Urine volume to hydration volume ratio is associated with pharmacokinetics of high-dose methotrexate in patients with primary central nervous system lymphoma.

Received: 3 June 2021 | Accepted: 30 September 2021

DOI: 10.1002/prp2.883

ORIGINAL ARTICLE

Urine volume to hydration volume ratio is associated with pharmacokinetics of high-dose methotrexate in patients with primary central nervous system lymphoma

Tetsuichiro Isono[1] | Daiki Hira[1,2] | Aya Morikochi[1] | Tadateru Fukami[3] | Satoshi Ueshima[2] | Kazuhiko Nozaki[3] | Tomohiro Terada[1] | Shin-ya Morita[1]

[1]Department of Pharmacy, Shiga University of Medical Science Hospital, Otsu, Shiga, Japan

[2]College of Pharmaceutical Sciences, Ritsumeikan University, Kusatsu, Shiga, Japan

[3]Department of Neurosurgery, Shiga University of Medical Science, Otsu, Shiga, Japan

Correspondence
Shin-ya Morita, Department of Pharmacy, Shiga University of Medical Science Hospital, Seta Tsukinowa-cho, Otsu, Shiga 520-2192, Japan.
Email: smorita@belle.shiga-med.ac.jp

Daiki Hira, College of Pharmaceutical Sciences, Ritsumeikan University, 1-1-1 Noji-Higashi, Kusatsu, Shiga 525-8577, Japan.
Email: hirad@fc.ritsumei.ac.jp

Present address
Daiki Hira and Tomohiro Terada, Department of Clinical Pharmacology and Therapeutics, Kyoto University Hospital, Kyoto, Japan

Funding information
This work was partly supported by JSPS KAKENHI Grant Number 20H01038.

Abstract

High-dose methotrexate (HD-MTX)-based chemotherapy is the first-line treatment for primary central nervous system lymphoma (PCNSL), but is associated with severe adverse effects, including myelosuppression and renal impairment. MTX is primarily excreted by the kidneys. Renal function calculated using serum creatinine (Scr) derived from muscle may be overestimated in elderly PCNSL patients. Therefore, we aimed to construct a population pharmacokinetic model in PCNSL patients and explore the factors associated with MTX clearance. Sixteen PCNSL patients (median age, 66 years) treated with HD-MTX were included, and serum MTX concentrations were measured at 193 points in 49 courses. A population pharmacokinetic analysis was performed using NONMEM. A Monte Carlo simulation was conducted, in which serum MTX concentrations were stratified into three groups of creatine clearance (Ccr) (50, 75, and 100 ml/min) with three groups of the urine volume to hydration volume (UV/HV) ratio (<1, 1–2, and >2). The final model was constructed as follows: MTX clearance = $4.90 \cdot (\mathrm{Ccr}/94.5)^{0.456} \cdot (\mathrm{UV/HV})^{0.458}$. In the Monte Carlo simulation, serum MTX concentrations were below the standard values (10, 1, and 0.1 μM at 24, 48, and 72 h, respectively, after the start of the MTX administration) in most patients with UV/HV >2, even with Ccr of 50 ml/min. Conversely, half of the patients with UV/HV <1 and Ccr of 50 ml/min failed to achieve the standard values. The present results demonstrated that the UV/HV ratio was useful for describing the pharmacokinetics of MTX in PCNSL patients.

Abbreviations: AKI, acute kidney injury; ALL, acute lymphoblastic leukemia; BSA, body surface area; BW, body weight; CCB, calcium channel blockers; Ccr, creatinine clearance; CG, Cockcroft-Gault; CI, confidence interval; CL, clearance; CWRES, conditional weighted residuals; eGFR, estimated glomerular filtration rate; GOF, goodness of fit; HV, hydration volume; IIV, individual variability; IPRED, individual predicted concentrations; LEV, levetiracetam; LOQ, the limit of quantification; MTX, methotrexate; NONMEM, non-linear mixed effect modeling; NSAIDs, Non-Steroidal Anti-inflammatory Drugs; OFV, objective function value; PCNSL, primary central nervous system lymphoma; PK, pharmacokinetic; PPI, proton pump inhibitors; PRED, predicted concentrations; Q, intercompartmental clearance; Scr, serum creatinine, UV, urine volume; V1, the volume of the central compartment; V2, the volume of the peripheral compartment; VPC, visual predictive check.

KEYWORDS
creatinine clearance, high-dose methotrexate, hydration volume, population pharmacokinetics, primary central nervous system lymphoma, urine volume

1 | INTRODUCTION

The incidence of primary central nervous system lymphoma (PCNSL) has been increasing in recent years, accounting for 4.9% of all brain tumors.[1] PCNSL frequently develops in the elderly, with 50% of patients being 65 years or older at the time of onset.[2] The first-line treatment for PCNSL is high-dose methotrexate (HD-MTX)-based chemotherapy, followed by whole-brain irradiation, which has significantly increased median survival time, and is strongly recommended as induction therapy for PCNSL.[3,4] The rapid and high-dose intravenous infusion of MTX in HD-MTX therapy increases the penetration of MTX into the central nervous system via the blood-brain barrier, resulting in stronger antitumor effects in the central nervous system.[5] However, this treatment is associated with severe adverse effects, such as renal damage and myelosuppression, due to increased systemic exposure.[6,7] Therefore, serum MTX concentrations need to be monitored during HD-MTX therapy. To the risk of adverse effects, it needs to be below the standard values, such as 10 μM at 24 h, 1 μM at 48 h, and 0.1 μM at 72 h after the initiation of its administration.[6,8] Since MTX is mainly excreted by the kidneys, the evaluation of renal function is important. Patients with PCNSL are older than those with acute lymphoblastic leukemia (ALL) and osteosarcoma, whereas the efficacy of HD-MTX for ALL and osteosarcoma is similar to that for PCNSL.[9,10] Although HD-MTX therapy is more toxic in elderly PCNSL patients as described above, less than 10% of patients develop grade 3–4 adverse events. Therefore, the monitoring and appropriate control of renal function and serum MTX concentrations will contribute to the more widespread application of HD-MTX therapy to elderly PCNSL patients.[11] The renal function generally declines with advancing age, and, thus, reduced MTX excretion in PCNSL patients may exacerbate renal impairment and result in a vicious cycle in which renal impairment further decreases MTX excretion. In addition, since the elderly often have a reduced muscle mass, renal function based on serum creatinine (Scr) levels derived from muscle may be overestimated.[12,13] A population pharmacokinetic analysis of HD-MTX-treated patients is useful for reducing the risk of adverse effects. Although various population pharmacokinetic analyses of HD-MTX patients have already been conducted, the target patients were those with ALL, osteosarcoma, or pediatric cancer.[10,14–18] Only one population pharmacokinetic analysis of adult PCNSL patients has been performed to date.[19] Previously reported clearance covariates were Scr and body surface area (BSA), and target patients included children. Difficulties are associated with predicting serum MTX concentrations by a population pharmacokinetic analysis based solely on Scr in PCNSL patients, many of whom are elderly.

