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Opportunistic dried blood spot sampling validates and optimizes a pediatric population pharmacokinetic model of metronidazole

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ABSTRACT Pharmacokinetic models rarely undergo external validation in vulnerable populations such as critically ill infants, thereby limiting the accuracy, efficacy, and safety of model-informed dosing in real-world settings. Here, we describe an opportunistic approach using dried blood spots (DBS) to evaluate a population pharmacokinetic model of metronidazole in critically ill preterm infants of gestational age (GA) ≤31 weeks from the Metronidazole Pharmacokinetics in Premature Infants (PTN_METRO, NCT01222585) study. First, we used linear correlation to compare 42 paired DBS and plasma metronidazole concentrations from 21 preterm infants [mean (SD): post natal age 28.0 (21.7) days, GA 26.3 (2.4) weeks]. Using the resulting predictive equation, we estimated plasma metronidazole concentrations (ePlasma) from 399 DBS collected from 122 preterm and term infants [mean (SD): post natal age 16.7 (15.8) days, GA 31.4 (5.1) weeks] from the Antibiotic Safety in Infants with Complicated Intra-Abdominal Infections (SCAMP, NCT01994993) trial. When evaluating the PTN_METRO model using ePlasma from the SCAMP trial, we found that the model generally predicted ePlasma well in preterm infants with GA ≤31 weeks. When including ePlasma from term and preterm infants with GA >31 weeks, the model was optimized using a sigmoidal Emax maturation function of postmenstrual age on clearance and estimated the exponent of weight on volume of distribution. The optimized model supports existing dosing guidelines and adds new data to support a 6-hour dosing interval for infants with postmenstrual age >40 weeks. Using an opportunistic DBS to externally validate and optimize a metronidazole population pharmacokinetic model was feasible and useful in this vulnerable population.

KEYWORDS pharmacokinetics, metronidazole, pediatric

External validation of pharmacokinetic (PK) models is essential for accurate and safe use of model predictions in real-world patient care (1, 2). External validation studies, in which the performance of published models is vetted in a separate data source (1), typically use traditional PK methods of intensive sampling and frequent blood draws. However, these methods are often not feasible or ethical in vulnerable populations such as critically ill infants (3, 4). As a result, existing PK models in this population are limited, and very few have undergone external validation (2, 5, 6). Without external validation, model-informed dosing may expose critically ill infants to sub- and/or supra-therapeutic concentrations (2), reduced efficacy and/or increased toxicity, and poor outcomes in real-world clinical care settings.

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Using dried blood spots (DBS) creates new opportunities for external validation studies. DBS are collected by applying a drop of blood to a filter paper, which is later extracted for analysis. Compared to traditional blood draws, collecting DBS markedly reduces blood volume requirements and eases the logistics of preparation, transport, and storage (7). Coupling DBS with opportunistic, or scavenged, sampling can reduce study burden while providing the data necessary for PK studies on infants (8). However, DBS also require comparability studies (7) and other added steps that can increase the complexity, imprecision, and potential for error. Thus, opportunities to use DBS to externally validate PK models in critically ill infants have been relatively underutilized.

The objective of this study was to use DBS to evaluate a previously published population PK (popPK) model of metronidazole in critically ill preterm infants (9). Metronidazole is a nitroimidazole antibiotic widely used to treat a variety of bacterial infections in patients of all ages (10, 11). In neonatal intensive care units, metronidazole is used to treat necrotizing enterocolitis and is among the most frequently administered medications (5, 12). Metronidazole distributes into several tissues, including red blood cells (13), and throughout the gastrointestinal tract, where it undergoes reductive activation of metabolites prior to excretion in urine and feces (11). The PK parameters of metronidazole undergo dramatic changes early in life (14), in part due to the ontogeny of drug-metabolizing enzymes such as CYP3A, underscoring the importance of model evaluation in infants of varying ages (15).

