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ORIGINAL ARTICLE

Population pharmacokinetics of cytarabine, etoposide, and daunorubicin in the treatment for acute myeloid leukemia

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Abstract
Purpose Interpatient variability in the pharmacokinetics (PK) of cytarabine, etoposide, and daunorubicin following body surface area–adjusted doses calls for studies that point to other covariates to explain this variability. The purpose of this study was to investigate such relationships and give insights into the PK of this combination treatment.
Methods A prospective population PK study of twenty-three patients with acute myeloid leukemia was undertaken. Plasma concentrations of patients were determined by high-pressure liquid chromatography. PK models were developed with NONMEM®; for daunorubicin, PK information from a prior study was utilized.
Results Baseline white blood cell count (bWBC) influenced the PK for all drugs. A small, statistically insignificant improvement in model fit was achieved when a relationship between bWBC and daunorubicin central volume of distribution was included. The volume increased 1.9% for each increase in bWBC by 1×10^6 cells/mL. The clearances of etoposide and cytarabine were significantly increased and decreased, respectively, by increased bWBC. Tenfold changes in bWBC were needed for these relationships to have potential clinical relevance. A decrease in creatinine clearance of 60 mL/min resulted in a decrease in etoposide clearance of 32%.
Conclusions Population-based models characterized the PK for all three drugs. bWBC was a significant covariate for etoposide and cytarabine and showed a trend for daunorubicin. Linking the significant bWBC relationships and the relationship between kidney function and etoposide clearance to clinical end points would support dose individualization. Patients with above-normal creatinine clearances and high bWBC may receive sub-optimal treatment due to elevated etoposide clearances.

Keywords Cytarabine · Etoposide · Daunorubicin · Population pharmacokinetics · NONMEM · Acute myeloid leukemia

Abbreviations

ADE	Cytarabine + Daunorubicin + Etoposide treatment
ALAT	Alanine aminotransferase
AML	Acute myeloid leukemia
Ara-C	Cytosine arabinoside
BLQ	Below limit of quantification
BSA	Body surface area
bWBC	Baseline white blood cell count

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cCrCL	Calculated creatinine clearance
Dnr	Daunorubicin
Eto	Etoposide
HPLC	High-pressure liquid chromatography
LLoQ	Lower limit of quantification
OFV	Objective function value
PK	Pharmacokinetic
RSE	Relative standard error
SCM	Stepwise covariate modeling
SD	Standard deviation
Se-Cr	Serum creatinine
v	Degrees of freedom
VPC	Visual predictive check

Introduction

Acute myeloid leukemia (AML) consists of a group of heterogenous, clonal, hematopoietic stem cell disorders, in which the ability of the neoplastic cells to normally proliferate and differentiate is lost. In the absence of treatment, the accumulation of non-functioning blast cells will inevitably lead to a fatal outcome within a matter of weeks [1]. The conventional induction chemotherapy regimens in the treatment for AML have, for the last decades, incorporated cytarabine (Ara-C) daunorubicin (Dnr) at various dosages and in some protocols etoposide (Eto) [2]. The treatment regimen that formed the basis for the present study is one of the induction treatments in the MRC-AML17 [webA] and MRC-AML15 [webB] trials, where some patients are randomized to all three drugs (the so-called ADE 10+3+5 treatment). Despite the many years of use, the optimal dose of all three drugs remains to be firmly established. The high interpatient variability in the PK of these drugs following standard body surface area (BSA)–adjusted doses calls for studies that may point to other clinically relevant covariates that can better explain at least a part of this variability.

The anti-metabolite cytarabine enters the cell and is phosphorylated to the active triphosphorylated state known as Ara-CTP. Ara-CTP exerts its cytotoxic effect in two ways: by DNA polymerase inhibition and by incorporation into the DNA string causing chain termination, which blocks DNA synthesis [3, 4]. Cytarabine is not given orally. After intravenous administration, the pharmacokinetics is most commonly described with a two-compartment model. The initial half-life is rapid (about 10 min) followed by a slower elimination half-life (about 2.5 h). Peak concentrations are about 500 ng/mL for 30 mg/m^2 intravenous injections and are about 25 times higher for 300 mg/m^2 injections [5]. Etoposide is frequently called an inhibitor of DNA topoisomerase II even though the mechanism of action is more a stabilization than an inhibition. When functioning correctly, topoisomerase II is able to regulate over- and under-winding of the DNA double helix by introducing transient breaks. In the presence of etoposide, these transient breaks are maintained through a stabilization of the covalent topoisomerase–DNA complex. This leads to an accumulation of permanent breaks in the DNA and cell death [6]. As for cytarabine, there is a biphasic pattern of elimination for etoposide after intravenous administration. The elimination half-life is about 6–8 h [5]. The cytotoxic effect of Dnr comes mainly from intercalation with DNA as well as interactions with cellular enzymes such as polymerases, helicases, and also topoisomerase II in a manner similar to etoposide [7, 8]. The disappearance of daunorubicin from plasma has a rapid initial half-life of about 6 min followed by a slower half-life of about 1.5–3.5 h and potentially a third half-life of 14–20 h [9, 10].

