ORIGINAL ARTICLE

Insights from a pharmacometric analysis of HDMTX in adults with cancer: Clinically relevant covariates for application in precision dosing

Manuel Ibarra[1] | Ryan Combs[2] | Zachary L. Taylor[3,4] | Laura B. Ramsey[3,4,5] | Torben Mikkelsen[6] | Randal K. Buddington[7] | Jesper Heldrup[8] | Jason N. Barreto[9] | Martin Guscott[2] | Jennifer Lowe[2] | Charles Hurmiz[10] | Suresh Marada[10] | Scott C. Howard[2,11] | Paula Schaiquevich[12,13]

[1]Department of Pharmaceutical Sciences, Faculty of Chemistry. Universidad de la República, Montevideo, Uruguay

[2]Resonance, Inc., Arlington, TN, USA

[3]Division of Research in Patient Services, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

[4]Division of Clinical Pharmacology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

[5]Department of Pediatrics, University of Cincinnati, Cincinnati, OH, USA

[6]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

[7]Department of Molecular and Cellular Physiology, LSU Health Sciences, Shreveport, LA, USA

[8]Childhood Cancer and Research Unit, University Children's Hospital, Lund, Sweden

[9]Department of Pharmacy, Mayo Clinic, Rochester, MN, USA

[10]Guardian Research Network, Spartanburg, SC, USA

[11]College of Nursing, University of Tennessee Health Science Center, Memphis, TN, USA

[12]Unit of Innovative Treatments, Hospital de Pediatria JP Garrahan, Buenos Aires, Argentina

[13]Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Buenos Aires, Argentina

Correspondence

Dr. Paula Schaiquevich, Unit of Innovative Treatments, Hospital de Pediatria JP Garrahan, Buenos Aires, Argentina.
Email: paulas@conicet.gov.ar

Aims: High-dose methotrexate (HDMTX) is an essential part of the treatment of several adult and paediatric malignancies. Despite meticulous supportive care during HDMTX administration, severe toxicities, including acute kidney injury (AKI), may occur contributing to patient morbidity. Population pharmacokinetics provide a powerful tool to predict time to clear HDMTX and adjust subsequent doses. We sought to develop and validate pharmacokinetic models for HDMTX in adults with diverse malignancies and to relate systemic exposure with the occurrence of severe toxicity.

Methods: Anonymized, de-identified data were provided from 101 US oncology practices that participate in the Guardian Research Network, a non-profit clinical research consortium. Modelled variables included clinical, laboratory, demographic and pharmacological data. Population pharmacokinetic analysis was performed by means of nonlinear mixed effects modelling using MonolixSuite.

Results: A total of 693 HDMTX courses from 243 adults were analysed, of which 62 courses (8.8%) were associated with stage 2/3 acute kidney injury (43 stage 2, 19 stage 3). A three-compartment model adequately fitted the data. Time-dependent serum creatinine, baseline serum albumin and allometrically scaled bodyweight were clinically significant covariates related to methotrexate clearance. External evaluation confirmed a satisfactory predictive performance of the model in adults receiving HDMTX. Dose-normalized methotrexate concentration at 24 and 48 hours correlated with AKI incidence.

Conclusion: We developed a population pharmacometric model that considers weight, albumin and time-dependent creatinine that can be used to guide supportive care in adult patients with delayed HDMTX elimination.

KEYWORDS

acute kidney injury, delayed elimination, high-dose methotrexate, pharmacokinetics

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1 | INTRODUCTION

Methotrexate (MTX), a folate antimetabolite that inhibits DNA synthesis and cellular replication, is an essential component of therapy for the treatment of numerous malignancies including acute lympho-blastic leukaemia (ALL), non-Hodgkin lymphoma (NHL), osteosarcoma and primary central nervous system (CNS) cancers in paediatric and adult patients. Intravenous infusions of doses higher than 500 mg/m^2, commonly referred to as high-dose MTX (HDMTX), are used for pro-phylaxis or treatment of patients with CNS involvement because of the ability of MTX to penetrate the blood–brain barrier at high doses.[1,2]

MTX disposition varies greatly among individuals, and prolonged exposure to high concentrations results in serious adverse events that can delay or interrupt subsequent anticancer therapy and compromise patient outcomes. Depending on the treatment protocol, quality of supportive care and other factors, between 0.5% and 30% of patients develop HDMTX-induced acute kidney injury (AKI),[3] which further impairs elimination, prolongs systemic exposure, and may result in therapy delay, dose reduction or even interruption of further MTX treatment, hampering treatment efficacy.[4] Unfortunately, it is difficult for clinicians to predict which patients are at higher risk of HDMTX-induced severe toxicities.

Model-informed analysis and Bayesian forecasting can support the individualization of clinical decisions, including MTX dose selection, optimization of folinic acid (leucovorin) rescue, enforced hydration and urine alkalinization, and provide guidance for the timing of other rescue therapies, such as glucarpidase administration, ulti-mately leading to safer HDMTX treatment. This approximation offers several advantages over the interpretation of a single concentration in relation to a specific target associated with efficacy or toxicity, allow-ing characterization and prediction of pharmacokinetic and pharmaco-dynamic outcomes at the individual level, integrating relevant patient characteristics and collected data. Therefore, adequate individualized MTX elimination forecasting may lead to better dose adjustment and supportive care for the patients, resulting in shorter hospital stays, improved quality of life and more convenient cost-effective therapy.

Model-informed precision dosing is supported by the population approach, which leverages individual characteristics as parameters to predict more precisely the pharmacokinetic and pharmacodynamic outcomes and quantify the risk of severe adverse events.[5] Several HDMTX population pharmacokinetic models have been reported for paediatric patients, mainly with ALL or osteosarcoma,[6-20] others were developed for adult patients,[21-30] and few have described HDMTX pharmacokinetics in children and adults simultaneously.[27,31-34] To date, these models reflect the high inter- and intraindividual variability linked to chemotherapeutic protocols and patient physiological and pathophysiological characteristics.

This study analysed retrospectively collected data from a large and heterogeneous adult patient population treated with HDMTX at multiple centres in the US for osteosarcoma, NHL or ALL to characterize HDMTX pharmacokinetics recognizing clinically relevant

What is already known about this subject

- High-dose methotrexate (HDMTX) is an essential part of the treatment of several adult and paediatric malignancies.
- Up to 30% of patients develop HDMTX-induced acute renal dysfunction.
- Model-informed precision dosing (MIPD) is emerging as an important approach to inform rescue therapy and dose adjustment in HDMTX.

What this study adds

- Bodyweight, time-varying creatinine and albumin were recognized as clinically relevant covariates for HDMTX pharmacokinetics in our adult cohort.
- 24- and 48-hour dose-corrected MTX concentrations are more sensitive in detecting AKI than partial AUCs and non-corrected concentrations.
- PopPK HDMTX models should be validated at each cen-tre before routine implementation of MIPD.

covariates using a model-based approach and relate MTX exposure to the occurrence of severe adverse events.

2 | METHODS

2.1 | Patient population

We included data from adults who received courses of HDMTX as part of standard of care treatment at 101 US community oncology programs that participate in the Guardian Research Network (GRN, https://www.GuardianResearch.org), a non-profit consortium of oncology practices in the US (see Supplementary Material for detailed description of data management). Eligibility criteria for this analysis included patients aged 18 years or older at the time of cancer diagno-sis (NHL, ALL or osteosarcoma) and administration of at least one course of HDMTX. All eligible patients with concentration-versus-time data were included in the analysis.

