Dosing considerations for preterm neonates: from pharmacometrics to clinical practice
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Citation

SECTION IV.

Fluconazole for the prevention and treatment of invasive candidiasis
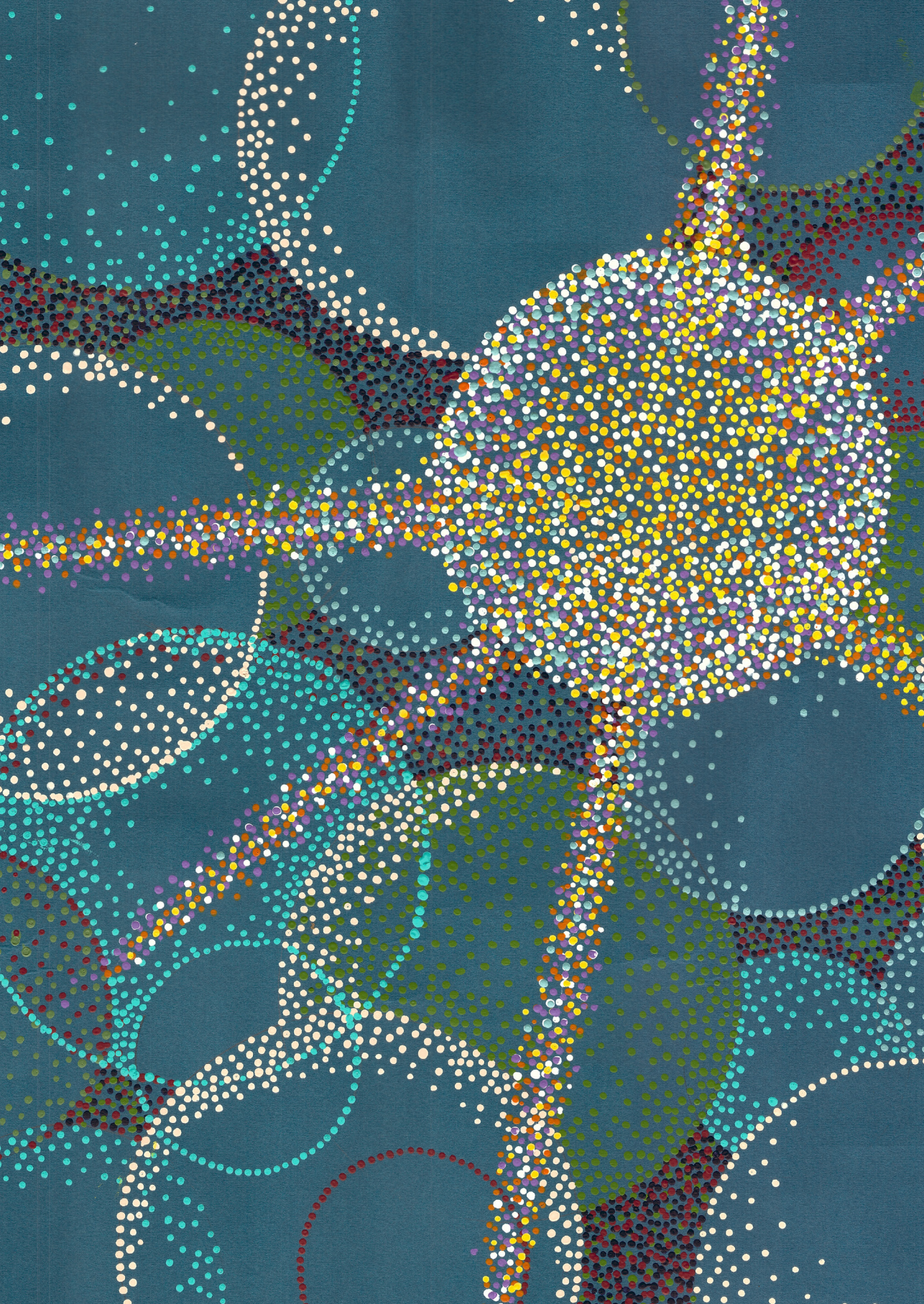
Optimization of fluconazole therapy for the treatment of invasive candidiasis in preterm infants

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ABSTRACT

Introduction
Fluconazole is an important antifungal in the prevention and treatment of invasive Candida infections in neonates, even though its use in preterm infants is still off-label. Here, we performed a population pharmacokinetic study on fluconazole in preterm neonates in order to optimize dosing through the identified predictive patient characteristics.

Methods
Fluconazole concentrations obtained from preterm infants from two studies were pooled and analysed using NONMEM V.7.3. The developed model was used to evaluate current dosing practice. A therapeutic dosing strategy aiming to reach a minimum target exposure of 400 and 200 mg*h/L per 24 h for fluconazole-susceptible *C. albicans* meningitis and other systemic infections, respectively, was develope*d*.

Results
In 41 preterm neonates with median (range) gestational age 25.3 (24.0-35.1) weeks and median PNA at treatment initiation 1.4 (0.2-32.5) days , 146 plasma samples were collected. A one-compartment model described the data best, with an estimated clearance of 0.0147 L/h for a typical infant of 0.87 kg with a serum creatinine concentration of 60 µmol/L and volume of distribution of 0.844 L. Clearance was found to increase with 16% per 100 g increase in actual bodyweight, and to decrease with 12% per 10 µmol/L increase in serum creatinine concentration once PNA was above 1 week. Dose adjustments based on serum creatinine and daily dosing are required for therapeutic target attainment.

Conclusion
In preterm neonates, fluconazole clearance is best predicted by actual bodyweight and serum creatinine concentration. Therefore, fluconazole dosing should not only be based on bodyweight but also on creatinine concentration to achieve optimal exposure in all infants.

INTRODUCTION

Fluconazole is an important antifungal in the prevention and treatment of invasive Candida infections, affecting 4-8% of infants with a birthweight below 1000 g.

With *C. albicans* still being a common pathogen for invasive candidiasis, treatment aims to obtain a target exposure to fluconazole. The pharmacodynamic index for fluconazole is defined as the area under the plasma concentration-time curve per 24-hour period (AUC_{24h}) over minimally inhibitory concentration (MIC) ratio to be above 100. The clinical breakpoint for in vitro susceptibility of *C. albicans* treated with fluconazole is 2 mg/L.[1] This would translate the need for obtaining a minimal target AUC_{24h} of 200 mg*h/L for a systemic infection in a worst case scenario. In the case of Candida meningitis the target AUC_{24h} is increased to 400 mg*h/L to account for reduced tissue penetration into the cerebrospinal fluid.[2]

Currently, there is no licensed dosing regimen for treatment of invasive candidiasis with fluconazole in preterm neonates. For term neonates with invasive candidiasis a maintenance dose of 6 to 12 mg/kg is recommended every 72h for neonates with a postnatal age (PNA) 0-14 days, every 48 h for neonates with a PNA 14-28 days and daily for infants with a PNA above 28 days.[3] To ensure a minimal AUC_{24h} of 400 mg*h/L on day 1, a loading dose of 25 mg/kg was suggested by Piper et al.[4] Recently, Leroux et al. proposed a daily dose adjusted to gestational age (GA), based on a study with 18 neonates.[5] More studies on fluconazole PK in preterm infants are lacking and therefore the regimen designed for term neonates is frequently applied to preterm infants. It is, however, not known whether preterm infants receive optimal exposure to fluconazole when treated as term infants.

In the current study, a population pharmacokinetic (PK) model was developed to evaluate which patient characteristics are predictive of the PK of fluconazole in preterm infants. This model was then used to evaluate currently used dosing strategies and develop a therapeutic dosing regimen for preterm infants. Fluconazole doses were selected aiming for a target AUC_{24h} of 400 mg*h/L based on a suspected worst-case scenario of Candida meningitis and aiming for a target AUC_{24h} of 200 mg*h/L based on a systemic invasive candidiasis with a maximum MIC of 2 mg/L.

