the variants including dbSNP. A total of 4 genes involved in MTX metabolism were analysed, which included 5,10-methylenetetrahydrofolate reductase (MTHFR, 665G>A, rs1801133), the solute carrier organic anion transporter 1B1 (SLCO1B1, 1865+4846T>C, rs11045879), ATP binding cassette subfamily B member 1 (ABCB1, 3435A>G, rs1045642), and methionine synthase reductase (MTRR, 66A>G, rs1801394).

2.3 Risk stratification

The patients who were less than 1 year old were assigned to infant (Inf) group, the others were classified as initial low-risk (LR), intermediate-risk (IR) or high-risk (HR) groups, based on age, white blood cell (WBC) count, the biologic features of the blasts and CNS status. Patients with age more than 10 years, WBC[?]50$\times 10^9$/L, T-cell immunophenotype (T-ALL), the t (9;22), t (1;19) and Ph-like gene rearrangement, testicular leukemia, the first cerebrospinal fluid sample was CNS2 (<5 leukocytes/μL with identifiable blasts) or CNS3 ([?]5 leukocytes/μL with identifiable blasts or the presence of cranial nerve palsy) status were defined as IR group. Patients were classified as HR ALL if they had an MLL gene rearrangement, hypodiploidy, the t (17;19) gene rearrangement, or the early T-cell precursor (ETP) ALL immunophenotype. All others were designated initial LR.

The final risk status was re-adjusted based on the response to remission induction therapy. Patients were adjusted to IR ALL if their BM contained 5 or more leukemic blasts on day 15 of remission induction but reached complete remission (CR) at the end of induction therapy. Patients did not reach CR at the end of induction therapy were classified as HR ALL. MRD[?]1% on TP1 with MRD[?]0.1% on TP2 for B immunophenotype (B-ALL) and MRD[?]1% on TP2 for T-ALL was assigned to final HR ALL. MRD<0.01% both on TP1 and TP2 for B-ALL was considered as final LR ALL, while the remaining patients were classified as the final IR ALL.

Treatment and HD-MTX administration

Before the year 2019, patients were treated with the National Protocol of Childhood Leukemia in China (NPCLC-ALL2008) protocol as previously described [1] [15]. After January 2019, this protocol was modified to Zhejiang University Children's Hospital ALL 2019 (ZJCH-ALL-2019). NPCLC-ALL2008 included 5, 7 and 9 courses of HD-MTX for LR, IR and HR groups, respectively, while in ZJCH-ALL-2019, the HD-MTX courses for IR and HR groups were reduced to 6 and 7, respectively. The early intensification with 3 successive courses of etoposide and cytarabine for HR ALL was eliminated in ZJCH-ALL-2019. The duration of therapy was reduced to 2 years for LR and IR ALL, 2.5 years for HR ALL in ZJCH-ALL-2019. The treatment protocol of Inf group was Interfant-99 [16].

The HD-MTX of 3 g/m^2 for the LR and 5 g/m^2 for both the IR and HR was performed after the consolidation therapy. Each course was infused continuously over 24 hours along with a triple intrathecal therapy, followed by leucovorin rescue (15 mg/m^2) at 42h after the start of MTX infusion. The leucovorin rescue was given every 6 hours for a total of 3 to 6 doses. Hydration and urinary alkalinization were implemented. The plasma concentrations of MTX were determined by fluorescent polarization immunoassay at 24h, 48h and 72h after the start of MTX infusion. Extra MTX concentration monitoring was carried out every other day until the level was below 0.3 μmol/L. The MTX given dosages were adjusted to achieve the target 24h MTX concentrations of 33 μmol/L for LR ALL and 65 μmol/L for both the IR and HR ALL [14]. MTX excretion delay was defined as an MTX concentration higher than 1.0 μmol/L at 48h, which was an indication for prolonged leucovorin rescue. The leucovorin rescue was performed until the MTX concentration was below 0.3 μmol/L. The dosages of leucovorin were adjusted based on the 48h concentration of MTX when patient encountered an MTX excretion delay. More detailed procedures for HD-MTX administration were described previously [17]. No patient received cranial radiation therapy.

The MTX exposure toxicities were evaluated after each HD-MTX course, which included: hematological tox-

icity, hepatotoxicity, nephrotoxicity, neurotoxicity, gastrointestinal toxicity and mucositis, etc. The toxicity grading was recorded according to the Common Terminology Criteria for Adverse Events (CTCAE v5.0).