2.2 | Drug administration and sampling

MTX was administered as 3-, 4-, or 24-hour infusions according to the disease and treatment protocol. Infusion lengths were grouped as short infusions (SI, $n = 466$) for those with HDMTX administered for 4 hours or less, and long infusions (LI, $n = 227$) for those administered

over 24 hours. Dosages ranged from 0.5 to 13.4 g/m^2 in the SI group and from 0.5 to 7.2 g/m^2 in the LI group.

Blood plasma MTX concentrations were sampled up to 400 hours from the beginning of the infusion. Most samples corresponded to standard HDMTX monitoring times collected at: 24, 48 and 72 hours from the start of the infusion; however, additional samples were also obtained at various intermediate times and many samples were collected at the end of SI. Since these data were collected during routine MTX therapeutic monitoring, an increase in the proportion of patients with delayed elimination is expected to characterize the dataset for times beyond 72 hours due to the censoring of MTX levels from patients with normal elimination. To reduce the impact of this data censoring on the population analysis, only levels reported up to 100 hours after the start of the infusion were considered for model development.

2.3 | Safety data

Except for AKI, all adverse events were assessed for each HDMTX course and graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.[35] AKI was graded according to the Acute Kidney Injury Network (AKIN) criteria[36] and incidence was determined based on serum creatinine. Description of the timing for each laboratory analysis used in the study is described in the Supplementary Material.

2.4 | Pharmacokinetic modelling

Data were analysed using nonlinear mixed effects modelling implemented in MonolixSuite® 2020R1 (Lixoft SAS, Antony, France) to estimate HDMTX population and individual pharmacokinetic parameters and assess the quantitative impact of individual characteristics. The stochastic approximation expectation maximization algorithm (SAEM) was used combined with a Markov chain Monte Carlo method (MCMC) for the estimation of model parameters.

Two- and three-compartment distribution models with first-order and nonlinear capacity-limited (Michaelis–Menten) elimination were tested as structural models describing plasma concentration-versus-time data. Inter-individual (IIV) and inter-occasion (IOV) variability were tested (see Supplementary Material).

The analysis was performed using log-transformation of MTX concentrations. Different models were evaluated to describe the residual unexplained variability, including an exponential and a combined error model.

The model-building process was driven by several diagnostic metrics, the corrected Bayesian Information Criterion (BIC), and the uncertainty in parameter estimation expressed as relative standard error (RSE). Basic goodness-of-fit plots in addition to simulation-based diagnostics, including prediction-corrected visual predictive check (pcVPC) and normalized prediction distribution errors (NPDE), were also taken into account.

2.5 | Covariate analysis

Individual and treatment characteristics recorded at each HDMTX course evaluated as potential covariates on the base model included: size descriptors (body surface area or BSA and bodyweight or BW), baseline serum creatinine (SCRb), time-varying serum creatinine (SCRt), age, sex, baseline liver enzymes (alanine aminotransferase or ALT, aspartate aminotransferase or AST), body mass index (BMI), body fat percentage, average blood urea nitrogen (BUN), average urine pH at each MTX course, total bilirubin (Tbili), albumin (ALB), ethnicity, AKI in the previous course of HDMTX, course number, concomitant administration of antibiotics, diuretics, proton pump inhibitor administration, previous administration of platinum-based antineoplastic drugs, and HDMTX infusion length categorized in LI and SI. Data management for the covariate parametrization and analysis is described in the Supplementary Material. Inclusion of continuous variables as model covariates was performed with centring SCRb and SCRt to the expected values reported by Junge et al.,[37] while AGE was centred on 60 years, ALB on 4 mg/dL, BUN on 14 mg/dL and Tbili on 0.3 mg/dL.

Correlation among individual characteristics was assessed to avoid collinearity. Individual covariates were added in a forward stepwise approach if we observed a reduction of the objective function value (OFV) by at least 3.84 units ($P < .05$) and a decrease in the IIV of the estimate parameter, if the estimated parameter was significantly different from 0 ($P < .05$, Wald test), and according to graphic diagnostics.

2.6 | Missing data

As SCR has been extensively reported as a key covariate on MTX CL,[6,9,11,13,15,21,23-25,28,30-33,38] the following imputation rules were implemented: when SCR was unavailable for a given patient in any HDMTX course, the value was imputed as the mean value for healthy adult subjects (0.92 mg/dL and 0.77 mg/dL for male and female patients, respectively). If SCR was absent for a given HDMTX course but available on any other occasion, a mean value for that subject was imputed. For the inclusion of SCR as a time-varying covariate, imputation was performed for intermediate missing data using linear interpolation between previous and posterior values or directly imputing the nearest reported SCR value (if only a previous or a posterior SCR observation was available).

2.7 | External model evaluation

Two datasets were used for external validation: the dataset supporting the population model developed by Kawakatsu et al.[33] and a dataset provided by Barreto et al.[3] as detailed in Supplementary Tables S1 and S2.

The global fit of the final developed model was evaluated graphically with pcVPCs and basic goodness-of-fit plots. The relative bias (rBias) and precision (relative root mean square error, rRMSE) of

model-predicted MTX concentrations were calculated as described in the Supplementary Material.[39,40] We defined an acceptable bias and imprecision of 25%, based on: (i) the most widely implemented analytical method for MTX quantification (Fluorescence Polarization Immunoassay TDx/TDxFLx, Abbott Laboratories) claims an allowable error of 10% in terms of relative deviation (accuracy) and coefficient of variation (precision); (ii) there is an additional experimental error given by the sampling time and additional sample preparation (e.g., dilution); and (iii) such bias and imprecision are considered acceptable for the use of population pharmacokinetic models in dose forecasting of HDMTX in clinical practice.

2.8 | Exposure-toxicity analysis

Individual parameter estimates were obtained for the final model and used along with individual characteristics to simulate individual MTX concentration-versus-time data and the following exposure metrics were computed applying non-compartmental analysis (NCA) to the simulated concentrations obtained using a simulation time step of 0.1 hour: area under the plasma concentration-versus-time curve between zero and the end of infusion (AUC_{0-inf}), between zero and 24 hours (AUC_{0-24}), between zero and 48 hours (AUC_{0-48}), and between zero and 72 hours (AUC_{0-72}) after the start of the infusion; MTX plasma levels at 24, 48 and 72 hours after the start of the infusion; and the maximum MTX concentration (C_{max}). Simulations and NCA were performed using MonolixSuite® modules Simulx and Pkanalix, respectively.

The analysis of the predictors of AKI was performed by logistic regression using R 4.1.1[41] and included clinical, biochemical, demographic and MTX-exposure parameters.

3 | RESULTS

A total of 318 adult patients with courses of HDMTX were identified in the GRN dataset. Of those, 243 patients with 693 courses had available MTX concentrations and were therefore included in the present analysis. Five patients received at least one dose of glucarpidase during an HDMTX course. MTX concentrations reported after the time of glucarpidase administration were not considered in the pharmacokinetic analysis.

An average of three samples per course (range: 1–8) was available. Observations included data reported as below the lower limit and above the upper limit of quantification. In addition, reported MTX levels below 0.04 μM were flagged as censored data. Both left and right censored data were handled using the M4 method as described by Beal.[42]

Patient demographic and clinical data as well as a description of concomitant medication are detailed in Table 1. MTX dosage ranged from 0.5 to 13.4 g/m² given over 3 to 24 hours.

A total of 143 patients with 466 HDMTX courses corresponding to 67% of the total dataset received MTX at dosages between 0.5 and

13.4 g/m² infused over 3 to 6 hours (short infusions) while 115 patients in 227 courses (33%) received MTX at dosages between 0.5 and 7.2 g/m² infused over 24 hours (long infusions).

