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ORIGINAL ARTICLE

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Population pharmacokinetic study of methotrexate in patients with lymphoid malignancy

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Abstract *Purpose*: A population pharmacokinetic model was developed to describe dose-exposure relationships of methotrexate (MTX) in adults with lymphoid malignancy; this is in order to explore the interindividual variability in relationship with the different physiopathological variables. The final model was applied to the Bayesian estimation of MTX concentrations using two blood samples. *Methods*: Fifty-one patients receiving 136 courses of MTX (1–6 per patient) were included in this study. The data was analysed using NONMEM software. A linear two-compartment model with linear elimination best described the data. Setting mean parameters values and variabilities to population values, we obtained Bayesian prediction of MTX pharmacokinetic parameters and concentrations. The predictive performance was evaluated by comparing the Bayesian estimated and observed concentrations and the Bayesian estimated parameters with the individual final model estimated parameters. *Results*: The population pharmacokinetic parameters and the inter-subject variablities expressed as coefficient of variation were: the total body clearance CL, 7.1 l h^{-1} (22%), the volume of the central and peripheral compartments V1, 25.1 l (22.5%), V2, 2.7 l (64%), respectively, and the transfer constant Q, 2.7 (51%) l h^{-1}. Inter-course variability was only significant on CL. Age and serum creatinine had significant effects on CL and was included in the final model. A good correlation was obtained between Bayesian estimated and experimental concentrations ($r^2 = 0.85$).

Keywords Population pharmacokinetics · Methotrexate · Lymphoid malignancy

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Introduction

Methotrexate (MTX) is an analog of aminopterine, a folinic acid antagonist, used as an anticancer agent since 1948. High dose methotrexate regimen (HDMTX: 1–8 g/m^2 IV injection) with folinic acid rescue is used in the treatment of lymphoid malignancy (Burkitt's lymphoma, large cell lymphoma and acute lymphoblastic leukemia) [1–4]. HDMTX enhances the prognosis of these patients by preventing the neuromeningeal localization of the lymphoid malignancy. The therapeutic drug monitoring is essential for clinical management of patients receiving HDMTX. This is due to the wide inter and intra individual variabilities, as well as the well established relationship between HDMTX efficacy, toxicity and pharmacokinetics. Concerning its efficacy, the plasma maximal concentration (C_{max}) and the area under the time-concentration curve (AUC) are the major prognosis factors in histological response and disease free survival [5–7]. In addition, a steady state of concentration greater than 16 μmol l^{-1} is associated with decrease risk of relapse [8]. On the other hand, the systemic toxicity has been demonstrated to be directly

related to MTX plasma concentrations, exposure time and the AUC [9, 10]. It also depends on to the dose adjustment and administration schedule of folinic acid rescue. This explains the importance of the strict monitoring of plasma concentrations during hospitalization until reaching a level below the threshold value of 0.05 μmol l^{-1}.

In our institution, HDMTX monitoring consists on measuring MTX concentration every 24 h until it falls bellow 0.05 μmol l^{-1}. This allows the determination of the folinic acid doses and schedule. However, this predefined sampling strategy can result in additional hours of hospitalization in patients whose concentration will reach 0.05 μmol l^{-1} before the next sampling time but also in many unnecessary controls for patients whose concentration will remain a longer time before reaching this threshold.

The objectives of our study were to (1) develop a population pharmacokinetic model of HDMTX in patient with lymphoid malignancy, (2) investigate the influence of different physiopathological factors on the HDMTX pharmacokinetics in order to identify the population at high risk (delayed clearance), (3) to develop a Bayesian tool for individual pharmacokinetic prediction. This would enable the clinician to adjust the folinic acid rescue dose and schedule and to predict hospitalization duration so as to improve the patient health care provision.

Materials and methods

Patients and treatment

This study included hospitalized large cell lymphoma, Burkitt's lymphoma and acute lymphoblastic leukemia patients receiving HDMTX and treated in the hematology and neurology departments of Pitié Salpétrière hospital.