Therefore, the purpose of the present study was to establish a population pharmacokinetic model for HD-MTX in patients with PCNSL and explore the factors associated with MTX clearance in addition to Scr.

2 | MATERIALS AND METHODS

2.1 | Study design

The present study was designed as a single-center, retrospective observational clinical study for investigating the pharmacokinetics of MTX. The study protocol was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee at Shiga University of Medical Science (Approval Number R2020-086). Japanese adult inpatients with PCNSL who received HD-MTX at Shiga University of Medical Science Hospital between July 2015 and June 2020 were enrolled in the present study. Written informed consent was waived because of the anonymous nature of the data. As an ethical consideration, participants had been provided with the opportunity to opt out from this research based on written information posted on the homepage of Shiga University of Medical Science Hospital. To prevent renal impairment by MTX, HD-MTX was administered after urine alkalinization with intravenous sodium bicarbonate. The dose of MTX was fixed at 3500 mg/m^2 and administered in a 4-h continuous intravenous infusion. Hydration and alkalinization were continued for 3 days along with an oral carbonate dehydratase inhibitor (acetazolamide 250 mg) every 12 h. The protocol of hydration was standardized as 3000 ml/day from day 1 to day 4 of MTX administration. If the monitored serum MTX concentration at each time point exceeded the standard values (10 μM at 24 h, 1 μM at 48 h, and 0.1 μM at 72 h), either 3000 ml/day was added on day 5 without increasing HV, or HV was increased in the range of 3500 ml/day to 4000 ml/day. Only a patient with acute kidney injury (AKI) received a high dose of hydration (21 500 ml/day).

Twenty-four hours after the administration of MTX, calcium folinate rescue was administrated intravenously (15 mg) every 6 h until serum MTX concentrations were below 0.1 μM on or after 72 h.

2.2 | Data collection

MTX doses, serum MTX concentrations, Scr, urine volume (UV), and the following demographics were extracted from electronic medical records at Shiga University of Medical Science Hospital: age, weight, height, gender, hydration volume, the number of MTX

chemotherapy cycles, and concomitant drugs (Non-Steroidal Anti-inflammatory Drugs (NSAIDs), proton pump inhibitors (PPI), levetiracetam (LEV), and calcium channel blockers (CCB)). Serum MTX concentrations at 4, 24, 48, and 72 h after the infusion were assessed for most patients. The assessment of Scr measurements was generally repeated every 2 days until day 4 and weekly thereafter. UV in 1 day was measured by clamping the indwelling bladder catheter in all patients. Saline volume for the dilution of drugs or oral water intake was not included in HV because saline volume for the dilution of drugs were very small compared to the scheduled HV and the oral water intake was not recorded in patients with PCNSL. The ratio of UV to HV was shown as UV/HV. Creatinine clearance (Ccr) in each patient was calculated using the Cockcroft-Gault formula (CG formula) based on gender, age, body weight (BW), and Scr.[20] The combination of NSAIDs,[21] PPI,[22] LEV,[23] or CCB[24] with MTX has been associated with delayed MTX excretion and altered transporter activities.

2.3 | Sampling and assays

Serum MTX concentrations were measured using the ABBOTT ARCHITECT® analyzer i1000SR fluorescence polarization immunoassay (Abbott Laboratories). The limit of quantification (LOQ) was 0.04 μM. Serum MTX concentrations below LOQ were fixed at 0.02 μM (LOQ/2).[25,26]

2.4 | Population pharmacokinetic analysis

A population pharmacokinetic analysis was performed using nonlinear mixed effect modeling (NONMEM) program version 7.5.0 (Icon Development Solutions). The first-order conditional estimation method was used throughout the model-building procedure. Two- and three-compartment structural models with an exponential residual error model were considered. Two-compartment structural models were selected as the base model after considering objective function value (OFV) and goodness-of-fit (GOF) plots (ADVAN3 TRANS4). The following pharmacokinetic parameters were estimated: the volume of the central compartment (V1), clearance (CL), intercompartmental clearance (Q), and the volume of the peripheral compartment (V2). An exponential relationship was employed to model inter-individual variability (IIV) for pharmacokinetic parameters. Differences between the observed concentrations in individuals and their respective predictors were considered. A stepwise covariate modeling procedure was implemented using the two-compartment structural model. The stepwise inclusion of a covariate was based on a decrease in NONMEM OFV > 3.841 ($p < .05$). An OFV decrease of more than 3.841 from the basic structural model ($p < .05$, chi-squared test) was considered to be significant during the covariate screening process. The influences of covariates were investigated using the following equations:

Continuous variable

$$P_i = \theta_1 \cdot (\mathrm{COV}_i/\mathrm{COV}_{\mathrm{median}})^{\theta_5} \quad (1)$$

Categorical variable

$$P_i = \theta_1 \cdot \theta_5^{\mathrm{COV}_i} \quad (2)$$

where P_i represents a pharmacokinetic parameter of ith patients, COV_i and $\mathrm{COV}_{\mathrm{median}}$ denote the covariate of the ith patients and the median of the covariate, and θ_1 and θ_5 represent population mean estimates. Equation (1) represents continuous variables, such as Scr and UV, while Equation (2) denotes categorical variables, including concomitant drugs. $\mathrm{COV}_i = 1$ means that a concomitant drug is used. The diagnostic criteria for GOF included a decrease in OFV of at least 3.841, a reduction in unexplained inter-patient variability, randomly distributed conditional weighted residuals (CWRES), and a closer relationship between the predicted and observed concentrations. The full model was built by incorporating significant covariates and the final model was developed using a backward deletion method. The coefficients in the full model were excluded from the model one at a time, and an increase in OFV of more than 6.635 from the full model ($p < .01$, chi-squared test) was considered to be significant.

2.5 | Model evaluation

The following GOF plots were used to investigate the models: the relationship between the observed and population-predicted value (PRED) or individual-predicted value (IPRED), and the relationship between CWRES and time after dose or PRED. The final model was also assessed using a visual predictive check (VPC) and non-parametric bootstrap analysis to investigate the robustness of the final model. In the VPC analysis, 1000 hypothetical data sets were simulated by random sampling using the NONMEM program. The median and 90% prediction interval of the simulated concentrations were plotted using OBS. The bootstrap was used to investigate the ability to predict data. The bootstrap method was performed with Perl-speaks-NONMEM (version 7.5.0).[27] Individual data were randomly sampled to produce another dataset with the same size as the original dataset. In the bootstrap analysis, the median values and 95% prediction intervals of the parameters estimated using 1000 replication data sets were compared with population parameters obtained by the final model. The model was considered to be validated if no significant differences were observed.