MTX concentration–time data were best described by a three-compartment model with first-order elimination using an exponential residual model ($\Delta OFV = -256$, Supplementary Table S2). Inclusion of IOV was not supported by the data and therefore model building was performed considering each HDMTX course independently. Then, IIV accounted for both between-patient and between-course variability and was precisely estimated for MTX clearance (CL) and for the volume of distribution of the central compartment (V1).

All MTX disposition parameters were scaled by bodyweight using a three-quarters power model for elimination and distribution clearances (CL, Q1 and Q2) and a linear model for volumes of distribution (V1, V2, V3). These effects were centred so that the typical reported values corresponded to a 70 kg patient. Including an effect of bodyweight on pharmacokinetic disposition parameters improved the fit of the data, changing the OFV by −7 points. On the contrary, accounting for the effect of BSA did not improve the model ($\Delta OFV = 9$). Thus, a three-compartment model including the effect of bodyweight on all parameters was considered as the base model.

In the univariate analysis, we identified 10 covariates to be significantly related to MTX CL, including SCRb, SCRt, AGE, Tbili, previous administration of platins, concomitant diuretics, concomitant tyrosine kinase inhibitors (TKI, dasatinib and imatinib), infusion length, BUN, ALB, and AKI in the previous HDMTX course (complete information of the model building process is detailed in Supplementary Table S3). Among these covariates, the following significant correlations were identified ($P < .05$, $R^2 > 0.3$) and therefore considered in the covariate model building so as to avoid collinearity: AGE with ALB, SCRb with BUN, and SCRt with BUN. Accounting for the effect of SRCt on CL led to a better fit than including SCRb or BUN and thus SCRt was retained in the model. Subsequently, the effects of ALB, Tbili, previous platin-based therapy, AKI in the previous HDMTX course, AGE, infusion length, concomitant TKIs and concomitant diuretics on CL were tested in the forward analysis. Despite a significant reduction in MTX clearance for administration at shorter infusion lengths (−12%), for occasions with previous AKI (−8%), and for patients with increasing age, the clinical impact was considered to be low and therefore these effects were excluded from the final model. Of the remaining individual characteristics, only the effect of ALB on MTX CL was retained. Thus, the final model for CL is shown in the following equation:

$$CL_i = CL_{pop} * \left(\frac{WT_i}{70}\right)^{0.75} * \left(\frac{SCR_{pop}}{SCR_i t}\right)^{\beta_{SCRt}} * \left(\frac{ALB_i}{4}\right)^{\beta_{ALB}}$$

were CL_i is the ith individual predicted MTX CL, CL_{pop} the typical MTX CL (i.e., the predicted MTX CL for a subject with bodyweight 70 kg, SCR equal to SCRpop, and ALB equal to 4 mg/dL), WT_i the ith individual bodyweight, $SCR_i t$ the SCR for the ith SCR value at time t, β_{SCRt} the coefficient for the effect of SCRt on CL_i, ALB_i the ith individual average

TABLE 1 Patient demographic and clinical data and concomitant medication

Panel A: Comparison of patient characteristics and baseline laboratory values by duration of high-dose methotrexate infusions			
	All patients ($n = 243$)	2 to 6 h ($n = 140$)	24 h ($n = 103$)
Sex			
Female	108	70	38
Male	135	70	65
Malignancy type			
Acute lymphoblastic leukaemia	14	2	12
Non-Hodgkin lymphoma	173	122	51
Others	56	16	40
Age at the time of the first methotrexate dose (years)[a]	60 (18–88)	64 (18–88)	52 (20–76)
Weight (kg)[a]	79.9 (43.9–161.6)	80.6 (43.9–161.6)	77.79 (47.1–145.8)
Height (cm)[a]	170.2 (142.2–200.7)	170.2 (142.2–198.1)	170.2 (152.4–200.7)
Body surface area (m^2)[a]	1.92 (1.43–2.72)	1.92 (1.44–2.72)	1.94 (1.43–2.55)
Initial dose of HDMTX[a] (mg/m^2)	3174 (511–13 419)	4237 (511–13 419)	1731 (521–7256)
Serum creatinine at baseline (mg/dL)[a]	0.74 (0.20–1.78)	0.71 (0.20–1.42)	0.78 (0.32–1.78)
Missing (n)	4	3	1
Aspartate aminotransferase at baseline (U/L)[a]	19 (4–291)	19.5 (4.0–146.0)	19 (6–291)
Missing (n)	33	18	15
Alanine aminotransferase at baseline (U/L)[a]	30 (5–451)	30 (8–320)	31 (5–451)
Missing (n)	49	29	20
Total bilirubin at baseline (mg/dL)	0.3 (0.1–1.7)	0.3 (0.1–1.7)	0.3 (0.1–1.3)
Missing (n)	8	5	3
Albumin at baseline (g/dL)	3.3 (1.9–4.7)	3.3 (2.3–4.4)	3.3 (1.9–4.7)
Missing (n)	29	15	14
Panel B: Concomitant medications used during high-dose methotrexate infusions			
	All courses ($n = 693$)	2 to 6 h ($n = 466$)	24 h ($n = 227$)
Tyrosine kinase inhibitors			
No	673 (97%)	466 (100%)	207 (91%)
Yes	20 (3%)	0 (0%)	20 (9%)
Prior therapy with platins (up to 45 days before HDMTX)			
No	673 (97%)	451 (97%)	222 (98%)
Yes	20 (3%)	15 (3%)	5 (2%)
Proton pump inhibitors			
No	496 (72%)	354 (76%)	142 (63%)
Yes	197 (28%)	112 (24%)	85 (37%)
Diuretics			
No	593 (86%)	408 (88%)	185 (81%)
Yes	100 (14%)	58 (12%)	42 (19%)
Antibiotics			
No	665 (96%)	447 (96%)	218 (96%)
Yes	28 (4%)	19 (4%)	9 (4%)

blood albumin, and β_{ALB} the coefficient for the effect of ALB on CL_i. For the volume of distribution, no significant covariate effect was detected beyond bodyweight.

Table 2 summarizes the population pharmacokinetic modelling results, and Figure 1 shows the prediction-corrected visual predictive check (pcVPC) for the final model. Additional goodness-of-fit plots (Supplementary Figures S1–S4), a scheme with highlights of covariate model building (Supplementary Figure S5) and a table detailing quantitative aspects of this process (Supplementary Table S3) are included in the Supplementary material.

