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The population pharmacokinetics of high-dose methotrexate in infants with acute lymphoblastic leukemia highlight the need for bedside individualized dose adjustment: a report from the Children's Oncology Group

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Abstract

Background: Infants with acute lymphoblastic leukemia (ALL) treated with high-dose methotrexate (MTX) may have reduced MTX clearance (CL) due to renal immaturity, which may predispose them to toxicity. The objective of this study was to develop a population pharmacokinetic (PK) model of MTX in infants with ALL.

Methods: A total of 672 MTX plasma concentrations were obtained from 71 infants enrolled in the Children's Oncology Group (COG) Clinical Trial P9407. Infants received MTX 4 g/m² intravenously for four cycles during weeks 4–12 of intensification. A population PK analysis was performed using NONMEM® version 7.4. The final model was evaluated using a non-parametric bootstrap, a visual predictive check, and simulations were performed to evaluate MTX dosage and the utility of a bedside algorithm for dose individualization.

Results: MTX was best characterized by a two-compartment model with allometric scaling. Weight was the only covariate included in the final model. The coefficient of variation for interoccasion variability (IOV) on CL was relatively high at 25.4%, compared to the

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6.1 Conflicts of Interest

RJ Beechinor, PA Thompson, RC Vargo, MF Hwang, LR Bomgaars, J Gerhart, Z Dreyer, and D Gonzalez declare that they have no conflicts of interest.

6.2 Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

6.3 Informed Consent

Informed consent was obtained from parents/guardians in accordance with federal and institutional guidelines.

interindividual variability for CL and central volume of distribution, 10.7% and 13.2%, respectively. Simulations identified that 21.1% of simulated infants benefitted from bedside dose adjustment, and adjustment of MTX doses during infusions can avoid supratherapeutic concentrations.

Conclusion: Infants treated with high-dose MTX demonstrated a relatively high degree of IOV in MTX CL. The magnitude of IOV in the CL of MTX suggests that use of a bedside algorithm may avoid supratherapeutic MTX concentrations resulting from high IOV in MTX CL.

Keywords

methotrexate; infants; pediatrics; pharmacokinetics; population modeling; NONMEM; acute lymphoblastic leukemia; cancer; interoccasion variability

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common form of pediatric cancer, representing 25% of all cancers diagnosed in children under 15 years of age [1,2]. Over the past several decades there have been significant improvements in the prognosis of pediatric ALL, and 5-year overall survival for contemporary treatment protocols has approached 90% [3]. Despite this improvement, the outcomes of infants diagnosed at less than 1 year of age have been consistently worse [4]. The explanation for this lack of improvement in the clinical outcomes of infant ALL has not been fully elucidated. However, there are features of infant ALL thought to be responsible for its poor prognosis; these include both the greater frequency of high-risk genetic mutations as well as higher tumor burden at presentation [5–7]. In addition to these factors, physiological differences between infants and older children may lead to alterations in drug disposition and pharmacokinetics (PK) of chemotherapy that may affect infant outcomes. This has led to the development of infant specific protocols designed to increase the intensity of therapy in these subjects [3,4,7].

Methotrexate (MTX) is an anti-neoplastic agent used commonly in a variety of childhood malignancies, and is a crucial component to ALL treatment protocols [8,9]. MTX is a folate analogue that competitively inhibits the activity of dihydrofolate dehydrogenase (DHFR), causing depletion of purines and thymidylate which halts DNA synthesis causing cell death [10,11]. This effect is amplified in rapidly dividing cells, leading to MTX's S-phase specific cytotoxicity [8]. Due to the high expression of DHFR in ALL cells, high-dose MTX is one therapeutic strategy utilized to saturate DHFR and maximize cytotoxicity [12,13]. However, high-dose MTX requires both therapeutic drug monitoring (TDM) and significant supportive care to avoid excessive exposure to MTX which has been shown to cause nephrotoxicity, myelotoxicity, mucositis, neurological complications, and other adverse effects [14,15].