To evaluate the metronidazole popPK model in critically ill infants, we first performed a comparability analysis of paired DBS and plasma metronidazole concentrations. The resulting predictive equation allowed us to estimate plasma metronidazole concentrations from DBS collected in a separate, larger, opportunistic study. We then used the estimated plasma metronidazole concentrations to evaluate and optimize the metronidazole popPK model.

MATERIALS AND METHODS

Study design and sample collection

We externally evaluated a previously published popPK model of metronidazole in critically ill preterm infants with gestational age (GA) ≤31 weeks and suspected intra-abdominal infection, initially developed with data from the Metronidazole Pharmacokinetics in the Premature Infants (PTN_METRO, NCT01222585) study (9). This model is referred to as the "PTN_METRO model" henceforth. In the PTN_METRO study, 111 plasma metronidazole samples were collected and used for popPK modeling. In addition, 42 paired metronidazole DBS and plasma concentrations were collected and used for the comparability analysis reported herein.

To evaluate the PTN_METRO model, we used DBS collected as part of the Antibiotic Safety in the Infants with Complicated Intra-Abdominal Infections (SCAMP, NCT01994993) trial (16). The SCAMP trial was a multicenter, prospective, partially randomized, open-label trial of antimicrobials, including metronidazole, in critically ill term and preterm infants with complicated intra-abdominal infections. Inclusion and exclusion criteria were previously reported (16). Metronidazole was administered as a 30-minute intravenous infusion with a 15-mg/kg loading dose followed by a 7.5-mg/kg maintenance dose at 24 hours. Subsequent maintenance doses were administered at intervals defined by post-menstrual age (PMA, defined as GA plus chronologic age in weeks) according to published guidelines (9, 10, 16–18) as follows: 7.5 mg/kg every 12 hours for PMA 23–<34 weeks, every 8 hours for PMA 34–40 weeks, and every 6 hours for PMA >40 weeks (17). DBS were collected on FTA DMPK Type C cards opportunistically alongside clinical care, guided by an optimal PK collection scheme (i.e., DBS collected when standard-of-care assessments aligned with a timepoint in the optimal PK collection scheme: 0–15 minutes and 1–2 hours after the end of infusion; followed by 4–6 or 8–10 hours after or 0–30 minutes before the next dose, depending on the dosing interval; and elimination sample 12–18, 16–24, or 24–36 hours after the end of the final dose). DBS

were shipped to the Pediatric Trials Network central laboratory (OpAns, LLC, Durham, NC) for analysis.

DBS analysis

DBS underwent new analysis using a similar methodology as previously reported (9). Metronidazole was extracted from DBS 6 mm punch using a methanol solvent and quantified using a validated liquid chromatography-tandem spectrometry assay with the Agilent 1200 series, Poroshell 120 SB C18 (30 × 2.1 mm id, 2.7 um; Agilent Technologies, Santa Clara, CA) and a gradient mobile phase. The validation range was 50 to 50,000 ng/mL with a linear quantification over the 1,000-fold validation range. Quality control samples included nominal concentrations of 150, 4,000 and 40,000 ng/mL. Accuracy and precision assessed using five determinations at theoretical levels of 150, 4,000, and 40,000 ng/mL were within the Food and Drug Administration bioanalytical assay validation criteria (i.e., ±15%) (19).

Comparability analysis

Paired plasma and DBS samples from the PTN_METRO study were analyzed using simple linear regression and mixed-effect (random intercept) models using both raw and log-transformed concentrations. Bias and imprecision were calculated using median percentage prediction error (MPPE) and median absolute percentage prediction error (MAPE), respectively. MPPE and MAPE <15% were considered acceptable. The regression model with the lowest MPPE and MAPE was selected to estimate the plasma metronidazole concentrations (ePlasma) from DBS samples collected in the SCAMP trial. Calculations were:

$$\begin{aligned}\text{MPPE} &= \text{median}\left[100\% \times (\text{CONCplasma\_PRED} - \text{CONCplasma\_OBS})/\text{CONCplasma\_OBS}\right]\\ \text{MAPE} &= \text{median}\left[100\% \times |(\text{CONCplasma\_PRED} - \text{CONCplasma\_OBS})|/\text{CONCplasma\_OBS}\right]\end{aligned},$$

where CONCplasma_OBS is the observed plasma concentration and CONCplasma_PRED is ePlasma.