2.6 | Monte Carlo simulation

Monte Carlo simulations were conducted using the final population pharamacokinetic model to assess the impact of the UV/HV ratio and Ccr on MTX excretion. Using the NONMEM program, 200 MTX

concentrations at 24, 48, and 72 h after its administration were simulated for patients with various Ccr (50, 75, and 100 ml/min) and UV/HV (<1, 1–2, and >2).

2.7 | Statistical analysis

Data are expressed as medians unless otherwise indicated. In multiple comparisons against a control group, significant differences were evaluated using the Kruskal–Wallis test, followed by IBM SPSS Statistics version 27. A probability value of less than .01 was considered to be significant.

3 | RESULTS

3.1 | Patient characteristics

Sixteen patients treated with HD-MTX for 49 courses were included in the population pharmacokinetic analysis. MTX concentrations were measured at 193 points (3–6 samples per course). Eighteen out of 193 points were below LOQ (0.04 μM) and fixed at half of LOQ (0.02 μM).[25,26] Patient characteristics are summarized in Table 1. MTX doses were fixed at 3500 mg/m^2, and the actual doses based on BSA ranged between 4970 and 6612 mg. The majority of PCNSL patients were elderly (median age of 66 years). Since hydration included only saline, UV was slightly higher than HV.

TABLE 1 Patient characteristics in the population pharmacokinetic study

	Median (range)
MTX concentrations measured	193
Number of courses	49
Gender (male/female)	12/4
Age (years)	66 (49.0–85.0)
Height (m)	1.66 (1.48–1.76)
Body weight (kg)	61.5 (48.4–77.4)
Body surface area (m^2)	1.68 (1.42–1.89)
Serum creatinine (mg/dl)	0.65 (0.20–0.81)
Creatinine clearance (ml/min)	91.3 (51.6–257.0)
MTX dose (mg)	5768 (4970–6612)
MTX dose per body surface area (mg/m^2)	3500
Number of courses per patient	3 (1–7)
Urine volume (ml)	4760 (1226–8166)
Hydration volume (ml)	3000 (1500–21 500)
Concomitant use	
Proton pump inhibitor	9
Levetiracetam	6
Calcium channel blocker	8

3.2 | Population pharmacokinetic analysis

The time after dose versus MTX concentrations is shown in Fig. S1. It was not a one-compartment model because semi-log plots were not a straight line. After 48 and 72 h, some points exceeded the standard values. MTX concentration-time data were best described by a two-compartment model with first-order elimination (OFV = 247.149). In addition, since there were few blood-sampling points (median of 4 points), two-compartment structural models were selected. Although IIV estimation parameters (CL and V1) were not significant (OFV = 245.802), IIV estimation parameters (CL and V2, CL and Q) were significant (OFV = 223.616, 222.298, respectively). Since the latter had a large ω^2 of Q (0.395), IIV estimation parameters were CL and V2. The OFV of an exponential error model was less (OFV = 196.659) than that of a combined error model with an exponential/additive component (OFV =223.616); however, ω^2 of Q was >1 in an exponential error model. The OFV of an additional error model did not converge. As described above, a combined error model was selected. The model building process is summarized in Table 2. The screening of the different covariates showed that Scr, Ccr, eGFR, age, and BSA reduced OFV, and Ccr exerted a stronger effect. CL was markedly influenced by UV, HV, and UV/HV, and UV/HV was significantly higher than UV or HV. BW, the number of MTX chemotherapy cycles, and concomitant drugs (NSAIDs, PPI, LEV, or CCB) did not induce a significant decrease in OFV. The effect of NSAIDs was not able to be analyze because none of the patients was administered aspirin or other non-steroidal anti-inflammatory drugs in the present study. There were no significant covariates in the volume of distribution. As a result, Ccr and UV/HV were selected and used as the full model. Ccr and UV/HV both had large effects among the indicators of renal function. Ccr or UV/HV in the full model was excluded from the model one at a time, and OFV increased by more than 6.635 from the full model; therefore, the full model was set as the final model. Although multicollinearity was considered between Ccr and UV/HV, no correlation was found between the two parameters (Fig. S2). Therefore, Ccr and UV/HV were both incorporated into the final model. Covariance between inter-individual variability for CL and that for V2 was 27.0%, and the correlation coefficient between individual CL and V2 was .621. The final model was shown by the following equation: $CL = 4.90 \cdot (Ccr/94.5)^{0.456} \cdot (UV/HV)^{0.458}$.

3.3 | Model evaluation

GOF plots for the base and final models are shown in Figure 1. In the final model (Figure 1C,D), PRED and IPRED correlated more strongly with OBS than those in the base model (Figure 1A,B). No systematic deviation was observed in the relationship between CWRES and time after dose or PRED in the final model (Figure 1E,F). Predictive accuracy was lower in the lower concentration area (<0.1 μM) than that in the higher concentration area because concentrations below the LOQ were fixed

TABLE 2 Summarized pharmacokinetic model building steps (on CL)

No.	Equation of CL	OFV	ΔOFV versus Base model	*P* value
Base model	θ_1	223.616		
1	$\theta_1 \cdot (\text{Scr}/0.65)^{\theta_5}$	209.894	−13.722	<.01
2	$\theta_1 \cdot (\text{Age}/66)^{\theta_5}$	211.676	−11.94	<.01
3	$\theta_1 \cdot (\text{Ccr}/94.5)^{\theta_5}$	184.178	−39.438	<.01
4	$\theta_1 \cdot (\text{eGFR}/87.7)^{\theta_5}$	204.067	−19.549	<.01
5	$\theta_1 \cdot (\text{BSA}/1.68)^{\theta_5}$	219.543	−4.073	<.05
6	$\theta_1 \cdot (\text{BW}/61.5)^{\theta_5}$	222.654	−0.962	n.s.
7	$\theta_1 \cdot (\text{UV}/4760)^{\theta_6}$	−69.827	−293.443	<.01
8	$\theta_1 \cdot (\text{HV}/3000)^{\theta_6}$	−118.658	−342.274	<.01
9	$\theta_1 \cdot (\text{UV}/\text{HV})^{\theta_6}$	−127.322	−350.938	<.01
10	$\theta_1 \cdot (1 - \theta_7 \cdot \text{MTXNUM})$	222.951	−0.665	n.s.
11	$\theta_1 \cdot \theta_8(\text{PPI})$	223.500	−0.116	n.s.
12	$\theta_1 \cdot \theta_8(\text{LEV})$	220.667	−2.949	n.s.
13	$\theta_1 \cdot \theta_8(\text{CCB})$	220.725	−2.891	n.s.
Full (=Final) model	$\theta_1 \cdot (\text{Ccr}/94.5)^{\theta_5} \cdot (\text{UV}/\text{HV})^{\theta_6}$	−160.148	−383.764	<.01

Note: Equations show the clearance of methotrexate.

The minimum value of OFV is listed (−2 log likelihood) for each NONMEM run.