Based on the physiologic differences between infants and older children, the PK of MTX in infants may be altered due to several factors. Firstly, MTX is primarily eliminated by renal excretion, as adult studies have shown that approximately 70–90% of each dose is excreted unchanged in urine [15,16]. Infants are known to have both delayed and variable maturation of renal tubule function, glomerular filtration rate, and renal blood flow [17–20]. Therefore, elimination of MTX in younger infants may be significantly delayed compared to older

children, which could increase toxicity. Additionally, MTX is known to have delayed elimination in patients with extracellular fluid accumulations [14]. This may affect drug disposition in infants, who are known to undergo significant changes in total body water content from approximately 75% during the neonatal period to 55% in adulthood [21,22].

Despite these concerns, to date there are limited published PK analyses describing the disposition of MTX in this vulnerable patient population. Moreover, few studies have included infants <6 months of age when these physiological changes are most relevant, and none have used population PK to characterize the disposition of methotrexate in this patient population [21,23,24]. The use of population PK offers several unique advantages over conventional PK analyses, most notably the ability to quantify the effects of patient covariates on drug exposure using sparse PK sampling [25–27]. A non-compartmental analysis with a portion of the data used for the current study was previously published [28], but a population PK analysis has not been previously performed. The objectives of this study were to develop a population PK model of MTX in infants with ALL, to characterize the impact of interoccasion variability (IOV) on this model, and to use simulations to evaluate the impact of various doses on MTX exposure.

2. METHODS

2.1 Patient Population

MTX concentrations were obtained from infants enrolled in the Children's Oncology Group (COG) Clinical Trial P9407, a portion of which were enrolled in a PK sub-study. The details of the study design, inclusion and exclusion criteria, treatment protocol, and PK sampling were described previously [28]. Briefly, this study included subjects with newly diagnosed ALL, <366 days postnatal age (PNA), and >36 weeks gestational age at birth with congenital ALL. Exact dates of infusions were not available for all subjects, therefore age at infusion was imputed based on expected age using the study protocol. This study was approved by the institutional review board and informed consent was obtained from parents/guardians in accordance with federal and institutional guidelines. All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration [28].

2.2 Drug Administration and Supportive Care

All patients enrolled received the same chemotherapy regimen, an intensified induction including high-dose MTX, and supportive care as described previously [28]. Subjects received their first cycle of MTX at week 4 of induction/intensification. High-dose MTX was administered as a 24 hour (h) intravenous (IV) infusion, with a 200 mg/m² loading dose over 20 min, followed by a 3.8 g/m² dose over the remainder of the 24 h. This dosing regimen was repeated at week 5 of induction intensification and in consolidation at weeks 11 and 12 of therapy. Standard supportive care for high-dose MTX was provided, including hydration with alkalized IV fluids and leucovorin rescue. MTX plasma concentrations are reported here as micromoles/L (μM) [28].

2.3 Pharmacokinetic Sampling

Beginning with the first cycle of MTX given at week 4, standard MTX monitoring was performed for all subjects at the end of drug infusion and 24 h later. Subsequent concentrations were monitored every 12–24 h until the MTX concentration was $<0.18 \mu\text{M}$. This standard monitoring was also performed during weeks 5, 11, and 12 of therapy. In addition to this, subjects enrolled in the PK sub-study had intensive MTX PK sampling at 1, 6, 12, and 23 h after the start of MTX infusions [28].

2.4 Bioanalytical Assay

Samples obtained as part of standard MTX monitoring were analyzed in the clinical laboratories of the treating hospitals. The most common assays used for quantification of MTX included fluorescence polarization immunoassay and the enzyme-multiplied immunoassay [29,30]. For the PK sub-study, samples were analyzed at the central study laboratory at Texas Children's Hospital using a high-performance liquid chromatography (HPLC) method [31]. For all MTX samples included in this analysis, a conservative lower limit of quantification (LLQ) of $0.05 \mu\text{M}$ was assumed, which is used commonly in clinical practice [14,28].

2.5 Population Pharmacokinetic Model Development

Population PK analyses were performed using NONMEM® version 7.4 (ICON Development Solutions; Ellicott City, MD, USA) using MTX concentration versus time data for all infants with MTX concentration data. The effect of log-transforming MTX concentration data was investigated. The first-order conditional estimation (FOCE) method with interaction was applied for all model runs. All data manipulation and visualization of diagnostic plots were executed using R (version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria), and RStudio (version 0.99, RStudio, Boston, MA, USA), with the packages lattice, latticeExtra, and ggplot2 [32–34]. Model development was guided by run minimization, successful covariance steps, objective function value (OFV) changes for each nested model, plausibility and precision of parameter estimates, evaluation of eta and epsilon shrinkage, reduction in residual variability, and manual inspection of diagnostic plots including visual prediction checks (VPCs).