Fifty-one patients with median age of 62 years were recruited between October 2003 and July 2005. A total number of 136 courses (1–6 per patient) were available for analysis. The patients with any of the following conditions were excluded: renal dysfunction (serum creatinine >3 N), hepatic dysfunction (ASAT, ALAT and GGT values >3 N), respiratory insufficiency, pregnancy and breastfeeding. Any treatments known to be modifying: the MTX renal excretion (cisplatin, salicylates) or the MTX plasma protein binding (barbiturates, anxiolytics, phenytoin and salicylates) were contraindicated. Informed consent was obtained from all patients. Each patient included in this study only at one HDMTX course and then the data for other courses were collected from the patient files and no extra tests or blood sampling were done during these courses. Clinical and hematological tolerance were closely observed. The number of courses was determined by the physician.

HDMTX administration

Pre-hydration and urine alkalinization with intravenous sodium bicarbonate were performed and the HDMTX was administered only if the urine was alkalinized to a pH >7. To maintain hydration and alkalinization a continuous perfusion of 1.4% sodium bicarbonate and (5% glucose, 2 g l^{-1} KCl 4 g l^{-1} NaCl, 30–40 ml/kg/24 h) was administered from the start of the HDMTX infusion up to 72 h. The dose of HDMTX (from 1 to 8 g/m^2) was administered as an intravenous infusion of 1–6 h. Folinic acid rescue 50 mg intravenous adjusted to MTX concentrations according to protocol guidelines [11] was given at 24 h after the start of HDMTX infusion and repeated every 6 h for 72 h or until MTX concentration falls bellow 0.05 μmol l^{-1}.

This study was approved by the Ethic Committee of Pitié-Salpêtrière hospital (CCPPRB) according to the requirement of French law on clinical research.

Measurement of HDMTX concentrations

Several blood samples were collected from all patients. The usual protocol sampling was at H24 and H48 h from the beginning of the infusion. Two supplementary blood samples were collected for this study: at the end of the infusion and between H8 and H12 from the beginning of the infusion. Eventual follow up plasma level determination was done at H72, H96 or more, if the MTX was not yet eliminated (MTX concentration remains >0.05 μmol l^{-1}). The first two supplementary blood samples were obtained only during the duration of patients' participation to the clinical study (one cycle only).

Methotrexate concentrations were determined by EMIT (enzymatic multiplied immunoassay technique), with a centrifugal COBAS®analyser. The limit of quantification was (LOQ) of 0.03 μmol l^{-1}. Quality control assessments were done during the whole study period. As 42 concentrations were below the quantification limit (BQL), we have deleted all but the first in each continuous series of BQL observations, fixed the remaining (first) one to 0.015 μmol l^{-1}; DV = LOQ/2. Then an additive plus proportional error model was used with the standard deviation (SD) of the additive part fixed to ($LOQ/2^2$). This is to preserve the information that might add the LOQ values to the model.

Population model building

Data were analysed using the nonlinear mixed effect modelling software program NONMEM (version V, level 1.1, double precision) with the DIGITAL FORTRAN compiler [12]. The first order conditional estimate (FOCE) method was used for the parameters estimation. After investigation of two and three compartment

models, the time course of MTX concentrations was described by a linear two-compartment model with linear elimination. The model parameters were the volume of the central compartment V1, the total body clearance CL, the inter-compartment clearance Q and the volume of the peripheral compartment V2.

Several error models were investigated (i.e., proportional, exponential and additive error models) to describe inter-subject (ISV) and residual variabilities. The random effect was modelled with unstructured (block) covariance. The inter-occasion variability (IOV) was also considered.