OFV was significantly lower in the full model than in Models 3 and 6 ($p < .01$).

Abbreviations: BSA, body surface area; CCB, calcium channel blocker; Ccr, creatinine clearance; eGFR, estimated glomerular filtration rate; HV, hydration volume; LEV, levetiracetam; MTXNUM, number of MTX chemotherapy cycles; n.s., Not significant; PPI, proton pump inhibitor; Scr, serum creatinine; UV, urine volume.

at 0.02 μM. CWRES was within an acceptable range (−3.03 to 3.16), with a mean and variance that were very close to zero and unity, respectively. The final model estimates are shown in Table 3. IIV (Shrinkage) for CL and V2 were 23.5% (13%) and 16.1% (20%), respectively. The final model was also assessed by 1000 bootstrap resamplings. The median values of the bootstrap procedure were similar to the parameter estimates obtained from the original dataset. The final model was further evaluated using a VPC analysis (Fig. S3). The VPC analysis generally indicated the reasonable predictability of the final model.

As shown in Figure 2, when Ccr values were set to the typical value (from 10 to 300 ml/min), the population mean of CL in patients with UV/HV = 3 was 9.63 ± 2.97 L/h, and 2.27-, 1.65-, and 1.20-fold higher than in patients with UV/HV = 0.5, 1, and 2, respectively.

3.4 | Monte Carlo simulation

To simulate serum MTX concentrations using the final population pharmacokinetic parameters, data sets were divided into three groups according to UV/HV as a virtual patient population as follows: patients with UV/HV <1, patients with UV/HV = 1–2, and patients with UV/HV >2. As shown in Figure 3A, the median predicted concentration in UV/HV <1 at 24 h decreased from 12.3 to 5.5 μM when Ccr values changed from 50 to 100 ml/min. Additionally, the median predicted concentration in Ccr = 50 ml/min at 24 h decreased from 12.3 to 4.3 μM when UV/HV changed from <1 to >2. The median predicted concentrations in UV/HV >2 were significantly lower than those in UV/HV <1 and UV/HV = 1–2 at all Ccr values ($p < .001$). Furthermore, the median predicted concentrations in UV/HV >2 were below the standard values at 24 h (10 μM), 48 h (1.0 μM), and 72 h (0.1 μM), regardless of Ccr values (Figure 3B,C). The median predicted concentrations in Ccr ≥100 ml/min were also below the criteria values regardless of the UV/HV ratios.

4 | DISCUSSION

The population pharmacokinetic analysis conducted in the present study revealed that UV/HV has a major impact on MTX excretion in PCNSL patients in addition to Ccr. Bootstrap and VPC analyses indicated that the robustness and accuracy of the final model were acceptable.

Since MTX is mainly excreted unchanged from the kidneys, serum MTX concentrations are slightly higher in patients with impaired renal function.[12,14,16] Therefore, eGFR (estimated glomerular filtration rate), Ccr, and Scr have been identified as important factors affecting MTX pharmacokinetics in various population pharmacokinetic analyses.[12,14,28,29] Scr and eGFR were also significant in the present study; however, the most important factor was Ccr. Therefore, Ccr based on Scr influences MTX pharmacokinetics in PCNSL patients. In addition to previous findings, the present study demonstrated for the first time that a higher UV/HV ratio was associated with greater MTX excretion. Other covariates, such as BSA,[30] BW,[18] and age,[18] have been reported to affect the pharmacokinetics of MTX. In the present study, we also analyzed BSA, BW, and age in a covariate analysis, and age and BSA,

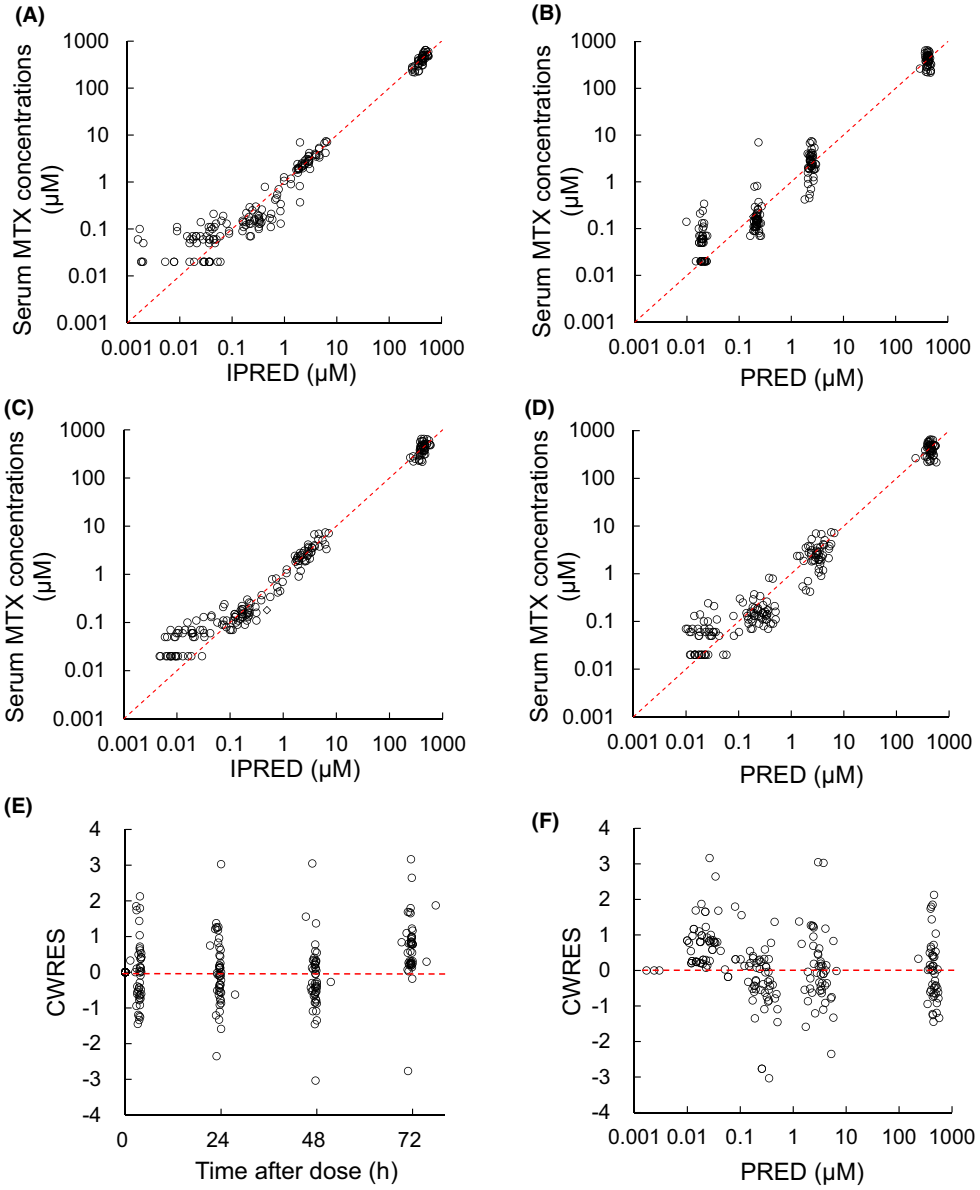

FIGURE 1 Scatter plots of the goodness-of-fit for the base model and final model. Observed concentrations versus individual-predicted (IPRED) concentrations for the base model (A); observed concentrations versus population-predicted (PRED) concentrations for the base model (B); observed concentrations versus IPRED concentrations for the final model (C); observed concentrations versus PRED concentrations for the final model (D); conditional weighted residuals versus time after the dose (E); conditional weighted residuals versus PRED concentrations (F). Open circles indicate observed values. Each dotted line shows a line of identity