In a previous study, it was shown that the baseline white blood cell count (bWBC) was inversely correlated with the plasma concentration of Dnr [11]. Interestingly, a similar effect has been described for Ara-C in a constant infusion regimen [12], whereas other investigators did not find this correlation for patients receiving higher doses of Ara-C (1,000–3,000 mg/m^2) [13]. Studies in other types of cancer have shown a correlation between serum creatinine (se-Cr) [14, 15] or calculated creatinine clearance (cCrCL) [16] and etoposide PK parameters, thus advocating that kidney function should be considered when dosing etoposide.

The purpose of the present study was to investigate these and other covariate relationships further in patients receiving all three drugs in the ADE 10+3+5 treatment regimen. Models for the three drugs were developed with the software NONMEM®, including data for observations below the limit of quantification with the M3 method [17] and for daunorubicin with the inclusion of information from a similar prior PK study with the subroutine PRIOR [11, 18].

Materials and methods

Patients

Twenty-four new patients suffering from AML (fourteen men and ten women) were included in the study. The patients were diagnosed according to the WHO classification with more than 20% myeloblasts in the bone marrow. The patients were recruited from two hospitals in Denmark: Rigshospitalet and Herlev Hospital. One female patient was excluded from the PK analysis because she only received Dnr and Ara-C treatment and because of erroneous registration of blood sampling times. Four

Table 1 Selected continuous covariates for the twenty-three patients included in the PK analysis

	Mean	Range
Age (years)	52.3	22–68
Weight (kg)	79.4	46–117
Body surface area (m^2)	1.91	1.5–2.2
Serum creatinine (μmol/L)	74.1	49–140
Calculated creatinine clearance (mL/min)	116	47–189
Bilirubin (μmol/L)	9.87	2–55
Baseline white blood cell count ($\times 10^6$ cells/mL)	26.9	0.9–167
Alanine aminotransferase (U/L)	40.0	8–152

patients received two series of treatment with approximately 1 month in between. The patient demographics from the twenty-three patients included in the analysis can be found in Table 1. The project was approved by the Danish national ethics committee (Journal no. H-A-2008-129), and all patients signed informed consent forms before entering the trial.

Treatment

The twenty-three patients included in the data analysis received 10 days of cytarabine (100 mg/m^2 b.i.d. on days 1–10), 3 days of daunorubicin (50 mg/m^2 q.d. on days 1, 3, and 5), and 5 days of etoposide (100 mg/m^2 q.d. on days 1–5). Ara-C was given as i.v. push over 5 min, while both Dnr and Eto were given as 1-h infusions. The order of the infusions was not strictly decided by the protocol, but by the nurse. All patients were provided adequate transfusion therapy and were treated for the multitude of complications that occurs in this patient population as per normal procedures at the two hospitals.

Patient samples

Blood samples (4 mL) were collected through central venous catheters into heparinised tubes containing tetrahydrouridine (0.1 mg THU/mL blood) [19]. Between eight and ten samples were collected per patient per series of treatment on different days of treatment. The specific sample times differed for each patient depending on the timing and order of the infusions. This resulted in a total number of observations of 162, 177, and 109 for Ara-C, Eto, and Dnr, respectively. Blank samples were collected immediately before treatment. All samples were stored at 4° C and, within 4 h after the sample was taken, plasma was separated by centrifugation at 2,500×g for 15 min. Plasma was kept at −20°C until analysis. The stability of the storage conditions was validated in the HPLC method.