The influence of continuous covariates was modelled according to the following equation, using CL for example,

$$\mathrm{CL} = \mathrm{TVCL} \times \{\mathrm{age}/\mathrm{median}\ (\mathrm{age})\}^{\theta_{\mathrm{age}}}$$

where TVCL is the typical value of CL for a patient with the median covariate value and θ_{age} is the estimated influential factor for age. Such covariates included: bodyweight, height, body surface area, age, serum creatinine, creatinine clearance (calculated according to the Cockcroft and Gault formula), ASAT, ALAT, Gamma GT, total bilirubin, alkaline phosphatase, proteinemia and lactate dehydrogenase. The influence of gender was modelled according to the following equation,

$$\mathrm{CL} = \mathrm{TVCL} \times \theta^{\mathrm{GENDER}},$$

where 0 or 1 denotes male or female gender respectively. Covariates were selected and retained in the population model if (1) their effect was biologically plausible, (2) they produced a minimum reduction of 4 U of the objective function value (OFV) and (3) they produced a reduction in the variability of the pharmacokinetic parameter, assessed by the associated inter-subject variability. Then, all covariates identified as potentially relevant were included in a full model. The final model was then obtained by dropping all non-significant covariates i.e., producing a reduction of less than 8 U of the OFV.

To evaluate the goodness-of-fit, the following graphs were compared: observed concentrations versus predictions (PRED-OBS), weighted residuals (WRES) versus time and weighted residuals versus PRED (WRES-PRED) as well as the corresponding graphs issued from the POSTHOC estimation step. Diagnostic graphics and distribution statistics were obtained using the R program [13].

The accuracy and robustness of the final population model were then assessed using a bootstrap method [14]. From the original data set of patients, 1,000 bootstraps were drawn with re-sampling. For each of the 1,000 bootstrap sets, the population parameters were estimated. Then the mean, median and SD of these 1,000 estimates were compared to those obtained in the original population set. The model was considered validated if no significant differences were observed.

Bayesian estimation

The Bayesian estimation was performed in 32 patients, from the original population, using two or three samples strategy. For each patient a file was generated from the original data set including the entire population and for these patients only the sets of sampling times to be tested was determined. The choice of the sampling time was based on the available observations. All the possible two and three sampling time combinations were tested. Individual predictions were obtained using the "POSHOC" option without the estimation step (MAXEVAL = 0), setting mean parameters values, and variabilities to population values obtained previously.

The predictive performance of the Bayesian method was evaluated by comparing the Bayesian predicted and observed concentrations and by comparing individual parameters with the ones obtained using the Bayesian approach. The evaluating criteria was the bias estimated by mean error (me), which assesses the accuracy of estimation [15]:

$$\mathrm{me} = 1/\mathrm{n} \times \sum \mathrm{pe_i};$$

where n is the number of observations, pe_i is the prediction error:

$$\mathrm{pe_i} = \mathrm{prediction} - \mathrm{observation}.$$

The precision of the prediction was estimated by the root mean squared error (rmse):

$$\mathrm{mse} = 1/\mathrm{n} \sum (\mathrm{Pe_i})^2 \text{ and } \mathrm{rsme} = \sqrt{\mathrm{mse}}$$

The limited sampling strategy was validated by means of the program OSP-Fit [16]. Given the population pharmacokinetic parameters, the theoretical optimal sampling times were determined, based on the random search and stochastic gradient algorithms. A constraint was that only two samples were allowed from the clinical staff.

Results

Data description

Fifty-one patients (28 males and 23 females) were included in the study (where 44 patients with large cell lymphoma, 3 patients with Burkitt's lymphoma and 2 patients with acute lymphoblastic leukemia (see Table 1). A total of 496 MTX concentrations were obtained. A wide interpatient variability was noticed at each time normalized concentration. Figure 1 shows that the concentrations at 24 h ranged between (0.05–14.26 μmol l^{-1}) and at 48 h (0.015–1.62 μmol l^{-1}). The number of samples was 2–11 samples per course. Several patients did not reach the threshold of 0.05 μmol l^{-1} before 72 h, thus their monitoring time was increased.