Validation

The PTN_METRO model was first evaluated using estimated plasma metronidazole concentrations (ePlasma) derived from DBS samples collected from SCAMP trial participants with GA ≤31 weeks, to be consistent with the inclusion criteria of the PTN_METRO study. The PTN_METRO model is a one-compartment model with covariates PMA on clearance (CL) and weight (WT) on the CL and volume of distribution (V). The predictive performance of the PTN_METRO model was evaluated by fixing the structural model and parameter estimates, as well as fixing the structural model and re-estimating parameters. For both approaches, evaluation was performed at both the individual and population levels. At the individual level, prediction error ($\text{PE}_i = \text{observed}_i - \text{predicted}_i$), mean prediction error ($\text{MPE} = \frac{\sum_{i=1}^{N} \text{PE}_i}{N}$), and root mean square error ($\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} \text{PE}_i^2}{N}}$) were calculated using the predicted and observed metronidazole concentrations. At the population level, 1,000 metronidazole concentrations per timepoint were simulated and compared to observed concentrations using prediction-corrected visual predictive checks (pcVPCs), which were normalized using the median prediction for each bin across time after dosing to account for influential covariates. The predictive performance was also evaluated using normalized prediction errors (NPDEs) for 1,000 simulated versus observed concentrations and visual assessment of the distribution of NPDEs versus the predicted concentration and time after dosing. Nonparametric bootstrapping of 1,000 replicates was used to generate 95% confidence intervals (*CIs*) for parameter estimates for the refitted model using ePlasma concentrations.

Optimization

ePlasma concentrations from the SCAMP trial were merged with plasma concentrations from the PTN_METRO study; using this combined data set, covariates were re-evaluated using the PTN_METRO model base one-compartment model without the covariate of PMA on CL, but including the linear relationship between WT and CL and V using a fixed exponent as follows:

$$\mathrm{CL}_i = \mathrm{CL} * \mathrm{WT}^1$$
$$\mathrm{V}_i = \mathrm{V} * \mathrm{WT}^1 \quad ,$$

where WT denotes the body weight of an individual participant; CL and V are body weight normalized CL and V, respectively; and CL_i and V_i are the estimates for each participant. Potential covariates including GA, PMA, postnatal age (PNA), sex, race, serum creatinine concentration, albumin concentration, and concomitant administration of the CYP3A inducer and/or CYP3A inhibitor were first explored in plots of covariate values versus population-typical value PK parameters (Eta). Covariates which showed a trend and had biological plausibility were then systematically evaluated for inclusion in the model using a forward inclusion [P < ~0.05 and reduction in objective function (dOFV) > ~3.84 per 1 degree of freedom] and backward elimination (P < ~0.005 and dOFV > ~7.88 per 1 degree of freedom) approach. The final model, referred to as "optimized model" henceforth, was evaluated based on minimization, diagnostic plots, plausibility and precision of parameter estimates, dOFV, shrinkage, bootstrapping (1,000 replicates), and pcVPCs.

Statistical analyses, data manipulation, and visualization were conducted using R (3.4.1, R Foundation for Statistical Computing, Vienna, Austria) and/or Stata (15.1, College Station, TX, USA). PK analyses were conducted using the NONMEM (7.4) first-order conditional estimate method with interaction, ADVAN1 TRANS2 subroutines, with Pirana (2.8.1) run management, Perl-speaks-NONMEM (3.6.2) for visual predictive checks, and bootstrapping and R package (npde) for NPDEs.