but not BW, were significant. However, since age and physical size were included in Ccr calculated from the CG formula,[20] age and BSA were not included in the full model. We did not investigate gender differences because the number of female ($n = 4$) was much smaller than male ($n = 12$) and there might be a multicollinear relationship in CCr calculated from the CG formula (multiplied by 0.85 in female).[20] A previous study on children also found that the number of MTX chemotherapy cycles before the infusion of MTX significantly affected its clearance.[14] However, the number of MTX chemotherapy cycles was not significant in this study. In the elderly, the effect of cycles may be negligible. Therefore, MTX clearance in the first course may be applicable to the second and subsequent courses.

Serum MTX concentration are high in patients with impaired renal function.[12,14,16] Therefore, the dosage administered needs to be adjusted based on renal function. Since UV is an index of renal function, it is included in the formula for inulin clearance and Ccr by 24-h urine collection.[31,32] These clearance values are generally not utilized in clinical practice because they are complex, expensive, and inaccurate.[31–33] Therefore, renal function is often evaluated by measuring Scr and Ccr using the CG formula,[20] and eGFR by Scr, age, BW, and BSA. However, since Scr is derived from muscle, Scr in elderly patients with a reduced muscle mass, such as sarcopenia or frailty, may be lower than that in young patients, resulting in the overestimation of renal function. Previous studies suggested that

TABLE 3 Population pharmacokinetic parameter estimates of methotrexate in the final model

		Final model		Bootstrap (n = 1000)		
Parameter		Estimates	95% CI	IIV% (shrinkage %)	Median	95% CI
CL (L/h)	θ_1	4.900	4.02–5.78	23.5 (13)	4.90	4.00–6.75
V1 (L)	θ_2	9.010	5.76–12.26	—	9.08	4.31–17.03
V2 (L)	θ_3	5.730	3.83–7.63	16.1 (20)	5.59	0.77–7.73
Q (L/h)	θ_4	0.669	0.42–0.69	—	0.63	0.043–0.93
Ccr on CL	θ_5	0.456	0.22–0.69	—	0.45	0.12–0.93
UV/HV on CL	θ_6	0.458	0.39–0.53	—	0.43	0.03–0.54
σ (CV)						
Proportional (%)		25.259	25.2–25.3	— (6)	24.842	18.1–34.7
Additive (μM)		0.050	0.049–0.050	— (6)	0.047	0.006–0.062

Note: σ values denote intra-individual variability.

95% CI values were derived from asymptotic SE produced by NONMEM.

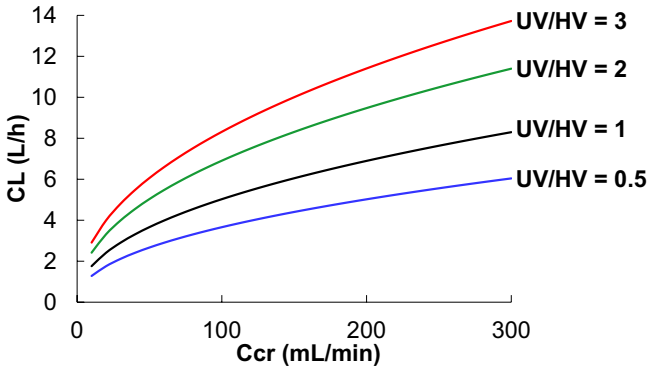

FIGURE 2 Impact of creatinine clearance and the urine volume/hydration volume ratio on methotrexate clearance. Correlation between the population mean estimates of methotrexate and creatinine clearance (Ccr) in the final model. Blue, black, green, and red lines indicate population mean estimates for a typical patient with a urine volume/hydration volume ratio = 0.5, 1, 2, and 3, respectively

renal function may not be accurately evaluated.[12,13] In the present study, some patients had Ccr higher than 150 ml/min. Furthermore, the clearance of MTX for a typical patient (Ccr value of 94.5 ml/min) in the present study was estimated to be 4.9 L/h (81.7 ml/min). The protein binding of MTX in serum has been reported to be in the range from 49 to 97%[34,35] and the renal clearance corrected by unbound MTX concentration is estimated to be in the range from 160 to 2723 ml/min. The estimated MTX clearance is significantly higher than the Ccr, which is an index of glomerular filtration rate in renal function. Therefore, in estimating MTX clearance, it is necessary to consider not only Ccr but also the contribution of proximal tubular secretion.

The incidence of PCNSL is high in the elderly, 50% of whom are 65 years or older at the time of onset.[2] In consideration of brain fragility in elderly patients, a treatment regimen without whole-brain irradiation has been assessed in Phase II trials.[36,37] In the present study, the majority of PCNSL patients were elderly with a median age of 66 years, and an accurate evaluation of renal function was required. Although the toxicity of HD-MTX therapy in the elderly is a concern, less than 10% of patients developed grade 3–4 adverse events.[5] Under properly managed serum MTX concentrations and renal function, HD-MTX-based chemotherapy is tolerable and effective as a remission induction therapy for PCNSL.[11] Many population pharmacokinetic analyses of HD-MTX patients have been conducted to date; however, the majority involved ALL, osteosarcoma, and pediatric cancers.[10,14–18] Only one analysis examined adult PCNSL patients,[19] which facilitated the estimation of serum MTX concentrations in PCNSL patients. However, in that study, the clearance covariate was assessed from Scr, and renal function may not have been accurately evaluated. Furthermore, the target patients included children.