Table 1 Baseline characteristics

Characteristics	Median or *n*	Interquartile[a]
MTX dose	3	
Gender (male/female)	28/23	2.9–3.1
Body weight (kg)	67	55–76
Age (years)	62	39–72
Height (cm)	169	163–172
Body surface area (m^2)	1.8	1.6–1.9
Serum creatinine ($\mu mol\ l^{-1}$)	71	63–88
Creatinine clearance ($ml\ min^{-1}$)	106	54–149
Protein ($g\ l^{-1}$)	64	60–68
Albumin ($g\ l^{-1}$)	35	35–37
ASAT ($IU\ l^{-1}$)	23	19–29
ALAT ($IU\ l^{-1}$)	33	21–47
Alkaline phophatase ($IU\ l^{-1}$)	68	64–74
GGT ($IU\ l^{-1}$)	40	22–59
Total bilirubin ($\mu mol\ l^{-1}$)	9	6–12
Lactate dehydrogenase ($IU\ l^{-1}$)	410	370–481

[a]25th–75th percentile

Population pharmacokinetics

The MTX time-concentrations courses were best described by a linear two-compartment model. The three-compartment model led to the imprecise parameter estimates and did not improve the diagnostic plots.

Inter-subject variabilities could be estimated for CL, V1, V2 and Q and were best described using an exponential error model. A full omega matrix was tested; addition of covariances terms between CL and V1 and between *Q* and V2 decreased significantly the OFV and were accurately estimated.

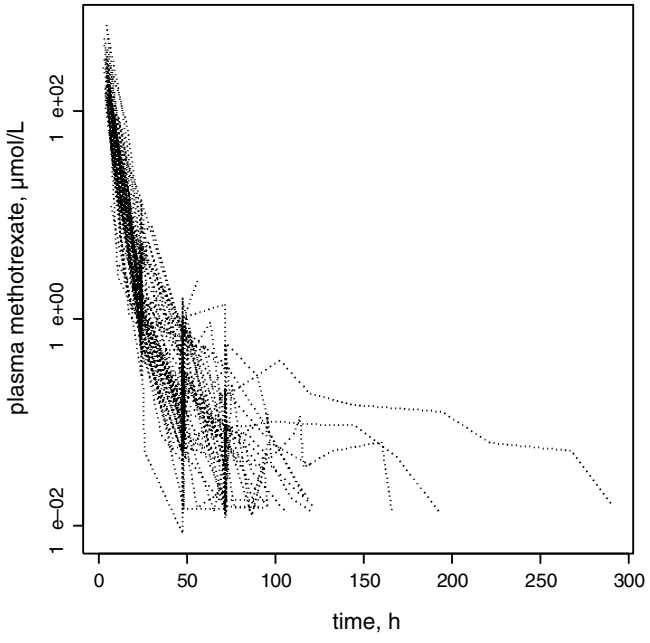

Fig. 1 Dose normalised MTX concentrations from a total number of 136 courses in 51 patients (range 1–6 course per patient)

The residual variability was best described by a combined model with an exponential component and an additive component fixed to the square of the LOQ^2.

This part was added due to the wide range of quantified concentrations including very low concentrations. Inter-occasion variability was investigated on CL, V1, Q and V2 and was only significant on CL, resulting in a 95 U decrease of the OFV, it also decreased the residual variability from 58 to 46%.

The correlation between predicted and observed MTX concentration was 0.87, and the correlation between individual predicted and observed MTX concentration was 0.94 (see Fig. 2).

In the final model, only age and serum creatinine had significant effect on CL and were retained. The incorporation of age and serum creatinine decreased the intersubject variability by 5 and 3%, respectively, and decreased the OFV (see Fig. 3). The final equation describing their effect on the CL is:

$$CL = TVCL \times \{62/(age)\}^{\theta age} \times \{67/(scr)\}^{\theta scr},$$

where scr denotes serum creatinine

None of the other covariates had any significant effect on *Q*, V1 or V2. The model building process for covariates is summarized in Table 2.

The final model was subjected to a bootstrap analysis (1,000 replicates). Table 3 summarizes the results presented as medians and 95% confidence intervals (2.5th and 97.5th percentiles). Bootstrap medians and parameter estimates from the original dataset were reasonably similar and indicated acceptable precision.