High-pressure liquid chromatography sample analysis

The HPLC analysis of the plasma samples has been described in detail elsewhere [19]. All three drugs were quantified simultaneously with this fully validated method. Briefly, the plasma samples were diluted 1:1 with 0.05 M HCl and were placed on Waters Oasis MCX solid-phase extraction columns in order to remove interfering substances. The remanence after evaporation was redissolved in mobile phase A (KH_2PO_4, pH 2.0) before injection into the HPLC. Separation was performed with an Acclaim Polar Advantage II (4.6 × 150 mm, 3 μm) C_{18} column and a gradient elution program with the mobile phases A and B (acetonitrile). Ara-C (LLoQ 13 ng/mL) was quantified by ultraviolet detection at 280 nm, while Eto (LLoQ 52.5 ng/mL) and Dnr (LLoQ 15 ng/mL) were quantified by fluorescence detection at excitation and emission wavelengths of 230/328 nm and 490/555 nm, respectively. The overall precision of the method (% relative standard deviation) was within 0.2–13.5%.

Population pharmacokinetic modelling

The software NONMEM VI version 2.0 was used for the nonlinear mixed effects PK model building. The estimation method was the first-order conditional estimation algorithm with the Laplacian method. This was chosen because the M3 method for incorporation of samples below the limit of quantification (BLQ) in the analysis was employed. The M3 method maximizes the likelihood for all data and treats BLQ observations as censored. The likelihoods of BLQ observations to be true BLQ observations are calculated simultaneously. The M3 method is considered the least biased way to handle BLQ observations [17, 20, 21]. It was applied through the use of the F_FLAG functionality and the PHI function as described by Ahn et al. [20].

The choice of one model over another was based on differences between objective function value (OFV) for nested, structural models (a decrease of >5.99, corresponding to $P < 0.05$ for two degrees of freedom was considered significant). Further, plausibility of the parameter estimates, the magnitude of their relative standard errors, graphical assessments and, especially for the covariates, potential clinical relevance were considered. The clinical relevance would be considered possible if the PK parameter changed more than 20% over the observed span of the given covariate [22]. PsN (http://psn.sourceforge.net/) was used to execute the NONMEM runs, calculate visual predictive checks (VPC), run bootstrap analyses, and run the stepwise covariate model building (SCM). 500 data sets were simulated for the VPCs, and 200 data sets were generated for the bootstrap. The R-based program Xpose4 (http://xpose.sourceforge.net/) was used to visualize the VPC runs. Basic goodness-of-fit plots

were not made because of the non-randomly missing data. The residuals and weighted residuals will not be calculated with the M3 method for individuals who have both continuous dependent variables and a likelihood, i.e., those with a BLQ observation.

The relatively sparse data in this study for Dnr analysis combined with the availability of information from a previous PK analysis with Dnr [11] led to the investigation of the PRIOR function in the Dnr model development. This method, in which the prior parameter estimates and their uncertainty are considered, may stabilize parameter estimation with the new data by imposing a penalty function on the OFV. The penalty of the prior information was derived from a normal-inverse Wishart distribution. For the random effect parameters (ω), the degree of informativeness from the prior is quantified by the assigned degrees of freedom, v, with a higher number increasing the basic penalty [18]. It is not possible to use the log likelihood ratio test to compare models directly (i.e., by ΔOFV) when changes are made in the PRIOR information. To avoid this problem, the OFV contribution for the current data set was investigated and compared between the two models with PRIOR by fixing the parameter estimates to those found with and without the covariate relationship included and then run without re-estimation (MAXEVAL = 0).

The covariates included in the evaluations were gender, age, weight, BSA, se-CR, cCrCL, bilirubin, bWBC, and alanine aminotransferase (ALAT) (Table 1). The cCrCL was calculated with the Cockcroft-Gault formula [23]. In the forward SCM, the most significant covariate was added in each step until the full forward model was built. A P value <0.05 was required for inclusion (corresponding to a decrease of >3.84 in OFV). In the backward SCM, the covariates were removed from the full forward model one at a time, and a stricter P value of 0.01 (corresponding to a decrease of >6.63 in OFV) was required to retain the covariate in the model. The continuous covariates were tested on the PK parameters clearance (CL) and central volume (Vc) in two different ways, firstly by a linear inclusion, and, if significant, secondly by a piecewise linear inclusion as follows:

$$\mathrm{VP_i} = \theta_\mathrm{p} \times \mathrm{e}^{\eta_\mathrm{i}} \times \left(1 + \theta_\mathrm{j} \times (\mathrm{COV_i} - \mathrm{median_{COV_i}})\right)$$

where $\mathrm{VP_i}$ is the individual value of population parameter P, θ_p is the estimate for the median patient, $\mathrm{e}^{\eta_\mathrm{i}}$ is the exponential function that describes the interindividual variability, and θ_j is the parameter estimate describing the change in the PK parameter for an individual with covariate $\mathrm{COV_i}$. In the piecewise inclusion, each $\mathrm{VP_i}$ is estimated similarly but with two different θ_j for estimations above and below the median covariate value, respectively. The only categorical covariate, gender, was tested as percentage change for women compared with men:

$$\text{If male:} \quad \mathrm{VP_i} = \theta_\mathrm{p} \times \mathrm{e}^{\eta_\mathrm{i}}$$

$$\text{If female:} \quad \mathrm{VP_i} = \theta_\mathrm{p} \times \mathrm{e}^{\eta_\mathrm{i}} \times \left(1 + \theta_\mathrm{j}\right)$$