RESULTS

Comparability

A total of 42 paired plasma and DBS samples with quantifiable concentrations and adequate DBS size from 21 infants in the PTN_METRO study were used for the comparability analysis (Table S1). Mean (SD) concentrations for metronidazole were 11,702 (5,391) ng/mL for plasma and 9,965 (4,631) ng/mL for DBS. Concentrations were consistently lower in DBS than in plasma, and this finding was true across the range of concentrations. MPPE and MAPE were <9% for all regression methods, with simple linear regression having the lowest MPPE and MAPE of 1.7% and 3.2%, respectively. The following linear regression model was therefore chosen:

$$\mathrm{C}_{\mathrm{plasma}} = 1.11 * \mathrm{C}_{\mathrm{DBS}} + 253$$

where C_{plasma} is measured plasma concentration and C_{DBS} is the DBS concentration from the PTN_METRO study and R^2 = 0.86 (Fig. S1). This model was used to estimate ePlasma from DBS samples from the SCAMP trial, where ePlasma = C_{plasma} and C_{DBS} is the DBS concentration.

Validation

In the SCAMP trial, 122 infants treated with metronidazole contributed a total of 446 DBS (Table S1); 47 DBS were not included due to inadequate spot size (34), incorrect or missing time (9), abnormally high concentrations after receiving standard-of-care doses prior to enrollment (3), and abnormally high concentrations 144 hours after the last dose (1). The median (range) number of DBS samples contributed per infant was 3 (1–7).

Validation was performed using DBS from 59 of 122 infants in the SCAMP trial with GA ≤31 weeks, who contributed a total of 209 DBS.

Diagnostic plots of ePlasma concentrations versus population predictions for SCAMP trial participants with GA ≤31 weeks, using the PTN_METRO model with fixed structural model and fixed or re-estimated parameters (as reported in Table S2), showed no specific trends (Fig. S2). For the PTN_METRO model with fixed structural model and fixed parameters, the median PE was 337 ng/mL, MPE 439 ng/mL, and RMSE 6,980 ng/mL. For the PTN_METRO model with fixed structural model and re-estimated parameters, the median PE was 663 ng/mL, MPE 1,013 ng/mL, and RMSE 6,980 ng/mL. pcVPCs demonstrated similar central tendency and distribution between the PTN_METRO model with fixed parameters and re-estimated parameters (Fig. 1). The percentages of observations falling outside the 90% prediction interval were nearly identical at 20/193 (10.4%) versus 18/193 (9.3%) for the PTN_METRO model with fixed parameters and re-estimated parameters, respectively. The NPDE showed a trend of under-prediction at lower concentrations for the PTN_METRO model with fixed parameters; this trend resolved after re-estimating parameters (Fig. S3).

Optimization

After combining plasma metronidazole concentrations from the PTN_METRO study and all ePlasma concentrations from the SCAMP trial, including participants with GA >31 weeks, re-estimation of the model parameters using the PTN_METRO model resulted in lower estimates for CL, V, and exponent of PMA on CL (Table S2). To optimize the PTN_METRO model using the combined data set, the PTN_METRO one-compartment base structural model with a linear relationship between WT and both CL and V was used, and covariates were re-evaluated for inclusion on both CL and V. The covariate selection process is summarized in Table S3. The final optimized model parameter estimates are shown in Table 1. Eta shrinkage for CL and V was 8% and 29%, respectively,