Parameter	Value	Standard error	Relative standard error (%)
CLpop (L/h)	5.92	0.44	7.38
β_{SCRt}	0.694	0.0434	6.25
β_{ALB}	0.594	0.103	17.3
V1 (L)	14.4	1.73	12.0
Q2 (L/h)	0.571	0.127	22.2
V2 (L)	3.55	0.570	16.1
Q3 (L/h)	0.0365	0.00542	14.9
V3 (L)	2.09	0.505	24.1
IIV CL (%)	25.4	1.60	6.23
IIV V1 (%)	90.0	4.70	5.22
Residual variability	0.751	0.0154	2.06

TABLE 2 Population pharmacokinetic parameter estimates for the final model of high-dose methotrexate

Abbreviations: CLpop, population estimate of clearance; V1, V2, V3: volume of the central and peripheral compartments, respectively; Q2, Q3: intercompartmental clearances; IIV CL, IIV V1: inter-individual variability in clearance and volume of distribution in the central compartment, respectively.
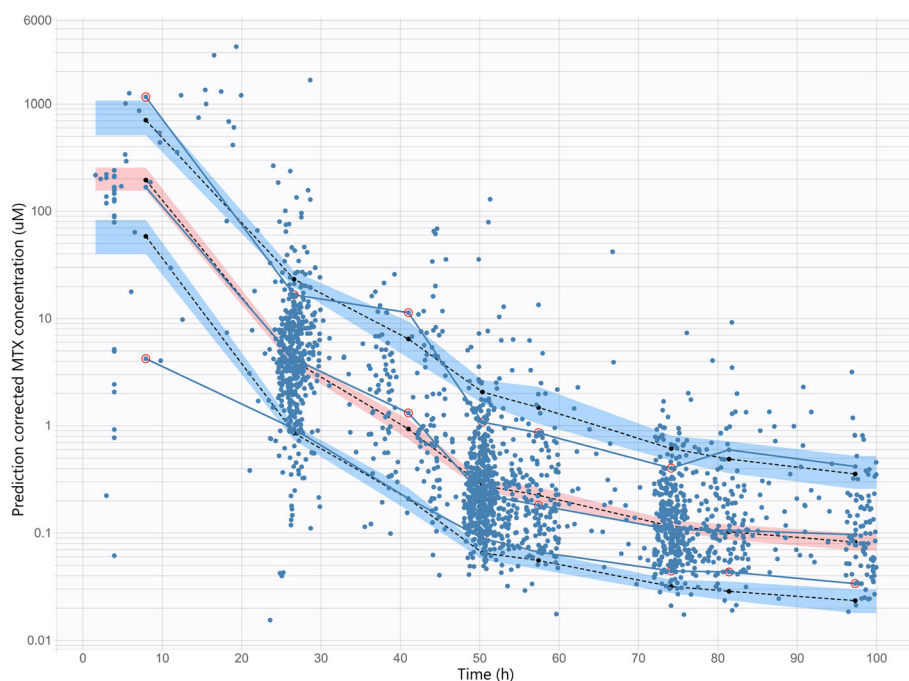
FIGURE 1 Prediction-corrected visual predictive check (pcVPC) for the final model. Blue dots represent observed MTX levels, blue lines represent the 5th, 50th and 95th percentiles for the observed MTX levels, dashed black lines represent the 5th, 50th and 95th percentiles for the simulated MTX levels, while the shaded areas correspond to the prediction interval for each percentile (blue for 5th and 95th, pink for the median). Dots with red outline show misspecifications (observed percentiles outside the corresponding prediction interval).

3.1 | External model validation

Two external datasets obtained from different clinical centres were used to assess the predictive performance of the model. For the dataset from Kawakatsu et al.,[33] consisting of 135 patients and 454 HDMTX courses from adult patients, a priori rBias and rRMSE (i.e., prediction based only on individual characteristics) were −3.73% and 66.4%, respectively. Both metrics improved substantially a posteriori (after accounting for MTX concentrations to estimate individual parameters) resulting in rBias and rRMSE of −0.047% and 17.6%, respectively. In the case of the dataset from Barreto et al.[3] (80 patients and 80 HDMTX courses), a priori rBias and rRMSE were 6.78% and 84.1%, respectively, whereas a posteriori rBias and rRMSE were 3.34% and 57.7%, respectively. Even though the a priori rBias of both datasets was far below the threshold of 25% established as an accurate model predictive performance, in both cases a priori rRMSE was higher than the cut-off (>25%) indicating high uncertainty for model predictions if MTX blood concentrations are not considered. However, the a posteriori rRMSE (i.e., considering patient MTX data) was substantially improved in both datasets but reached an acceptable value only for the Kawakatsu et al. dataset. The pcVPCs for the model fit on both datasets (Supplementary Figure S6) are in accordance with these metrics. Overall, these results indicate that, despite an excellent mean prediction error, the model's predictive performance for HDMTX concentrations might be centre-dependent, most probably related to differences in HDMTX clinical management.

TABLE 3 Comparison of methotrexate course characteristics, toxicity and exposure by length of high-dose methotrexate infusions

	All courses (n = 693)	2 to 6 h (n = 466)	24 h (n = 227)	P-value
Dosage (mg/m^2)				
Median (range)	3431 (511–13 419)	3533 (511–13 419)	1004 (521–7256)	<0.001[a]
Dose (mg)				
Median (range)	6300 (948–27 240)	7052 (955–27 240)	1950 (948–15 520)	<0.001[a]
Length of hospital stay				
Median (range)	4.46 (0.15–26.66)	4.35 (0.15–23.05)	5.08 (0.99–26.66)	0.058[a]
Acute kidney injury grade (AKIN criteria)				
0	501 (72%)	329 (71%)	171 (75%)	0.512[b]
1	130 (19%)	91 (19%)	40 (18%)	
2	43 (6%)	31 (7%)	12 (5%)	
3	19 (3%)	15 (3%)	4 (2%)	
Blood bilirubin increased				
0	621 (90%)	431 (92%)	190 (84%)	<0.001[b]
1	44 (6%)	25 (5%)	19 (8%)	
2	24 (3%)	9 (2%)	15 (7%)	
3	4 (1%)	1 (0%)	3 (1%)	
Neutrophil count decreased				
0	240 (64%)	153 (67%)	87 (60%)	<0.001[b]
1	78 (21%)	53 (23%)	25 (17%)	
2	13 (3%)	10 (4%)	3 (2%)	
3	9 (2%)	2 (1%)	7 (5%)	
4	33 (9%)	11 (5%)	22 (15%)	
Missing (n)	320	237	83	
Platelet count decreased				
0	295 (45%)	266 (59%)	29 (14%)	<0.001[b]
1	154 (24%)	127 (28%)	27 (13%)	
2	39 (6%)	25 (6%)	14 (7%)	
3	38 (6%)	15 (3%)	23 (11%)	
4	127 (19%)	16 (4%)	111 (54%)	
Missing (n)	40	17	23	
Alanine aminotransferase increased				
0	391 (58%)	256 (56%)	135 (60%)	0.636[b]
1	204 (30%)	141 (31%)	63 (28%)	
2	37 (5%)	26 (6%)	11 (5%)	
3	43 (6%)	28 (6%)	15 (7%)	
4	3 (0%)	3 (1%)	0 (0%)	
Missing (n)	15	12	3	
Aspartate aminotransferase increased				
0	455 (66%)	293 (63%)	162 (72%)	0.031[b]
1	180 (26%)	129 (28%)	51 (23%)	
2	16 (2%)	15 (3%)	1 (0%)	
3	29 (4%)	18 (4%)	11 (5%)	
4	9 (1%)	8 (2%)	1 (0%)	
Missing (n)	4	3	1	

(Continues)

TABLE 3 (Continued)

	All courses (*n* = 693)	2 to 6 h (*n* = 466)	24 h (*n* = 227)	*P*-value
AUC$_{0-inf}$ (µM*h)				
Mean (range)	2709 (256–13 166)	3426 (256–13 166)	1238 (289–5808)	<0.001[c]
AUC$_{0-24}$ (µM*h)				
Mean (range)	2548 (246–12 658)	3282 (255–12 658)	1041 (246–4870)	<0.001[c]
AUC$_{0-48}$ (µM*h)				
Mean (range)	2677 (255–13 033)	3392 (255–13 033)	1210 (288–5728)	<0.001[c]
AUC$_{0-72}$ (µM*h)				
Mean (range)	2696 (256–13 091)	3411 (256–13 091)	1227 (289–5781)	<0.001[c]
C$_{max}$ (µM)				
Mean (range)	427 (12–2175)	604 (43–2175)	64 (12–256)	<0.001[c]

Abbreviations: AUC$_{0-inf}$, AUC$_{0-24}$, AUC$_{0-48}$, AUC$_{0-72}$: area under the concentration-versus-time profile between the start and the end of infusion, and 24, 48 and 72 hours after the start of the infusion, respectively; C$_{max}$: MTX concentration at maximum level.
[a]A *t*-test was applied for comparison;
[b]Pearson's chi-squared test was considered for comparison;
[c]Wilcoxon rank-sum test.