METHODS

Patients and Samples

Data from two cohorts were combined. Patients in cohort 1 were included in the DI-NO-study (Drug dosage Improvement in Neonates, NCT 02421068) between 2014 and 2017, which was designed to evaluate the PK and pharmacodynamics of frequently used off-label drugs in preterm neonates based on opportunistic sampling. Infants with a GA below 32 weeks and an indication for one of the nine study drugs were included and treated according to standard of care. Prophylactic fluconazole therapy consisted of 3 mg/kg twice weekly, and therapeutic doses were 6-12 mg/kg with a loading dose of 12 or 25 mg/kg. Blood samples of 0.2 mL were collected in EDTA-tubes and withdrawn from an indwelling arterial catheter or with routinely scheduled samples for clinical purposes. The local ethics committees approved the protocol and written informed consent from parents/legal guardians was obtained (MEC-2014-067).

Cohort 2 consisted of patients submitted to the neonatal intensive care unit of the Radboud Medical Centre Nijmegen or University Medical Centre Groningen who required treatment with fluconazole. Patients received 6 to 12 mg/kg once daily or every two days. Plasma samples were collected opportunistically or for therapeutic drug monitoring (local target trough concentration 10-50 mg/L.

Bioanalytical analysis

Fluconazole plasma concentrations from both cohorts were measured in the same laboratory using a validated assay using liquid chromatography coupled with tandem mass spectrometry (validated range 0.0302 to 30.21 mg/L, coefficient of variation intra-assay: 2.8%, inter-assay: 1.5%).

Population PK model

The population PK model was developed in NONMEM version 7.3 (ICON Development Solutions, Ellicott City, MD, USA) supported by Perl-speaks-NONMEM version 4.7.0. Model development was based on comparison of the objective function value (OFV) for nested models, along with numerical and graphical model performance, i.e. relative standard error [RSE] of < 50% and evaluation of goodness of fit (GOF) plots that included individual predicted and population predicted concentrations vs observed concentrations, conditional weighted residuals (CWRES) vs times after dose and observed concentrations. Actual bodyweight, GA, PNA, postmenstrual age, gender and being small for gestational age (SGA) were evaluated as covariates, and, where applicable and available, serum creatinine concentration (S_{cr}) and co-administration with ibuprofen or antibiotics (tobramycin, gentamycin or vancomycin) within 1 week of a fluconazole dose. Covariates were first selected based on a visual trend with interindividual variability (IIV)

where possible, and/or evaluation of GOF plots split for the covariate of interest. Selected covariates were examined in a stepwise manner and added based on significant improvement in OFV (p < 0.01 and p < 0.001 for forward inclusion and backwards exclusion, respectively), a decrease in IIV and improved (split) goodness-of-fit. Bodyweight was interpolated linearly if not measured daily and S_{cr} observations were interpolated in a forward manner. For patients without any S_{cr} observation (n=15), the median of the available serum creatinine (60 µmol/L) was imputed. Only S_{cr} observed from a PNA higher or equal to 8 days were used in the covariate analysis, since during the first week the S_{cr} concentrations may also reflect maternal S_{cr}.[6] The final model was evaluated with a bootstrap and a normalized prediction distribution error analysis (NPDE), both based on 1000 simulations.[7]

Evaluations of the current and proposed dosing regimens

Using the final model, exposure to fluconazole was evaluated for typical preterm infants with a GA of 25.5 weeks whose treatment was initiated at a PNA of 3, 10 or 17 days. Median birthweight and actual bodyweight were extracted from https://www.growthcalculator.org/ based on a male infant and S_{cr} was set at 60 µmol/L once PNA was above 1 week. For the evaluation of the term neonate dosing regimen as advised by the Dutch Paediatric Formulary for term neonates[8] and the label[3], treatment was initiated with a loading dose of 25 mg/kg, followed by 12 mg/kg every 72 h when PNA was below 15 days, 12 mg/kg every 48 h for PNA 15-28 days and 12 mg/kg daily when PNA was above 28 days. Loading- and maintenance doses were optimized to achieve 100% target attainment in all patients across the S_{cr} and actual bodyweight range (30, 50, 80 or 100 µmol/L and 700 – 1500 g with 100 g increments, respectively). Target attainment after the loading dose was defined as a minimal AUC_{24h} of 400 mg*h/L on day 1 of treatment based on the worst-case scenario of the infant suffering from Candida meningitis. Target attainment of the maintenance dose was defined for two scenarios: the first assumes that the infant does have confirmed Candida meningitis or is awaiting lab results for this diagnosis and requires a minimal AUC_{24h} of 400 mg*h/L. The second scenario assumes that a lower exposure suffices because of a negative lumbar puncture and an MIC < 2 mg/L, requiring a minimal AUC_{24h} of 200 mg*h/L. To evaluate the exposure to fluconazole following the optimized dosing regimen S_{cr} was set at either 30, 50, 80 or 100 µmol/L. 1000 simulations were run for each preterm infant of which the median concentrations were extracted and individual AUC_{24h} was calculated.

The exposure to fluconazole following prophylactic treatment of 3 and 6 mg/kg[8,9] twice weekly, initiated at the first day of life, for infants with a GA of 24, 25.5 and 27 weeks was simulated over 30 days. S_{cr} was set at 60 µmol/L once PNA was above 1 week and 1000 simulations were run for each preterm infant. Due to the lack of a PK target dose optimization was not performed for prophylactic treatment.

RESULTS

Population

Data from a total of 41 infants were available, 28 in cohort 1 and 13 in cohort 2, and included 146 plasma samples. Median GA was 25.3 weeks (range 24.0 – 35.1) weeks, and median birthweight was 0.76 (range 0.49 – 2.00) kg. Population characteristics are presented in Table 1.

Table 1. *Patient characteristics (median (range)) and dosing information of the two study cohorts, and the total study population. *Of time-varying characteristics (current weight, postnatal age during treatment, postmenstrual age, plasma creatinine concentrations and urine output) the median of the individual medians is given.*

Patient characteristics	Cohort 1		Cohort 2	Total population
	Prophylactic indication	Therapeutic indication	Therapeutic indication	
Number of neonates	24	4	13	41
Gender (male/female)	10/14	1/3	7/6	18/23
Gestational age (weeks)	25.0 (24.0-27.1)	25.0 (24.3-26.4)	26.1 (24.6-35.1)	25.3 (24.0-35.1)
Birth weight (kg)	0.73 (0.49-0.99)	0.76 (0.54-0.95)	0.94 (0.64-2.00)	0.76 (0.49-2.00)
Small for gestational age (n (%))	3 (13%)	1 (25%)	1 (8%)	5 (12%)
Current weight during treatment (kg)*	0.82 (0.50-1.58)	0.90 (0.66-1.35)	1.00 (0.70-2.20)	0.87 (0.50-2.20)
Postnatal age at treatment initiation (days)	0.9 (0.2-16.0)	21.2 (3.9-32.5)	10.0 (3.0-18.0)	1.4 (0.2-32.5)
Postmenstrual age during treatment (weeks)*	27.4 (24.1-28.4)	28.2 (25.7-31.3)	28.4 (26.0-38.9)	26.7 (24.1-38.9)
Number of patients with at least one available plasma creatinine observation (%)	23 (96%)	3 (75%)	0 (0%)	26 (63%)
Serum creatinine concentration during treatment (µmol/L)*	61 (22-127)	58 (32-63)	Unknown	60 (22-127)
Urine output during treatment (mL/h/kg)*	4.1 (2.8-4.8)	7.3 (7.3-7.3)	Unknown	4.2 (2.8-7.3)
Co-administration of ibuprofen (number of neonates (%))	19 (79%)	4 (100%)	Unknown	23 (56%)
Time after last ibuprofen dose at fluconazole plasma sample time (h)	126 (13-1140)	173 (1-815)	Unknown	130 (1-1140)
Co-administration of antibiotics (number of neonates (%))	21 (75%)	0 (0%)	Unknown	21 (51%)
Time after last antibiotics dose at fluconazole plasma sample time (h)	41 (1-409)	-	Unknown	41 (1-409)

