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Population Pharmacokinetics of Metronidazole Evaluated Using Scavenged Samples from Preterm Infants

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Pharmacokinetic (PK) studies in preterm infants are rarely conducted due to the research challenges posed by this population. To overcome these challenges, minimal-risk methods such as scavenged sampling can be used to evaluate the PK of commonly used drugs in this population. We evaluated the population PK of metronidazole using targeted sparse sampling and scavenged samples from infants that were ≤32 weeks of gestational age at birth and <120 postnatal days. A 5-center study was performed. A population PK model using nonlinear mixed-effect modeling (NONMEM) was developed. Covariate effects were evaluated based on estimated precision and clinical significance. Using the individual Bayesian PK estimates from the final population PK model and the dosing regimen used for each subject, the proportion of subjects achieving the therapeutic target of trough concentrations >8 mg/liter was calculated. Monte Carlo simulations were performed to evaluate the adequacy of different dosing recommendations per gestational age group. Thirty-two preterm infants were enrolled: the median (range) gestational age at birth was 27 (22 to 32) weeks, postnatal age was 41 (0 to 97) days, postmenstrual age (PMA) was 32 (24 to 43) weeks, and weight was 1,495 (678 to 3,850) g. The final PK data set contained 116 samples; 104/116 (90%) were scavenged from discarded clinical specimens. Metronidazole population PK was best described by a 1-compartment model. The population mean clearance (CL; liter/h) was determined as $0.0397 \times (\text{weight}/1.5) \times (\text{PMA}/32)^{2.49}$ using a volume of distribution (V) (liter) of $1.07 \times (\text{weight}/1.5)$. The relative standard errors around parameter estimates ranged between 11% and 30%. On average, metronidazole concentrations in scavenged samples were 30% lower than those measured in scheduled blood draws. The majority of infants (>70%) met predefined pharmacodynamic efficacy targets. A new, simplified, postmenstrual-age-based dosing regimen is recommended for this population. Minimal-risk methods such as scavenged PK sampling provided meaningful information related to development of metronidazole PK models and dosing recommendations.

Preterm infant pharmacokinetic (PK) studies are exceptionally scarce due to the research challenges posed by this population, such as limited blood volume for PK sampling, difficulty in timing of PK samples due to the critical medical condition of the infants, difficult access to obtain samples, and low rates of informed parental consent. Feasibility issues also are imposed by traditional PK study designs, which require large numbers of specifically timed PK blood samples. These difficulties have encouraged investigators to explore novel minimal-risk methods to evaluate the PK of antimicrobials in this population. One proposed method is the use of scavenged samples left over from the normal clinical care of infants. The use of scavenged samples for PK studies in preterm infants offers several advantages over traditional timed PK trials. These include avoiding the need for heel sticks specifically for the study, higher rates of parental consent due to perceived minimal risk, availability of several samples per infant, and avoidance of time-specific PK sampling (23). This method, combined with collection of timed PK samples (collected specifically for study purposes), has proven successful in the evaluation of fluconazole PK in preterm infants (23). To our knowledge, the use of scavenged samples for PK analysis of other antimicrobials has not been evaluated.

Metronidazole is approved by the U.S. Food and Drug Administration for the treatment of adults with serious infections caused by susceptible anaerobic bacteria but is not approved for use in children. In spite of this, metronidazole is extensively used "off-label" in children to treat anaerobic intra-abdominal infections (i.e., perforated appendicitis) (21). In young infants, its use is typically restricted to treatment of rare cases of anaerobic bacteremia, central nervous system infections, and complicated intra-abdominal infections such as necrotizing enterocolitis (3, 18). Because infection in young infants with very low birth weight (<1,500 g) is associated with devastating outcomes, including death and neurodevelopmental impairment, appropriate dosing recommendations for agents such as metronidazole for this population are needed.

Metronidazole dosing recommendations for preterm infants listed in commonly used reference sources for pediatric practitioners are based on a combination of birth weight and postnatal age (PNA) (20) or birth gestational age (BGA) and postmenstrual age (PMA) (19). These recommendations are derived from small, single-center studies and have not been prospectively evaluated. In

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TABLE 1 Dosing schemes used to assess pharmacodynamic target achievement[a]

Regimen source or type	BW (kg)	BGA (wk)	PNA (days)	PMA (wk)	Loading dose (mg/kg)	Maintenance dose (mg/kg)	Dosing interval (h)
The Harriet Lane Handbook	<1.2	NA	≥7	NA	NA	7.5	24
	1.2–2	NA	≥7	NA	NA	7.5	12
	≥2	NA	≥7	NA	NA	15	12
Neofax	NA	NA	≤28	≤29	15	7.5	48
	NA	NA	>28	≤29	15	7.5	24
	NA	NA	≤14	30–36	15	7.5	24
	NA	NA	>14	30–36	15	7.5	12
	NA	NA	≤7	37–44	15	7.5	24
	NA	NA	>7	37–44	15	7.5	12
	NA	NA	ALL	≥45	15	7.5	6
PMA-based dosing regimen	NA	≤32	NA	<34	15	7.5	12
	NA	≤32	NA	34–40	15	7.5	8
	NA	≤32	NA	>40	15	7.5	6

[a] BW, birth weight; GA, gestational age; PMA, postmenstrual age; PNA, postnatal age; NA, not applicable.

addition, these dosing regimens are cumbersome due to the different combinations of maturation components required to choose the most appropriate dose. PMA-based dosing offers an advantage over current recommendations by simplifying dosing regimens and potentially providing desired metronidazole concentrations over a wider age range. PMA-based dosing has not been systematically evaluated for metronidazole; however, it has proven successful for other therapeutics in preterm infants (11, 13, 15).

The present study was conducted to assess the population PK of intravenous metronidazole using scavenged samples collected from preterm infants ≤32 weeks BGA.

MATERIALS AND METHODS

Study design. PK samples for this analysis were obtained from the Antimicrobial PK in High-Risk Infants trial sponsored by the Pediatric Pharmacology Research Unit. This trial was a multicenter, prospective, open-label PK study of antimicrobial agents commonly used in the neonatal intensive care unit. Infants with a BGA of ≤32 weeks and <120 days of age receiving intravenous metronidazole as part of their routine medical care were enrolled. Metronidazole dosing was determined by the routine clinical practice in each unit, and no exclusion criteria were used. To evaluate the effect of maturation on metronidazole PK, infants were stratified at enrollment by BGA: <26 weeks, 26 to 29 weeks, and 30 to 32 weeks. The study was approved by the institutional review boards at each institution, and informed consent was obtained from a parent or guardian prior to enrollment.