Statistical analysis

All statistical analyses were performed with the SPSS 25 software. The survival rates of EFS and overall survival (OS) were estimated with Kaplan-Meier analysis and log-rank test. Comparison of continuous and categorical variables was performed by Mann-Whitney U test and Chi-square test, respectively. Logistic regression analysis was used to examine the relationship between MTX concentration and related clinical factors. Survival related variables which showed significant impact in the univariate analysis were added to a multivariate Cox regression model to assess their added impact value. A P -value < 0.05 (two tailed) was considered to be statistically significant.

Results

3.1 Patients characteristics and outcomes

A total of 246 patients (139 males and 107 females) with a total of 1435 courses of HD-MTX treatment were analysed. The final data used was updated on December 31, 2021. The median follow-up time was 2.9 years (ranging from 1.8 to 4.2 years).

The cohort 246 ALL patients included 223 B-ALL and 23 T-ALL patients. Inf group included 14 patients (5.7%), while the remaining patients were assigned to LR group (88 patients, 35.8%), IR group (96 patients, 39.0%) and HR group (48 patients, 19.5%), respectively. On TP1, 237 patients (96.3%) reached complete remission (CR), while all patients (100%) reached CR on TP2. A total of 16 relapses occurred, including 10 isolated bone marrow, 3 isolated CNS (2 B-ALL and 1 T-ALL), 3 combined BM and CNS relapses (all 3 were T-ALL patients), no testicular relapse. The 3-year EFS and OS rates were 91.4±1.9% and 95.2±1.4%, respectively (Figure 1A). T-ALL patients had significant worse outcomes than the B-ALL (Figure 1B). LR group had an excellent survival, whose EFS and OS rates were 97.7±1.6% and 100%, respectively (Figure 1C, 1D). Cox regression analysis result showed that T-ALL was the most significant factor on prognosis (Hazard ratio 10.06, 95% confidence interval: 4.158-24.330, P<0.001).

24h MTX concentrations

In the total of 1435 HD-MTX courses, the median 24h MTX concentrations were 42.0 μmol/L (13.4-155.0 μmol/L) for LR patients and 70.0 μmol/L (17.0-369.2 μmol/L) for non LR patients. In the 439 courses of LR-ALL, 310 courses reached the target 24h MTX concentration (70.6%), while in the 996 courses of non LR-ALL, only 601 courses did (60.3%, P<0.001). There was no significant difference between the courses of B-ALL and T-ALL which reached the target 24h MTX concentration (63.6% vs. 62.6%, P>0.05). Only 75 patients (30.5%) had all the courses reached the target 24h MTX concentration. Among them, there were more LR patients than the non LR patients (42/88 LR and 33/158 non LR patients, P<0.001).

The chi-squared test showed that the polymorphisms of SLCO1B1, MTRR and MTHFR genes had correlations with whether or not the 24h MTX concentration could reached the target concentration. For the SLCO1B1 (1865+4846T>C) polymorphism, TT, TC and CC genotypes were observed in 36.5%, 45.0% and 18.5% of the patients, respectively; and the percentage of courses which reached the target 24h MTX concentration were 66.7%, 60.0%, and 56.9% (P=0.017), respectively. Over half of the patients (153/246; 62.2%) carried the AA genotype of MTRR (66A>G), while 83/246 (33.7%) and 10/246 (4.1%) patients carried the AG and GG genotypes, respectively. The patients with AA genotype of MTRR had 66.3% courses reached the target 24h MTX concentration, while the percentage of AG genotype was only 58.6% (P=0.018). For the MTHFR (665G>A) polymorphism, the courses which reached the target 24h MTX concentration of genotype GG, GA and AA were 66.7%, 62.2%, and 57.8% (P=0.048), respectively. However, the polymorphisms of SLCO1B1, MTRR, and MTHFR genes showed no relationships with the outcomes of patients (Figure 2). Only the difference for OS rates with the genotype of ABCB1 (3435A>G) reached the statistically significance. The OS rate in patients with the AA genotype of ABCB1was significantly lower than those with both the GG and AG genotypes (P<0.05, Figure 2G), respectively.