TABLE 4 Model-estimated dose-normalized MTX concentrations at 24 and 48 hours for short and long HDMTX infusions, with and without acute kidney injury. Results shown as median predicted values (interquartile range). The *P*-value corresponds to the comparison between with and without AKI

	Short infusions with AKI	Short infusions without AKI	*P*-value	Long infusions with AKI	Long infusions without AKI	*P*-value
C24/D (µM/g/m^2)	1.82 (0.99–7.00)	0.82 (0.49–2.00)	0.00362	27.3 (22.8–31.7)	18.9 (13.8–26.9)	0.00181
C48/D (µM/g/m^2)	0.165 (0.078–0.844)	0.064 (0.039–0.143)	0.0253	1.92 (0.53–4.60)	0.265 (0.152–0.595)	0.0147

3.2 | Exposure-toxicity analysis

There were no therapy-related deaths. Stage 2/3 AKI developed in 62 courses and was detected during the first HDMTX course in 30 patients (13.2% considering first-courses only, *n* = 227), and 14 (8.5%) and 5 (4.5%) during the second (*n* = 164) and third courses (*n* = 111), respectively. In 32 courses, patients experienced stage 2/3 AKI and did not proceed to the next course (4.6% of total courses). In the other 30 courses when patients developed stage 2/3 AKI but continued HDMTX, 21 patients were given a similar dosage to that administered for the course immediately before the renal injury event but nine of the cases received a reduced HDMTX dosage of between 11.6% and 57.9%.

The most common severe toxicity was stage 2/3 AKI in 9.9% patients administered with short HDMTX infusions (less than 6 h) while haematological toxicity manifested as grade 3/4 platelet decrease (59%) and neutrophil decrease (12.8%) was the most frequent severe adverse event observed in patients that received HDMTX in long infusions (Table 3, Supplementary Table S3).

The exclusion of 75 out of 314 patients due to absence of MTX plasma concentrations may introduce a bias to the reported proportions of adverse events, since this loss of information could be attributed to a clinical decision of not monitoring MTX levels given a good clinical evolution informed with serum creatinine values. However, this variable was also missing in more than 70% of the excluded patients, preventing the estimation of AKI incidence. Nonetheless, in excluded patients with enough data, this variable showed similar results to those reported for the patients included in the study.

As shown in Table 4, we found a significant correlation between dose-normalized MTX concentrations at 24 hours (C24/D) and at 48 hours (C48/D) with the incidence of AKI for patients receiving MTX at both short and long infusion lengths. These parameters were associated with an increased risk of stage 2/3 AKI in both short (OR 1.16, 95% CI 1.09–1.24, and OR 1.57, 95% CI 1.25–2.03, respectively; *P* < .05) and long infusion HDMTX courses (OR 1.08, 95% CI 1.02–1.14, and OR 1.72, 95% CI 1.36–2.24, respectively; *P* < .05). Boxplots showing the distribution of these variables are included in Supplementary Figures S7 and S8. No association was observed between the development of stage 2/3 AKI and MTX AUC.

4 | DISCUSSION

We developed a population pharmacokinetic model for HDMTX therapy in adult patients with different malignancies treated at multiple clinical centres in the US and we identified individual patient characteristics with clinical relevance in MTX pharmacokinetics. To our knowledge, this is the largest sample size used for MTX

pharmacokinetic modelling in adults. In addition to time-varying serum creatinine, we found that MTX CL increased with individual albumin levels showing the importance of albumin monitoring in MTX dose management.

The pharmacokinetics of MTX was adequately described with a three-compartment model consistent with previous reports in children[6] and adults,[21,23,26] despite most authors reporting that a two-compartment model was adequate enough to describe MTX disposition.[18,22,24,29,31,32] The discrepancy may be attributed to the available data used for model building in each case as HDMTX therapeutic drug monitoring usually ends 72 hours after the start of the infusion.[43] Although our original dataset included MTX plasma observations up to 14 days post-dose, we analysed data up to 100 hours after the start of the infusion to reduce the impact of data censoring with normal elimination. Data recorded at longer times could be a result of drug release from 'third spaces' like pleural effusions and ascitic fluid.[44] Our final model characterized MTX disposition in three phases, with half-lives of 1.5 hours, 4.9 hours and 40 hours, acknowledging the existence of a third compartment with a slow disposition half-life. The typical CL for a 70 kg patient with normal SCR and albumin was estimated at 5.9 L/h, similar to published reports in adults[21-25,27,29,30] but lower than in other studies.[26,31,32] The estimated volume of distribution at steady state was 20 L for a 70 kg patient, which is slightly above the extracellular water volume (20–35% of bodyweight). Although we observed within-patient variability in the different courses, our data did not support the discrimination between IOV and IIV in the model but allowed the quantification of both as one random effect on CL and on V1. We observed high IIV in V1 (90%), for which bodyweight remained in the final model as the only covariate. For CL, we identified three covariates that explained 39% of the IIV parameter.

Although MTX dosing is usually based on body surface area (BSA), we found a better correlation between MTX disposition and bodyweight, as reported elsewhere.[19,23,31,45] For MTX CL, this correlation was described with an allometric model using the three-quarters coefficient.[46,47] Given the non-proportional relation between BSA and bodyweight,[48] a direct interpretation of this finding may be that BSA-guided dosing could be inaccurate. However, this is not reflected in our population since there was similar mean drug exposure throughout the BSA range.

HDMTX CL showed an inverse relation with SCRt, in line with a strong dependence on renal function for drug elimination. The implementation of SCRt to describe the temporal change in MTX CL achieved a superior data description compared to only accounting for the baseline value (SCRb). Moreover, this approximation provides higher flexibility for changes in MTX elimination to be estimated during the same course of drug administration.

A novel contribution of this analysis is the quantitative characterization of the correlation between albumin and MTX CL. As a weak acid, MTX circulates in the bloodstream 50–60% bound to albumin, and only the unbound drug enters cells by transport and diffusion. First, we highlight the importance of hypoalbuminaemia as it was present in 68% of the courses in our cohort. Hypoalbuminaemia is a frequent characteristic reported in cancer patients that can be a reflection of the extent of the disease, resulting in higher vascular permeability and protein leaking into extracellular spaces. Also, reduced albumin synthesis or augmented proteolysis secondary to the release of proinflammatory cytokines has been reported.[49-51] Lastly, hypoalbuminaemia may result from previous treatment with asparaginase.[52,53] The impact of hypoalbuminaemia on individual MTX CL was significant: mean MTX CL reductions of 15, 25 and 34% are expected based on our estimations for blood albumin levels of 3.0, 2.5 and 2.0 g/dL, respectively. The underlying mechanisms for the relation between hypoalbuminaemia and reduced MTX CL have yet to be defined. Nonetheless, it may result in local kidney toxicity and reduced CL consequent to a higher unbound fraction. Lower serum albumin has been reported to be related to renal hypoperfusion[54] and was found to be an independent predictor of AKI, indicating that hypoalbuminaemia may causally contribute to renal failure.[55-57] Overall, these findings may have an important impact on HDMTX treatment management and, therefore, we urge clinicians to routinely assess serum albumin levels prior to HDMTX to inform dosing and reduce the probability of drug-induced toxicity.