Table 1. *Continued.*

Patient characteristics	Cohort 1		Cohort 2	Total population
	Prophylactic indication	Therapeutic indication	Therapeutic indication	
Number of plasma samples per neonate (n (range))	6 (1-13)	5 (2-7)	1 (1-2)	5 (1-13)
Duration of fluconazole treatment (days)	22 (6-67)	8 (2-19)	5 (2-18)	12 (2-67)
Loading dose (mg/kg) [number of patients]	–[0]	18.2 (11.1-27.0) [4]	- [0]	18.2 (11.1-27.0) [4]
Maintenance dose (mg/kg)	2.9 (1.9-5.8)	10.1 (2.5-12.6)	11.1 (5.4-15.1)	3.01(1.9-15.1)
Time of fluconazole plasma sample after last dose (h)	29.2 (0.6-270)	7.7 (0-42.7)	23.8 (23.0-24.0)	23.8 (0-270)
Fluconazole plasma concentration (mg/L)	2.61 (0.04-13.6)	22.00 (4.38-29.60)	16.05 (10.90-26.00)	3.09 (0.04-29.60)

Population PK model

Data were best described by a one-compartment model with a proportional error. Actual bodyweight was found to be most predictive of CL with a linear relationship (p < 0.001 [-65 points in OFV]), explaining 21% of the interindividual variability (IIV) in CL. Second, CL was found to decrease with increasing S_{cr} observed from a PNA of 8 days or higher (p< 0.001 [-23 points in OFV]), explaining 7.3% percent of IIV on clearance (CL). The relationship between CL and actual bodyweight and S_{cr} is visualized in Figure 1. Actual bodyweight was also found to predict V_d with a linear relationship (p < 0.001 [-17 points in OFV]). Addition of this relationship decreased IIV on V_d from 37% to 9% after which IIV on volume of distribution (Vd) could be removed from the model. The increase in CL and V_d with actual bodyweight could be described with one parameter, which was not significantly different to estimating two separate parameters (p > 0.05 [+0.3 points in OFV]). Individual CL and V are estimated by Equation 1 and 2, respectively, where CL_{ind} is the individual predicted CL, $Weight_{ind}$ is the actual bodyweight, $S_{cr,ind}$ is the most recent measured S_{cr} and V_{ind} is the individual predicted V. Parameter estimates of the final model and the respective bootstrap estimates are presented in Supplementary Table 1. The goodness-of-fit plots of the final model (Supplementary Figure 1) and results of the NPDE analysis (Supplementary Figure 2) show that the model describes the data well across different bodyweights and S_{cr}.

$$\text{(Eq. 1) } CL_{ind} \text{ (L/h)} = 0.0147 \text{ L/h} * (1+ 1.56 \,(Weight_{ind} - 0.87 \text{ kg}))* (1- 0.0118(S_{cr,ind} - 60 \text{ µmol/L}))$$

$$\text{(Eq. 2) } V_{ind} \text{ (L)} = 0.844 \text{ L} * (1+ 1.56(Weight_{ind} - 0.87 \text{ kg}))$$
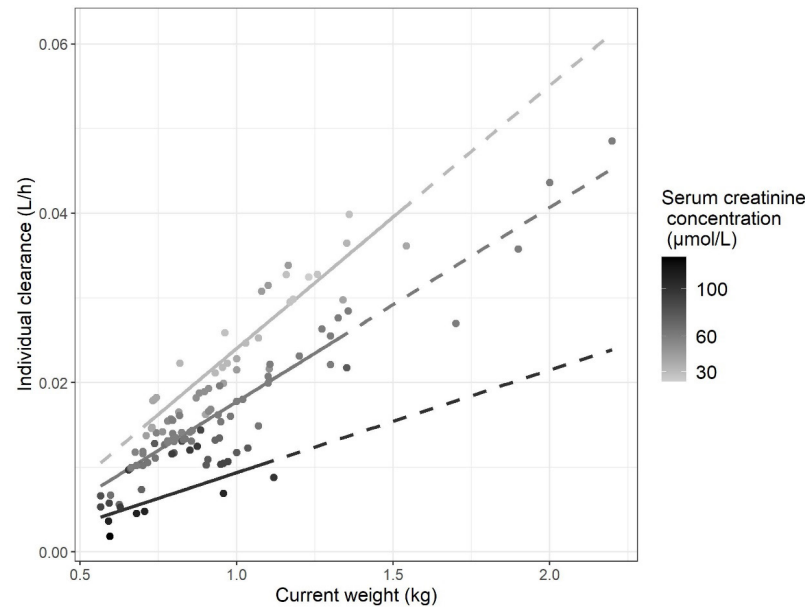
Figure 1. *Clearance of fluconazole versus current body weight with colour intensity increasing with serum creatinine concentrations. Dots represent the individual post hoc clearance values and the solid lines represent the estimated relationship between clearance and actual body weight for an infant with a serum creatinine concentration of either 30, 60 or 100 μmol/L. Dashed lines represent expected clearance values for combinations of current weight and serum creatinine concentration not present in the current study population.*

Figure 2. *Concentration- time profiles (upper panel) and corresponding area under the curve per 24 hours (AUC24h) of fluconazole in a typical infant (gestational age 25.5 weeks and serum creatinine concentration 60 μmol/L) when the dosing interval of the maintenance dose is adjusted to postnatal age (PNA) as currently advised, that is, every 72 hours if PNA is below 14 days, every 48 hours if PNA is 14–28 days and daily if PNA is above 28 days. Treatment was initiated at a PNA of 3, 10 or 17 days, presented in the left, middle and right panels, respectively, and consisted of a loading dose of 25 mg/ kg and a maintenance dose of 12 mg/kg. The typical infant has a gestational age of 25.5 weeks and is expected to follow median growth for a male, resulting in current weights of 738, 771 and 856 g at PNAs 3, 10 and 17 days, respectively. A serum creatinine concentration of 60 μmol/L was assumed to remain constant throughout the treatment period. (A) Median fluconazole concentration of 1000 simulations versus time since first dose, and (B) AUC24h of 1000 simulations. The horizontal line in (B) represents a target AUC24h of 400 mg*hour/L as target for suspected Candida meningitis.*