Both one and two-compartment structural models were evaluated. The base model assumed a standard allometric scale based on total body weight (WT). A single exponential value of 0.75 was assumed for clearance (CL) and intercompartmental clearance (Q). A single exponential value of 1.0 was assumed for central volume of distribution (V_c) and peripheral volume of distribution (V_p) [17,35]. Estimation of these exponents was also investigated. Standard PK equations were applied for a two-compartment model using the ADVAN3 TRANS4 subroutines in NONMEM®. Inter-individual variability (IIV) was assessed for all PK model parameters using an exponential relationship as shown below for a two-compartment model (Eq. 1–4).

$$CL_i = \theta_{CL,std} \times \left(\frac{WT_i}{70kg} \right)^{0.75} \times \exp(\eta_{i,CL}) \quad (1)$$

$$V_{c,i} = \theta_{V_c,std} \times \left(\frac{WT_i}{70kg} \right)^{1.0} \times \exp(\eta_{i,V_c}) \quad (2)$$

$$V_{p,i} = \theta_{V_p,std} \times \left(\frac{WT_i}{70kg} \right)^{1.0} \times \exp(\eta_{i,V_p}) \quad (3)$$

$$Q_i = \theta_{Q,std} \times \left(\frac{WT_i}{70kg} \right)^{0.75} \times \exp(\eta_{i,Q}) \quad (4)$$

where CL_i , $V_{c,i}$, $V_{p,i}$, and Q_i are the individual values of CL, V_c , V_p and Q, respectively; $\theta_{CL,std}$, $\theta_{V_c,std}$, $\theta_{V_p,std}$, and $\theta_{Q,std}$ are the respective parameter values for a subject with a body weight of 70 kg; WT_i is the individual subject weight; $\eta_{i,CL}$, η_{i,V_c} , η_{i,V_p} and $\eta_{i,Q}$ are random effect parameters assumed to be symmetrically distributed with a mean equal to zero and variance estimated as ω_{CL}^2 , $\omega_{V_c}^2$, $\omega_{V_p}^2$ and ω_Q^2 which describe each individual's variation from the population estimate. Covariance between these variability estimates was evaluated for each parameter.

In addition to the IIV, IOV was also characterized for each PK parameter as follows (Eq. 5)

$$Parameter\ Value_{i,k} = Typical\ Value \times \exp(\eta_i + \kappa_i) \quad (5)$$

where i indicates the i^{th} individual, k indicates the k^{th} occasion, the typical value is the mean value of the parameter in the population, η_i is the random effect accounting for IIV, and κ_i is the random effect account for IOV [36,37]. Each treatment cycle was treated as a different occasion, and IOV was assumed to be the same across occasions. Both additive, exponential, and combined residual error models were tested.

Covariates available for analysis including weight, age, body surface area (BSA), and sex. Covariates were evaluated for the model in a stepwise fashion based on changes in the OFV first by forward inclusion ($P < 0.05$ and $OFV > 3.8$) followed by backward elimination ($P < 0.001$ and OFV of > 10.8). Both continuous and categorical covariates were tested using the power model normalized as follows (Eq. 6–7):

$$P_{i,j} = \theta_{pop,std} * \left(\frac{cov_i}{cov_m} \right)^{\theta_{cov}} \quad (6)$$

$$P_{i,j} = \theta_{pop,std} * \theta_{cov}^{CATEGORICAL} \quad (7)$$

where $P_{i,j}$ indicates the j^{th} parameter estimate for the i^{th} individual, $\theta_{pop,std}$ indicates the population parameter values for a subject with a body weight of 70 kg, cov_i indicates the individual covariate value for the i^{th} individual, cov_m indicates the median population covariate value, θ_{cov} is the parameter indicating the covariate effect, and $CATEGORICAL$ is a categorical variable which can take on values of zero or one.

2.6 Model Evaluation

The precision of the final population PK model parameter estimates were evaluated using non-parametric bootstraps with 1000 replicates to generate 95% confidence intervals of each parameter using the percentile method [38]. VPCs were run on key models to assess model performance and robustness. VPCs were performed with Perl-speaks-NONMEM (version 3.6.2) and visualized in R using xpose4 [39,40].