Multivariate logistic regression analysis was used to evaluate whether the patient characteristics (gender, age, risk groups, karyotypes, fusion gene subtypes, the doses of hydration and alkalinization, MTX doses, hepatic and renal functions before the MTX infusion, genetic polymorphisms, etc.) were associated with the MTX concentration at 24h. In the LR group, only SLCO1B1 genotype was associated with the MTX concentration at 24h (Figure 3A). More patients with age older than 10 years had more courses reached the target 24h MTX concentration than the infants and the patients aged between 1 to 10 years (75.4%, 68.4%, and 61.5%, respectively, P=0.001). In the non-LR group, only age was associated with the MTX concentration at 24 hours (Hazard ratio 2.27, 95% confidence interval: 1.561-3.289, P<0.001).

However, the 3-year EFS and OS of patients had courses failed to reach the target 24h MTX concentration were comparable to those who did not have (EFS: 92.7±2.1%, vs. 88.1±4.0%, P=0.286; OS: 95.8±1.5%, vs. 93.4±3.2%, P=0.592), either in the different risk groups (Figure 4A) or with different percentage of HD-MTX courses which failed to achieving the target 24h MTX concentration (Table 2).

MTX excretion delay

MTX excretion delay was observed in 211 (211/1435, 14.7%) courses from 125 patients, including single occurrence in 70 patients, and more than once in 55 patients. MTX excretion delay was more frequent in non-LR-ALL patients (56.3%, 89/158, in non-LR group, vs. 40.9%, 36/88, in LR group, P=0.020). 19 of 23 T-ALL patients (82.6%) had MTX excretion delay, which were much more than that of B-ALL patients (106/223, 47.5%, p=0.001). The higher patient's age was closely associated with MTX excretion delay. The percentage of patients in the [?]10 years and <10 years group with MTX excretion delay was 74.1% (20/27) and 47.9% (105/219), respectively (P=0.010). However, after multivariate logistic regression analysis, the difference failed to reach a statistically significance (P=0.148, Figure 3C).

Surprisingly, none of the polymorphisms of SLCO1B1, MTRR, MTHFR and ABCB1 genes showed a correlation with MTX excretion delay. Logistic regression analysis results showed that only MTX concentration at 24h was associated with MTX excretion delay (P<0.001). T-ALL was also associated with MTX excretion delay in the non-LR group (Figure 3C). Other factors such as gender, karyotypes, fusion gene subtypes, the doses of hydration and alkalinization, hepatic and renal functions before MTX infusion, had no relationship with the MTX excretion delay (P > 0.05).

All the six CNS relapses (3 isolated CNS, and 3 combined BM and CNS relapses) occurred in the patients with MTX excretion delay, while no CNS relapse was observed in those without (6/119 vs. 0/121, P=0.015). The 3-year EFS rate of patients with MTX excretion delay was 88.5%+-2.9%, which was probably worse than that of the patients without (94.3+-2.3%) (P=0.078, Figure 4B). The 3-year EFS rates were comparable between the patients with only single time and more than once MTX excretion delay (Figure 4D). The patients with more than 20% of MTX excretion delay courses had significant worse EFS and OS rates (83.8%+-5.0% vs. 93.7%+-1.8% for EFS, P=0.014, and 88.8%+-4.3% vs. 97.2%+-1.1% for OS, P=0.010, Figure 4E, 4F).

MTX-related toxicity

MTX-related toxicities were described in Table 1. No treatment-related death was encountered during all the HD-MTX courses of this cohort. Two patients with one course each of MTX excretion delay had transient convulsion, while none was encountered in patients without MTX excretion delay group. No other neurotoxicity occurred. The hematopoietic toxicity (56.9%) was observed in 120 out of 211 courses with MTX excretion delay, which was significantly higher than that in those without the delay (399/1224, 32.6%, P<0.001). Grade 4 neutropenia happened in 64 courses (64/1435, 4.5%), requiring platelet transfusion in 26 courses (26/1435, 1.8%) or red blood cell transfusion in 16 courses (16/1435, 1.1%) with MTX excretion delay. MTX-related toxicities, such as oral mucositis, vomiting, diarrhea, hyperbilirubinemia, and nephrotoxicity were significantly more common in the courses with MTX excretion delay (Table 1).