Parameterization of the relationship between cCrCL and total etoposide CL was tested and compared to a parameterization with cCrCL and etoposide CL split into a renal and a non-renal part. This was done by TVCL = ($\theta_1 + \theta_2$*cCrCL)*bWBC, where θ_1 represents non-renal clearance and θ_2*cCrCL represents renal clearance.

Results

The final population PK parameter estimates based on successful bootstrap runs and their relative standard errors for all three drugs can be seen in Table 2. All final models were based on two-compartment pharmacokinetics with an additive residual error model on the log scale, which is similar to a proportional error model on the normal scale. The objective function values for the one- and two-compartment models for all three drugs clearly showed a significant improvement in model fit for the two-compartment models. For Dnr, the OFV decreased by 10.524, while it decreased 67.7 and 48.637 for Ara-C and Eto, respectively. The VPC double panel plots of the incorporation of BLQ samples showed good ability of the models to quantify the observations above BLQ and characterize the proportion of samples below BLQ for all three drugs (Figs. 1, 2, 3) [21]. For Eto, there was a total of 28 BLQ observations, while for Ara-C there were 53 and for Dnr 17. None of the models identified age, BSA, weight, or serum creatinine as significant covariates for CL or Vc. No correlations were evident between the clearances of the three drugs.

For Ara-C, gender was found to be a significant covariate on CL, with women having a 9% higher clearance than men. However, the relative standard error of the parameter describing the relationship between gender and CL was high (87%) in the present study and the 95% confidence interval of the estimate from the bootstrap overlapped zero. CL of Ara-C correlated inversely with bWBC (i.e., a high bWBC was associated with a low CL). The CL was typically decreased by 17% for a patient with a bWBC of ten times the upper value of the normal range (3–9 × 10^6 cells/mL), i.e., 90 × 10^6 cells/mL, compared with a patient with a bWBC of 9 × 10^6 cells/mL. The correlation between bWBC and CL gave a drop in OFV of 8.9 (P value 0.003). However, the interindividual variability only decreased slightly (48–45%).

The amount of Dnr data in this study was relatively sparse (92 observations from 23 patients). All samples taken more than 9 h after infusion had concentrations below LLoQ (17 observations). Therefore, information from an earlier PK analysis was included in the model

Table 2 Population PK estimates obtained from bootstrap analysis with the final models for the three drugs

	Etoposide		Cytarabine		Daunorubicin	
	TVP (RSE %)	IIV CV% (RSE %)	TVP (RSE %)	IIV CV% (RSE %)	TVP (RSE %)	IIV CV% (RSE %)
Population pharmacokinetics						
CL (L/h)	0.99 (15)[a]	28 (32)	272 (1.9)	45 (5.3)	129 (0.98)	42 (5.7)
	1.17 (15)[b]					
Vc (L)	9.13 (4.1)	22 (49)	62.8 (2.5)	70 (5.2)	453 (2.8)	105 (3.5)
Q (L/h)	1.14 (11)		13.7 (4.1)		152 (2.8)	
Vp (L)	4.75 (7.2)		75.4 (32)		911 (1.8)	
Res. error (%)	54.8 (1.7)		75.7 (1.3)		76.1 (0.64)	
Covariates						
CL-bWBC	0.00185 (3.7)		−0.0020 (5.1)			
CL-cCrCL	0.00996 (7.8)					
Vc-GENDER	−0.2793 (5.1)					
Vc-bWBC					0.0185 (2.5)	

CL clearance, *Vc* central volume, *Q* intercompartmental clearance, *Vp* peripheral volume, *Res. error* residual error, *bWBC* baseline white blood cell count, *cCrCL* calculated creatinine clearance, *TVP* typical value of parameter, *IIV* inter-individual variability, *RSE %* relatives standard error in percent