The following information was collected for covariate analysis: BGA, PNA, PMA, weight, sex, race, serum creatinine, and ethnicity. Covariates that exhibited time-dependent changes (e.g., weight and PNA) were permitted to change with time, and the actual value in the data set reflects the observations made at each patient visit. Missing weights were assigned, with the last recorded value being carried forward for up to 7 days. Serum creatinine was recorded when obtained for clinical care. Missing serum creatinine values were assigned based on an exponential model of serum creatinine and PMA derived from the data (16).

PK sample collection. A sparse sampling approach was followed in this study. Samples were divided into two types: scavenged and blood draw. Scavenged samples were defined as samples obtained without obtaining additional blood from the infant. These samples were collected from the clinical laboratory from discarded blood (heparinized or EDTA tubes) obtained for routine clinical care. Blood draw samples were defined as samples obtained by collection of extra blood from the infant. Each blood draw was approximately 0.3 ml of blood collected in EDTA Microtainers. PK sample collection was planned at the following time points: immediately prior to metronidazole infusion, immediately after the completion of infusion (time zero), approximately 1 to 1.5 h after completion of infusion (1 to 1.5 h), approximately 3 to 4 h after completion of infusion (3 to 4 h), and immediately prior to infusion of the next dose. The following information was collected for all PK samples: draw date, time, type of sample (i.e., scavenged versus blood draw), specimen number, collection tube type, and estimated sample volume. The scavenged sample information was collected from original source documents (nursing documentation from the medical record). Both accuracy and completeness of information regarding scavenged samples were assessed through queries after metronidazole concentration data were obtained. The duration of metronidazole infusion was performed according to site routine clinical care. Samples were refrigerated or placed on ice immediately after collection and then centrifuged at 1,500 × *g* and 4°C for 10 min. Plasma was removed and stored at −70°C. Samples from all sites were shipped on dry ice to Duke University Medical Center, where they were stored at −70°C prior to analysis. Samples were stored for a maximum of 32 months prior to analysis.

Bioanalytical assay. A liquid chromatography-tandem mass spectrometry assay for metronidazole detection in human plasma suitable for small plasma volumes was developed and validated (5). Briefly, sample analysis was performed on an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems–ABSciex, Foster City, CA) operated with electrospray ionization (TurboV source using a TurboIonspray probe). Instrument parameters were optimized for the metronidazole transition (172.1→128.1 *m/z*). Chromatographic separation was achieved using a reverse-phase C_{18} Aquasil column (Thermo Fisher, Waltham, MA) with a flow rate of 0.75 ml/min using a gradient mobile phase. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in methanol. Analytical data were acquired by Analyst software 1.4.1 (Applied Biosystems–ABSciex, Foster City, CA). The lower limit of quantitation of metronidazole in plasma was 0.05 mg/liter. Intraday and interday coefficients of variation were <9.5% at concentrations ranging from 0.05 to 25 mg/liter.

Population PK analysis. PK data were analyzed with a nonlinear mixed-effect modeling approach using the computer program NONMEM (version 7) in conjunction with WINGS for NONMEM version 7.03 (University of Auckland, Auckland, New Zealand). Output was summarized using STATA 10 (StataCorp, College Station, TX). The first-order conditional estimation method with interaction was used

TABLE 2 Clinical data by gestational age group

Characteristic	Value[a] for group with gestational age at birth (wk)		
	<26	26–29	30–32
n	13	14	5
Gestational age (wk)	24 (22–25)	28 (26–29)	31 (30–32)
Postnatal age (days)	53 (7–97)	32 (0–97)	33 (8–71)
Postmenstrual age (wk)	32 (24–39)	32 (28–43)	36 (32–40)
Weight (g)	1,410 (678–2,537)	1,510 (850–3,611)	1,658 (1,230–3,850)
Female	6 (46)	9 (64)	2 (40)
White race	4 (31)	10 (71)	2 (40)
Hispanic	1 (8)	2 (14)	0 (0)
Serum creatinine (mg/dl)	0.5 (0.3–4.7)	0.5 (0.2–1.2)	0.2 (0.1–0.6)
Dose (mg/kg)	7.6 (4.2–14.2)	7.8 (6.2–15.1)	9.0 (6.5–15.4)
Dosing frequency (h)	11.9 (5.9–48.2)	12 (5.9–48)	12.3 (11.2–26.7)

[a] Data are given in the formats median (range) for continuous data and *n* (%) for categorical data.

for all model runs. One- and two-compartment structural PK models were evaluated. Interindividual random effects were evaluated on clearance (CL) and volume of distribution (*V*). An exponential model for interindividual variance was used, and a proportional error model was deemed appropriate to describe residual variability. The potential impact of clinical covariates on PK parameters was explored if a relationship was suggested by visual inspection of scatter and box plots (continuous and categorical variables, respectively) of individual Bayesian estimates obtained from the base model and CL interindividual variability against covariates. The following covariates were evaluated: weight (kg), BGA (weeks), PNA (days), PMA (defined as GA plus PNA in weeks [PNA/7]), serum creatinine, race, sex, and ethnicity. A serum creatinine model including an indicator variable (CR) that excluded a potential outlier with a serum creatinine of 4.7 mg/dl was also evaluated. Once covariates were identified during the model-building process, covariate testing was performed via standard forward-addition–backward-elimination methods. Potential covariates that reduced the objective function by more than 3.84 ($P < \sim 0.05$) were included in the subsequent multivariable analysis. A forward inclusion with backward elimination approach was used during the multivariable step, and a reduction of 6.63 ($P < \sim 0.01$) was required for retention of a covariate in the final model. Continuous covariates were scaled to their median values. Empirical Bayesian estimates of individual infant PK parameters were generated from the final model

