Dosing considerations for preterm neonates: from pharmacometrics to clinical practice

Engbers, A.G.J.

The effect of ibuprofen exposure and patient characteristics on the closure of the patent ductus arteriosus in preterm infants

Authors: Aline G.J. Engbers, Swantje Völler, Robert B. Flint, Sebastiaan C. Goulooze, Johan de Klerk, Elke H.J. Krekels, Monique van Dijk , Sten Willemsen, Irwin K.M. Reiss, Catherijne A.J. Knibbe, Sinno H.P. Simons

Clin Pharmacol Ther (2022) 112, pp. 307–315.

5

ABSTRACT

Spontaneous closure of the ductus arteriosus depends on gestational age (GA) and might be delayed in preterm infants, resulting in patent ductus arteriosus (PDA). Ibuprofen can be administered to enhance closure, but the exposure-response relationship between ibuprofen and the closure of PDA remains uncertain. We investigated the influence of patient characteristics and ibuprofen exposure on ductus closure.

A cohort of preterm infants with PDA and treated with ibuprofen was analysed. Ibuprofen exposure was based on a previously developed population pharmacokinetic study that was in part based on the same study population. Logistic regression analyses were performed with ductus closure (y/n) as outcome, to analyse the contribution of ibuprofen exposure and patient characteristics.

In our cohort of 263 preterm infants (median GA 26.1 (range 23.7-30.0) weeks, birthweight 840 (365-1470) g) receiving ibuprofen treatment consisting of three doses that was initiated at a median postnatal age (PNA_{start}) of 5 (1-32) days, PDA was closed in 55 (21%) patients. Exposure to ibuprofen strongly decreased with PNA_{start}. Overall, the probability of ductus closure decreased with PNA_{start} (odds ratio (OR) 0.7 (0.6-0.8 95%CI)) and Z-score for birthweight ($Z_{Birthweight\text{-}for\text{-}GA}$)(OR 0.8 (0.6-1.0 95%CI), and increased with GA (OR 1.5 (1.1-1.9 95%CI)). For patients with PNA_{start} < 1 week, concentrations of ibuprofen, GA and $Z_{Birthweight\text{-}for\text{-}GA}$ predicted probability of ductus closure.

During a window of opportunity for ductus closure within the first days of life, probability of closure depends on GA, $Z_{Birthweight\text{-}for\text{-}GA}$, and ibuprofen exposure. Increased, yet unstudied dosages might increase the effectivity of ibuprofen beyond the first week of life.

INTRODUCTION

The ductus arteriosus usually closes within hours after birth of a term born infant, but in prematurely born neonates closure is often delayed or does not occur spontaneously, resulting in a patent ductus arteriosus (PDA). The consequent ductal left-to-right shunt can result in increased pulmonary blood flow and systemic hypoperfusion, which in turn can lead to comorbidities such as bronchopulmonary dysplasia intraventricular haemorrhage, necrotizing enterocolitis, and renal failure.[1]

Spontaneous closure of the ductus is an effect of multiple complex physiological mechanisms. A drop in prostaglandin E_2 (PGE_2) levels due to lost supply by the placenta after birth is one of these mechanisms. Because of higher sensitivity to PGE_2 earlier in pregnancy, this mechanism of ductus closure may be less effective in preterm infants. [2] Cyclo-oxygenase (COX) inhibitors can be used to pharmacologically stimulate closure of the ductus by decreasing PGE_2 production. Other suggested mechanisms that make preterm infants more prone for PDA are an increased nitric oxide sensitivity in combination with decreased expression of ion channels in the ductus that induce vasodilation, and an immature anatomy of the ductus that postpones anatomical closure.[2]