Simulations to evaluate term infant dosing in preterm infants

Figure 2 shows the concentration-time plots and exposure to fluconazole in a preterm neonate with median GA of 25.5 weeks and median S_{cr} of 60 μmol/L receiving the licensed dose for a term neonate, i.e. 12 mg/kg every 72 h when PNA is below 15 days, 12 mg/kg every 48 h for PNA 15-28 days and 12 mg/kg daily when PNA is above 28 days[3], with a loading dose of 25 mg/kg as recommended by Piper et al.[8] Despite varying exposure, an AUC_{24h} of 400 mg*h/L is achieved on the first day of treatment in all infants due to the loading dose of 25 mg/kg that is administered independent of PNA. The figure also shows that the PNA-dependent dosing intervals (i.e. 72 h for PNA 0-14 days, 48 h for PNA 14-28 days) lead to varying exposure upon different PNAs at treatment initiation, and to different AUCs per 24h in one patient. In addition to that, in the majority of patients the target AUC_{24h} of 400 mg*h/L is not reached on most of the days (Figure 2). Even larger variation in exposure is observed when variation in S_{cr} is taken into account (Supplementary Figure 3). Based on these results, a daily maintenance bodyweight-based dose – independent of PNA – seems necessary to reach an effective concentration over time.
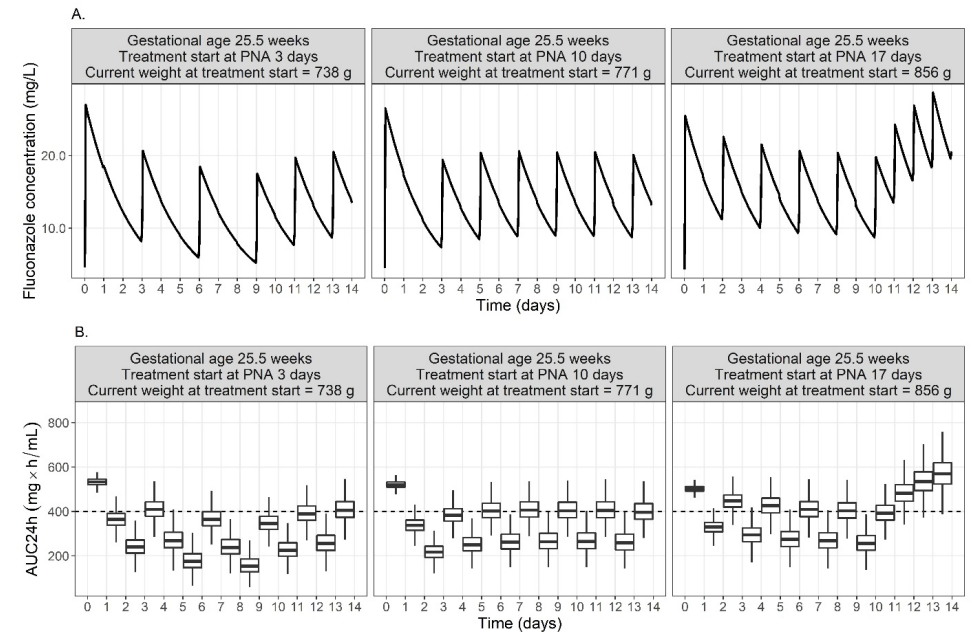
The designed model also allowed us to demonstrate the exposure to fluconazole upon prophylactic treatment with 3 or 6 mg/kg twice weekly, which is presented in Supplementary Figure 4.

Optimization of the dosing regimen

Figure 3 shows the exposure to fluconazole for a model-based PNA- and S_{cr} -based dosing regimen, which is presented in Figure 4, based on Supplementary Figure 5. This dosing regimen aims to achieve a target AUC_{24h} of 400 mg*h/L immediately after the loading dose and throughout the following treatment period for a worst-case scenario of suspected Candida meningitis. The figure shows that the target of 400 mg*h/L is achieved immediately after the loading dose. This minimum AUC is maintained using

the newly proposed daily maintenance doses in a child born at 25.5 weeks (median of the population, Figure 3), 24 or 28 weeks (on Supplementary Figure 6). As presented in Figure 4, during the first week of life a loading dose of 25 mg/kg and a daily maintenance dose of 12 mg/kg is advised for all infants, independent of S_{cr}. If treatment is initiated above 1 week of age, an increased loading dose of 30 mg/kg is suggested for infants with a S_{cr} below 70 µmol/L (Figure 4, left panel). Above 1 week of postnatal age the maintenance dose should be adjusted to S_{cr} as follows: 9 mg/kg if S_{cr} is above 100 µmol/L, 12 mg/kg is S_{cr} is between 70 and 99 µmol/L, 15 mg/kg if S_{cr} is between 40 and 69 µmol/L and 18 mg/kg if S_{cr} is below 40 µmol/L (Figure 4, Middle panel). Once a clean lumbar puncture confirms the absence of Candida meningitis and the determined MIC suggests that a lower target AUC_{24h} of 200 mg*h/L is sufficient, all maintenance doses can be reduced by 50% (Figure 4, right panel).

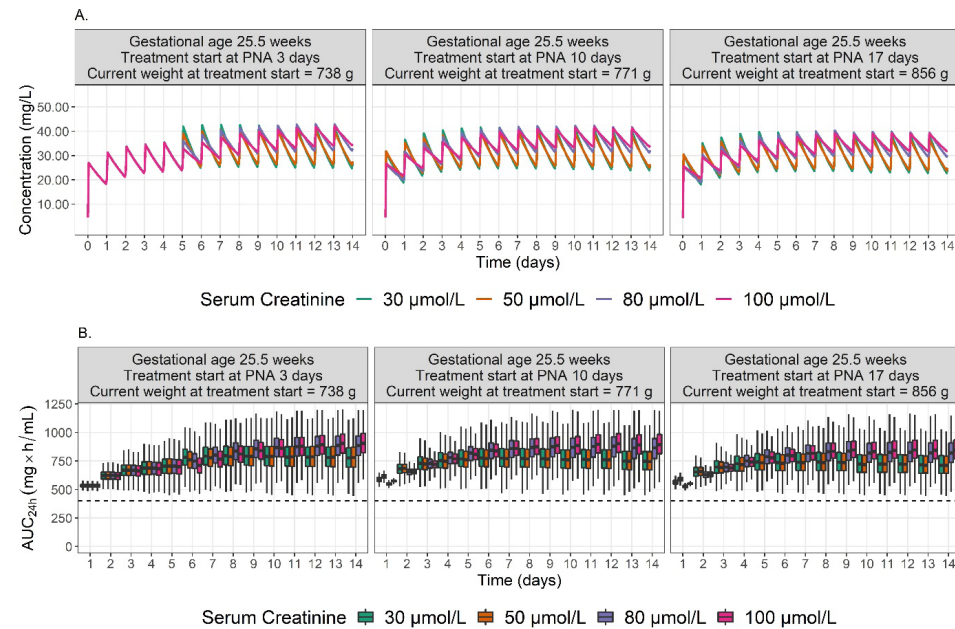*Figure 3.* Concentration- time profiles (upper panel) and corresponding area under the curve per 24 hours (AUC24h, lower panel) of fluconazole when the loading and maintenance doses are adjusted to postnatal age (PNA) and serum creatinine concentration as proposed in figure 4, when aiming for a target AUC24h of 400 mg*hour/L. (A) The median concentration- time profile of 1000 simulations and (B) the corresponding AUC24h. Results are shown for a typical individual with a gestational age of 25.5 weeks and median birth weight and growth, and treatment was initiated at day 3 (left panel), 10 (middle panel) or 17 (right panel). Serum creatinine concentration was set at either 30, 50, 80 or 100 µmol/L and was assumed to remain constant during the treatment period.
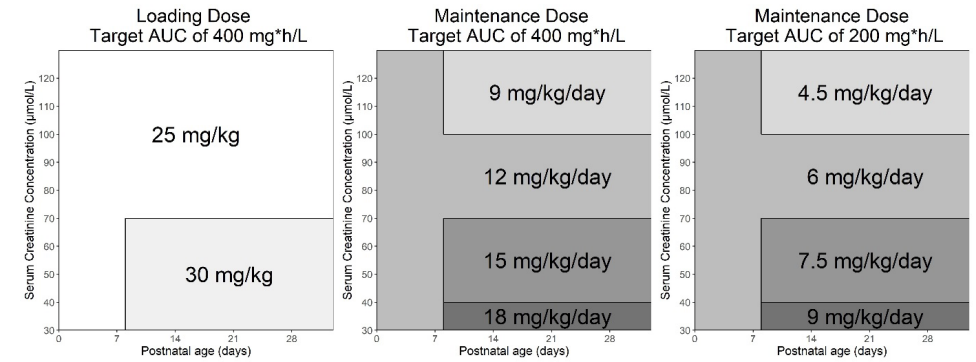

Figure 4. Proposed model- based dose adaptation based on postnatal age and serum creatinine concentrations. Left: proposed loading dose aiming for a target area under the curve per 24 hours (AUC24h) of 400 mg*hour/L for worst- case scenario of suspected Candida meningitis or an unknown minimally inhibitory concentration (MIC). Middle panel: proposed maintenance dose when aiming for a target AUC24h of 400 mg*hour/L. Right panel: proposed maintenance dose when aiming for a target AUC24h of 200 mg*hour/L that can be used for pathogens with an MIC <2 mg/L.