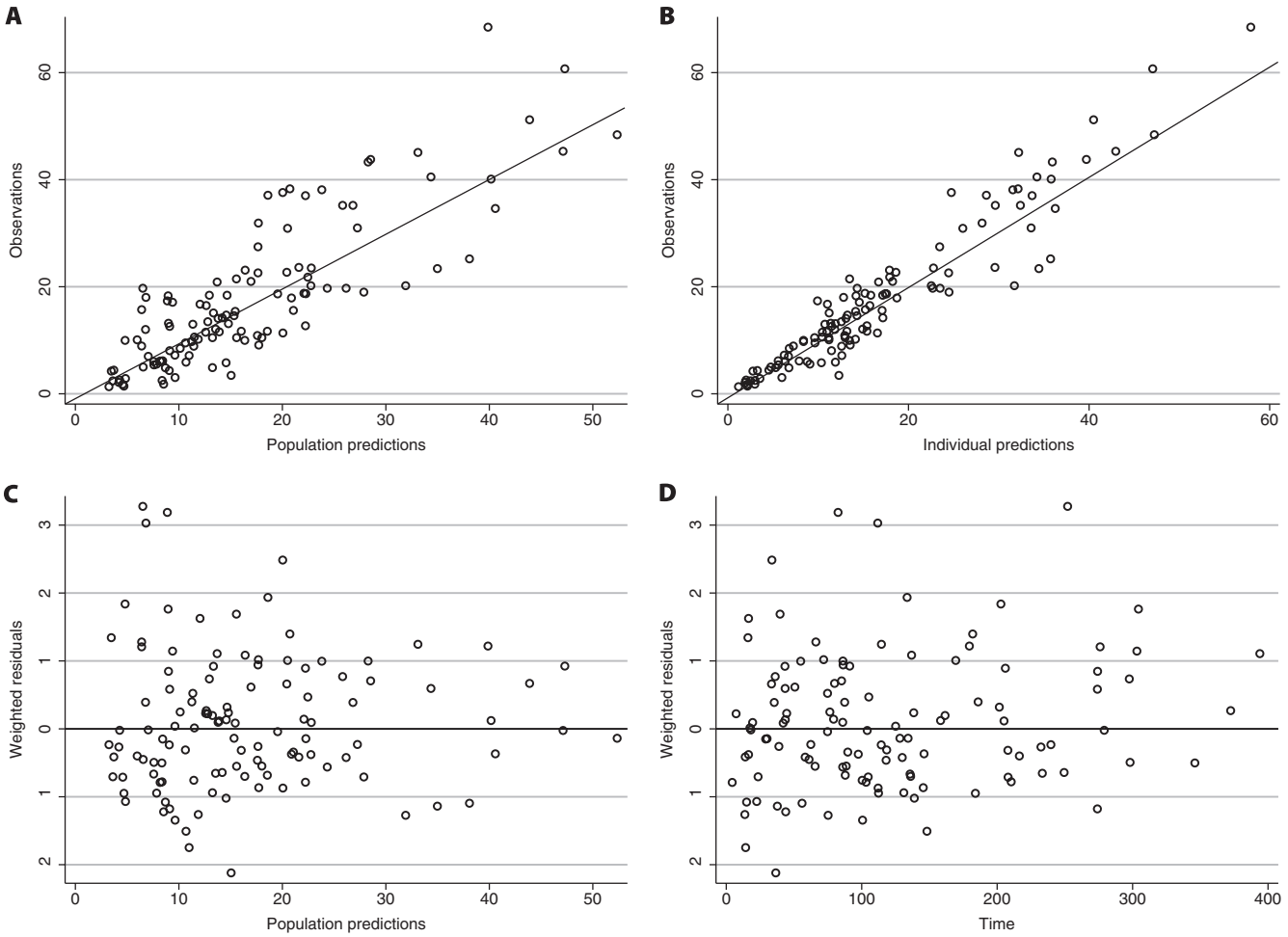

FIG 1 Final population PK model diagnostic plots, showing observed versus population predictions (A) and individual predictions (B) and weighted residuals versus population predictions (C) and time (D). In panels A and B, the line of identity is included as a reference. For weighted residuals, a solid line at $y = 0$ is included as a reference.

TABLE 3 Model-building process[a]

Base model and univariable analysis	Population model	OFV	ΔOFV
V	$V = \theta_V \times (\text{wt}/1.5)$	498	
CL base model	$\text{CL} = \theta_{\text{CL}} \times (\text{wt}/1.5)$	498	
PMA	$\text{CL} = \theta_{\text{CL}} \times (\text{wt}/1.5) \times (\text{PMA}/32)^{\theta\text{CL-PMA}}$	484.5	−13.5
PNA	$\text{CL} = \theta_{\text{CL}} \times (\text{wt}/1.5) \times (\text{PNA}/57)^{\theta\text{CL-PNA}}$	486.4	−11.6
SCR	$\text{CL} = \theta_{\text{CL}} \times (\text{wt}/1.5) \times (0.5/\text{SCR})^{\theta\text{CL-SCR}}$	483.7	−14.3
SCR + indicator	$\text{CL} = \theta_{\text{CL}} \times (\text{wt}/1.5) \times (0.5/\text{SCR})^{\theta\text{CL-SCR} \times \text{CR}}$	487.2	−10.8
Multivariable model			
CL, PMA, and SCR	$\text{CL} = \theta_{\text{CL}} \times (\text{wt}/1.5) \times (\text{PMA}/32)^{\theta\text{CL-PMA}} \times (0.5/\text{SCR})^{\theta\text{CL-SCR}}$	480.8	−3.7

[a] CL, clearance; OFV, objective function value; PMA, postmenstrual age; PNA, postnatal age; SCR, serum creatinine (mg/dl); *V*, volume of distribution. Weight is in kg.

using the *post hoc* subroutine. Bias introduced by scavenged specimens was evaluated in the random-effects error model of the final population model using a fixed effect parameter (θ^{SCAV}), as well as different residual variability estimates for blood draws and scavenged samples. An indicator variable was used to identify scavenged samples.

Model evaluation. Models were evaluated based on successful minimization, goodness-of-fit plots, precision of parameter estimates, bootstrap procedures, and visual predictive check. The precision of the final population PK model parameter estimates were evaluated using nonparametric bootstrapping (1,000 replicates) to generate the 95% confidence intervals for parameter estimates. For the visual predictive check, the final model was used to generate 1,000 Monte Carlo simulation replicates of metronidazole exposure, and simulated results were compared with those observed in the study. The number of observed concentrations outside the 90% prediction interval for each time point was quantified.

Assessment of dose-exposure relationship. Surrogate pharmacodynamic (PD) targets for metronidazole against anaerobic bacteria are poorly defined (10). Therefore, for target exposure, a MIC of 8 mg/liter at steady state was chosen as the surrogate efficacy target. This MIC target is consistent with the Clinical and Laboratory Standards Institute-recommended MIC susceptibility breakpoint of metronidazole for anaerobic organisms (4). Metronidazole trough concentrations at steady state were predicted for each subject using individual empirical Bayesian estimates from the final model and dosing prescribed in the study per routine medical care. The proportion of subjects in the study who met the PD target