[a] Non-renal clearance

[b] Renal clearance for a patient with cCRCL of 116 mL/min

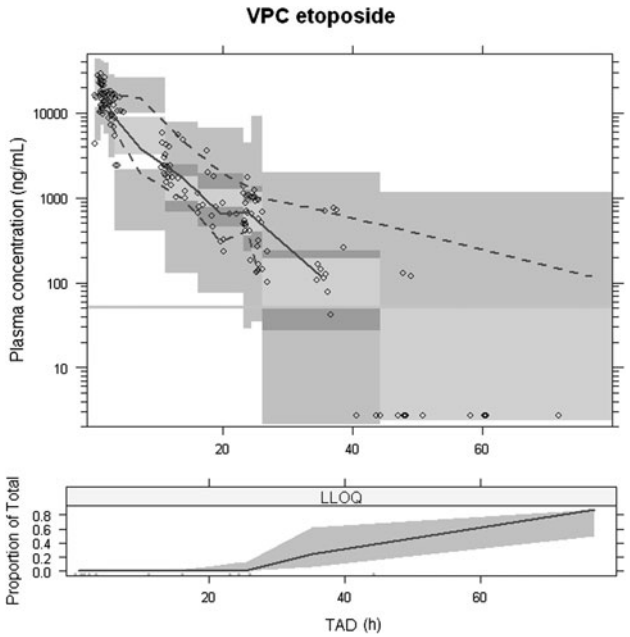

Fig. 1 VPC double panel plot for Eto with 90% prediction intervals. TAD, time after dose. *Top panel* observed data (*circles*), median (*full line*), 5th and 95th intervals (*dotted lines*) of the observations, and 95% confidence intervals for the median (*light gray area*) and the 5th and 95th percentiles (*darker gray areas*) of the simulated data. The *light* and *darker gray* areas may overlap resulting in *very dark gray* areas. *Bottom panel* observed fraction of BLQs (*full line*) and 95% confidence interval for fraction of BLQ in simulated data sets (*light gray area*)

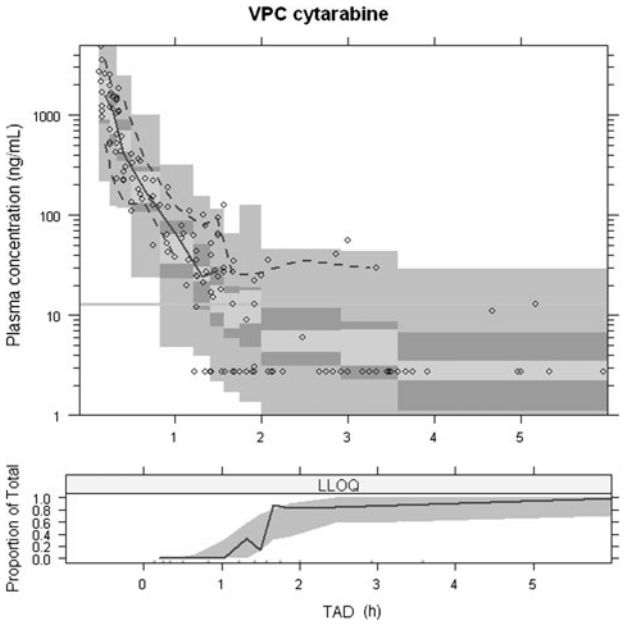

Fig. 2 VPC double panel plot for Ara-C. See Fig. 1 for explanation of graphs

building process with the PRIOR subroutine. However, the initial SCM covariate testing was performed on the model without prior information (model A). The fully automated SCM cannot handle the PRIOR function due to the varying number of THETAS, a number that must be defined in the