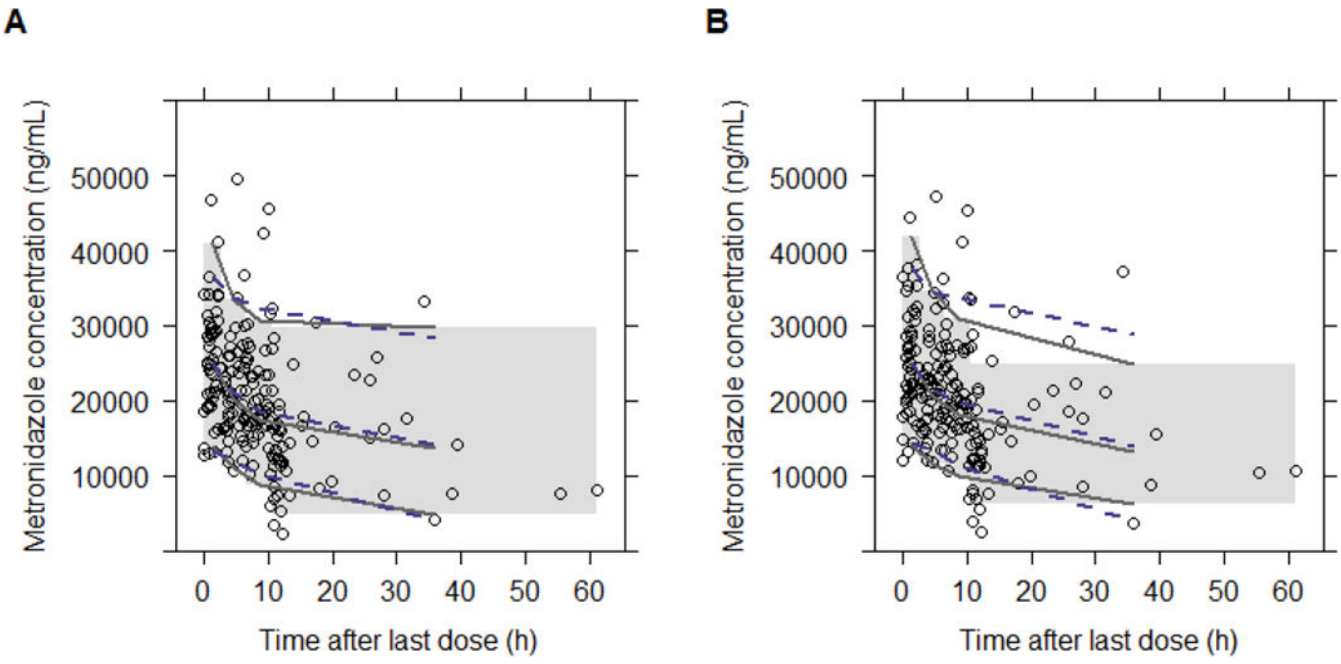

FIG 1 Prediction-corrected visual predictive check for external validation of the PTN_METRO population pharmacokinetics model (9) of metronidazole using estimated metronidazole concentrations from critically ill infants in the SCAMP trial (16) with gestational ages ≤ 31 to match the PTN_METRO study demographics. Panel A shows the original PTN_METRO model with fixed parameters, and Panel B shows the original PTN_METRO model with re-estimated parameters. The shaded region denotes the 90% prediction interval of the simulated data. The solid lines from the bottom to the top represent the predicted 5th, 50th, and 95th percentiles. The dashed lines from the bottom to the top represent observed 5th, 50th, and 95th percentiles. Abbreviations: PTN_METRO, Metronidazole Pharmacokinetics in Premature Infants; SCAMP, Antibiotic Safety in Infants with Complicated Intra-Abdominal Infections.

TABLE 1 Optimized population pharmacokinetic model parameter estimates for metronidazole derived from plasma and estimated from plasma in two studies (9, 16) of critically ill infants with intra-abdominal infections[a]

Parameter	Estimate	RSE (%)	2.5th percentile	Bootstrap[b] median	97.5th percentile
Structural model					
$CL = \theta_{CL} * WT * [PMA^{Hill}/(TM50^{Hill}+PMA^{Hill})]$					
$V = \theta_V * WT^{\theta WT\text{-}V}$					
θ_{CL} (L/kg/h)	0.036	4	0.033	0.036	0.039
θ_V (L/kg)	0.853	3	0.802	0.857	0.921
TM50 (weeks)	25.6	2	24.4	25.6	26.5
Hill	7	37	8.4	15.9	41.0
$\theta_{WT\text{-}V}$	0.763	8	0.645	0.752	0.876
Interindividual variability (%CV)					
CL	35.6	21	28.4	35.5	43.4
V	25.4	31	16.5	24.8	31.6
Residual error					
Proportional error (%)	19.1	13	16.5	18.9	21.1