2.7 Model Simulations

For all simulations, a virtual population of 1000 infants was created assuming all subjects were of full gestational age, and a similar weight distribution as our study population. Monte Carlo simulations were performed to assess the frequency of subtherapeutic and supratherapeutic MTX concentrations with doses of 2, 4, 6, and 8 g/m² of MTX given IV over 24 h. For each dose, MTX concentrations were simulated at 2, 6, 8, 24, 42, 48, 72, 96, 120, 144, 168, 216, and 240 h. Simulations were repeated to generate four cycles of simulated MTX concentrations for each subject. Target methotrexate concentrations were defined based on previous literature suggesting that methotrexate concentrations < 16 µM at 24 h are associated with relapse and are subtherapeutic, and methotrexate concentrations > 100 µM at 24 h, > 1.0 µM at 48 h, and > 0.1 µM at 72 h are associated with increased toxicity and are supratherapeutic [41,42].

The R package mlxR was used to simulate a “bedside algorithm” for individualizing high-dose MTX exposure similar to those proposed previously [43,44]. This algorithm adjusts the rate of MTX infusion according to MTX concentrations obtained at 2 and 6 h after start of a 24 h IV infusion of 4 g/m² of MTX. Simulations maintained an infusion length of 24 h which is known to be essential to MTX’s antitumor activity. At 2 h, the infusion rate was decreased by 50% in subjects with MTX concentrations >100 µM. At 6 h, the infusion rate was decreased by 25% for subjects with MTX concentrations between 75 – 100 µM, and the infusion rate was decreased by 50% in subjects with MTX concentrations >100 µM. The percent of subjects requiring dose adjustment was calculated, and the median 24 h concentration was compared between subjects that did or did not require dose adjustments.

3. RESULTS

3.1 Patient Data

A total of 690 MTX concentrations were available from 71 subjects who underwent 229 cycles of high-dose MTX treatment for ALL. There were 2.6% (18/690) MTX concentrations below LLQ of 0.05 μM , all of which were excluded [45]. The PK profile of all 71 subjects stratified by cycle is shown in Figure 1. Intensive MTX PK sampling was performed in 24.0% (17/71) of subjects enrolled, and 92.0% (65/71) of subjects had PK data for more than one occasion. Of subjects who had intensive PK monitoring, 94.1% (16/17) of these intensive collections occurred during cycle 1. There were no differences in the baseline demographics between subjects in the PK sub-study and the routine clinical care group (Table 1). For the majority of MTX cycles, subjects received MTX doses close to 4 g/m^2 . However, for 14.4% (33/229) of cycles patients received doses $<3.5 \text{ g}/\text{m}^2$ (median 2.99 g/m^2 , range 2.0 – 3.48 g/m^2), and for 1.3% (3/229) of cycles subjects received doses $>4.5 \text{ g}/\text{m}^2$ (median 5.63 g/m^2 , range 5.34–5.63). The reason for dose modification in these subjects is unknown to us.

3.2 Population Pharmacokinetic Analysis

MTX plasma concentrations were best characterized by a two-compartment model with linear elimination. Data was log-transformed as model minimization was not achievable using raw concentrations, likely due to the wide range of concentrations. After allometrically scaled total body weight was incorporated into CL, V_c , V_p , and Q terms, no other covariates were found to be significant. Visual inspection of the eta plots revealed a weak association between age and CL, but incorporating this as a covariate reduced the OFV by <3.8 . Visual inspection of the empirical Bayesian estimates (EBEs) for CL did not reveal a correlation with age or treatment cycle (Electronic Supplemental Materials). Estimation of the allometric scaling factors for CL and volume of distribution terms did not improve the OFV, increased the shrinkage of IIV estimates, and resulted in estimates similar to fixed exponents, 0.80 and 0.98 respectively, and therefore these were fixed. The final model included estimation of the IIV on both CL and V_c , as well as IOV on CL. A correlation between the IIV of CL and V_c was noted in diagnostic plots, however this was not included in the final model as estimation of this term resulted in a relatively small correlation coefficient (0.383), and did not allow for a successful covariance step. Estimation of IIV and IOV on other model parameters was not included in the final model due to inflation of the eta shrinkage values to $>60\%$ for IIV estimates. The final model included a single proportional residual error model for all MTX concentrations.