Evaluation of the Bayesian pharmacokinetic parameter prediction

The Bayesian prediction based on two or three early (before H48) blood sampling strategy could not give a precise prediction of the following concentrations values. The addition of H48 in a two samples strategy [H24, H48] improved the Bayesian prediction. The choice of this limited sampling strategy was confirmed by the use of the program OSP-Fit. Given the final population model, with fixed allowed time range between 1 and 96 h post-infusion, the two theoretical optimized sampling times were 21.9 and 42.3 h post-infusion, which compares well with our proposal of [H24 and H48].

The mean and standard error of estimate (SE) values of the Bayesian parameters were: CL, 7.1 (1.7) $l\ h^{-1}$; V1, 25.7 (2.6) l; *Q*, 0.14 (0.03) $l\ h^{-1}$; V2, 2.7 (1.2) l. These values were calculated using population characteristics and the two theoretical sampling at H24 and H48, (see Table 4). These values were close to those determined using the reference method of CL, 7.1 (0.6) $l\ h^{-1}$; V1, 25.1(2.8) l; *Q*, 0.15 (0.03) $l\ h^{-1}$; V2, 2.7 (0.4) l, respectively. A satisfactory correlation was obtained between all the observed concentrations and those predicted by the Bayesian two point [H24, H48] estimates ($r^2 = 0.84$, Fig. 4).