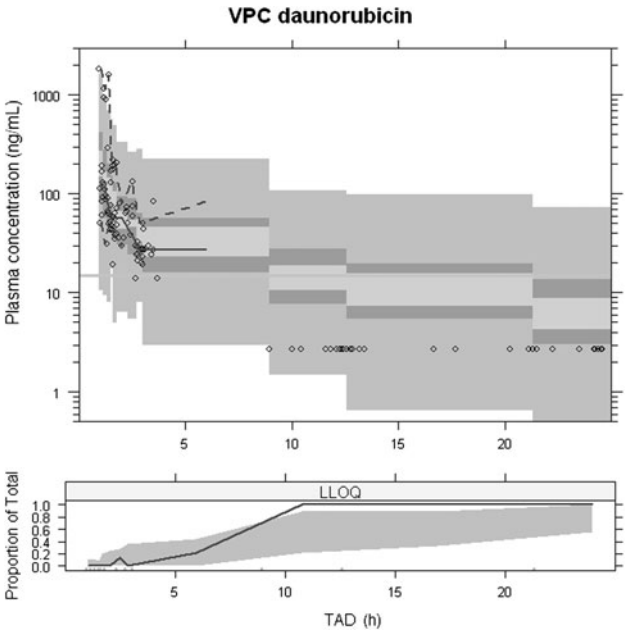

Fig. 3 VPC double panel plot for Dnr. See Fig. 1 for explanation of graphs

model before running. With model A, no covariates were recognized as significant with the SCM; for instance, the covariate bWBC on Vc only caused a ΔOFV of −1.63. The OFV based on the runs without parameter re-estimation (MAXEVAL = 0, see methods section) was 2.9 units lower when the Vc-bWBC covariate relationship was included, while the interindividual variability for Vc decreased from 115 to 105%. The reduction in OFV indicated that there was also support for this relationship in the current data. For comparison, the differences in parameter estimates and uncertainties, along with the parameter estimates from the prior study [11], can be seen in Table 3. The Vc was increased by 1.9% for each increase in bWBC of 1×10^6 cells/mL. The median value was set to the value from the previous PK study (39×10^6 cells/mL).

Bilirubin was found to be a significant covariate in the SCM for Eto. However, only two patients had values (29 and 55 μmol/L) above what was considered the normal range of bilirubin (4–25 μmol/L). When the patient with a bilirubin concentration of 55 μmol/L was excluded from the analysis, bilirubin was no longer a significant covariate. For this reason, bilirubin was not included as a covariate in the final model. Several other covariates were significant in the SCM for Eto. ALAT, cCrCL, and bWBC were significant covariates on CL; ALAT as a piecewise linear model and the other covariates as linear inclusions. It was chosen not to include ALAT in the final model, since the θ_j (below median) was negative (−0.08) and the θ_j (above median) was positive (0.03) signifying a U-shaped relationship between ALAT and CL, which is difficult to explain physiologically. The two parameters also had high standard errors (227 and 109%, respectively). In the final model, gender was a significant categorical covariate for Vc, with women having a lower Vc than men, and cCrCL and bWBC were covariates on CL (Table 2). A decrease in cCrCL from the median of 116 mL/min (within normal range) to 60 mL/min (below normal range) gave a decrease in total CL of 30%, and conversely an increase from 116 to 160 mL/min (above normal range) gave an increase in total CL of 24%. The time course in plasma concentration and AUC of Eto for these changes in a typical individual is illustrated in Fig. 4. The corresponding $AUC_{0–24}$ values (calculated with the trapezoidal rule) for an otherwise typical patient with cCrCL of 60 mL/min was 122.5 mg/L h; for the mean cCrCL: 90.1 mg/L h; and for a cCrCL of 160 mL/min, it was 74.0 mg/L h. An increase in bWBC from 9×10^6 to 90×10^6 cells/mL gave a typical increase in 15% in CL. The inclusion of these covariates caused a total decrease in OFV of 13.7, a decrease in interindividual error for CL from 33 to 28% and for Vc from 33 to 22%.

The impact of dividing the total clearance into a non-renal clearance and a renal clearance was tested. The OFV only decreased by 0.052 for the split clearance model, but it

Table 3 Comparison of parameter estimates for the Dnr model building process

	Prior study[a]	Only present study	Prior study + Vc-bWBC[a]	Only present study + Vc-bWBC	Present study with PRIOR subroutine	Present study with PRIOR subroutine + Vc-bWBC
CL (L/h)	115	267	114	260	138	129
Vc (L)	373	198	412	187	350	453
Q (L/h)	252	135	254	133	235	152
Vp (L)	1,120	223	1,120	211	1,080	911
Res. error (%)	52.4	89.8	51.7	86.5	77.8	76.1
Vc-bWBC	–	–	0.0138	0.00038†	–	0.0185§

For abbreviations, see Table 2. Addition of bWBC on Vc was not significant without prior information (†) but showed a small improvement in fit when prior information was included (§)

[a] Data from Bogason et al. [11]