The diagnostic plots for the final population PK model are shown in Figure 2. These plots demonstrate some misspecification in the population predictions, which is improved with the incorporation of IIV and IOV in the individual predictions. Additionally, while panel C appears to show misspecification occurring at concentrations observed at $>200 \text{ h}$ after dosing, this is likely due to the small number of MTX observations. The final model PK parameters, standard errors, and results of the bootstrap analysis are shown in Table 2. Overall, there was a relatively high degree of IOV on CL (25.4%) compared to both the IIV on CL (10.7%) and IIV on V_c (13.2%). All parameter estimates fell within 10% of the

median bootstrap estimates suggesting reasonable precision of parameter estimates. Four example subjects who demonstrated a high degree of IOV in their methotrexate CL are shown in Figure 3. The results of the VPC are shown in Figure 4, and demonstrate that the model captures the observed variability well. Notably, 11.9% (80/672) of the observations fell outside of the 90% prediction interval, suggesting a slight underestimation of the random effects. Few MTX observed concentrations fell below the 5% prediction interval, suggesting the final model prediction for CL may be slightly overestimated. These results were similar when a VPC was performed focusing solely on the first 72 h after infusion (Electronic Supplemental Materials).

3.4 Model Simulations

The results of the dose ranging simulation are shown in Table 3. None of the dosing regimens evaluated resulted in simulated 24 h MTX concentrations $<16 \mu\text{M}$. Additionally, compared to the study dose of 4 g/m^2 , escalating doses of 6 g/m^2 and 8 g/m^2 resulted in a substantial increase in the percentage of simulated supratherapeutic MTX concentrations. The percent of subjects with supratherapeutic MTX concentrations at 72 h ($>0.1 \mu\text{M}$) was high for all simulated doses, similar to the exposures in our subjects who received 4 g/m^2 . This suggests further research may be needed to define supratherapeutic concentrations at 72 h in this patient population.

Simulation of a bedside algorithm showed that without dosage adjustment, 21.1% (211/1000) of infants receiving a single cycle of 4 g/m^2 would be expected to achieve supratherapeutic MTX concentrations, similar to published reports [43,46]. A total of 214 dose adjustments were made, of which 1.87% (4/214) were a 50% decrease in infusion rate at 2 h due to a simulated methotrexate concentration $>100 \mu\text{M}$, 29.0% (62/214) were a 50% decrease in infusion rate at 6 h due to a simulated methotrexate concentration $>100 \mu\text{M}$, and 69.2% (148/214) were a 25% decrease in infusion rate at 6 h due to a simulated methotrexate concentration between 75 and $100 \mu\text{M}$. All four simulated infants who required dose adjustment at 2 hours also required adjustment at 6 hours, and therefore 21.1% (211/1000) of simulated infants had their dose modified by the algorithm. As shown in Figure 5, dose adjustment using this bedside algorithm resulted in similar median (25th percentile – 75th percentile) 24 h MTX concentrations between subjects requiring dose adjustment and those that did not, $58.8 \mu\text{M}$ (50.5 – 69.2) and $59.1 \mu\text{M}$ (49.5 – 69.7), respectively. These results suggest that the application of a simple algorithm to adjust MTX infusion rate may make targeted MTX exposure more feasible, and can overcome the challenge of IOV in MTX CL between cycles.

4. DISCUSSION

TDM of high-dose MTX remains an essential practice for identifying subjects at risk for MTX toxicity and informing supportive care measures [14,47]. Previous research has shown that individualization of MTX exposure using TDM can reduce the incidence of MTX toxicity and improve patient outcomes [41,46]. Individualization of MTX exposure is challenging due to the known within subject and between subject variability of MTX CL, which may be better characterized using population PK modeling [24,48,49]. Therefore, the

objective of our study was to develop a population PK model of MTX in infants with ALL, and to quantify the effects of IOV between cycles on this PK model.