DISCUSSION

In this study we successfully characterized the PK of fluconazole in a large cohort of preterm infants that received multiple and varying doses. Using the developed model and in absence of a dosing regimen for preterm infants in the label, we evaluated the performance of the recommended current dosing regimen for term infants, and adapted this to a suggested optimized dosing regimen for treatment of preterm infants.

Renal function was found to be an important predictor of fluconazole CL in addition to body weight after the first week of life. Recently, in adults, a dose adaptation based on renal function was suggested.[10] Whereas for adults renal function is usually represented by glomerular filtration rate, the best marker for renal function in preterm infants remains a topic of debate. In our study, we used absolute S_{cr} observations[9,11–14] with 63% of the infants having one or more S_{cr} observations. As during the first week of life, S_{cr} might reflect maternal creatine, only S_{cr} observations taken on or after PNA day 8 were included in the analysis. Using this approach the effect of S_{cr} on CL was highly significant with CL increasing by 12% when S_{cr} decreases from 60 to 50 µmol/L. Estimated model parameters and covariate relationships are well in line with previously published fluconazole PK reports[4,5,9,13,14]. Momper et al. also identified S_{cr} as predictor of CL, albeit it with a non-linear relationship where the largest effect of S_{cr} was predicted for the lowest S_{cr} observations, and the decrease in CL plateaus at high S_{cr} observations.[9] It is likely that this plateau was identified due to a wider range in S_{cr} in their study (8.8 to 318 µmol/L vs. 22 to 127 µmol /L in the current population). Recently, van Donge et

al. developed a population model for S_{cr} to get a better understanding of (gestational) age-dependent S_{cr} reference values.[15] Upon further expanding of this method, such models could be of great value in future investigations of S_{cr}-based renal function as covariate in preterm infants. Ibuprofen was found as predictor of CL of other renally cleared drugs but was not more predictive than S_{cr} in our study, probably because the effects of ibuprofen are incorporated in the observed S_{cr}.[16,17]

In this study we optimized the therapeutic dosing regimen for a clinical scenario where the infant is suspected to have invasive candidiasis or Candida meningitis and requires effective treatment immediately, without time to await microbiological blood-culture results. Therefore, treatment is designed to achieve optimal exposure for the worst-case scenario, i.e. Candida meningitis caused by a pathogen with an MIC of 2 mg/L but with confirmed susceptibility, corresponding to a target AUC_{24h} of 400 mg*h/L. We propose a loading dose and daily maintenance dose to maintain optimal exposure, which was also the conclusion of Leroux et al.[5] The Infectious Diseases Society of America already recommends 12 mg/kg daily for neonatal candidiasis but without a loading dose, while our and previous results strongly advise upon a loading dose.[4,5,18] We propose to adjust both the loading- and maintenance dose to S_{cr}, while Leroux et al. found GA as best predictor for fluconazole CL and therefore adjusts the maintenance dose to GA.[5] In the present study GA was not found as predictor of CL, but might be represented in S_{cr} since renal function is expected to increase with GA.[15] According to our model, infants with good renal function (S_{cr} < 70 μmol/L) require a loading dose of 30 mg/kg to reach an AUC_{24h} of 400 mg*h/L on day 1 of treatment if treatment is initiated above 1 week of PNA. This increased loading dose has not been previously studied in preterm infants, but might be comparable to the loading dose of 25 mg/kg with which no safety concerns were found.[4,5] Leroux et al. increased the maintenance dose to 20 mg/kg for infants with a GA ≥ 32 weeks, which did not result in any fluconazole-associated safety concerns. These results suggest that no safety concerns are to be expected for the S_{cr}-based dose administered daily (Figure 4) that we propose in our study, which has a maximum of 18 mg/kg, but safety should be monitored with e.g. liver function tests when applying the suggested dosing strategy. Most studies, including Leroux et al., aim for a target AUC_{24h} of 400 mg*h/L usually based on an MIC of 4 or even 8 mg/L. [4,5,14,19] The variety in reported MIC's and target MIC:AUC_{24h} indicate that consensus is lacking, and that the future might lie in more individualized therapy using biomarkers. [20]While fluconazole is usually well tolerated, unnecessary overexposure is considered unwanted. Therefore, we advise to decrease the maintenance dose once the need for a lower target AUC_{24h} is confirmed by the lab results, e.g. as a result of a clean lumbar puncture or an MIC < 2 mg/L, and to seek alternative treatment if the MIC is determined to be > 2 mg/L (Figure 4, right panel). Because no S_{cr} observations were available from infants weighing more than 1.5 kg the developed dosing regimen is applicable for

preterm infants with a maximum actual bodyweight of 1.5 kg.