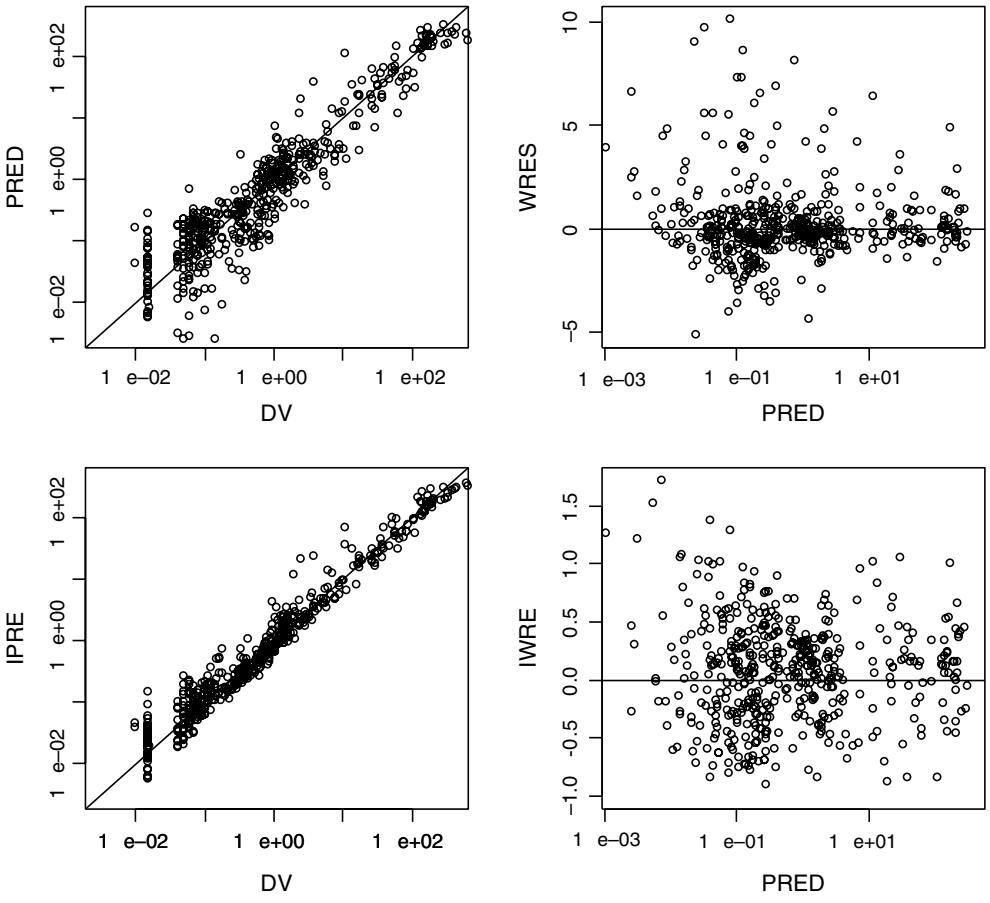

Fig. 2 *Top* Log observed concentrations versus log predictions (PRED-OBS), log weighted residuals versus log PRED (WRES-PRED). *Bottom* Log observed concentrations versus log individual predicted predictions (IPRED-OBS), log individual weighted residuals versus log individual prediction (IWRES-PRED)

The performance of the Bayesian estimation in pharmacokinetic parameter prediction, in the subset of 32 patients from the original population, was expressed by bias (mean prediction error and 95% CI) and precision (rmse and 95% CI) and the results are given in Table 4. Using the predicted individual Bayesian time concentration curves, the time to reach the HDMTX threshold 0.05 μmol l^{-1} was determined graphically and compared to the actual time. In 81% of the cases the time was predicted correctly.

This Bayesian procedure allowed the estimation of pharmacokinetic parameters with biases not significantly different from zero (*t* test showed that CIs included zero), and with a good precision.

Discussion

In the present study we developed a population pharmacokinetic model for adults with lymphoid malignancies. In agreement with the previous studies, we observed a high inter and intra individual pharmacokinetic variability for HDMTX [17–19]. The two-compartment model revealed to be the best model describing the pharmacokinetics of HDMTX. This model was previously used by many authors [18–21]. The clearance and central volume of distribution typical values CL, 7.1(0.6) l h^{-1}; V1, 25.1 (2.8) l estimated by NONMEM were close to values reported in other population pharmacokinetic studies as the values reported by Rousseau et al. [19]; CL, 7.4(3.2) l h^{-1}; V1, 18.2 (9.9) l and by Pignon et al. [22]; V1, 21.8(7.9) l; CL, 5.8 (1.3) l h^{-1}.

In the final model HDMTX clearance decreased with markers of renal function, age and serum creatinine. Many studies have also shown a correlation between age and HDMTX clearance. Bacci et al. [23] found a significant relationship between the delayed elimination of HDMTX (concentration >5 mol l h^{-1} after 24 h) and the patients' age. Another study by Donelli et al. [24] revealed that the elimination half-lives were greater in the older group and the clearance was inversely proportional to age.

Several studies have also established a correlation between HDMTX elimination and renal function and confirmed the necessity of rigorous serum creatinine monitoring as a predictor of methotrexate elimination [25, 26]. Skäry et al. [27], have demonstrated in a study on 247 children a correlation between the number of days with serum creatinine level ≥1.5 times the normal and the HDMTX elimination time thus the authors proposed elevation serum creatinine level by