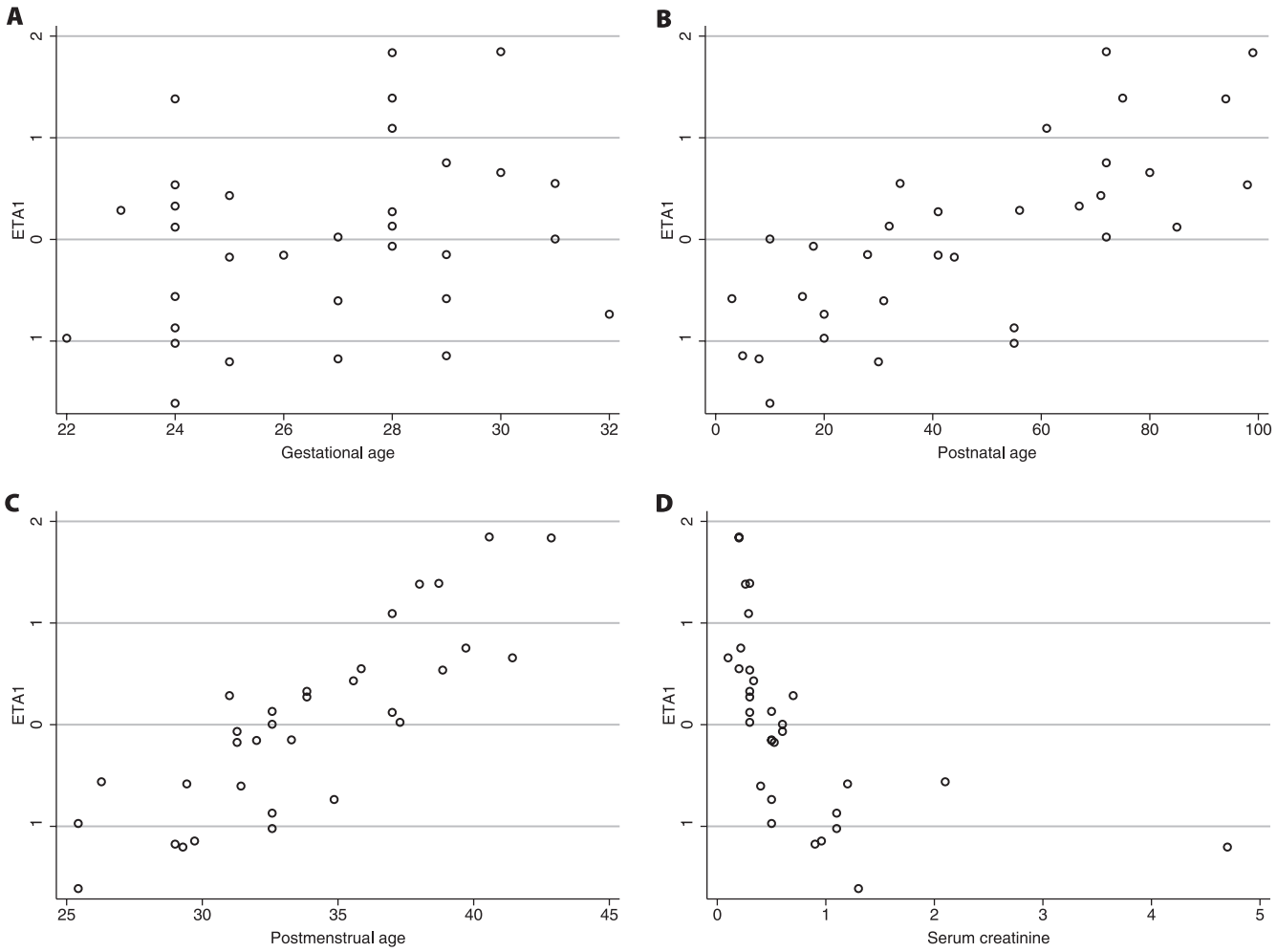

FIG 2 Base model scatter plots of CL empirical Bayesian estimates and BGA (A), PNA (B), PMA (C), and serum creatinine (D).

TABLE 4 Final population pharmacokinetic model parameter estimates[a]

Parameter	Symbol	Point estimate	% RSE	Bootstrap CI		
				2.5%	Median	97.5%
CL (liters/h)	θ_{CL}	0.0397	10.9	0.0307	0.0398	0.0483
V (liters)	θ_{V}	1.07	15.0	0.85	1.12	1.37
CL, PMA	$\theta_{CL\text{-}PMA}$	2.49	29.8	1.01	2.57	4.20
SCAV	θ_{SCAV}	0.713	12.3	0.581	0.721	0.899
Interindividual variance						
CL (CV%)	ω^2_{CL}	42.5	28.5	24.2	40.4	52.8
Residual variance (CV%)						
Blood draws	σ_1^2	13.5	24.5	0.3	13.3	15.7
Scavenged	σ_1^2	29.0	17.9	23.7	27.8	34.9

[a] CI, confidence interval; CL, clearance; CV, coefficient of variation; PMA, postmenstrual age; RSE, relative standard error; *V*, volume of distribution; SCAV, scavenged samples.

was calculated by BGA group. In addition, Monte Carlo simulations (n = 1,000) using the final population PK model were used to explore dose-exposure relationships of commonly used metronidazole dosing recommendations listed in *Neofax 2011* (19) and *The Harriet Lane Handbook* (20), as well as a newly proposed, simpler dosing regimen based on PMA (Table 1). When a dosing range was recommended, the highest end of the range was chosen for the simulations. The proportion of simulated subjects who met the PD target was calculated by BGA group and PNA. Additionally, Monte Carlo simulations (n = 100) using the final population PK model fixed and random-effects estimates were performed to predict metronidazole concentrations in typical subjects receiving the newly proposed PMA-based dosing regimen, including a loading dose. These typical subjects included an infant with a PMA of 26 weeks (weight, 900 g), one with a PMA of 32 weeks (weight, 1,900 g), one with a PMA of 36 weeks (weight, 2,800 g), and one with a PMA of 41 weeks (weight, 3,800 g).

RESULTS

Study population. Thirty-three subjects from five centers were evaluated for analysis. One subject was excluded from the analysis because sampling was obtained during drug infusion and no other samples were collected. The overall median (range) BGA, PNA, PMA, weight, serum creatinine, and dose were 27 (22 to 32) weeks, 41 (0 to 97) days, 32 (24 to 43) weeks, 1,495 (678 to 3,850) g, 0.5 (0.1 to 4.7) mg/dl, and 8 (4 to 15) mg/kg, respectively (Table 2). The majority of subjects were female (17/32 [53%]). Sixteen (50%) were white, and 4 (9%) were Hispanic.

PK specimens. A total of 3 outlier concentrations (of a total of 119 [2.5%]) were removed from the analysis due to unreliability of sampling times related to time of flush (n = 2) or sample contamination (n = 1). The exclusion of these subjects and samples resulted in 32 subjects from 5 sites with 116 concentrations being used in the modeling process. The median time of PK sampling was 6.5 (0.5 to 24) hours after dosing, and the median concentration was 14.5 (1.31 to 68.5) mg/liter. An average of 3.6 samples per infant (range, 1 to 15) was collected, and the majority of PK samples were scavenged from the clinical laboratory (104/116, 90%).