The recommendation for prophylactic fluconazole treatment is dependent on the rate of invasive candidiasis of a medical centre, since beneficial effects are observed in centres with a high rate of infections while for centres with low numbers of invasive candidiasis the number needed to treat is very high.[21,22] A meta-analysis of placebo-controlled studies reported a reduction in invasive fungal infection upon prophylaxis with fluconazole, but did not identify a dose-effect.[22] While large variation in exposure to fluconazole is expected upon different dosing strategies and patient characteristics (Supplementary Figure 6), it is complicated to determine which strategy is best for all preterm infants. In conclusion, because fluconazole CL is well predicted by actual bodyweight and S_{cr} in preterm infants with a PNA above 1 week, both loading- and maintenance doses should be adjusted to S_{cr} once PNA is above 1 week to achieve adequate exposure.
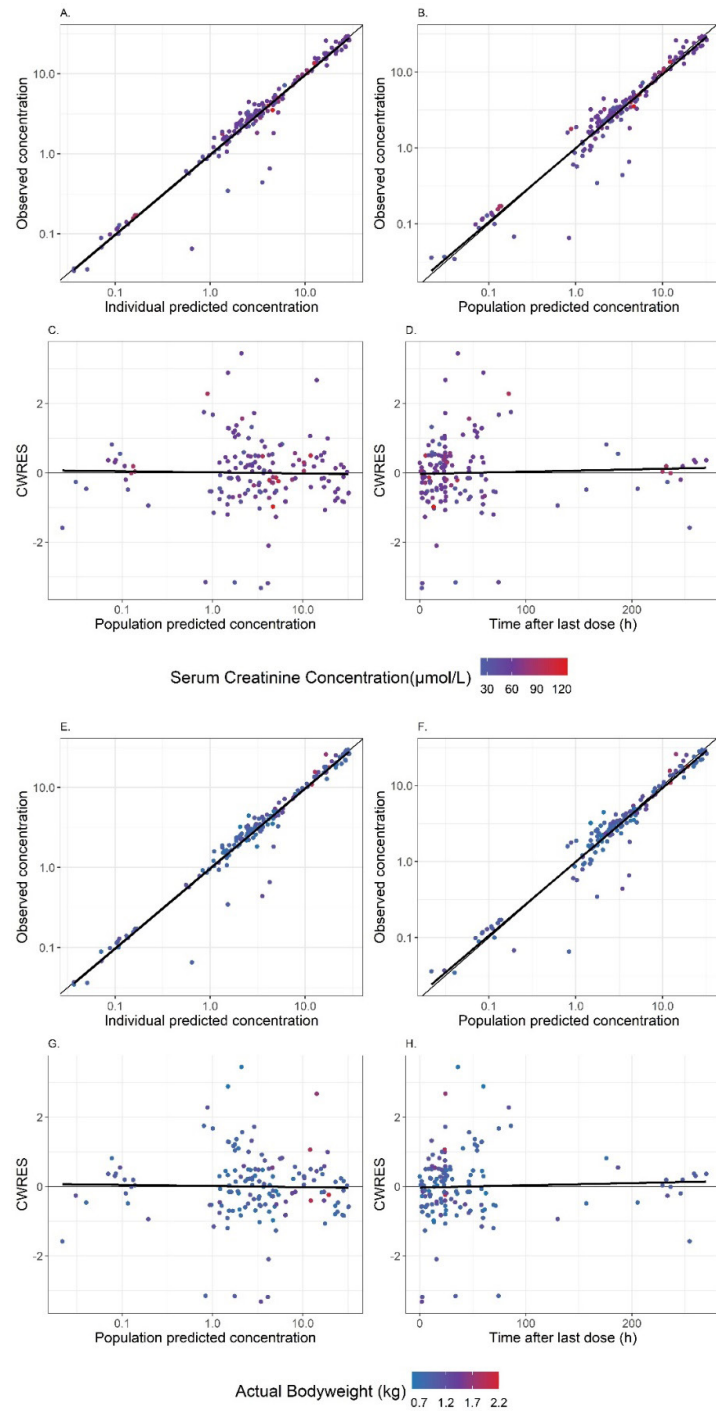
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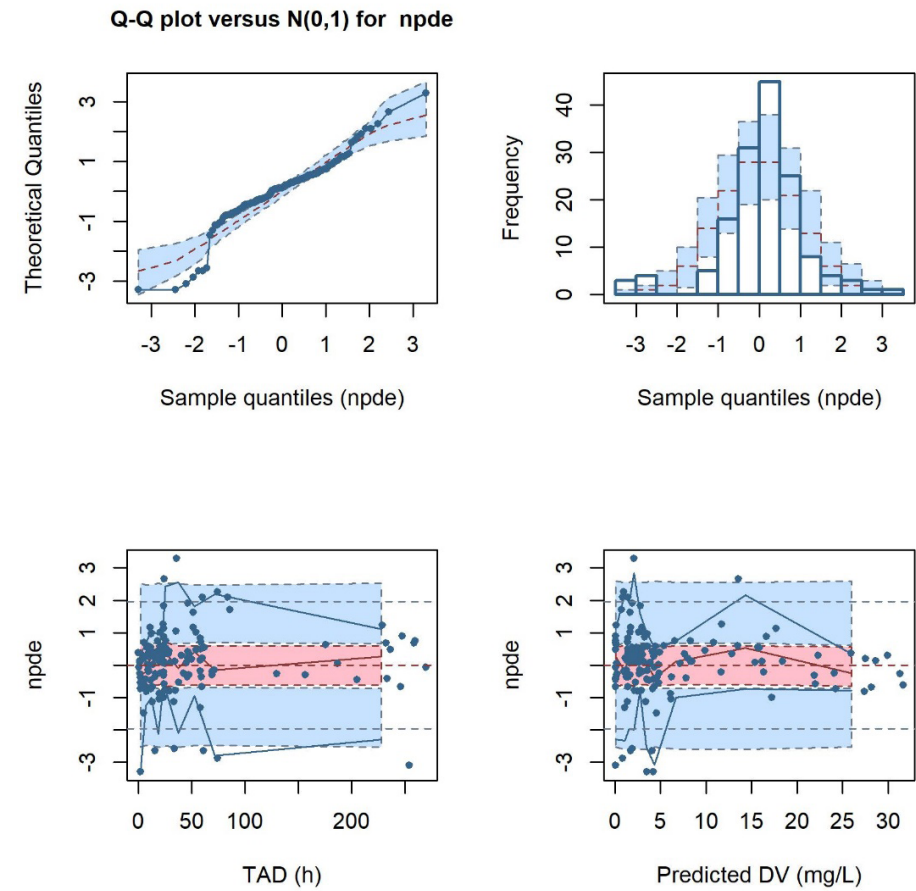
SUPPLEMENTAL MATERIALS

Supplementary Table 1. *Parameter estimates of the final model and the corresponding bootstrap estimates.*

|	Final parameter estimate (RSE %)	Bootstrap estimate (95% CI)
Structural model parameters		
$CL_{ind} = CL_{pop} * (1 + \theta_{Weight} (Weight_{ind} - Weight_{med})) * (1 + \theta_{Creatinine}(Creatinine_{ind} - Creatinine_{med}))$		
CL_{pop} (L/h)	0.0147 (4%)	0.0154 (0.0143 – 0.0168)
θ_{Weight}	1.56 (16%)	1.56 (1.07 – 1.98)
$\theta_{Creatinine}$	-0.0118 (19%)	-0.0069 (-0.0109 - -0.0028)
$V_{ind} = V_{pop} * (1 + \theta_{Weight} (Weight_{ind} - Weight_{med}))$		
V_{pop} (L)	0.844 (6%)	0.837 (0.749 – 0.933)
Variable model parameters [shrinkage]		
IIV on CL (%)	16.6 (19%) [26%]	16.8 (9.7 – 23.1)
Proportional residual error (%)	25 (15%)	25 (0.17 – 0.31)

RSE: relative standard error, CI: confidence interval, CL_{ind}: individual predicted clearance, CL_{pop}: population mean value of clearance for a neonate with a median weight of 0.87 kg and a median serum creatinine concentration of 60 μmol/L, θ_{weight}: slope parameter for clearance and volume of distribution with weight, $Weight_{ind}$: observed actual bodyweight in kg, $Weight_{med}$: median actual bodyweight of the population (0.87 kg), $\theta_{Crreatinine}$: slope parameter for clearance with serum creatinine concentration, $Creatinine_{ind}$: observed serum creatinine concentration in in μmol/L, $Creatinine_{med}$: median serum creatinine concentration (60 μmol/L), V_{ind}: individual predicted volume of distribution, V_{pop}: population mean value of volume of distribution for a neonate with a weight of 0.87 kg, IIV: inter-individual variability.

**Q-Q plot versus N(0,1) for npde**

Supplementary Figure 2. Normalized prediction distribution error (NPDE) analysis of the final model. Top left: quantile-quantile plots, top right NPDE quantiles with a normal distribution overlay, bottom left: NPDE versus time after dose (TAD) in hours, bottom right, NPDE versus predicted concentrations of fluconazole (DV). Global adjusted p-value: 9.6*10^{-7}(p<0.001).