The present study on elderly PCNSL patients demonstrated for the first time that UV/HV evaluations improved the descriptive ability of MTX pharmacokinetics in addition to creatinine-based renal function. MTX is administered with large volume of saline hydration containing sodium bicarbonate to prevent MTX accumulation in the tubules, and serum MTX concentrations were shown to be significantly lower in patients receiving greater hydration.[38,39] Therefore, it is reasonable to incorporate HV as a covariate in the model. In addition, in the case of decreased UV after administration of HD-MTX, a large volume of saline hydration is recommended to increase UV and reduce serum MTX concentrations.[40] HV and UV are inextricably linked, as UV increases in response to an increase in HV. In the present study, although UV was also a significant factor in increasing CL, UV/HV improved the model fit compared to evaluating each of UV and HV alone (Table 2). Therefore, UV/HV was incorporated as a covariate in the final model. The physiological implication by which UV/HV increases CL remains unclear. In the present study, there were inter- and intra-individual differences in UV/HV, suggesting that some factors other than glomerular filtration, such as tubular secretion, may be involved. This constructed descriptive model was able to represent

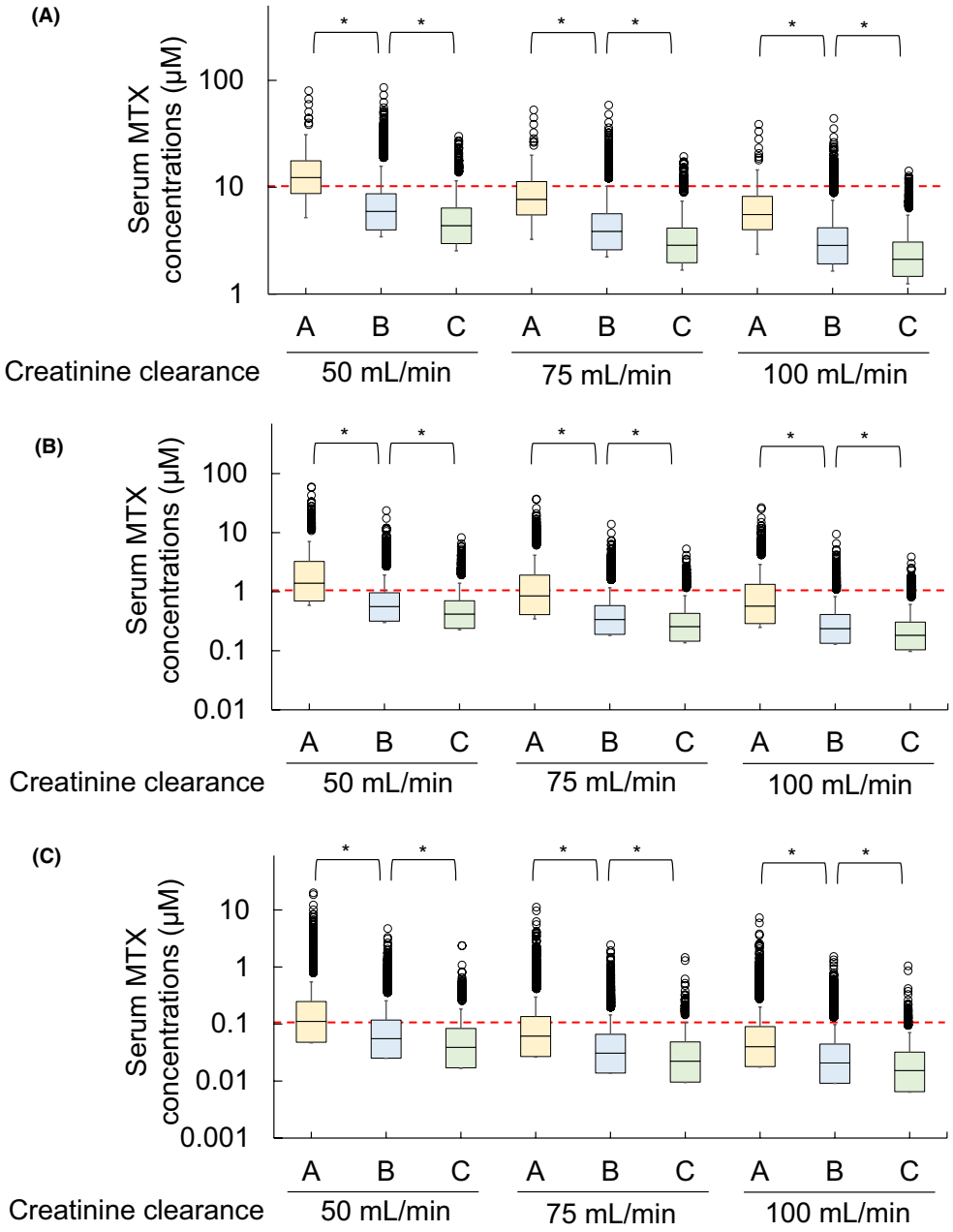

FIGURE 3 Simulations of serum concentrations of methotrexate at 24 h (A), 48 h (B), and 72 h (C) after dose administration in 200 replication data sets from 16 patients administered 3500 mg/m^2. These simulations were conducted using the final model. Box-and-whisker plots are presented according to Tukey's style. Open circles show outliers. Three groups consisted of patients with a urine volume to hydration volume ratio <1 (A), 1–2 (B), and >2 (C). The red dotted lines indicate standard lines (10 μM at 24 h, 1 μM at 48 h, and 0.1 μM at 72 h after the start of the methotrexate administration). *$p < .001$ by the Kruskal–Wallis test, followed by Dunn's multiple comparison test

the inter- and intra-individual differences in MTX clearance by UV/HV. UV/HV reflects the variation in urine volume relative to hydration volume and is an empirically useful index for evaluating MTX pharmacokinetics only by measuring UV during the MTX administration period. In addition, the effect of hydration on serum MTX concentrations has not been evaluated quantitatively.[38,39] The present study supports the importance of determining UV/HV. Furthermore, a decrease in UV/HV may help identify patients with early AKI after HD-MTX administration, even before creatinine increases. On the other hand, whether urine volume reflects renal impairment is controversial. Some reports suggest that MTX does not cause non-oliguric renal impairment.[41] Others suggest that measuring urine volume is important to prevent adverse effects,[40,42] which is supported by the results of the present study. Further study may be needed to clarify the mechanism of UV/HV interaction with MTX CL. In addition, we did not specify the oral water intake, which suggests that the oral water intake varies among individuals. Because, if the oral water intake changes, the amount of water entering the body can vary greatly from patient to patient even with the same amount of HV, we should not ignore the effect of the oral water intake on clearance. It is necessary to

construct the population pharmacokinetic model with HV including the intake volume of fluids in the future.

In Monte Carlo simulations, we visualized the effects of Ccr and UV/HV to achieve less than the standard values (10, 1, and 0.1 μM) at 24, 48, and 72 h, respectively, after the administration of MTX. As shown in Figures 2 and 3, UV/HV markedly affected CL. Even if patients have Ccr = 50 ml/min and UV/HV >2, the MTX concentration may be below the standard values, which is expected to reduce the risk of adverse effects due to MTX. On the other hand, since the MTX concentration is unlikely to decrease below the standard values with Ccr = 50 ml/min and UV/HV <1, further efforts are needed to reduce the risk of adverse effects.