[a]θ_{CL}, typical value for CL; θ_V, typical value for V; $\theta_{WT\text{-}V}$, exponent of body weight on V; CL, clearance; CV, coefficient of variation; Hill, Hill coefficient in sigmoidal maturation function; PMA, postmenstrual age (weeks); TM50, maturation half-life calculated as a function of PMA (weeks); V, volume of distribution; WT, body weight.
[b]1000 bootstrap runs were performed, 99.5% converged to >3 significant digits.

and epsilon shrinkage was 17%. Diagnostic plots revealed no obvious trend (Fig. S4). pcVPCs showed reasonable fit between observed and predicted concentrations, with 7% of observed concentrations outside of the 90% prediction interval (Fig. 2). The NPDE distribution was normal and centered on 0 (Fig. S5). Using a 1,000-set bootstrap analysis, 99.7% of data sets converged to >3 significant digits, and the median of all estimates was within 5% of population estimates from the original data set. Comparing the optimized model to the PTN_METRO model fitting the same data, IIV on V was lower (25.4% vs 28.4%), IIV on CL was essentially unchanged (35.6% vs. 36.1%), as was the residual error (19.7% vs. 19.7%).

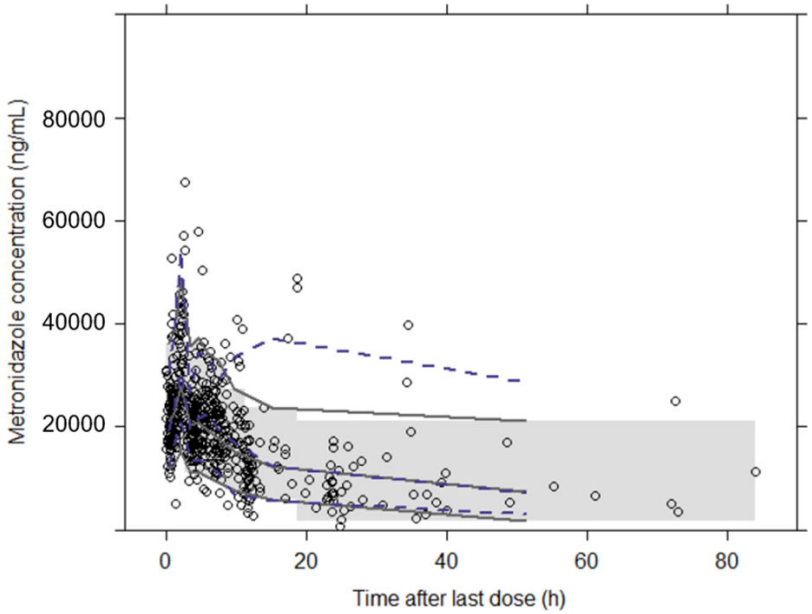

FIG 2 Prediction-corrected visual predictive check for an optimized population pharmacokinetics model of metronidazole in critically ill term and preterm infants. The shaded region and solid lines denote the 90% prediction interval based on 1,000 simulations.

DISCUSSION

Opportunistic DBS sampling yielded sufficient quantity and quality of data to externally validate a previously published popPK model of metronidazole in critically ill preterm infants. Furthermore, ePlasma concentrations derived from DBS were used to optimize the model to include more mature preterm infants and term infants. The optimized model adequately characterized the PK of metronidazole in infants of 22.7–41.0 weeks GA and 0–80 days PNA (23 to 48 weeks PMA). These findings support existing age-based dosing guidelines for metronidazole in term and preterm infants: 15 mg/kg loading dose followed by maintenance doses of 7.5 mg/kg every 12 hours for PMA <34 weeks and 8 hours for PMA 34–40 weeks (9, 10, 14, 16–18). Additionally, findings address the ambiguity in maintenance dosing for infants with PMA >40 weeks within existing guidelines by supporting the maintenance dosing of 7.5 mg/kg every 6 hours for term and preterm infants with PMA >40 weeks. Because metronidazole is widely used in this population and the PK is known to change markedly early in life, defining and validating PK are especially important (9, 14, 20).