Supplementary Figure 1. Goodness-of-fit of the final model. CWRES: conditional weighted residuals. The colour of the dots represents the serum creatinine observations (A-D) or actual bodyweight (E-H).
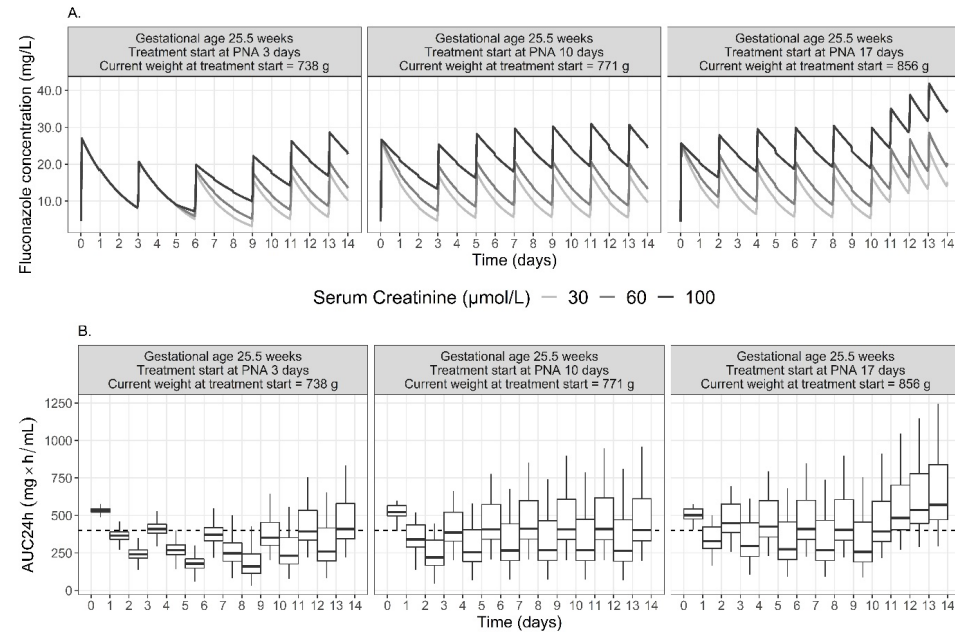
Supplementary Figure 3. *Exposure to fluconazole in a typical infant (gestational age 25.5 weeks, S_{cr} either 30, 60 or 100 µmol/L) when the dosing interval of the maintenance dose is adjusted to postnatal age as currently advised by the label[1] and Dutch Paediatric Formulary[3], i.e. every 72 h if postnatal age is below 14 days, every 48 h if postnatal age is 14-28 days and daily if postnatal age is above 28 days. Treatment was initiated at a postnatal age of 3, 10 or 17 days, presented in the left, middle and right panels, respectively, and consisted of a loading dose of 25 mg/kg and a maintenance dose of 12 mg/kg. The typical infant has a gestational age of 25.5 weeks and is expected to follow median growth for a male, resulting in current weights of 738, 771 and 856 g at postnatal ages 3, 10 and 17 days, respectively. Serum creatinine concentrations were assumed to remain constant throughout the treatment period. Figure 2.A: Median fluconazole concentration of 1000 simulations versus time since first dose, and Figure 2.B: area under the curve per 24 h (AUC_{24h}) versus time since first dose of all serum creatinine concentrations combined (1000 simulations per S_{cr}). The horizontal line in Figure 2.B represents a target AUC_{24} of 400 mg*h/L for suspected Candida meningitis.*
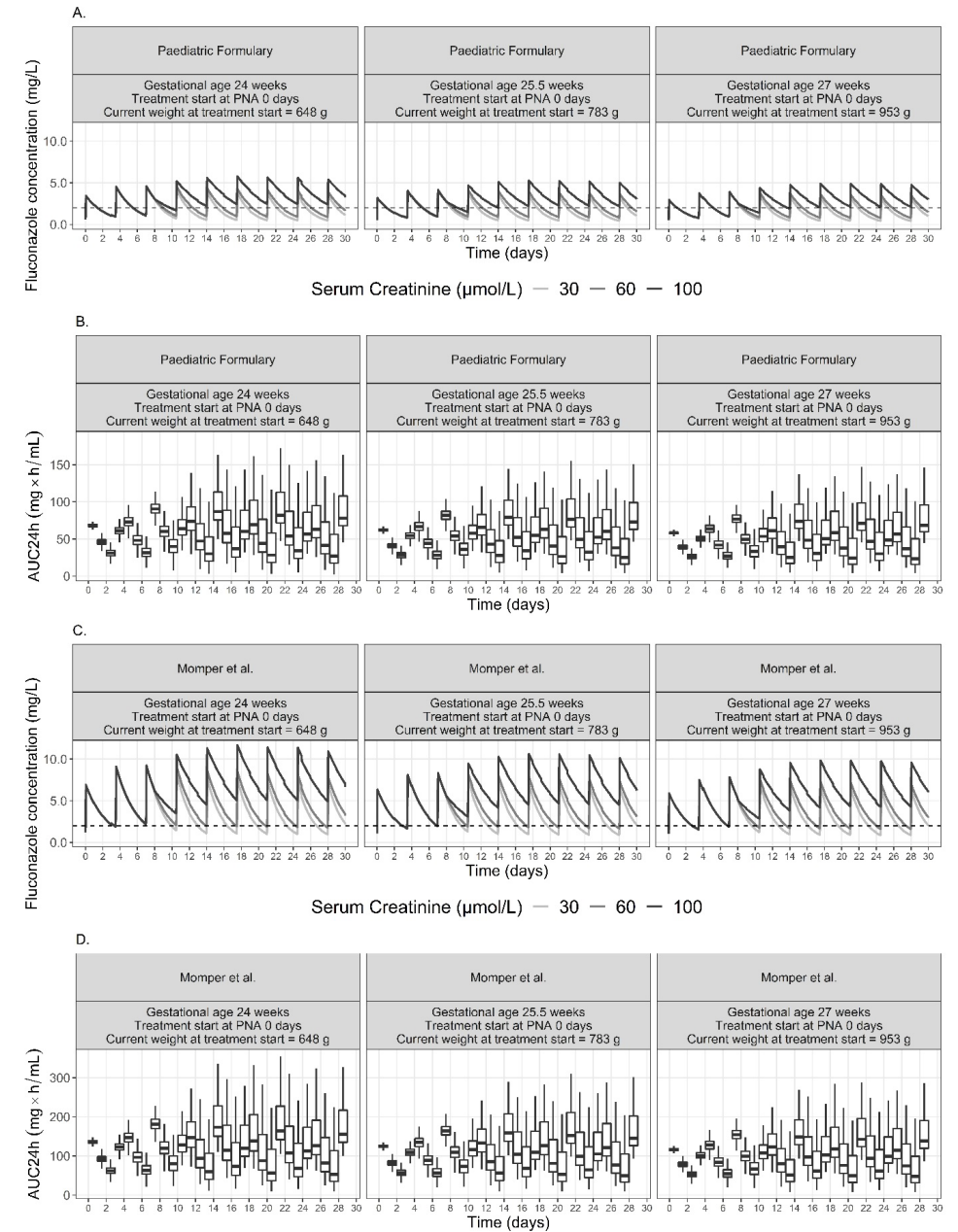
Supplementary Figure 4. *Exposure to prophylactic fluconazole treatment as currently advised by the Dutch Paediatric Formulary (A. & B.), and as suggested by Momper et al. (C. & D.). The Dutch Paediatric Formulary recommends 3 mg/kg twice weekly, and Momper et al. recommend 6 mg/kg twice weekly, aiming for a trough concentration of 2 mg/L (dashed line in A. & C.). A. and C. represent the median concentration of 1000 simulations, and B. and D. represent the corresponding area under the curve per 24h of all serum creatinine concentrations combined (each 1000 times simulated).*