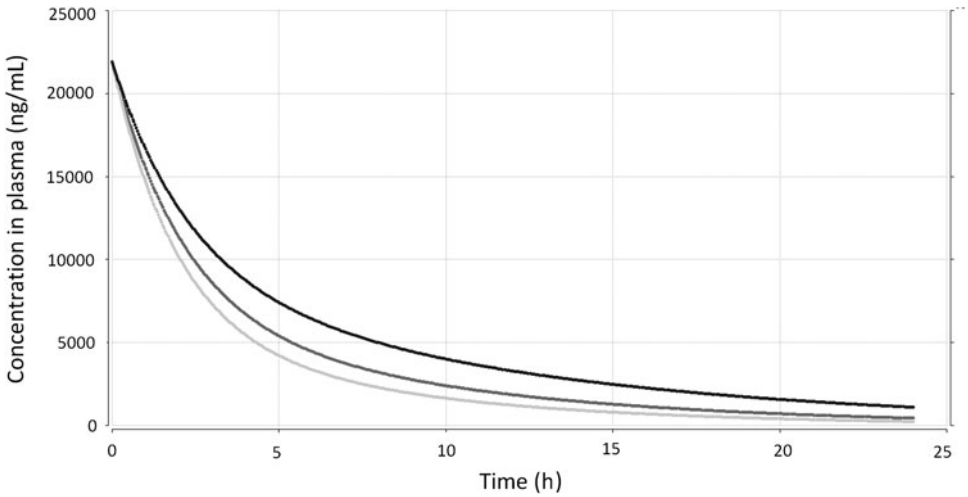

Fig. 4 Calculated time course for Eto concentrations in plasma for a typical individual with total CL values calculated from different cCrCL values. *Top black line* cCrCL = 60 mL/min, *middle dark gray line* cCrCL 116, *bottom light gray line* cCrCL = 160 mL/min. The corresponding AUC_{0-24} values (calculated with the trapezoidal rule) were: *top line* 122.5 mg/L h, *middle line* 90.1 mg/L h, and *bottom line* 74.0 mg/L h

was chosen as the final model because of the mechanistic interpretation. The final parameter estimates suggested that renal clearance in a patient with cCRCL of 116 mL/min constituted 54% of the total clearance, which is somewhat higher than previously published data (24–40%) [24, 25].

Discussion

The M3 method for BLQ observations was used in all three final models. This was chosen because of a good fit as demonstrated by the VPC double panel plots (Figs. 1, 2, 3). Furthermore, previous studies have supplied evidence for the reduction in parameter biases with the M3 method [17, 20, 21]. The relatively small misspecification for the Dnr BLQ samples after approximately 10 h and onwards could be explained by the use of the PRIOR function. Instead of using the M3 method, the prior study used the measured values between LLoQ and limit of detection (LoD) directly in the model building, while samples below LoD were omitted without consideration in the analysis [11]. The use of the PRIOR subroutine in the final model for Dnr was decided for several reasons: A) the scientific gain from the added knowledge from the prior PK study on the sparse data in the present study, B) the relatively small change in parameter estimates with or without the prior information, C) the reduced residual error with the prior information, and D) the further indication of bWBC as a covariate on Vc. The number of degrees of freedom, ν, was tested with different values, with only little change in the parameter estimates (data not shown). Therefore, the value of ν in the final model was set to 2 (the lowest possible value), since a low value of ν may be more appropriate to compensate for the choice of distribution (i.e., inverseWishart) [18].

The correlation between Ara-C clearance and bWBC was in the opposite direction of what was found in a simpler linear regression test by Fleming et al. [12]. Ara-C is metabolised both intra-cellularly in white blood cells and in plasma to the inactive metabolite Ara-U 4. Based on the inverse CL-bWBC relationship in the present study, it could be argued that the major part of this metabolism takes place outside the white blood cells. A higher degree of deamination in plasma compared to white blood cells has been proposed previously [26]. Furthermore, the bWBC needs to be quite elevated before it is potentially clinically relevant. The very high values of bWBC are, however, not values that are uncommon in patients with AML. In the present study, four of the twenty-three patients had bWBC above 70×10^6 cells/mL. Gender was a significant covariate for Ara-C clearance in the present study and has also previously been included in patients with AML [12, 13]. In spite of this, it was not included in the final model. The evidence is diluted by the fact that the two previous studies provide conflicting data. The study by Burk et al. [13], with high-dose, 3-h infusion Ara-C treatment, found an increased CL in female patients, and Fleming et al. [12], with intermediate dose, continuous infusion Ara-C, found an increased CL in the male population. Weight may in part explain gender differences, but neither study included weight as a covariate.