To date, this is the largest population PK model of high-dose MTX in infant ALL, and the first to include infants as young as 2 months PNA. Our PK dataset consisted of MTX plasma concentrations collected as part of routine care combined with MTX plasma concentrations from infants enrolled in a PK sub-study [28]. The PK sub-study data significantly improved model fitting by increasing the total number of MTX concentrations, and by providing MTX concentration data drawn during the infusion which are informative for MTX parameter estimation. Additionally, our PK dataset contained multiple cycles of MTX concentrations, which allowed us to quantify the within subject variability in PK parameters across cycles as IOV.

A two-compartment model best characterized the PK of MTX in the 71 infants in our study, which is consistent with previous reports of high-dose MTX in both children and adults [46,48,50–57]. Our model applied theory based allometry to scale CL and volume of distribution parameters to a standard weight of 70 kg [17,58]. The resulting final model CL and V_c estimates scaled to a 70 kg adult are similar to published reports in adults [52,59,60]. Additionally, a retrospective case-control study comparing the PK of high-dose MTX in pediatric ALL subjects applied an identical methodology for allometric scaling of CL and V_d terms [51]. The final parameter estimates scaled to a 70 kg adult from this previously published model were a CL of 13 L/h/70kg and a V_c of 46 L/70kg, which compare favorably to our estimates of 11.0 L/h/70 kg and 63.4 L/70kg, respectively. Our weight-normalized final model estimate of CL was 0.273 L/h/kg, which falls within the range of reported adult and pediatric literature values of 0.117 – 0.374 L/h/kg. Similar to our estimate for CL, our final model estimate of V_c of 0.910 L/kg falls within the range of reported values of 0.356 – 1.27 L/kg in adults and pediatrics [30,48,50,55–57,61].

We did not identify age as a covariate impacting CL, which has been described previously [46,48,62–64]. However, the impact of age on MTX CL has been reported inconsistently, as there are multiple published PK analyses which have found no impact of age on CL of MTX in pediatric subjects [50,54,57]. The effect of renal maturation, reflected in modeling PNA as a covariate on methotrexate CL, would be expected to be highest in both premature infants as well as full-term infants <6 months of age [17–20]. Therefore it is possible that we were unable to identify PNA as a significant covariate on MTX CL because our data only included 28.4% (65/229) of cycles with infants <6 months of age, and premature infants were excluded from this study.

Our study demonstrated a similar magnitude of IOV in the CL of MTX compared to previous studies performed in children [48,51,65]. This variation in the CL of MTX across cycles may be explained by changes in disease progression, drug-drug interactions, or unmeasured covariates which vary across cycles. The magnitude of IOV in the CL of MTX suggests that Bayesian adaptive dosing algorithms may be optimized by obtaining MTX concentrations during the current cycle infusion, rather than concentrations measured from previous cycles.

Simulations revealed that dosing regimens of 2–8 g/m² provide sufficient MTX exposure based on the 24 h MTX target of 16 µM [41]. However, increasing doses of high-dose MTX above 4 g/m² may result in untoward renal toxicity, as >15% of all simulated infants would have experienced suprathreshold MTX concentrations. Application of the final population PK model using a bedside dosing algorithm revealed the potential clinical impact of our protocol, as our simulated individualization strategy reduced suprathreshold MTX concentrations in 21.1% of simulated subjects. Future prospective studies individualizing MTX exposure using a similar approach are needed to evaluate the impact of such a bedside algorithm on patient outcomes.

Our study has several limitations. First, our study data included limited covariates, and notably we did not have markers of renal function. Despite this, all infants were screened for adequate organ function prior to each MTX treatment cycle, including a creatinine CL >70 mL/min/1.73 m². Therefore it is unlikely markers for renal function would have improved model fitting [48,66]. Other missing potential covariates included; serum albumin, urine pH, pharmacogenomic data, and concomitant drug use [67–70]. A second limitation is that the exact age of each infant during MTX infusion was not available, and therefore there may have been discrepancies between ages used for modeling and true age during infusion. A third limitation is that the MTX concentrations we chose as targets for our simulation have not been prospectively validated. Therefore, future studies are still needed to elucidate the relationship between MTX concentrations and both efficacy and toxicity. A fourth limitation of our analysis is that intensive PK sampling was not evenly distributed across treatment cycles, and this may limit our ability estimate true IOV in MTX CL across treatment cycles. Despite these limitations, our results add to the dearth of literature on the PK of MTX in infants with ALL, and provide additional evidence of the potential benefit of individualized dosing strategies.