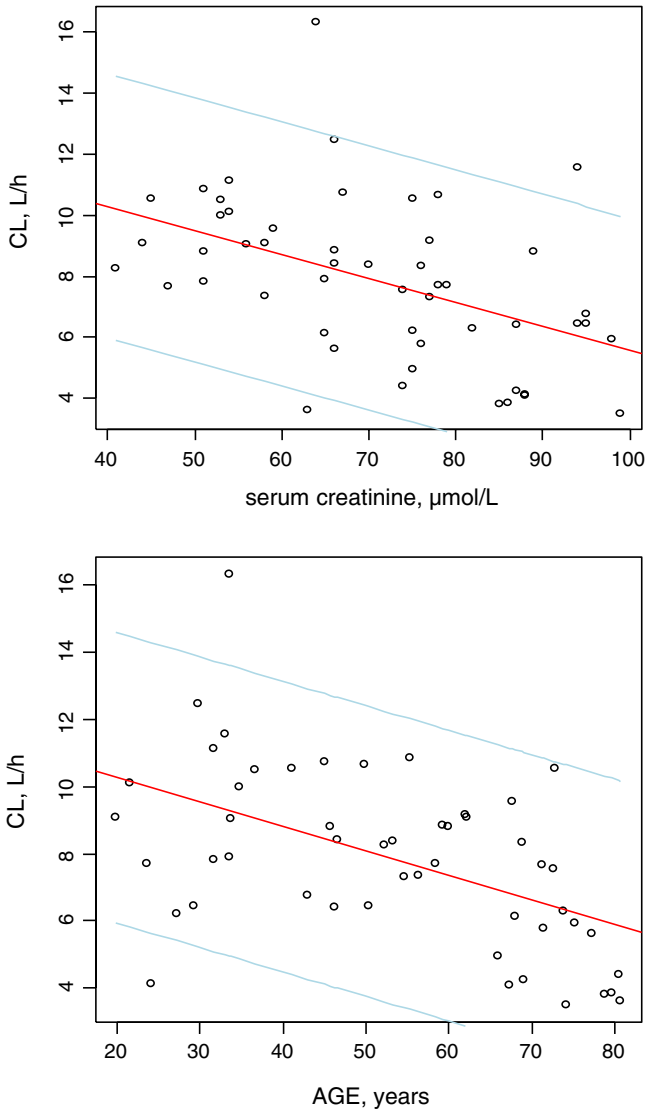

Fig. 3 Individual predicted MTX clearance versus age and serum creatinine (*SCR*)

≥50% as an early predictor of delayed HDMTX elimination.

Otherwise our modelling did not show any effect of gender, bodyweight, body surface area, ASAT, ALAT, GGT, total bilirubin, alkaline phosphatase, proteinemia, on the pharmacokinetic parameters. The effect of these factors was previously investigated by different authors using different approaches and similar conclusions were reported [19, 21, 24].

The Bayesian prediction of the individual time-concentration curves would be helpful in decreasing the number of blood sampling and the follow-up period. The Bayesian method provided a reasonable predictive performance and could be applied to routine clinical care. The two sampling strategy including only H24 and H48 gave a satisfactory prediction of HDMTX pharmacokinetics with a non-significant bias. Earlier sampling strategies did not reach the desired accuracy and precision and we preferred not to increase the number of blood samples.

This approach may have advantages over the current monitoring as it may allow early identification of patients with delayed elimination, so it can save those patients from numerous unnecessary blood samplings. It is also an alerting tool so that the proper measures (increasing the dose of folinic acid, urine alkalinization and intravenous hydration) could be taken earlier. However, the present results must be confirmed and tested prospectively in a larger population.

Conclusion

The pharmacokinetic parameters of HDMTX were accurately estimated. Serum creatinine and age influenced the HDMTX clearance, so they should be taken into account in the choice of the dose regimen. Our Bayesian approach enabled a satisfactory estimation of

Table 2 Summary of the model building

Steps	OFV	ΔOFV	Description of each step	Significant Y/N
	−90		Base NONMEM model without covariates	
1	−90	0	Effect of BW, BSA and Height, on CL and V1	N
2	−90	0	Effect of ASAT, ALAT, GGT, albumin, alkaline phosphatase, Total Bilirubin, Protein and LDH on CL	N
3	−98	−8	Effect of gender on V1	Y
4	−102	−12	Effect of age on CL	Y
5	−120	−30	Effect of serum creatinine on CL	Y
6	−129	−39	Full model with effect of serum creatinine, age on CL and gender on V1	
7	−129	−39	Final model with the effect of serum creatinine and age on CL	

BW body weight, *BSA* body surface area, *CL* total body clearance, *V1* volume of the central compartment, *LDH* lactate dehydrogenase

Table 3 Parameter estimates from the final population model and results of the bootstrap analysis