No covariate to the volume of distribution was observed. Since the dose was fixed (3500 mg/m^2) for all target patients, the effects of BSA and BW may have been concealed.

Previous studies reported the involvement of various transporters in the excretion of MTX, and fluctuations in CL due to genetic polymorphisms.[43–45] Many Japanese individuals have mutations in *ABCG2* and *SLCO1B1* (*OATP1B1*).[46,47] MTX clearance was found to be reduced in patients with *SLCO1B1* mutations.[43] These issues were not examined in the present study; therefore, further research is needed on the effects of genetic polymorphisms in transporters on MTX pharmacokinetics. In addition, we investigated the effects of concomitant drugs. The combination of PPI,[22] LEV,[23] or CCB[24] was previously reported to be associated with delayed MTX excretion and altered transporter activities. However, other studies demonstrated that the combined use of PPI did not affect the excretion of MTX.[48] In the present study, the combined use of PPI did not have any significant effects, similar to LEV or CCB. It is important to note that the antiepileptic drug LEV did not affect the clearance of MTX. PCNSL patients often develop epilepsy due to brain disorders,[49] requiring the administration of antiepileptic drugs that do not affect MTX clearance.

A limitation of this study is the small number of patients enrolled (male/female; 12/4) although the total MTX concentrations measured was 193. Since no significant effects of concomitant drugs and other factors were observed in these patients, further comprehensive analysis including concomitant drugs need to be conducted in a larger number of patients.

5 | CONCLUSIONS

The present study demonstrated that newly constructed population pharmacokinetic parameters in PCNSL patients appropriately reflect the pharmacokinetic characteristics of MTX, and that the UV/HV ratio was useful for describing pharmacokinetics in PCNSL patients. In addition, the UV/HV ratio may be associated with the excretion of MTX not only in PCNSL patients, but also in ALL, osteosarcoma, and pediatric cancer patients. Therefore, we intend to examine the relationships of UV/HV ratio with the pharmacokinetics of MTX in all diseases.

DISCLOSURE

None of the authors have any conflicts of interest to declare that may be relevant to the contents of this manuscript.

AUTHORS' CONTRIBUTIONS

All authors had access to the data and a role in writing this manuscript. Tetsuichiro Isono, Daiki Hira, Aya Morikochi, Tomohiro Terada, and Shin-ya Morita contributed to the study conception and design. Tetsuichiro Isono, Daiki Hira, Aya Morikochi, Tadateru Fukami, and Kazuhiko Nozaki were involved in the collection of data. Tetsuichiro Isono, Daiki Hira, Aya Morikochi, and Satoshi Ueshima analyzed the data. Tetsuichiro Isono and Daiki Hira drafted the manuscript. Tadateru Fukami, Satoshi Ueshima, Kazuhiko Nozaki, Tomohiro Terada, and Shin-ya Morita critically revised the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

The study protocol was approved by the Institutional Review Board of Shiga University of Medical Science (Approval Number R2020-086). As an ethical consideration, participants were provided with the opportunity to opt out from the study based on written information posted on the homepage of Shiga University of Medical Science Hospital.

DATA AVAILABILITY STATEMENT

All data analyzed during this study are included in this published article and supplemental materials.

ORCID

Tetsuichiro Isono https://orcid.org/0000-0001-5771-3819
Daiki Hira https://orcid.org/0000-0001-8344-2469
Shin-ya Morita https://orcid.org/0000-0003-4079-707X

REFERENCES

1. Brain Tumor Registry of Japan (2005-2008). *Neurol Med Chir*. 2017;57(suppl 1):9-102. doi:10.2176/nmc.sup.2017-0001
2. Panageas KS, Elkin EB, DeAngelis LM, Ben-Porat L, Abrey LE. Trends in survival from primary central nervous system lymphoma, 1975–1999: a population-based analysis. *Cancer*. 2005;104(11):2466-2472. doi:10.1002/cncr.21481
3. Hiraga S, Arita N, Ohnishi T, et al. Rapid infusion of high-dose methotrexate resulting in enhanced penetration into cerebrospinal fluid and intensified tumor response in primary central nervous system lymphomas. *J Neurosurg*. 1999;91(2):221-230. doi:10.3171/jns.1999.91.2.0221
4. O'Brien PC, Roos DE, Pratt G, et al. Combined-modality therapy for primary central nervous system lymphoma: long-term data from a Phase II multicenter study (Trans-Tasman Radiation Oncology Group). *Int J Radiat Oncol Biol Phys*. 2006;64(2):408-413. doi:10.1016/j.ijrobp.2005.07.958
5. Jahnke K, Doolittle ND, Muldoon LL, Neuwelt EA. Implications of the blood-brain barrier in primary central nervous system lymphoma. *Neurosurg Focus*. 2006;21(5):1-11. doi:10.3171/foc.2006.21.5.12
6. Stoller RG, Hande KR, Jacobs SA, Rosenberg SA, Chabner BA. Use of plasma pharmacokinetics to predict and prevent methotrexate