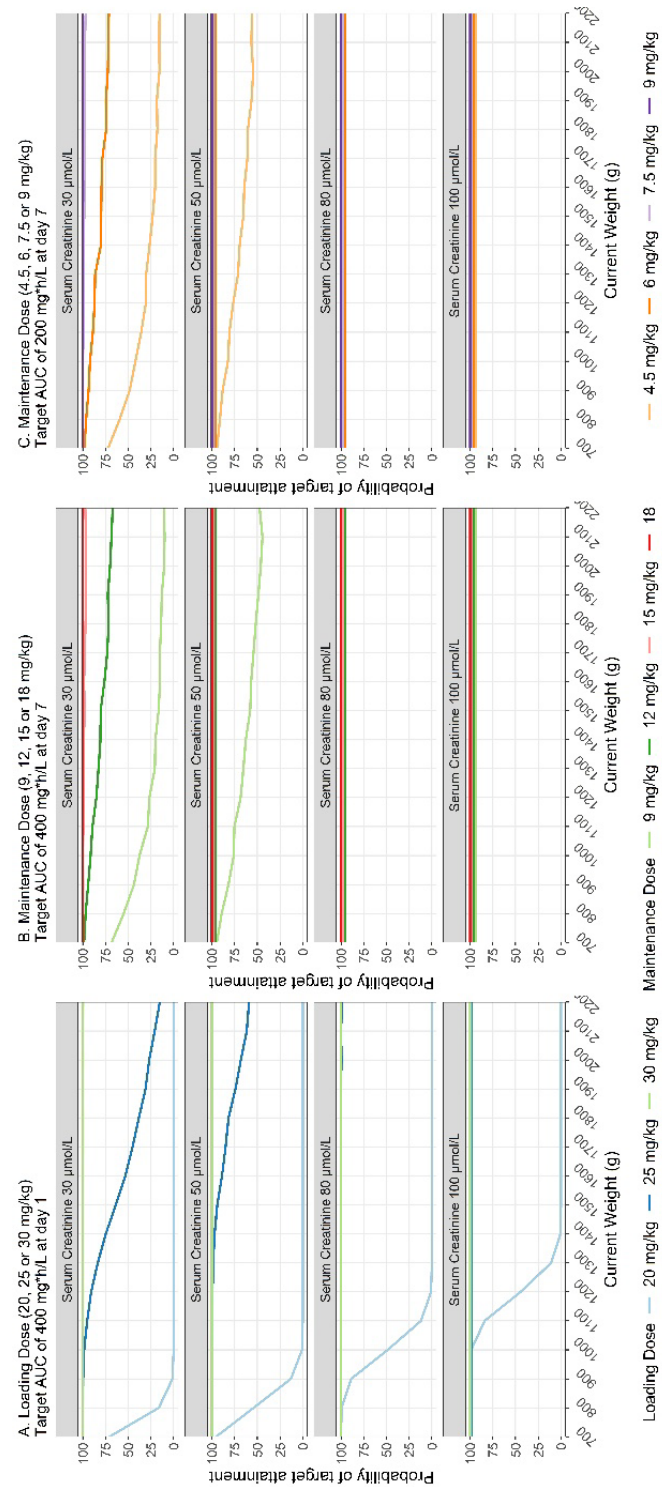
Supplementary Figure 5. *Probability of target attainment of examined loading- and maintenance doses across the weight range and for infants with a serum creatinine concentration of either 30, 50, 80 or 100 μmol/L. A.: Target attainment on the first day of treatment aiming for an AUC$_{24h}$ of 400 mg*h/L, upon a loading dose of either 20, 25 or 30 mg/kg. B.: Target attainment on the seventh day of treatment aiming for an AUC$_{24h}$ of 400 mg*h/L, upon a loading dose of 25 mg/kg and a maintenance dose of either 9, 12, 15 or 18 mg/kg. C.: Target attainment on the seventh day of treatment aiming for an AUC$_{24h}$ of 200 mg*h/L, upon a loading dose of 25 mg/kg and a maintenance dose of either 4.5, 6, 7.5 or 9 mg/kg. Each combination of current weight and S$_{cr}$ was simulated 1000 times and the lines represent the percentage of each combination achieving the target AUC$_{24h}$. Current weight and S$_{cr}$ were assumed to remain constant during the treatment period.*
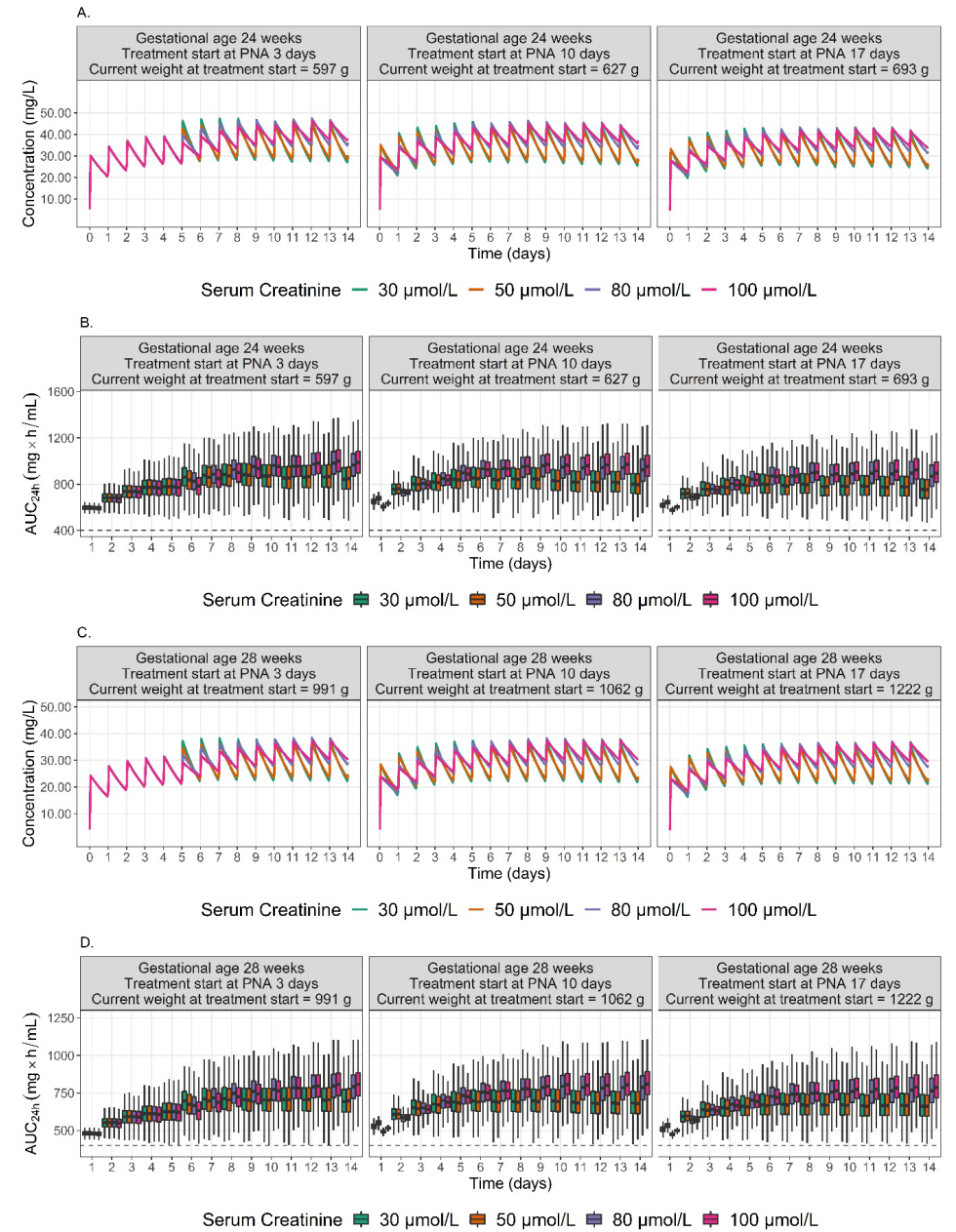
Supplementary Figure 6. *Performance of the developed model-based dosing regimen as depicted in Figure 4 in typical infants with a gestational age of 24 (A. and B.) or 28 (C. and D.) weeks. The concentration-time profiles (A. and C.) and corresponding area under the curve per 24 h (AUC$_{24}$, B. and D.) are presented for infants with a S$_{cr}$ of 30, 50, 80 or 100 μmol/L, each simulated 1000 times. Treatment was initiated at a postnatal age of 3 (left panel), 10 (middle panel) or 17 (right panel) days. Median birth weight and growth for the corresponding gestational ages were assumed.*