Besides other drugs such as acetaminophen and indomethacin, the COX-inhibitor ibuprofen is the most frequently used for the treatment of PDA.[3,4] The effects of ibuprofen have been compared to placebo or to alternative treatments such as indomethacin. In a meta-analysis, of all currently studied treatment strategies, oral ibuprofen treatment with 20 mg/kg on the first day of treatment, followed by 10 mg/kg daily on day 2 and 3 was shown the most effective for PDA closure.[5] This analysis did not identify patient characteristics such as gestational age (GA) or postnatal age (PNA) as confounding factors, but might have been limited by the fact that these characteristics were not always reported in the examined studies.[6] The probability of spontaneous closure increases with GA, and as a result the effectiveness of ibuprofen may appear higher in infants with a lower GA compared to studies in infants with a higher GA.[7] Moreover, most studies reported on scenarios in which ibuprofen treatment is started within the first few days of life. The clearance of ibuprofen increases with PNA, resulting in a lower exposure to ibuprofen [8,9] These factors should be considered simultaneously for the evaluation of the efficacy of ibuprofen.

The aim of this study was to quantify the contribution of patient characteristics and ibuprofen exposure to the closure of PDA in a large population of preterm infants with varying patient characteristics and ibuprofen dosing regimens. The overall population was evaluated as well as a subset of the population in which ibuprofen was initiated during the first week of life.

METHODS

Patients and data

Data on a clinical cohort of preterm infants (n=298) who all received ibuprofen for the treatment of PDA were available for analysis. Data from part of this cohort (n=43) were also used for the development of a recently published population pharmacokinetic (PK) model that is described in more detail in the next section.[8] All patients had confirmed PDA based on an echocardiographic ultrasound prior to treatment initiation. Treatment with ibuprofen was initiated if the PDA was judged as hemodynamically significant (hsPDA) by the clinical team (paediatric cardiologist and neonatologist) according to the local protocol and there was no contra-indication for ibuprofen (i.e., hepatic or renal failure, severe thrombocytopenia or other known clotting disorders, recent IVH or other bleeding, sepsis, suspected or confirmed necrotizing enterocolitis, or severe hyperbilirubinemia). For infants born before June 2015, the protocol for ibuprofen treatment consisted of a loading dose of 10 mg/kg bodyweight on day 1 followed by 2 maintenance doses of 5 mg/kg on day 2 and 3 (also written as 10-5-5 mg/kg). After June 2015, a new dosing regimen was applied that accounts for the maturation of ibuprofen clearance with postnatal age (PNA)[9]. Loading and maintenance doses were adapted to PNA , resulting in 10-5-5 mg/kg for PNA < 70 hours, gradually increasing per day up to 20-10-10 mg/kg if PNA was above 196 hours. Ibuprofen was administered intravenously or orally using same dosages, and an ibuprofen cycle consisting of three doses could be repeated as judged clinically deemed necessary. Typically, an ultrasound was made after the third dose to assess the status of the PDA. To prevent unnecessary burden for the patient the attending physician could postpone or cancel an ultrasound after three doses if the outcome was expected to be clinically irrelevant (apparent signs for open ductus such as a persistent murmur or low diastolic blood pressure or no signs of a clinically relevant ductus). If necessary, ibuprofen treatment could be continued for an additional 3 to 6 days. For the purpose of this analysis, all ultrasounds were re-assessed by one experienced clinician (JdK) who determined whether PDA was closed or not. Additionally, the diameter of the ductus, the diameter of the left pulmonary artery, the ratio between the diameter of the ductus and the left pulmonary artery, the maximal diastolic velocity, ratio between atrium to aortic valve ratio, flow through the left pulmonary artery, and flow through the aorta were documented for each ultrasound. For each patient, the Z-score for birthweight ($Z_{Birthweight\text{-}for\text{-}GA}$) was calculated based on birthweight, GA and Dutch perinatal growth curves.[10] The cohort has been previously described by de Klerk *et al*.[11].