toxicity. *N Engl J Med*. 1977;297(12):630-634. doi:10.1056/nejm197709222971203
7. 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
8. Nirenberg A, Mosende C, Mehta BM, Gisolfi AL, Rosen G. High dose methotrexate with citrovorum factor rescue: predictive value of serum methotrexate concentrations and corrective measures to avert toxicity. *Cancer Treat Rep*. 1977;61(5):779-783. https://pubmed.ncbi.nlm.nih.gov/302143/
9. Sakura T, Hayakawa F, Sugiura I, et al. High-dose methotrexate therapy significantly improved survival of adult acute lymphoblastic leukemia: a phase III study by JALSG. *Leukemia*. 2018;32(3):626-632. doi:10.1038/leu.2017.283
10. 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*. 2005;44(4):406-411. doi:10.1080/02841860510029770
11. Zhu J-J, Gerstner ER, Engler DA, et al. High-dose methotrexate for elderly patients with primary CNS lymphoma. *Neuro Oncol*. 2009;11(2):211-215. doi:10.1215/15228517-2008-067
12. Baxmann AC, Ahmed MS, Marques NC, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol*. 2008;3(2):348-354. doi:10.2215/CJN.02870707
13. Burkhardt H, Bojarsky G, Gretz N, Gladisch R. Creatinine clearance, Cockcroft-Gault formula and cystatin C: estimators of true glomerular filtration rate in the elderly? *Gerontology*. 2002;48(3):140-146. doi:10.1159/000052832
14. Zhang W, Zhang Q, Tian X, et al. Population pharmacokinetics of high-dose methotrexate after intravenous administration in Chinese osteosarcoma patients from a single institution. *Chin Med J*. 2015;128(1):111-118. doi:10.4103/0366-6999.147829
15. 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
16. Ye M, Fu Q, Li P, 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
17. 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
18. 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
19. 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
20. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16(1):31-41. doi:10.1159/000180580
21. Thyss A, Kubar J, Milano G, Namer M, Schneider M. Clinical and pharmacokinetic evidence of a life-threatening interaction between methotrexate and ketoprofen. *Lancet*. 1986;1(8475):256-258. doi:10.1016/S0140-6736(86)90786-5
22. Suzuki K, Doki K, Homma M, et al. Co-administration of proton pump inhibitors delays elimination of plasma methotrexate in high-dose methotrexate therapy. *Br J Clin Pharmacol*. 2009;67(1):44-49. doi:10.1111/j.1365-2125.2008.03303.x
23. Bain E, Birhiray RE, Reeves DJ. Drug-drug interaction between methotrexate and levetiracetam resulting in delayed methotrexate elimination. *Ann Pharmacother*. 2014;48(2):292-296. doi:10.1177/1060028013511951
24. Inose R, Takahashi K, Nanno S, Hino M, Nagayama K. Calcium channel blockers possibly delay the elimination of plasma methotrexate in patients receiving high-dose methotrexate therapy. *J Chemother*. 2019;31(1):30-34. doi:10.1080/1120009X.2018.1544194
25. Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn*. 2001;28(5):481-504. doi:10.1023/A:1012299115260
26. Bergstrand M, Karlsson MO. Handling data below the limit of quantification in mixed effect models. *AAPS J*. 2009;11(2):371-380. doi:10.1208/s12248-009-9112-5
27. Lindbom L, Pihlgren P, Jonsson N. PsN-Toolkit - a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Comput Methods Programs Biomed*. 2005;79(3):241-257. doi:10.1016/j.cmpb.2005.04.005
28. 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
29. 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
30. 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
31. Gerber DE, Grossman SA, Batchelor T, Ye X. Calculated versus measured creatinine clearance for dosing methotrexate in the treatment of primary central nervous system lymphoma. *Cancer Chemother Pharmacol*. 2007;59(6):817-823. doi:10.1007/s00280-006-0339-x
32. Bjornsson TD. Use of serum creatinine concentrations to determine renal function1. *Clin Pharmacokinet*. 1979;4(3):200-222. doi:10.2165/00003088-197904030-00003
33. Gaspari F, Perico N, Remuzzi G. Application of newer clearance techniques for the determination of glomerular filtration rate. *Curr Opin Nephrol Hypertens*. 1998;7(6):675-680. doi:10.1097/00041552-199811000-00009
34. Steele WH, Lawrence JR, Stuart JFB, McNeill CA. The protein binding of methotrexate in the serum of patients with neoplastic disease. *Cancer Chemother Pharmacol*. 1981;7(1):61-64. doi:10.1007/BF00258215
35. Bratlid D, Moe PJ. Pharmacokinetics of high-dose methotrexate treatment in children. *Eur J Clin Pharmacol*. 1978;14(2):143-147. doi:10.1007/BF00607446
36. Omuro A, Chinot O, Taillandier L, et al. Methotrexate and temozolomide versus methotrexate, procarbazine, vincristine, and cytarabine for primary CNS lymphoma in an elderly population: an intergroup ANOCEF-GOELAMS randomised phase 2 trial. *Lancet Haematol*. 2015;2(6):e251-e259. doi:10.1016/S2352-3026(15)00074-5
37. Fritsch K, Kasenda B, Schorb E, et al. High-dose methotrexate-based immuno-chemotherapy for elderly primary CNS lymphoma patients (PRIMAIN study). *Leukemia*. 2017;31(4):846-852. doi:10.1038/leu.2016.334
38. Christensen ML, Rivera GK, Crom WR, Hancock ML, Evans WE. Effect of hydration on methotrexate plasma concentrations in children with acute lymphocytic leukemia. *J Clin Oncol*. 1988;6(5):797-801. doi:10.1200/JCO.1988.6.5.797
39. Traivaree C, Likasitthananon N, Monsereenusorn C, Rujkijyanont P. The effect of intravenous hydration strategy on plasma

methotrexate clearance during intravenous high-dose methotrexate administration in pediatric oncology patients. *Cancer Manag Res*. 2018;10:4471-4478. doi:10.2147/CMAR.S172117

40. Howard SC, McCormick J, Pui C, Buddington RK, Harvey RD. Preventing and managing toxicities of high-dose methotrexate. *Oncologist*. 2016;21(12):1471-1482. doi:10.1634/theoncologist.2015-0164
41. Abelson HT, Fosburg MT, Beardsley GP, et al. Methotrexate-induced renal impairment: clinical studies and rescue from systemic toxicity with high-dose leucovorin and thymidine. *J Clin Oncol*. 1983;1(3):208-216. doi:10.1200/JCO.1983.1.3.208
42. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. *Oncologist*. 2006;11(6):694-703. doi:10.1634/theoncologist.11-6-694
43. Ramsey LB, Panetta JC, Smith C, et al. Genome-wide study of methotrexate clearance replicates SLCO1B1. *Blood*. 2013;121(6):898-904. doi:10.1182/blood-2012-08-452839
44. Chen M, Chen W, Liu P, et al. The impacts of gene polymorphisms on methotrexate in Chinese psoriatic patients. *J Eur Acad Dermatol Venereol*. 2020;34(9):2059-2065. doi:10.1111/jdv.16440
45. Vlaming MLH, van Esch A, van de Steeg E, et al. Impact of Abcc2 [multidrug resistance-associated protein (Mrp) 2], Abcc3 (Mrp3), and Abcg2 (breast cancer resistance protein) on the oral pharmacokinetics of methotrexate and its main metabolite 7-hydroxymethotrexate. *Drug Metab Dispos*. 2011;39(8):1338-1344. doi:10.1124/dmd.111.038794
46. Ueshima S, Hira D, Kimura Y, et al. Population pharmacokinetics and pharmacogenomics of apixaban in Japanese adult patients with atrial fibrillation. *Br J Clin Pharmacol*. 2018;84(6):1301-1312. doi:10.1111/bcp.13561
47. Nakanishi T, Tamai I. Genetic polymorphisms of OATP transporters and their impact on intestinal absorption and hepatic disposition of drugs. *Drug Metab Pharmacokinet*. 2012;27(1):106-121. doi:10.2133/dmpk.dmpk-11-rv-099
48. Reeves DJ, Moore ES, Bascom D, Rensing B. Retrospective evaluation of methotrexate elimination when co-administered with proton pump inhibitors. *Br J Clin Pharmacol*. 2014;78(3):565-571. doi:10.1111/bcp.12384
49. Riva M. Brain tumoral epilepsy: a review. *Neurol Sci*. 2005;26(suppl 1):40-42. doi:10.1007/s10072-005-0404-y

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Isono T, Hira D, Morikochi A, et al. Urine volume to hydration volume ratio is associated with pharmacokinetics of high-dose methotrexate in patients with primary central nervous system lymphoma. *Pharmacol Res Perspect*. 2021;9:e00883. doi:10.1002/prp2.883