Other important individual characteristics that showed a correlation with MTX CL but were not retained in the final model due to statistical reasons included concomitant administration of diuretics and TKIs, previous administration of platin-based chemotherapy, development of AKI in previous courses and age. The association with diuretics, specifically furosemide, and MTX toxicity is already described.[57] It is unclear whether the association of the administration of diuretics and MTX CL is because of the nephrotoxicity caused by diuretics or due to MTX-induced nephrotoxicity that triggers the clinical use of diuretics. The association between an increase in MTX CL and concomitant use of TKIs has also been described previously[58,59] and has caused some paediatric hospitals to hold TKIs from the start of HDMTX until it is cleared. This drug–drug interaction may be mediated by the SLCO1B1 transporter because both MTX and TKIs are substrates of the transporter and the TKIs may also regulate the transporter's function.[60-62] Previous administration of platin-based chemotherapy and prior AKI may predispose the patient to MTX-induced AKI requiring more frequent kidney function monitoring. We observed that patients with AKI showed a ∼20% reduction in CL for subsequent courses, putting them at higher risk for toxicity as detailed before.[4,63] Although age did have a small influence on MTX CL, it was not retained in the final model due to its correlation with creatinine.

Validation results indicate that the predictive performance of the model can be dependent on the evaluated population since variables such as the rescue protocol can influence MTX pharmacokinetics. The a priori use of the model (i.e., only using individual characteristics to predict the pharmacokinetic outcome) achieved an adequate mean prediction error but was imprecise. However, in the larger dataset reported by Kawakatsu et al.,[33] the model achieved a very good fit to the data after individual observations were considered. Thus, the model was successful in characterizing HDMTX disposition and we emphasize the need to measure MTX concentrations together with SCR and albumin to predict individual MTX pharmacokinetics.

HDMTX is associated with serious toxicities despite routine drug monitoring and supportive therapy. In adult patients, a wide range of AKI incidence is reported with values up to 60%.[64-66] In our population we observed that stage 2/3 AKI developed in 8.8% of the total courses with a higher incidence during the first and second infusions (13% for the first and 8.5% for the second course), which may be related to a genetic predisposition to AKI.[67] The clinical impact of developing stage 2/3 AKI is clear as half of the courses in which this severe event occurred, no further HDMTX courses were administered and 14% of the patients underwent a dose reduction that globally may decrease antitumour efficacy.

The incidence of stage 2/3 AKI was higher in short infusions compared to long infusions. Specifically, HDMTX-induced AKI episodes occurred in 0.5%–1.0% of courses in children with ALL receiving long infusions but doubled in patients with osteosarcoma most commonly treated with short HDMTX infusions.[4,67,68] The difference in the rate of nephrotoxicity could be explained by a saturation of renal elimination when high concentrations are reached during short infusions, resulting in direct tubular toxicity.

The relationship between HDMTX exposure and AKI has been previously reported. In our cohort, dose-corrected MTX concentrations at 24 hours (C24/D) and 48 hours (C48/D) were significantly correlated with the occurrence of stage 2/3 AKI, showing a superior performance over non-corrected concentrations (C24 and C48) which are often used to assess the individual MTX clearance without considering the dose nor the infusion length.

Limitations of the present study are inherent to the use of real-world data (RWD).[69] Available data were sparse and sampling times were variable throughout different courses, which may have increased experimental error. We mitigated missing data by imputing values, which may also have introduced error. These limitations have probably contributed to the high residual unexplained variability estimated in the final model. As this translates into uncertainty for individual predictions, translation into clinics should be done carefully including model validation stages using data from the targeted population. Our intention is to apply and further evaluate the developed population pharmacokinetic model in the clinical setting to provide real-time and individual patient-adapted guidance based on clinically relevant variables for adequate management of HDMTX infusions by caregivers.

5 | CONCLUSION

The developed population model characterized MTX pharmacokinetics in a large population of adult patients with various malignancies and dosing schemes. Bodyweight, time-varying serum creatinine and serum albumin explained significant interindividual and longitudinal variability in HDMTX pharmacokinetics and proved to be clinically relevant covariates to optimize MTX dosing and supportive care. This study expands the current knowledge of MTX population pharmacokinetics, a key step towards the development of a clinical pharmacotherapeutic decision support tool for adult patient management of HDMTX.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Manuel Ibarra https://orcid.org/0000-0002-0484-6367
Paula Schaiquevich https://orcid.org/0000-0002-2568-4731

REFERENCES

1. Rubenstein JL, Gupta NK, Mannis GN, Lamarre AK, Treseler P. How I treat CNS lymphomas. *Blood*. 2013;122(14):2318-2330. doi:10.1182/blood-2013-06-453084
2. Shapiro WR, Young DF, Mehta BM. Methotrexate: distribution in cerebrospinal fluid after intravenous, ventricular and lumbar injections. *N Engl J Med*. 1975;293(4):161-166. doi:10.1056/NEJM197507242930402
3. Barreto JN, Reid JM, Thompson CA, et al. Prospective evaluation of high-dose methotrexate pharmacokinetics in adult patients with lymphoma using novel determinants of kidney function. *Clin Transl Sci*. 2021;15(1):105-117. doi:10.1111/cts.13125
4. Howard SC, McCormick J, Pui C, et al. Preventing and managing toxicities of high-dose methotrexate. *Oncologist*. 2016;21(12):1471-1482. doi:10.1634/theoncologist.2015-0164
5. Ibarra M, Lorier M, Trocóniz IF. Pharmacometrics in precision dosing. In: *The ADME Encyclopedia*. Cham: Springer International Publishing; 2021:1-7. doi:10.1007/978-3-030-51519-5_175-1
6. Taylor ZL, Mizuno T, Punt NC, et al. MTXPK.org: a clinical decision support tool evaluating high-dose methotrexate pharmacokinetics to inform post-infusion care and use of glucarpidase. *Clin Pharmacol Ther*. 2020;108(3):635-643. doi:10.1002/cpt.1957
7. Shi Z, Liu Y, Gu H, et al. Population pharmacokinetics of high-dose methotrexate in Chinese pediatric patients with medulloblastoma. *Biopharm Drug Dispos*. 2020;41:101-110. doi:10.1002/bdd.2221
8. Desoky ESE, Ghazal MH, Singh RP, et al. Population pharmacokinetics of methotrexate in Egyptian children with lymphoblastic leukemia. *Pharmacol Pharm*. 2013;4(2):139-145. doi:10.4236/pp.2013.42020

9. Rühs H, Becker A, Drescher A, et al. Population PK/PD model of homocysteine concentrations after high-dose methotrexate treatment in patients with acute lymphoblastic leukemia. *PLoS ONE*. 2012; 7(9):1-8. doi:10.1371/journal.pone.0046015

10. Panetta JC, Roberts JK, Huang J, et al. Pharmacokinetic basis for dosing high-dose methotrexate in infants and young children with malignant brain tumours. *Br J Clin Pharmacol*. 2020;86(2):362-371. doi:10.1111/bcp.14160

11. Aquerreta I, Aldaz A, Giráldez J, Sierrasesúmaga L. Pharmacodynamics of high-dose methotrexate in pediatric patients. *Ann Pharmacother*. 2002;36(9):1344-1350. doi:10.1345/aph.1A446

12. Hui KH, Chu HM, Fong PS, Cheng WTF, Lam TN. Population pharmacokinetic study and individual dose adjustments of high-dose methotrexate in Chinese pediatric patients with acute lymphoblastic leukemia or osteosarcoma. *J Clin Pharmacol*. 2019;59(4):566-577. doi:10.1002/jcph.1349