Population PK model building. A 1-compartment model was the appropriate structural PK model for this data set (Fig. 1). Because few samples were obtained within the first few hours after dosing, intercompartmental clearance was not estimated, and a 2-compartment model did not provide a better fit to the data. Weight was included in the base CL and *V* models (Table 3). An estimation of the body size exponent (weight$^{\theta}$) was explored as a potential body size model for CL and *V*; however, it was excluded due to lack of improvement in model fit and imprecision around the exponent parameter estimate. After weight was incorporated into the base model, it was not possible to estimate interindividual variability in *V*. An evaluation of the model prior to the addition of weight as a covariate for *V* demonstrated high shrinkage in *V* interindividual variability (47%). This finding suggested that the *V* interindividual variability parameter estimate deviated from a normal distribution leading to nonparametric estimation of this parameter. However, once weight was reincorporated into the model, the *V* interindividual variability parameter was estimated with a value close to zero via nonparametric methods and was excluded during the model-building process. Age- and maturity-related covariates (PNA and PMA) as well as serum creatinine showed correlation with unexplained CL interindividual variability (Fig. 2). During the univariable evaluation (after inclusion of body weight), all age-related covariates as well as serum creatinine resulted in a significant decrease in the objective function value (Table 3). The largest drop in objective function value occurred when serum creatinine was added to the model. However, when the serum creatinine outlier indicator variable was used, the change in objective function was not as pronounced as that observed with the addition of maturation covariates. This suggested that the significant association between serum creatinine and CL was influenced by an outlier observation (Table 3). In the multivariable analysis, the addition of serum creatinine did not improve the model goodness of fit, nor did it significantly decrease the objective function value (Tables 3 and 4). When the bias of scavenged samples was evaluated in the error model of the final population PK model, underestimation of metronidazole concentrations by 30% (95% confidence interval, 10 to 42%) was observed (Table 4).

Population PK model evaluation. The final model had good

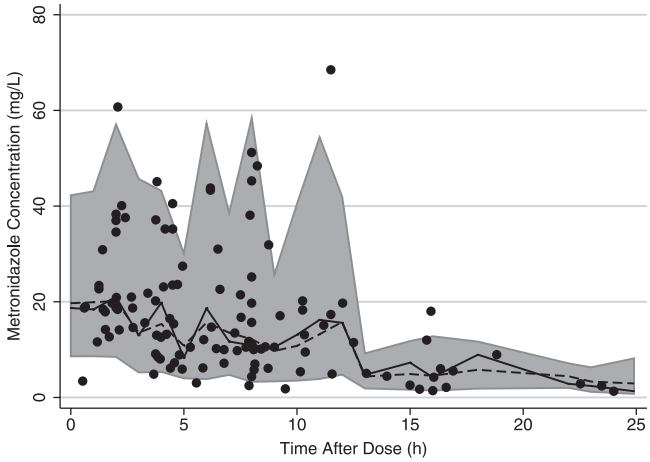

FIG 3 Visual predictive check of metronidazole concentrations versus time. Solid black circles represent observed concentrations. The shaded area represents the 90% prediction interval. Solid and dashed lines represent observed and predicted median concentrations, respectively.

TABLE 5 Individual empirical Bayesian pharmacokinetic parameter estimates by gestational age group

Gestational age	CL (liters/h)		CL (liters/h/kg)		*V* (liters)		*V* (liters/kg)		Half-life (h)	
	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI	Median	95% CI
<26 weeks	0.033	0.010, 0.187	0.024	0.010, 0.086	1.00	0.48, 1.81	0.71	NA	20.5	5.7, 49.9
26-29 weeks	0.040	0.012, 0.274	0.026	0.012, 0.076	1.08	0.60, 2.58	0.71	NA	18.6	6.5, 38.7
30-32 weeks	0.071	0.019, 0.285	0.029	0.015, 0.074	1.18	0.88, 2.75	0.71	NA	16.7	6.7, 31.1
Overall	0.041	0.012, 0.274	0.025	0.012, 0.076	1.06	0.54, 2.58	0.71	NA	19.1	6.5, 38.7

CI, confidence interval; CL, clearance; *V*, volume of distribution.

precision, as evidenced by relative standard errors around the parameter point estimates of 11 to 30% and by 95% confidence intervals generated by bootstrapping (1,000 simulated trials; 997 successful runs) (Table 4). Goodness-of-fit diagnostic plots for the final model are shown in Fig. 1. The visual predictive check revealed a good fit between observed and predicted metronidazole concentrations (Fig. 3). Only 7% (8/116) of observed concentrations were outside the 90% prediction interval. The two highest concentrations (>60 mg/liter), outside the 90% prediction interval, were observed in two subjects. The weight-adjusted estimated metronidazole clearance for these subjects was 0.012 liter/kg/h (5th percentile) and 0.020 liter/kg/h (25th percentile), respectively, and both subjects were <1 week of age.

Bayesian estimates of CL, *V*, and half-life. The median individual empirical Bayesian estimates for CL, *V*, and half-life were summarized by BGA group (Table 5). There was a trend toward increasing median metronidazole weight-normalized CL and decreasing half-life with increasing BGA group. Consistent with the PMA covariate influence in the final population PK model, weight-adjusted metronidazole CL increased with increasing PMA and decreased with increasing serum creatinine (Fig. 4).

Dose-exposure relationship. Over 70% of all infants included in this study achieved the PD target (steady-state trough concentrations > than an MIC of 8 mg/liter) (Fig. 5; Table 2). In contrast, <70% of subjects across BGA groups achieved the target when dosing recommendations from *Neofax 2011* and *The Harriet Lane Handbook* were used in simulated data sets (Fig. 5 and 6). The smallest proportion of preterm infants who achieved the PD target was observed in the 30- to 32-week BGA group and in infants with a PNA of >60 days (Fig. 5 and 6). In contrast, 90% of subjects regardless of BGA group and PNA achieved the target when the PMA-based dosing scheme was used in simulated data sets. Higher steady-state metronidazole trough concentrations were predicted by the PMA-based dosing regimen compared with the other regimens, and therapeutic concentrations (median) were achieved after a loading dose on the first day of therapy (Fig. 5 and 7).