Discussion

The pharmacokinetics and pharmacodynamics of MTX have been wildly studied by many researches. The

MTX influx to efflux by cells is primarily mediated by the reduced folate carrier 1[8] and ABC transporters[18], respectively. Intracellularly, MTX is metabolized into MTXPGs which cannot be pumped out by ABC transporters. MTXPGs inhibit the enzymes of folate cycle which are critical for DNA synthesis, repair, and cell replication [19]. The ability of accumulation of MTXPGs in vivo has been shown to vary among different ALL subtypes [6, 20], which influences the antileukemic effect and outcome of ALL patients[21]. Because the measurement of the intracellular levels of MTXPGs in bone marrow blasts during HD-MTX therapy is complicated [18, 22-24] and infeasible sometimes, the plasma concentrations of MTX are more commonly monitored instead[5, 25-34]. However, the plasma concentrations of MTX vary widely among different patients and even within the same patient on different cycles with the same doses of MTX. The clinical influence factors of MTX concentration and its relationship with survival in childhood ALL remain to be elucidated.

Due to the fact that optimal concentration of MTX in terms of both efficacy and safety for patients remains uncertain, the presumable peak concentrations of MTX for different risk groups for childhood ALL have been recommended: the 24h MTX target concentrations were set at 33 μmol/L for LR ALL and 65 μmol/L for either IR or HR ALL[14]. High risk patients with lower steady state MTX levels seems to have increased risk of relapse in early study[35, 36]. Whether these 24h MTX concentrations have impact on the outcome of childhood ALL remains undetermined. Our results in this study showed that only less than one third of the patients had all the HD-MTX courses reached the target concentration. Surprisingly, the 3-year EFS and OS rates of patients with or without reaching the target 24h MTX concentration were comparable. This indicated that the target 24h MTX concentration had no influence on the survival outcomes of ALL patients in this study. More studies are needed to evaluated the ideal target 24h MTX concentration during HD-MTX therapy in ALL patients.

In the non-LR group, patients [?]10 years old reach the target 24h MTX concentration more commonly, while in the LR group, although SLCO1B1 genotype is associated with the MTX concentration at 24h, no significant relationship is found after multivariate logistic regression analysis. Patients carried the CC genotype of SLCO1B1 (rs11045879) who had less courses reached the target 24h MTX concentration, got a worse EFS rate than those with the TT and TC genotypes, however, the difference failed to reach the statistical significance level (P=0.058). Trevino et al.[14] have showed that SLCO1B1 is an important determinant of methotrexate's pharmacokinetics and clinical effects, the SNPs including rs11045879, rs4149081, rs11045818, rs10841753, etc. are strongly associated with MTX clearance in childhood ALL. The rs11045897 CC and rs4149081 AA genotypes also showed to be indicators for high MTX plasma concentrations in children with ALL by Li et al.[37]. However, Liu et al.[38] found that only patients with CC genotype of SLCO1B1 rs10841753 had a lower MTX plasma level than those with the T allele (CT+TT), but not rs11045897, and the survival was worse in patients with the SLCO1B1 rs4149056 CC genotype than in those with TT or TC genotype. Thus, the relationship between SLCO1B1 polymorphisms and MTX response needs to be further validated.

The patients with MTX excretion delay had significant higher CNS related relapses and worse survival, one reason may be due to the prolonged leucovorin rescue. Too much leucovorin can reverse the antitumor effects of MTX [35]. The study by Skarby et al.[39] suggested that high doses of leucovorin increased the relapse risk by 22% in ALL children. Recently, Niinimaki et al. [40] showed that reduced dose of leucovorin rescue is not associated with increased toxicity. Therefore, the leucovorin rescue need more prudently administration and optimization. Other reasons to explain the MTX excretion delay with worse survival including omitting the 6-mercaptopurine during the courses with MTX excretion delay, reducing the doses of methotrexate after the courses with MTX excretion delay, and chemotherapy suspension because of the MTX-induced toxicities.

In this study, 82.6% of T-ALL patients had MTX excretion delay, which is much higher than B-ALL. Meanwhile, T-ALL patients had much more CNS relapses than B-ALL (four vs. two) and extremely sad survival which desperately need improvement. The COG ABFM regimen with escalating-dose MTX without leucovorin rescue plus pegasparagase (Capizzi-MTX) in T-ALL could be a good choice to improve their dismal outcomes[41].