Ibuprofen exposure and concentrations

A previously developed population PK model for ibuprofen in preterm infants [8] was used to calculate ibuprofen exposure upon the applied dosing strategies based on relevant patient characteristics. For oral administrations, 100% bioavailability was assumed and the absorption rate constant was set at 0.6562 h^{-1}.[12] Based on this PK model clearance keeps increasing with PNA while physiologically a plateau-function in clearance is expected at higher PNA[13]. Maximum PNA in the PK model dataset was 18 days. To prevent extrapolation of the maturation function outside the range in PNA of the patients that were included in the PK study , for infants in the current study with a PNA above 18 days (n=18) clearance was calculated as if their age was 18 days[8]. If one or more plasma samples of ibuprofen were available for an individual patient (n=150 plasma samples from 43 patients), the model was used to best fit the plasma-concentration time curve of that individual patient, also referred to as individual predicted exposure. If no concentration was available, the infants' characteristics (i.e. GA, PNA and SGA) were used to calculate the typical value for the population, also called population predicted exposure. Comparison of the demographics between the group with PK samples and the group without PK samples did not reveal any differences.

Ibuprofen exposure was examined using different metrics. First, area under the curve between the first dose and 24h after the third dose ($AUC_{0\text{-}72h}$) was tested based on the results of Hirt et al.[9] Second, the lowest ibuprofen concentration during the first three days of ibuprofen treatment of the patient ($C_{trough72h}$) was selected based on ibuprofen's mechanism of action that concerns COX-2 inhibition via competitive and reversible COX-2 binding[14]. Therefore, a certain minimal concentration would be needed to maintain COX-2 inhibition and achieve closure of the ductus[15]. $C_{trough72h}$ could be the trough concentration of either the first, second or third dose, dependent on which was the lowest. Additionally, average $C_{trough72h}$ ($C_{trough72h\_average}$) was defined as the average trough concentration after the first, second and third dose.

Logistic regression analysis

Only results upon the first ibuprofen treatment cycle that consisted of three doses were used. To account for variable dosing and ultrasound timing, patients who only received 1 or 2 ibuprofen doses, patients with more than 72 hours between 2 of the first 3 doses, and patients with an ultrasound made more than 1 week after the first dose were excluded to assure that closure (yes/no) happened within the interval where the effect of ibuprofen would still be relevant. If no ultrasound was made after three doses and ibuprofen treatment was continued, the ductus was assumed to be open 24 h after the third dose. Patient without at least one ultrasound after diagnosis were excluded.

Closure of the ductus (yes/no) approximately 24 h after the first ibuprofen treatment episode (3 ibuprofen doses) was the primary outcome for logistic regression. The influence of the patient characteristics GA, PNA at the start of treatment (PNA_{start}), postmenstrual age, sex, birthweight and $Z_{Birthweight\text{-}for\text{-}GA}$ on the primary outcome were analysed, as well as ibuprofen exposure using the following different measures: the

cumulative first 3 dose amounts, route of administration, $AUC_{0\text{-}72h,}$ $C_{trough72h}$, and $C_{trough72h\_average}$. First, a univariate analysis was performed to examine and compare predictive performance of all covariates. Second, a multivariate analysis was performed for which all relevant covariates were included as predictors. Insignificant predictors ($p>0.05$) were removed one-by-one to obtain the final model in which each predictor was significant ($p<0.05$).

Subgroup analysis

Finally, the multivariate analysis was repeated on a subgroup of the dataset in which the maximal PNA_{start} was 7 days because in clinical practice ibuprofen treatment is usually initiated during the first week of life[16].

For both multivariate analyses interaction terms were tested and collinearity was assessed by the variance inflation factor. Potential overparameterization of the final models was assessed by using a Hosmer-Lemeshow test.[17]

Simulation of dosing strategies

Coefficients of the final model were used to calculate the probability of closure for hypothetical preterm infants representative for the study population. GA was set at either 24, 25, 26, 27 or 28 weeks with median birthweight for each GA for Dutch male infants[10]. In simulations, these infants were treated with ibuprofen intravenously 10-5-5 mg/kg, 20-10-10 mg/kg. Population predicted $C_{trough72h}$ and $AUC_{0\text{-}72h}$ were obtained for each dosing strategy based on a previously developed population PK model in preterm infants [8].