DISCUSSION

In the present study, the scavenged sampling technique was used to successfully develop a population PK model and new metronidazole dosing recommendations for preterm infants. The newly proposed PMA-based dosing regimen compared very favorably against published schemes (19, 20); a higher proportion (~90%) of subjects achieved the therapeutic target in simulated data sets. The PMA-based dosing scheme was particularly effective in more mature (30 to 32 weeks) and older (PNA > 60 days) infants, where suboptimal metronidazole concentrations were achieved when published guidelines were used in simulated data sets. To achieve higher concentrations in more mature infants, a PMA cutoff of 34 weeks was chosen to increase the dosing frequency in the PMA-based dosing regimen. This decision was based upon examination of the relationship between weight-adjusted CL and PMA (Fig. 4) where, at 34 weeks, the rate of change in metronidazole CL increased. A higher proportion of subjects achieved the therapeutic target with the newly proposed regimen, while trough concentrations in each BGA group were similar to those observed with current

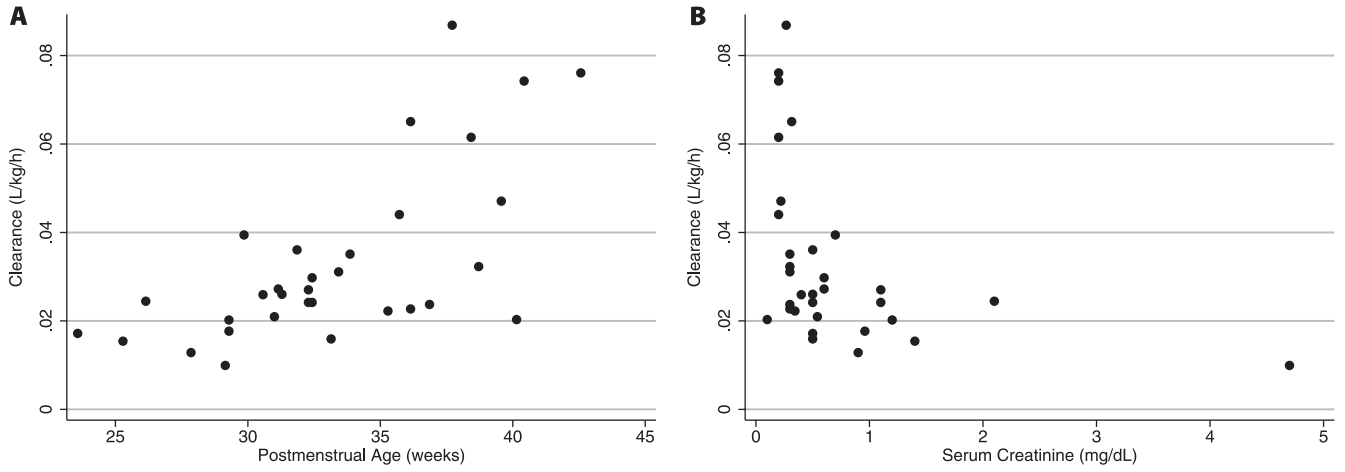

FIG 4 Weight-normalized metronidazole clearance versus postmenstrual age (A) and serum creatinine (B).

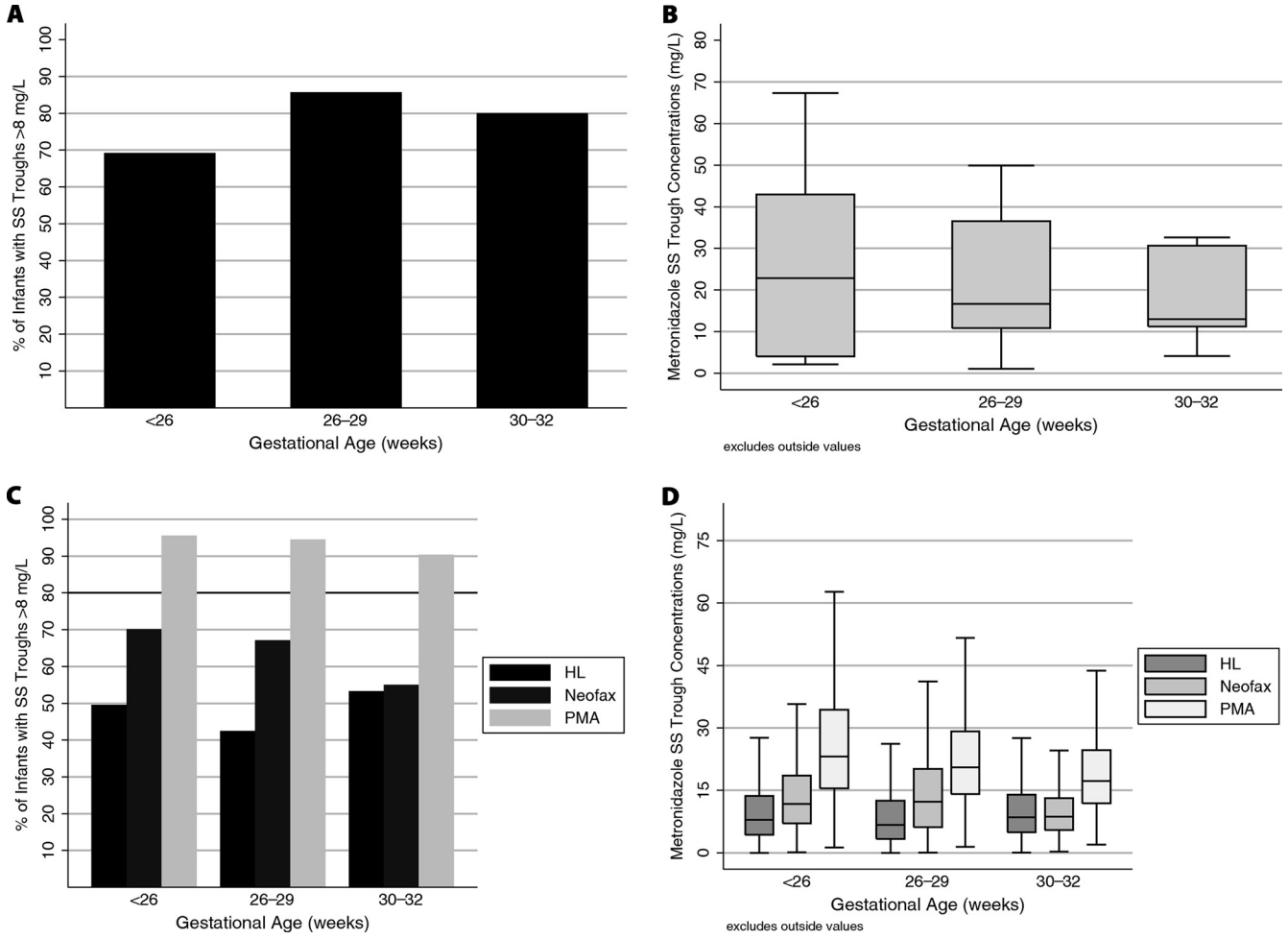

FIG 5 PD target attainment rates by gestational age group. (A) Proportion of study subjects who met the PD target with dosing prescribed during routine medical care; (B) predicted steady-state (SS) metronidazole trough concentrations in study subjects; (C) proportion of simulated subjects who met PD target with different dosing schemes; (D) predicted steady-state metronidazole trough concentrations in simulated subjects. The horizontal line in panel C represents an 80% success rate. HL, regimen derived according to *The Harriet Lane Handbook*; PMA, postmenstrual age dosing regimen.

routine medical care (Fig. 5). These data suggest that safety should not be different between the new dosing regimen and current clinical practice, but further prospective studies are warranted to verify this finding.