Parameters	Final model	Bootstrap[a]	
	Estimate (SE)	Median	2.5th–97.5th[b]
Fixed effects			
V1(l)	25.1 ± 2.8	25	20–30
CL (l h^{-1})	7.1 ± 0.6	7	5.7–8.4
Q (l h^{-1})	0.15 ± 0.03	0.13	0.09–0.19
V2 (l)	2.7 ± 0.4	2.7	1.9–4
AGE on CL	−0.22 ± 0.09	−0.19	−0.37–0.06
SCR on CL	−0.43 ± 0.10	−0.40	−0.64–0.23
Random effects			
Intersubject variability exponential model[c]			
ISV (V1) (%)	22.5 ± 17	30	11–45
ISV (CL) (%)	22 ± 12	20	9–32.5
ISV (Q) (%)	51 ± 32	56	6–91
ISV (V2) (%)	64 ± 29	66	29–89
Inter-occasion variability on CL (%)	16.5 ± 6	15.5	8.5–23
Residual variability combined model			
Exponential part (%)	46 ± 6	56	49–63
Additive part (μmol l^{-1})	0.015 fixed	NA	NA

SE Standard error of estimate, *CL* total body clearance, *V1* volume of the central compartment, *Q* inter compartment clearance, *V2* volume of the peripheral compartment, *SCR* serum creatinine, *ISV* intersubject variability, *NA* not applicable
[a]Statistics on 806 successful bootstrap runs (1,000 programmed)
[b]Non parametric 95% confidence interval based on the 2.5th–97.5th percentiles
[c]Correlations between ISV terms, corr(V1, CL) = 0.98 ± 0.50 and corr(*Q*, V2) = 0.79 ± 0.45

Table 4 Statistical analysis after Bayesian estimation of the MTX pharmacokinetic parameters and concentrations

	V1 (l)	CL (l h^{-1})	Q (l h^{-1})	V2 (l)	MTX (μmol l^{-1})
Mean (SD) [min–max]	25.672 (2.558) [19.849–29.978]	7.094 (1.666) [3.148–10.086]	0.144 (0.032) [0.102–0.230]	2.714 (1.176) [1.244–6.051]	[0.004–0.582]
Bias (95% CI)	0.746[a] (−0.890, 2.382)	0.127[a] (−0.365, 0.620)	−0.003[a] (−0.019, 0.013)	−0.155[a] (−0.575, 0.264)	−0.035 (−0.051, −0.019)
Precision (95% CI)	4.632 (3.763, 6.293)	1.381 (1.122, 1.877)	0.045 (0.036, 0.061)	1.183 (0.961, 1.608)	0.074 (0.063, 0.090)

CL total body clearance, *V1* volume of the central compartment, *Q* inter compartment clearance, *V2* volume of the peripheral compartment, *min* minimum value, *max* maximum value, *CI* confidence interval
[a]Not significantly different from 0

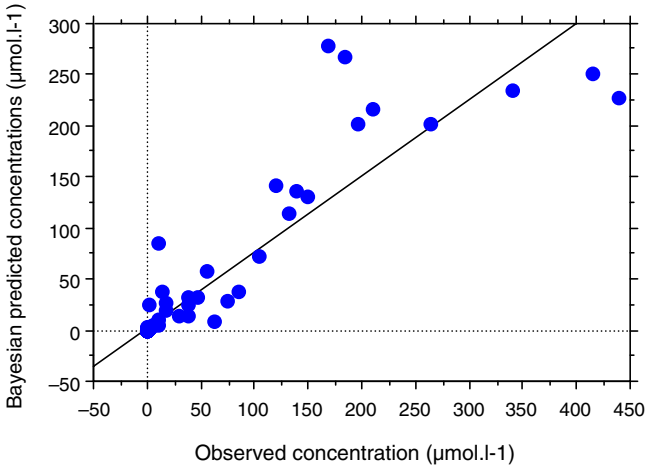

Fig. 4 Correlation between: experimental and Bayesian estimations of high dose MTX concentrations (μmol l^{-1}) using two sampling times H24 and H48 ($r^2 = 0.84$)

HDMTX concentrations. After prospective validation, this can be a valuable tool in the clinical practice as it uses limited sampling strategy, allows management and prediction of toxic events.

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