RESULTS

Patients and data

Of the total of 299 infants, at least one follow-up ultrasound was made in 295 patients. Upon diagnosis, the median diameter of the PDA was 2.2 (range 1.2-4.1) mm in this overall cohort. During the first follow-up ultrasound, performed at a median of 78 (60-71) hours after diagnosis and 71 (56-165) hours after the first dose, PDA was closed in 78 patients (26%). In the remaining patients, the median diameter of the PDA was 2.0 (0.5-4.0) mm. In 46 patients, a second follow-up ultrasound was made after repeated ibuprofen treatment cycles at 112 (72 – 3662) hours after the first ultrasound. In 7 of these patients, PDA was reported to be closed, while the median diameter in the other patients was 1.8 (0.6 – 3.0) mm.

Following the exclusion criteria, 263 preterm infants were included in the data analysis (Figure 1). In 55 infants (21%) the ductus arteriosus was closed after 3 ibuprofen doses,

of which in 52 patients treatment was initiated during the first week of life. For 114 patients the first follow-up ultrasound was postponed to beyond 3 doses and the ductus was assumed to have not closed after 3 doses. Ultrasounds made after more than 3 doses showed that in 103 (90%) of these patients the ductus was still open which justified the assumption. Patient characteristics of the analysed infants are presented in Table 1. Figure 2 shows the$C_{trough72h}$ and $AUC_{0\text{-}72h}$ versus PNA_{start}.

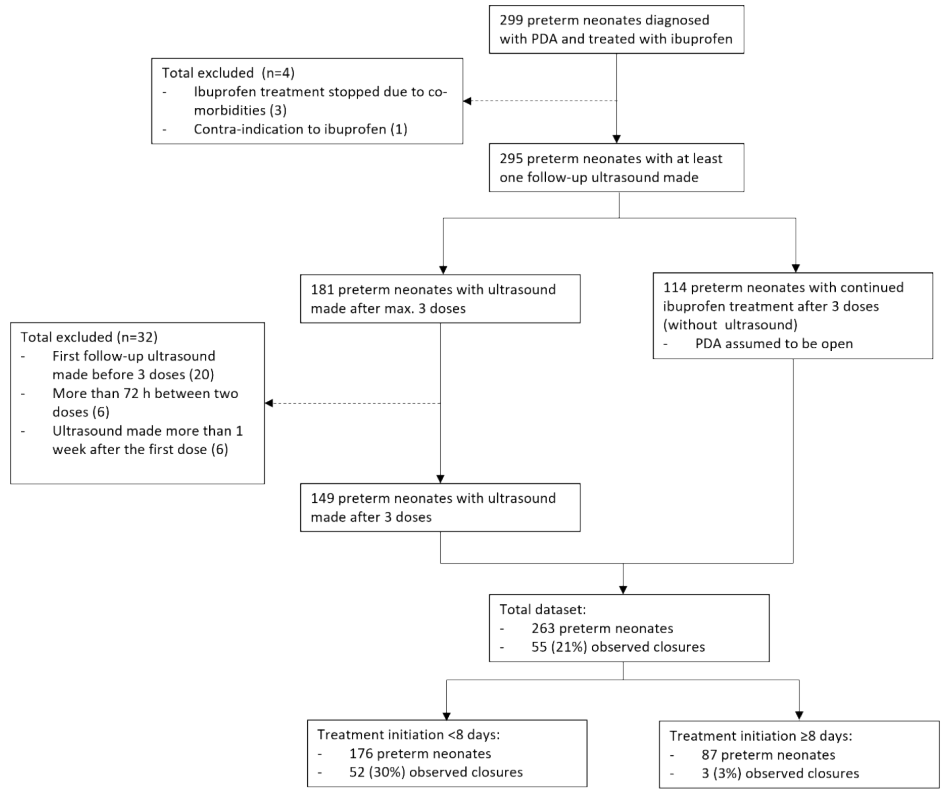


Figure 1. *Data selection flowchart.*

Univariate analysis

In the univariate analysis GA ($p=0.02$), PNA_{start} ($p=0.00006$), ($p=0.02$), $C_{trough72h}$ ($p=0.006$), $C_{trough\_average}$ ($p=0.002$) and $AUC_{0\text{-}72h}$ ($p=0.001$) significantly predicted closure of the ductus. PNA_{start} provided the biggest drop (30 points) in Akaike Information Criterion and was therefore the most predictive covariate. For every week increase in GA, the odds of ductus closure was found to increase with 30% (odds ratio (OR) 1.30 (1.04 – 1.64 95%CI)). For every day increase in PNA_{start} the odds of closure decrease with 23% (OR 0.77 (0.67 – 0.86 95%CI)). For every unit increase in $Z_{Birthweight\text{-}for\text{-}GA}$ the odds of closure decrease