In conclusion, we characterized the population PK of MTX in infants with ALL using a two-compartment model allometrically scaled by body size. Infants treated in our study demonstrated a relatively high degree of IOV in MTX CL across treatment cycles. These analyses suggest that individualization of MTX therapy may be improved by performing additional monitoring and dose adjustment during MTX infusions to account for the change in MTX CL occurring across treatment cycles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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KEY POINTS:

- High-dose methotrexate is a crucial component of treatment protocols for acute lymphoblastic leukemia (ALL), but few studies have characterized its disposition in infants.
- Infants with ALL demonstrated significant interoccasion variability in their clearance of methotrexate across treatment cycles.
- Monte Carlo simulations performed using a developed population pharmacokinetic model revealed the utility of bedside monitoring and dose adjustment to optimize methotrexate exposure.

Fig. 1.
Methotrexate plasma concentrations from 229 cycles in 71 infants subset by treatment cycle.
The x-axis represents time after the start of the 24 hour methotrexate infusion. The y-axis
represents plasma methotrexate on a log scale.

Fig. 2.

Diagnostic plots of the final population pharmacokinetic model. a) Population predicted concentrations versus observations, b) individual predictions versus observations, c) time after last dose versus conditional weighted residuals, and d) population predicted concentrations versus conditional weighted residuals. For a) and b), the dashed line represents the line of unity and the solid line represents a linear regression. For c) and d), the black line represents the LOESS curve, and the grey lines represent conditional weighted residuals (CWRES) values of -4 , -2 , 0 , 2 , and 4 .

Fig 3.

Four infants demonstrating high interoccasion variability in their clearance of methotrexate. Each subject received the same weight based dose of high dose methotrexate of 4 g/m^2 . The x-axis represents time after the start of the 24 hour methotrexate infusion. The y-axis represents plasma methotrexate concentrations on log scale.

Fig. 4.

Final model visual predictive check. A total of 80 points (11.9%) fell outside the 90% prediction interval. Dotted and solid lines represent the 5th, 50th, and 95th percentiles of the simulated data and observed data, respectively. Gray shaded areas represent the 90% prediction interval. The x-axis represents time after the start of the 24 hour methotrexate infusion. The y-axis represents plasma methotrexate concentrations on log scale.

Fig 5.

Simulated methotrexate (MTX) exposures in 1000 virtual infants using a bedside adjustment with the R package mlxR. This algorithm adjusts the rate of MTX infusion according to MTX concentrations obtained at 2 and 6 h after the start of a single 24 h IV infusion of 4 g/m² of MTX. At 2 h, the infusion rate was decreased by 50% in subjects with MTX concentrations >100 µM. At 6 h, the infusion rate was decreased by 25% for subjects with MTX concentrations between 75 – 100 µM, and the infusion rate was decreased by 50% in subjects with MTX concentrations >100 µM. The x-axis represents time after the start of infusion. The y-axis shows simulated MTX concentrations. The dark black line denotes the median MTX concentration, and the gray shaded region shows the 25th – 75th percentiles of simulated MTX concentrations. The left panel shows simulated MTX concentrations in subjects requiring bedside adjustment (211/1000), and the right panel shows simulated MTX concentrations in subject without dosage adjustment (789/1000).

Table 1

Demographic and clinical characteristics of infants included in the study

Study	^a Pharmacokinetic sub-study group (n=17)		^b Routine clinical care group (n=54)	
	Median	Range	Median	Range
^c Post-natal age (months)	8.5	2.9 – 12.9	7.0	1.0 – 12.9
^c Total body weight (kg)	8.6	4.5 – 11.9	7.6	3.0 – 12.1
^c Height (cm)	69	51 – 78	66.5	49.7 – 79.0
^c Body surface area (m ²)	0.40	0.24 – 0.48	0.36	0.20 – 0.48
Methotrexate dose (g)	1.5	0.62 – 1.96	1.4	0.60 – 2.2
Methotrexate dose (g/m ²)	4.0	2.0 – 4.5	4.0	2.0 – 5.6
Methotrexate C _{24h} (μM)	54.7	24.2 – 146.6	61.5	24.0 – 140.0
Methotrexate C _{48h} (μM)	0.2	0.07 – 7.0	0.20	0.06 – 6.7
Methotrexate C _{72h} (μM)	0.17	0.07 – 2.4	0.11	0.05 – 2.5