Because the present study did not evaluate efficacy or clinical outcomes, a surrogate PD endpoint was used for the study population receiving metronidazole as part of routine clinical care and in simulated data sets. In this study, the majority of subjects (>70%) receiving metronidazole during routine medical care achieved the surrogate PD target for efficacy. In contrast, when dosing recommendations published in commonly used pediatric sources were used in simulated data sets, a lower proportion of subjects achieved the surrogate PD target. This finding may be due to higher doses (more frequent administration) prescribed during routine medical care than in published regimens and suggests that prescribing practices in the neonatal intensive care unit are not driven by these sources (19, 20). Of five participating centers in this study, two used *Neofax 2011* as a reference for metronidazole dosing, two used LexiComp (17a), and one used unit-specific dosing guidelines. Of note, the scientific bases for dosing recommendations made by *The Harriet Lane Handbook* and *Neofax 2011* are limited due to small sample sizes and incomplete PK sampling.

In adults, metronidazole undergoes extensive hepatic metabolism with subsequent renal elimination (14); the elimination half-life is 8 h (8), 20% is protein-bound, and the apparent V ranges between 0.25 and 0.85 liter/kg (8). The hepatic metabolizing and renal elimination systems undergo ontogenic changes during infancy, resulting in increased CL with increasing size and age (2, 6). In young infants, metronidazole PK differs substantially from adults; the elimination half-life is 2- to 3-fold longer, and it decreases with increasing GA at birth and PNA (7, 9, 17, 22). Not surprisingly, in the present model, metronidazole CL increased proportionally with weight and disproportionally with PMA, and both covariates explained a substantial amount (~80%) of the CL interindividual variability. This finding is consistent with prior observations in a metronidazole population PK study of 32 preterm infants (PMA, 25 to 38 weeks) using dried blood spots, in which body weight and PMA explained ~93% of the CL interindividual variability (17). The population PK parameter estimates in the present study are also comparable to those estimated in the study using dried blood spots (17). Other smaller, single-center

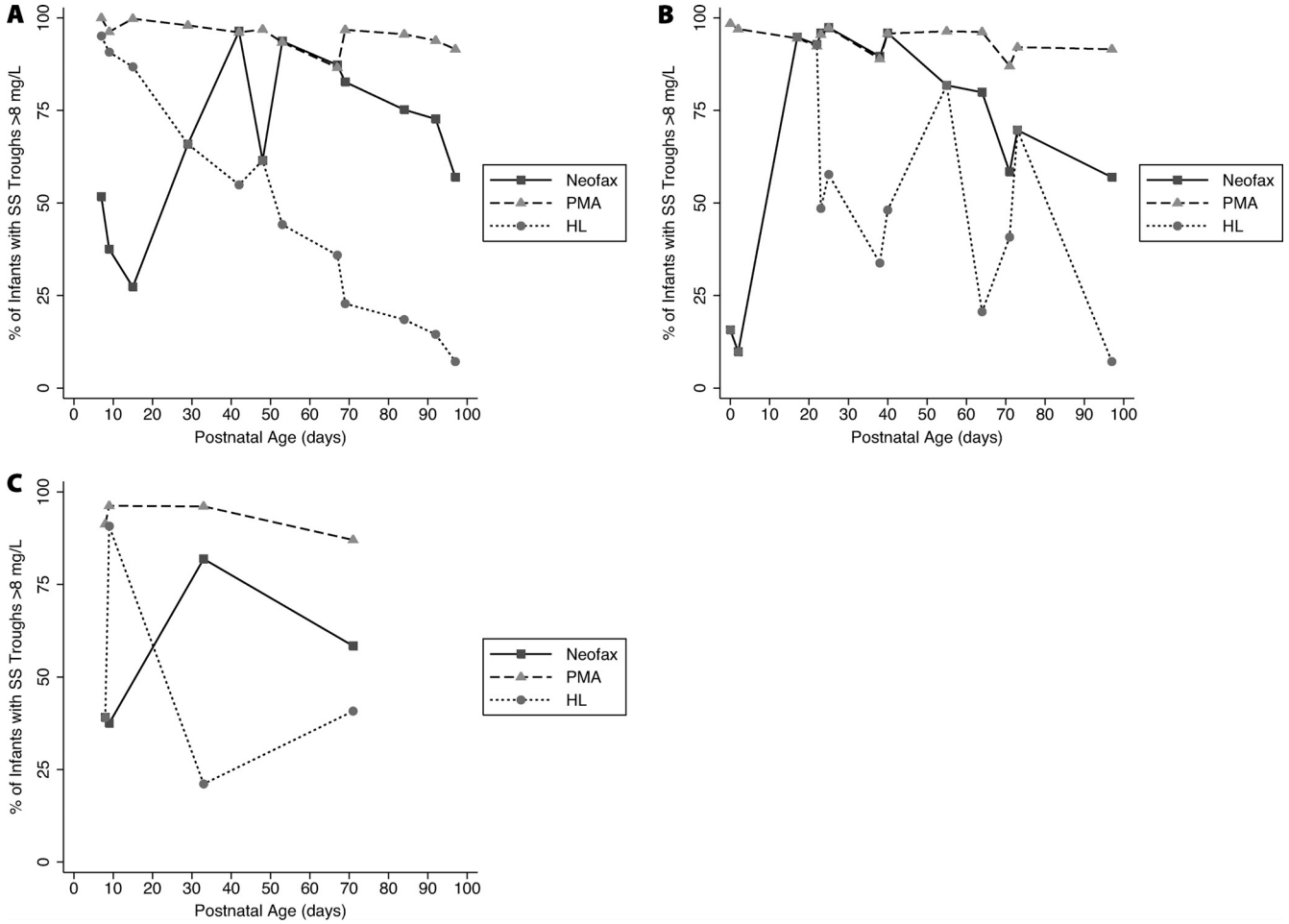

FIG 6 PD target attainment rates by gestational age group and postnatal age in simulated subjects in the <26-week (A), 26- to 29-week (B), and 30- to 32-week (C) BGA groups. HL, regimen derived according to *The Harriet Lane Handbook*; PMA, postmenstrual age dosing regimen.

studies have also shown similar associations between metronidazole CL and maturation and between metronidazole *V* and weight (7, 9).