with 21% (OR 0.79 (0.65- 0.97 95% CI). For every mg/L increase in $C_{trough72h}$ the odds of closure increase with 6% (OR 1.06 (1.02 – 1.195% CI)). For every 100 mg*h/L in AUC_{0-72h} the odds of closure increase with 8% (1.0008 (1.0003 – 1.001 95% CI)). Postmenstrual age, birthweight, sex, route of administration and cumulative dose were not predictive for closure of the ductus.

Table 1. *Summary of the patient characteristics of the patients in median and ranges.*

	Outcome after 3 doses ibuprofen			
	Open	**Closed**	**% Closed**	**Total**
Number of patients	208	55	21	263
· Male (n)	118	26	18	144
· Female (n)	90	29	24	119
Gestational age (weeks)	26.1 (23.7-29.4)	26.6 (24.0-30.0)*		26.1 (23.7-30.0)
Birthweight (g)	840 (365-1320)	840 (440-1470)		840 (365-1470)
Small for gestational age (n)	52	19	27	71 (27%)
Postmenstrual age (weeks)	27.6 (24.7-32.8)	27.6 (25.0-31.1)		27.6 (24.7-32.8)
Postnatal age at treatment initiation (days)	6 (1-33)	4 (2-17)**		5 (1-33)
Postnatal age at treatment initiation < 8 days (n)	128	52	29	180
Postnatal age at treatment initiation ≥ 8 days (n)	80	3	4	87
Z-score of birthweight for GA	-0.3 (-5.2 – 3.3	-0.7 (-4.7 – 1.5)*		-0.4 (-5.2 – 3.3)
Diameter PDA at diagnosis (mm)	2.3 (1.2 – 4.1)	2.0 (1.0 – 3.5)		2.2 (1.0 – 4.1)
Diameter PDA at first follow-up ultrasound (mm)	2.0 (0.5 – 4.0)	0.0 (0.0 -0.0)*		1.7 (0.0 – 4.0)
Time between diagnosis and first follow-up ultrasound (h)	78 (60 – 171)	87 (68 – 158)		85 (60 – 171)
Dosing regimen				
· 10-5-5 mg/kg	154	42	21	196
· 20-10-10 mg/kg	31	3	9	34
· Other	23	10	30	33
Route of ibuprofen administration (n)				
· I.V.	189	55	23	244
· Oral	19	-	0	19
AUC_{0-72h} (mg*h/L)	895 (51-2567)	1363 (232-2605)**		992 (51-2605)
$C_{trough72h}$ (mg/L)	3.3 (0.0-25.9)	12.8 (0.0-22.2)**		4.6 (0.0-25.9)

*: p-value < 0.05, **: p-value <0.01, based on Mann-Whitney test. AUC_{0-72h} is the area under the curve between the first dose and 24 h after the third dose. $C_{trough72h}$ is the minimal trough concentration of the first 3 doses.