The validity of the original PTN_METRO model in SCAMP trial participants is evidenced by adequate predictions of ePlasma concentrations from SCAMP trial participants with GA ≤31 weeks. Refitting the PTN_METRO model to ePlasma concentrations of SCAMP trial participants with GA ≤31 weeks did result in some changes, including an improved agreement between model predictions and observations, slightly lower estimates of CL and V (less than 10%), lower IIV on CL, a lower exponent of PMA on CL (27% lower), and higher IIV for V (greater than twofold). The lower IIV for CL may be related to the relatively homogeneous study population in the SCAMP trial. The difference in estimates of CL and V may be related to physiological changes associated with complicated intra-abdominal infection in SCAMP trial participants, including the development of perforation or ischemia of the bowel, third-spacing, intra-abdominal hypertension, multiple organ dysfunction (21, 22), and/or demographic differences (i.e., higher ages and weights in the SCAMP trial). When including ePlasma concentrations from SCAMP trial participants with GA >31 weeks, population mean parameter estimates for CL and V were similar, but the relationship between PMA and CL was better captured using a maturation function rather than power function, likely due to the wider age range in the SCAMP trial to define this relationship. Additionally, weight on volume of distribution was better represented with an estimated exponent. Ultimately, the optimized model adequately characterized the pharmacokinetics of metronidazole in infants across a wider range of ages than previously published.

A strength of this study is its design, which leverages a small cohort of paired plasma and DBS samples to enable an opportunistic PK study collecting DBS alone. Although DBS offer practical and logistical advantages (7, 8), these advantages must be weighed against analytical challenges (i.e., the potential need for novel assays, reference intervals, and decision to measure drug and/or metabolite concentrations in DBS) and the additional required step(s) for comparability studies (7). The utility of DBS may be limited in specific settings due to drug-related factors, i.e., erythrocyte partitioning, protein binding (13, 23), or large across-patient variability (24). Future PK studies of various therapeutics in vulnerable populations may benefit from planning an initial, small study collecting paired liquid matrix samples alongside DBS to establish feasibility and comparability. If feasibility and comparability are established, DBS could enable an array of PK studies, including modeling, external evaluation, and optimization.

One limitation of this study is the lack of hematocrit data to evaluate the dilutional effect of metronidazole partitioning into erythrocytes in DBS (13). However, simple linear regression modeled the relationship between DBS and plasma well, with very low measures of bias and imprecision. For other drugs, in which a simple model may fail to accurately estimate plasma concentrations from DBS, this approach may require adjustment for hematocrit and other variables. Furthermore, plasma concentrations were not collected in the SCAMP trial to validate the linear regression model. It remains possible that systematic differences in demographics, physiology, or pertinent covariates

across the two studies could bias results; however, the overall consistency of demographics (Table S1) and parameter estimates (Table S2) is reassuring.

Overall, we successfully estimated plasma metronidazole concentrations from DBS and subsequently conducted an external popPK model validation and optimization study collecting opportunistic DBS alone. This approach requires additional analytic steps but showed high feasibility and utility in a vulnerable population of critically ill infants. For future PK studies in vulnerable populations, we suggest collecting paired DBS and liquid matrix samples in early, smaller studies, conducting comparability analyses, and continuing to refine the comparability over time to maximize the advantages of DBS in future, larger studies.

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DATA AVAILABILITY

To help expand the knowledge base for pediatric medicine, the PTN is pleased to share data from its completed and published studies with interested investigators. For requests, please contact a PTN Program Manager (PTN-Program- Manager@dm.duke.edu).

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental Tables and Figures (AAC01533-23-s0001.docx). Supplemental Tables S1-S3 and supplemental Figures S1-S5.

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