The addition of serum creatinine to the multivariable CL model did not improve the goodness of fit, nor did it significantly explain remaining CL interindividual variability. Due to ontogenic changes in renal function among preterm infants, serum creatinine is strongly linked with maturational components such as PMA. This observation likely prevented us from distinguishing the effect of the maturational component in CL interindividual variability from serum creatinine in the CL model-building process. The population PK model developed in this study performed well during model evaluation and showed good precision around parameter estimates. After incorporation of weight as a covariate for *V*, the interindividual estimate on *V* was close to zero. It is plausible that in the subjects enrolled in this study, weight explained all the interindividual variability in *V*. It is also possible that the data were uninformative for estimating this parameter due to the use of sparse sampling (1).

The bias introduced by scavenged sampling was quantified in this study and resulted in an underestimation of metronidazole concentrations by ~30%. This finding suggests that collecting scavenged samples from the clinical laboratory is a viable strategy to describe metronidazole PK and is further supported by the environmental stability of metronidazole for up to 48 h (5, 12). However, during bootstrap procedures, imprecision was observed around the scavenged sample fixed parameter estimate (95% confidence interval, 10 to 42%). To more precisely estimate the amount of bias introduced by scavenged samples, a higher number of timed samples should be obtained. As expected, the residual variability estimated for scavenged samples was higher than that estimated for timed specimens. This finding could be due to higher documentation errors associated with sampling or dosing times extracted from the medical record after a scavenged sample was collected. In addition, information regarding the duration and conditions under which the samples remained in the clinical laboratory before freezing were not collected. Delays in these steps could add to the increased residual variability in scavenged samples.

In summary, the minimal-risk approach of scavenged PK sampling was an effective method to describe the population PK of metronidazole in preterm infants, to identify covariates that explain CL interindividual variability, and to provide dosing recommendations for this population. After scaling for size, incorporating PMA as a covariate increased the model fit and led to a newly proposed, simpler dosing regimen based on

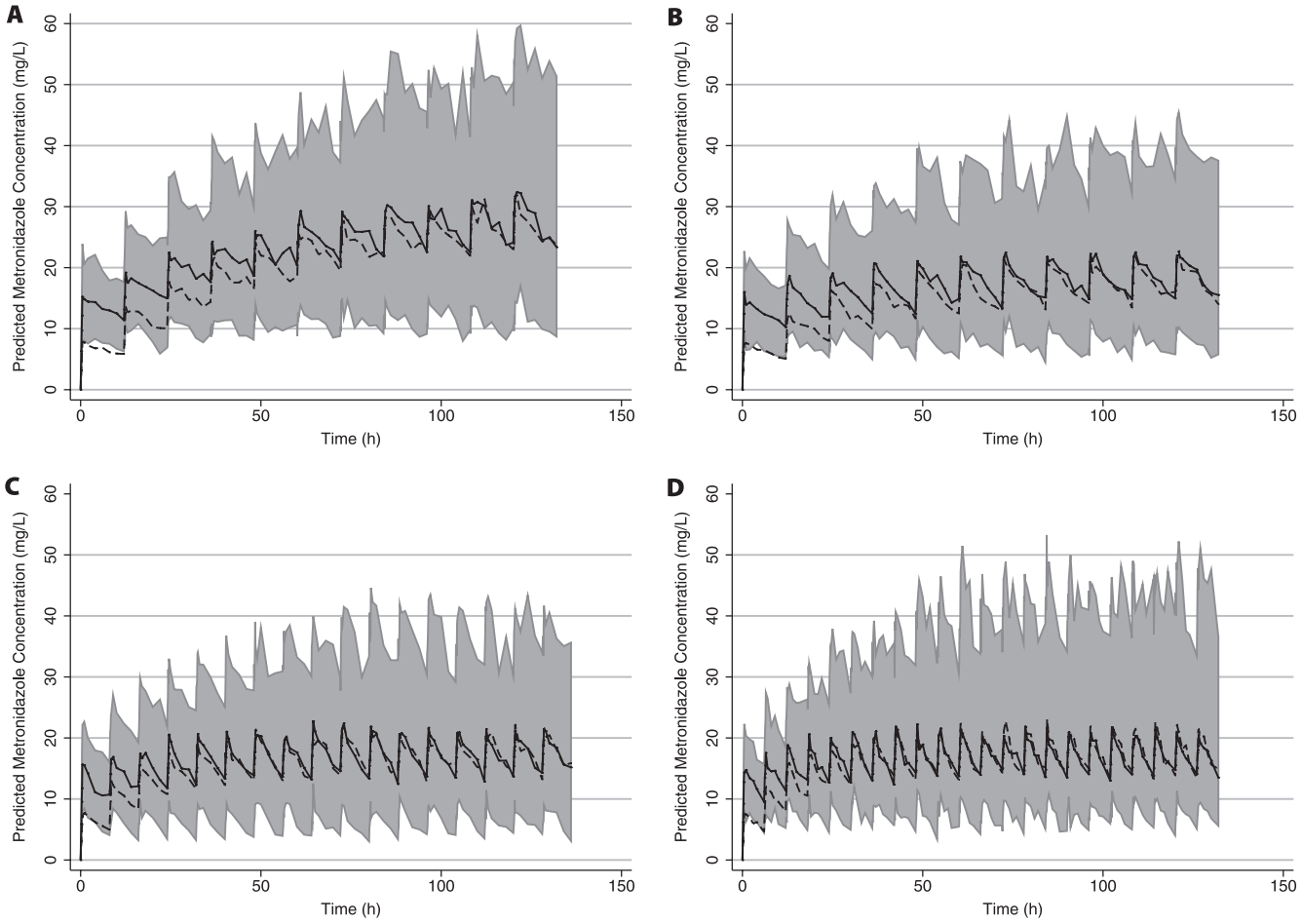

FIG 7 Simulated time-concentration profiles with the proposed PMA dosing regimen in typical subjects. (A) PMA, 26 weeks; weight, 900 g; (B) PMA, 32 weeks; weight, 1,900 g; (C) PMA, 36 weeks; weight, 2,800 g; (D) PMA, 41 weeks; weight, 3,800 g. The shaded area represents the 90% prediction interval around the loading dose simulations. Solid and dashed lines represent predicted median concentrations for loading versus no loading dose, respectively.

PMA. Future evaluations of this method should consider the physicochemical properties of the drug (i.e., drug stability), more detailed documentation of sample collection and storage conditions, and simultaneous collection of traditional plasma samples to fully assess the extent of bias introduced by scavenged sampling.

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