^a8 males; 9 females^b24 males; 30 females^cReported at first treatment courseMethotrexate dose = total dose given as a 24-hour infusion, C_{xh} = plasma concentration of methotrexate at x hours

Table 2

Final population pharmacokinetics model parameter estimates and bootstrap results

Parameter	Final Model	^a Bootstrap Analysis (N=1000)		
	Estimate ^b (RSE%)	5 th Percentile	Median	95 th Percentile
^c Fixed Effects				
$\theta_{CL, std}$ (L/h/70 kg)	11.0 (3.1)	10.4	11.0	11.6
$\theta_{V_c, std}$ (L/70 kg)	63.4 (5.1)	57.2	62.9	69.0
$\theta_{V_p, std}$ (L/70 kg)	13.6 (8.5)	10.6	13.3	15.6
$\theta_{Q, std}$ (L/h/70 kg)	0.13 (7.6)	0.11	0.13	0.15
^d Inter-individual variability (IIV)				
ω_{CL} (IIV as CV%)	10.7 (49.7)	5.23	10.7	14.7
ω_{V_c} (IIV as CV%)	13.2 (56.2)	5.74	12.7	18.7
^c Inter-occasion variability (IOV)				
$\omega_{CL, IOV}$ (IOV as CV%)	25.4 (21.1)	20.9	25.3	29.8
^e Residual variability (RV)				
Proportional Error (%)	37.5 (7.52)	34.9	37.2	39.6

^aA total of 951 runs (95.1%) successfully minimized and 1000 (100%) runs completed the covariance step^bRSE is the relative standard error defined as the standard error of the estimate divided by the final parameter estimate multiplied by 100

^cTheory based allometry was applied such that model parameters are standardized to a 70 kg adult assuming an exponent of 0.75 for clearance terms and 1.0 for volume of distribution terms; $\theta_{CL, std}$ is the population clearance standardized to a 70 kg adult, $\theta_{V_c, std}$ is the population central volume of distribution standardized to a 70 kg adult, $\theta_{V_p, std}$ is the population peripheral volume of distribution standardized to a 70 kg adult, $\theta_{Q, std}$ is the population intercompartmental clearance standardized to a 70 kg adult

^dModel estimates reported in CV%; ω_{CL} is the variance of the IIV for clearance, ω_{V_c} is the variance of the IIV for volume, $\omega_{CL, IOV}$ is the variance of the IOV on clearance.

^eProportional residual error coded as additive on logarithmic scale

Table 3.

Simulation results of 1000 virtual patients treated with four cycles of high dose methotrexate

Clinical Endpoint	Dose of Methotrexate Simulated			
	2 g/m ²	4 g/m ²	6 g/m ²	8 g/m ²
^a MTX C _{24h} (μM)	33.5 (27.9 – 40.0)	67.4 (55.7 – 79.8)	100.5 (83.7 – 119)	134.3 (111 – 159)
^a MTX C _{48h} (μM)	0.125 (0.0735 – 0.235)	0.250 (0.147 – 0.470)	0.375 (0.221 – 0.705)	0.501 (0.295 – 0.940)
^a MTX C _{72h} (μM)	0.0672 (0.0592 – 0.0983)	0.134 (0.0907 – 0.196)	0.202 (0.135 – 0.292)	0.267 (0.181 – 0.392)
^b Subtherapeutic at 24 h	0%	0%	0%	0%
^c Supratherapeutic at 24 h	0.3 %	6.28%	51.1%	85.5%
^c Supratherapeutic at 48 h	3.05%	9.43%	16.4%	23.3%
^c Supratherapeutic at 72 h	24.0%	69.3%	89.0%	95.6%
^d Time to MTX <0.05 μM	42 (42 – 72)	42 (42 – 48)	42 (42 – 96)	42 (42 – 120)

^aMTX C_x is simulated methotrexate concentration at time x reported as median (25th – 75th percentile)^bThe percent of subjects subtherapeutic at 24 hours (<16 μM)^cThe percent of subjects supratherapeutic at 24 hours (>100 μM), 48 hours (>1 μM) and 72 hours (>0.1 μM)^dReported in hours as median (minimum - maximum)