A weak trend was seen for the covariate relationship between bWBC and Dnr Vc. Albeit non-significant, the parameter relationship pointed in the same direction as previously reported (i.e., toward an inverse relationship). The previous PK study found this covariate relationship to be significant. Only this relationship was chosen to be tested on the full model including the PRIOR subroutine with full information from the previous study where bWBC was included. In this case, the addition of bWBC on Vc gave a further, slight improvement in model fit. Dose individualization based on Vc could be useful in general, since it has been shown that the peak concentration of Dnr is of importance for drug effect [27]. However, the influence of the bWBC on this parameter was not statistically

significant based on the current data. Other studies point toward AUC as the most important PK parameter for anthracycline myelotoxicity, which diminishes the usefulness of individualization based on Vc [28]. For these reasons, no dose adjustments based on this covariate can be recommended for daunorubicin.

The importance of kidney function in the dosing of Eto has been shown previously [14–16] and was further confirmed in this study with the inclusion of the renal CL-cCrCL relationship. These findings suggest that the dose of Eto should be lowered in patients with poor kidney function, but also that it could be considered to increase the dose in patients with a cCrCL that lies above the normal range. For these patients, an increased total CL may lead to sub-optimal treatment. Studies including clinical end points are needed before such conclusions can be drawn. Some issues should be noted when cCrCL is used as a covariate. Bias of the assays used for determining the Se-Cr has an impact on the cCrCL, especially for normal and above-normal values [29]. Low values of Se-Cr may be due to a decreased production of creatinine rather than an increased clearance. Therefore, Kirkpatrick et al. [30] assigned a Se-Cr value of 60 μmol/L to all values measured below 60 μmol/L. With the present data, this procedure resulted in a similar OFV as with using the actual measurements of Se-Cr (ΔOFV = 0.252). Problems have also been recognized for the use of the Cockcroft-Gault equation for estimating cCrCL. It leads to underestimations of cCrCL in healthy subjects with a cCrCL above normal, and it leads to an overestimation in obese patients [31, 32]. The issue with obese patients can be overcome by assigning an upper limit of cCrCL as in the work by Mould et al. [33], who capped the cCrCL for patients weighing more than 100 kg at 150 mL/min. However, in the present study, none of the patients with a weight above 90 kg had a cCrCL above 150 mL/min. If the underestimation effect in healthy subjects can be translated directly to AML patients, it only augments the risk of sub-optimal treatment in patients with high cCrCL. For these reasons, it was decided to use the cCrCL without any lower or upper limits. The possibility of sub-optimal treatment needs to be confirmed with further studies that include clinical end points such as disease remission or toxicity and maybe through the use of other equations or methods to estimate cCrCL as well.

It is known that Eto undergoes both hepatic metabolism and renal excretion [34]. Neither ALAT, as a measure of liver damage, nor bilirubin, as a surrogate for liver functionality, were included in the final model. The argument for including bWBC on CL is that an increased amount of white blood cells will remove more drugs from the plasma and that Eto to some extent may be metabolized within the white blood cells. This metabolic pathway has been described for both Ara-C and Dnr, but not for etoposide [4, 9, 35]. As discussed for Ara-C, a relatively large change in the magnitude of bWBC was also needed for the covariate relationship to be clinically important for Eto. The THETA for the Vc-Gender correlation was negative (−0.2793), which means that women typically had a Vc that was 32% lower than men. This is in agreement with a study of etoposide phosphate by Kaul et al. [36], but the magnitude of the relationship was higher in the present study. Others have not been able to show a correlation between gender and PK parameters [14–16]. The clinical relevance of this relationship is diminished by the fact that AUC supposedly is the most important PK parameter for Eto [28, 34]. It should be noted that although weight was not found to be a significant covariate in the SCM, women had a significantly lower weight ($P < 0.01$, two-tailed student's t test), and that the gender influence in part may be driven by the differences in weight between the two groups.

In conclusion, this study has provided further insight into the clinical pharmacokinetics of Eto, Ara-C, and Dnr after administration in combination. The recently suggested influence of bWBC on central volume of distribution of Dnr has been further indicated, but with insufficient strength to propose dose adjustments based on this relationship. It was shown that bWBC also influenced the PK of both Eto and Ara-C. Relatively, large changes in magnitude were needed for these relationships to have potential clinical relevance. Linking the influence of kidney function and bWBC on Eto clearance or AUC to disease remission or toxicity could potentially point toward dose individualization. This is the case not only for patients with a poor kidney function, but also for patients with an above-normal creatinine clearance, who may receive sub-optimal treatment due to an elevated clearance of Eto. Likewise, a study of a possible correlation between baseline white blood cell count, cytarabine clearance (or AUC), and clinical end points in a larger population would be relevant.

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