13. Gao X, Qian XW, Zhu XH, et al. Population pharmacokinetics of high-dose methotrexate in Chinese pediatric patients with acute lymphoblastic leukemia. *Front Pharmacol*. 2021;12:1-9. doi:10.3389/fphar.2021.701452

14. Beechinor RJ, Thompson PA, Hwang MF, et al. 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. *Clin Pharmacokinet*. 2019;58(7):899-910. doi:10.1007/s40262-018-00734-0

15. Aumente D, Buelga DS, Lukas JC, et al. Population pharmacokinetics of high-dose methotrexate in children with acute lymphoblastic leukaemia. *Clin Pharmacokinet*. 2006;45(12):1227-1238. doi:10.2165/00003088-200645120-00007

16. Medellin-Garibay SE, Hernández-Villa N, Correa-González LC, et al. Population pharmacokinetics of methotrexate in Mexican pediatric patients with acute lymphoblastic leukemia. *Cancer Chemother Pharmacol*. 2020;85(1):21-31. doi:10.1007/s00280-019-03977-1

17. Widemann BC, Balis FM, Kempf-Bielack B, et al. High-dose methotrexate-induced nephrotoxicity in patients with osteosarcoma: incidence, treatment, and outcome. *Cancer*. 2004;100(10):2222-2232. doi:10.1002/cncr.20255

18. Crews KR, Liu T, Rodriguez-Galindo C, et al. High-dose methotrexate pharmacokinetics and outcome of children and young adults with osteosarcoma. *Cancer*. 2004;100(8):1724-1733. doi:10.1002/cncr.20152

19. Colom H, Farré R, Soy D, et al. Population pharmacokinetics of high-dose methotrexate after intravenous administration in pediatric patients with osteosarcoma. *Ther Drug Monit*. 2009;31(1):76-85. doi:10.1097/FTD.0b013e3181945624

20. Faganel Kotnik B, Grabnar I, Bohanec Grabar P, Dolžan V, Jazbec J. Association of genetic polymorphism in the folate metabolic pathway with methotrexate pharmacokinetics and toxicity in childhood acute lymphoblastic leukaemia and malignant lymphoma. *Eur J Clin Pharmacol*. 2011;67(10):993-1006. doi:10.1007/s00228-011-1046-z

21. Arshad U, Taubert M, Seeger-Nukpezah T, et al. Evaluation of body-surface-area adjusted dosing of high-dose methotrexate by population pharmacokinetics in a large cohort of cancer patients. *BMC Cancer*. 2021;21(1):1-10. doi:10.1186/s12885-021-08443-x

22. Comandone A, Passera R, Boglione A, Tagini V, Ferrari S, Cattel L. High dose methotrexate in adult patients with osteosarcoma: clinical and pharmacokinetic results. *Acta Oncol (Madr)*. 2005;44(4):406-411. doi:10.1080/02841860510029770

23. Dupuis C, Mercier C, Yang C, et al. High-dose methotrexate in adults with osteosarcoma: a population pharmacokinetics study and validation of a new limited sampling strategy. *Anticancer Drugs*. 2008;19(3):267-273. doi:10.1097/CAD.0b013e3282f21376

24. Faltaos DW, Hulot JS, Urien S, et al. Population pharmacokinetic study of methotrexate in patients with lymphoid malignancy. *Cancer Chemother Pharmacol*. 2006;58(5):626-633. doi:10.1007/s00280-006-0202-0

25. Fukuhara K, Ikawa K, Morikawa N, Kumagai K. Population pharmacokinetics of high-dose methotrexate in Japanese adult patients with malignancies: a concurrent analysis of the serum and urine concentration data. *J Clin Pharm Ther*. 2008;33(6):677-684. doi:10.1111/j.1365-2710.2008.00966.x

26. Joerger M, Huitema ADR, Van Den Bongard HJGD, et al. Determinants of the elimination of methotrexate and 7-hydroxy-methotrexate following high-dose infusional therapy to cancer patients. *Br J Clin Pharmacol*. 2006;62(1):71-80. doi:10.1111/j.1365-2125.2005.02513.x

27. Mei S, Li X, Jiang X, Yu K, Lin S, Zhao Z. Population pharmacokinetics of high-dose methotrexate in patients with primary central nervous system lymphoma. *J Pharm Sci*. 2018;107(5):1454-1460. doi:10.1016/j.xphs.2018.01.004

28. Pai MP, Debacker KC, Derstine B, Sullivan J, Su GL, Wang SC. Comparison of body size, morphomics, and kidney function as covariates of high-dose methotrexate clearance in obese adults with primary central nervous system lymphoma. *Pharmacotherapy*. 2020;40(4):308-319. doi:10.1002/phar.2379

29. Watanabe M, Fukuoka N, Takeuchi T, et al. Developing population pharmacokinetic parameters for high-dose methotrexate therapy: implication of correlations among developed parameters for individual parameter estimation using the Bayesian least-squares method. *Biol Pharm Bull*. 2014;37(6):916-921. doi:10.1248/bpb.b13-00672

30. Yang L, Wu H, de Winter BCM, et al. Pharmacokinetics and pharmacogenetics of high-dose methotrexate in Chinese adult patients with non-Hodgkin lymphoma: a population analysis. *Cancer Chemother Pharmacol*. 2020;85(5):881-897. doi:10.1007/s00280-020-04058-4

31. Johansson ÅM, Hill N, Perisoglou M, Whelan J, Karlsson MO, Standing JF. A population pharmacokinetic/pharmacodynamic model of methotrexate and mucositis scores in osteosarcoma. *Ther Drug Monit*. 2011;33(6):711-718. doi:10.1097/FTD.0b013e31823615e1

32. Holmboe L, Andersen AM, Mørkrid L, Slørdal L, Hall KS. High dose methotrexate chemotherapy: pharmacokinetics, folate and toxicity in osteosarcoma patients. *Br J Clin Pharmacol*. 2012;73(1):106-114. doi:10.1111/j.1365-2125.2011.04054.x

33. Kawakatsu S, Nikanjam M, Lin M, et al. Population pharmacokinetic analysis of high-dose methotrexate in pediatric and adult oncology patients. *Cancer Chemother Pharmacol*. 2019;84(6):1339-1348. doi:10.1007/s00280-019-03966-4

34. Min Y, Qiang F, Peng L, Zhu Z. High dose methotrexate population pharmacokinetics and Bayesian estimation in patients with lymphoid malignancy. *Biopharm Drug Dispos*. 2009;30(8):437-447. doi:10.1002/bdd.678

35. US Department of Health and Human Services, National Institutes of Health NCI. Common Terminology Criteria for Adverse Events (CTCAE). v.5.0. 2017.

36. Makris K, Spanou L. Acute kidney injury: definition, pathophysiology and clinical phenotypes. *Clin Biochem Rev*. 2016;37(2):85-98.

37. Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffé method. *Clin Chim Acta*. 2004;344(1-2):137-148. doi:10.1016/j.cccn.2004.02.007

38. Joerger M, Huitema ADR, Krähenbühl S, et al. Methotrexate area under the curve is an important outcome predictor in patients with primary CNS lymphoma: a pharmacokinetic-pharmacodynamic analysis from the IELSG no. 20 trial. *Br J Cancer*. 2010;102(4):673-677. doi:10.1038/sj.bjc.6605559

39. Broeker A, Nardecchia M, Klinker KP, et al. Towards precision dosing of vancomycin: a systematic evaluation of pharmacometric models for Bayesian forecasting. *Clin Microbiol Infect*. 2019;25(10):1286.e1-1286.e7. doi:10.1016/j.cmi.2019.02.029

40. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. *J Pharmacokinet Biopharm*. 1981;9(4):503-512. doi:10.1007/BF01060893

41. R Core Team. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing; 2018. https://www.r-project.org/

42. Beal SL. Ways to fit a PK model with some data below the quantitation limit. *J Pharmacokinet Pharmacodyn*. 2001;28(5):481-504. doi:10.1023/A:1012299115260

43. Zhang Y, Sun L, Chen X, Zhao L, Wang X, Zhao Z, Mei S. A systematic review of population pharmacokinetic models of methotrexate. *Eur J Drug Metab Pharmacokinet*. 2022;47(2):143–164. doi:10.1007/s13318-021-00737-6

44. Wright KD, Panetta JC, Onar-Thomas A, et al. Delayed methotrexate excretion in infants and young children with primary central nervous system tumors and postoperative fluid collections. *Cancer Chemother Pharmacol*. 2015;75(1):27-35. doi:10.1007/s00280-014-2614-6

45. Odoul F, Le Guellec C, Lamagnère JP, et al. Prediction of methotrexate elimination after high dose infusion in children with acute lymphoblastic leukaemia using a population pharmacokinetic approach. *Fundam Clin Pharmacol*. 1999;13(5):595-604. doi:10.1111/j.1472-8206.1999.tb00366.x

46. Holford N, Heo Y-A, Anderson B. A pharmacokinetic standard for babies and adults. *J Pharm Sci*. 2013;102(9):2941-2952. doi:10.1002/jps.23574

47. Anderson BJ, Holford NHG. Mechanism-based concepts of size and maturity in pharmacokinetics. *Annu Rev Pharmacol Toxicol*. 2008; 48(1):303-332. doi:10.1146/annurev.pharmtox.48.113006.094708

48. Livingston EH, Lee S. Body surface area prediction in normal-weight and obese patients. *Am J Physiol Endocrinol Metab*. 2001;281(3):586-591. doi:10.1152/ajpendo.2001.281.3.e586

49. Soeters PB, Wolfe RR, Shenkin A. Hypoalbuminemia: pathogenesis and clinical significance. *J Parenter Enteral Nutr*. 2019;43(2):181-193. doi:10.1002/jpen.1451

50. Hohloch K, Ziepert M, Truemper L, et al. Low serum albumin is an independent risk factor in elderly patients with aggressive B-cell lymphoma: results from prospective trials of the German High-Grade Non-Hodgkin's Lymphoma Study Group. *eJHaem*. 2020;1(1):181-187. doi:10.1002/jha2.61

51. Gupta D, Lis CG. Pretreatment serum albumin as a predictor of cancer survival: a systematic review of the epidemiological literature. *Nutr J*. 2010;9(1):1-16. doi:10.1186/1475-2891-9-69

52. Yang L, Panetta JC, Cai X, et al. Asparaginase may influence dexamethasone pharmacokinetics in acute lymphoblastic leukemia. *J Clin Oncol*. 2008;26(12):1932-1939. doi:10.1200/JCO.2007.13.8404

53. Albertsen BK, Schrøder H, Ingerslev J, et al. Comparison of intramuscular therapy with Erwinia asparaginase and asparaginase Medac: pharmacokinetics, pharmacodynamics, formation of antibodies and influence on the coagulation system. *Br J Haematol*. 2001;115(4):983-990. doi:10.1046/j.1365-2141.2001.03148.x

54. Mizuno T, Hayashi T, Shimabukuro Y, et al. Lower blood pressure-induced renal hypoperfusion promotes cisplatin-induced nephrotoxicity. *Oncologia*. 2016;90(6):313-320. doi:10.1159/000446371

55. Wiedermann CJ, Wiedermann W, Joannidis M. Causal relationship between hypoalbuminemia and acute kidney injury. *World J Nephrol*. 2017;6(4):176-187. doi:10.5527/wjn.v6.i4.176

56. Yu MY, Lee SW, Baek SH, et al. Hypoalbuminemia at admission predicts the development of acute kidney injury in hospitalized patients: a retrospective cohort study. *PLoS ONE*. 2017;12(7):1-14. doi:10.1371/journal.pone.0180750

57. Wiczer T, Dotson E, Tuten A, Phillips G, Maddocks K. Evaluation of incidence and risk factors for high-dose methotrexate-induced nephrotoxicity. *J Oncol Pharm Pract*. 2016;22(3):430-436. doi:10.1177/1078155215594417

58. Pommert L, Liberio N, Ng JS, et al. Concurrent imatinib dosing with high-dose methotrexate leads to acute kidney injury and delayed methotrexate clearance in pediatric patients with Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia. *J Pediatr Hematol Oncol*. 2021;43(2):e296-e300. doi:10.1097/MPH.0000000000001816

59. Ramsey LB, Mizuno T, Vinks AA, O'Brien MM. Delayed methotrexate clearance in patients with acute lymphoblastic leukemia concurrently receiving dasatinib. *Pediatr Blood Cancer*. 2019;66(5):1-5. doi:10.1002/pbc.27618

60. Hayden ER, Chen M, Pasquariello KZ, et al. Regulation of OATP1B1 function by tyrosine kinase-mediated phosphorylation. *Clin Cancer Res*. 2021;27(15):4301-4310. doi:10.1158/1078-0432.CCR-21-0023

61. Ramsey LB, Bruun GH, Yang W, et al. Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. *Genome Res*. 2012;22(1):1-8. doi:10.1101/gr.129668.111

62. Hu S, Mathijssen RHJ, De Bruijn P, et al. Inhibition of OATP1B1 by tyrosine kinase inhibitors: in vitro-in vivo correlations. *Br J Cancer*. 2014;110(4):894-898. doi:10.1038/bjc.2013.811

63. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. *Oncologist*. 2006;11(6):694-703. doi:10.1634/theoncologist.11-6-694

64. DeFino CE, Barreto JN, Pawlenty AG, et al. Lack of drug interaction between levetiracetam and high-dose methotrexate in patients with lymphoma. *Pharmacotherapy*. 2021;41(5):430-439. doi:10.1002/phar.2516

65. Barreto JN, Peterson KT, Barreto EF, et al. Early, empiric high-dose leucovorin rescue in lymphoma patients treated with sequential doses of high-dose methotrexate. *Support Care Cancer*. 2021;29(9):5293-5301. doi:10.1007/s00520-021-06106-y

66. May J, Carson KR, Butler S, Liu W, Bartlett NL, Wagner-Johnston ND. High incidence of methotrexate associated renal toxicity in patients with lymphoma: a retrospective analysis. *Leuk Lymphoma*. 2014;55(6):1345-1349. doi:10.3109/10428194.2013.840780

67. Christensen AM, Pauley JL, Molinelli AR, et al. Resumption of high-dose methotrexate after acute kidney injury and glucarpidase use in pediatric oncology patients. *Cancer*. 2012;118(17):4321-4330. doi:10.1002/cncr.27378

68. Svahn T, Mellgren K, Harila-Saari A, et al. Delayed elimination of high-dose methotrexate and use of carboxypeptidase G2 in pediatric patients during treatment for acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2017;64(7):1-7. doi:10.1002/pbc.26395

69. Miksad RA, Abernethy AP. Harnessing the power of real-world evidence (RWE): a checklist to ensure regulatory-grade data quality. *Clin Pharmacol Ther*. 2018;103(2):202-205. doi:10.1002/cpt.946

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