Surprisingly, multivariate logistic regression analysis showed that only MTX concentration at 24h and T-ALL were associated with delayed MTX excretion, other factors such as age, gender, karyotypes, fusion gene

subtypes, the doses of hydration and alkalinization, hepatic and renal functions before the MTX infusion, the polymorphisms of SLCO1B1, MTRR, MTHFR and ABCB1 genes, showed no relationship with the delayed MTX excretion in our study. The factors related to delayed MTX excretion have been investigated in several studies, however, the results are controversial and no consensus has been achieved. Extravascular fluid collections, including ascites, pleural effusions[32], higher dose of MTX, higher total bilirubin, lower urine volume [33], creatinine clearance rate[17], and creatinine [42]etc. have been showed to be associated with delayed MTX excretion. The relationship between gene polymorphisms and delayed MTX excretion are even more confusing [43]. In an Egyptian study, ABCB1 rs1045642 was found to have a significant association with delayed MTX excretion [44], while in the Spanish study, it was not [45]. The MTHFR polymorphism such as C677T or A1298C, was reported to have association with delayed MTX excretion in some studies [5, 25], however, some other studies showed different findings [46, 47]. Thus, the relationship between the pharmacogenomics and MTX response warrants further study.

Overall, this study found that achieving the target 24h MTX concentration or not during the HD-MTX therapy is not a good indicator for the survival of ALL patients. Monitoring plasma MTX concentrations are helpful for the detection of MTX excretion delay, which will lead to obvious MTX-induced toxicities, higher incidence of CNS relapses and worse prognosis. More serious consideration should be taking into account in terms of the dose and the duration of the leucovorin rescue. Much more T-ALL patients had MTX excretion delay than those with B-ALL. The Capizzi approach of MTX therapy is worth of trial for the T-ALL patients to improve the dismal outcomes and a prospective randomized controlled trial has been scheduled in our center. MTX gene polymorphism studies failed to provide any definitive conclusion, which need more investigations.

The drawbacks of this study are as follows: (1) This is a retrospective study. Prospective randomized controlled trial with pharmacodynamics study is needed. (2) This study was performed in single center only and the number of patients is limited, multi-center study with more patients would be better. (3) This study has too short follow-up period, longer follow-up is needed.

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Data availability statement: The data of this study are available from the corresponding author upon reasonable request.

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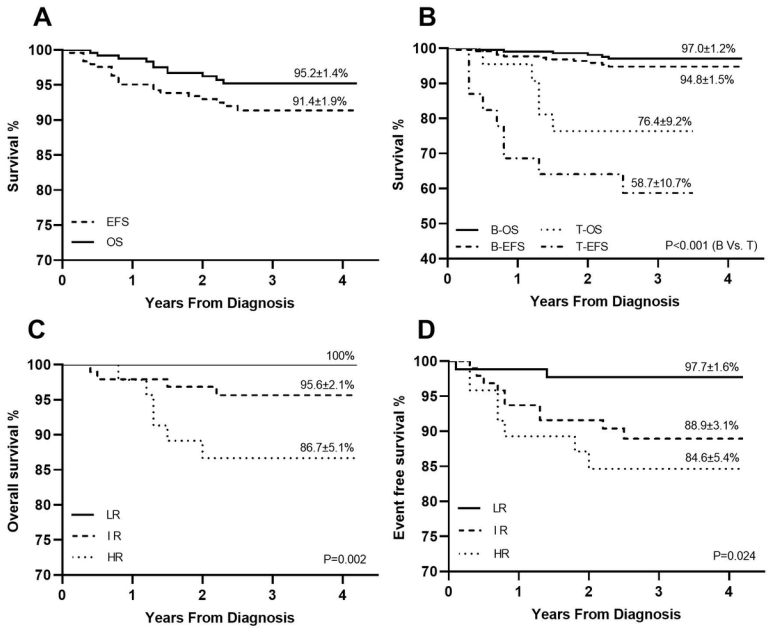

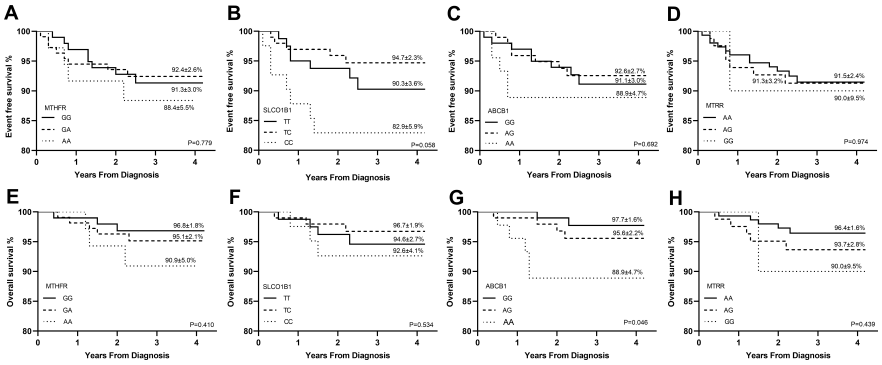

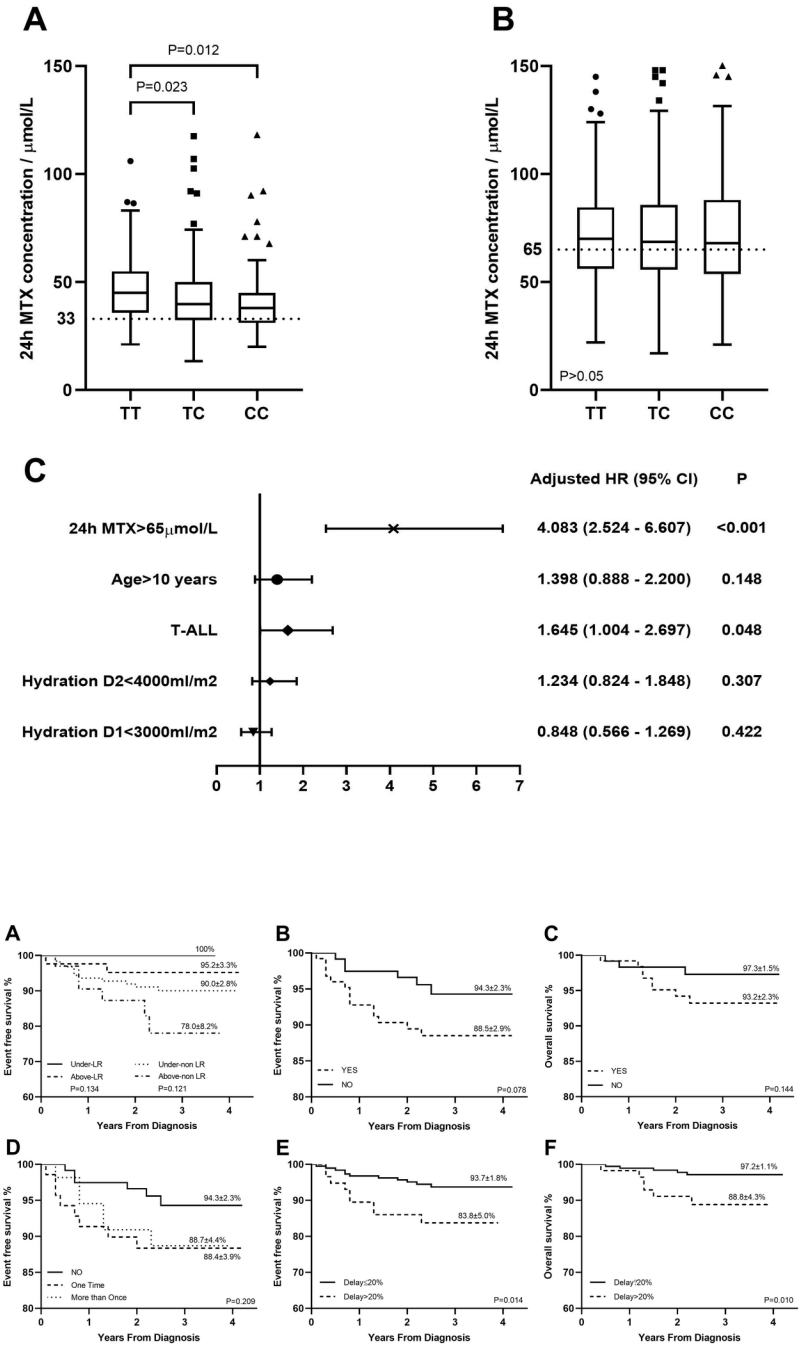

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