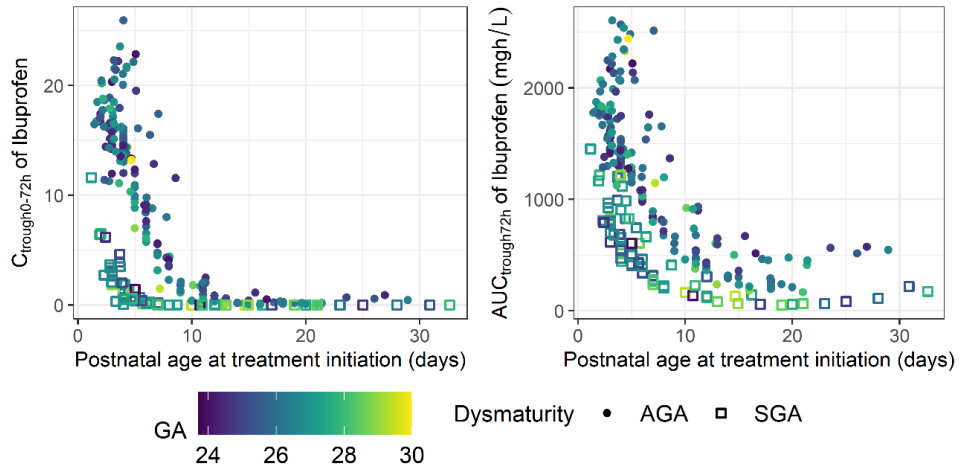


Figure 2. *Lowest ibuprofen trough concentration in the 72 hours after start of treatment(left) and ibuprofen area under the curve (right) for each patient included in the logistic regression analysis. Circles represent appropriate for gestational age infants, and squares represent small for gestational age infants. GA: gestational age, AGA: appropriate for gestational age, SGA: small for gestational age.*

Multivariate analysis complete dataset

GA, $Z_{Birthweight\text{-}for\text{-}GA}$, PNA_{start} and AUC_{0-72h} were included in the multivariate analysis. With both PNA_{start} and AUC_{0-72h} in the model, PNA_{start} was more predictive for ductus closure than AUC_{0-72h}, which no longer met the significance criterion (p=0.12) and was therefore removed from the model. Based on the final model, the odds of ductus closure increase with increasing GA (OR 1.47 (1.12 -1.94, 95% CI)) and decreases with increasing PNA_{start} (OR 0.74 (0.64 – 0.84, 95%CI)) and $Z_{Birthweight\text{-}for\text{-}GA}$ (OR 0.79 (0.63 – 1.00, 95% CI)). A Hosmer-Lemeshow test did not suggest a misfit of the model (p= 0.06). Based on these results, the probability of closure based on these patient characteristics can be calculated with Equation 1, and parameter estimates and corresponding odds ratios are presented in Table 2. In Figure 3 the probability of closure with PNA_{start} is visualized for infants with varying $Z_{Birthweight\text{-}for\text{-}GA}$. AUC_{0-72h} was initially selected because it was more predictive than $C_{trough72h}$ in the univariate analysis. Repetition of the multivariate with $C_{trough72h}$ instead of AUC_{0-72h} resulted in a similar conclusion regarding the impact of GA, PNA_{start} and ibuprofen exposure.

$$(Eq.\,1)\ Probability\ of\ closure = \frac{\exp\left(-9.91 - 0.30 * \mathrm{PNA}_{Start} + 0.38 * GA - 0.23 * Z_{\mathrm{Birthweight-for-GA}}\right)}{1 + \exp\left(-9.91 - 0.30 * \mathrm{PNA}_{Start} + 0.38 * GA - 0.23 * Z_{\mathrm{Birthweight-for-GA}}\right)}$$

Table 2. *Estimated odds by the logistic regression analysis with closure of the ductus after 3 ibuprofen doses as outcome measure. First, the logistic regression based on the complete study population is presented, and below the logistic regression based on the subset of the study population with a maximum postnatal age at treatment initiation of 7 days. $C_{trough72h}$ is the minimal trough concentration of the first 3 doses.*

	Estimated coefficient (95% C.I.)*	Estimated Odds ratio (95% C.I.)	P-value	Variance-inflation factor
Complete Study population				
Postnatal age of treatment initiation (days)	-0.30 (-0.45 – 0.18)	0.74 (0.64 – 0.84)	$0.15*10^{-4}$	1.07
Gestational age (weeks)	0.38 (0.12 – 0.66)	1.47 (1.12-1.94)	0.0058	1.12
Z-score for birthweight	-0.23 (-0.46 – 0.002)	0.79 (0.63 – 1.00)	0.048	1.06
Subgroup: maximum postnatal age at treatment initiation 7 days				
Gestational age (weeks)	0.57 (0.27 – 0.90)	1.77 (1.31 – 2.46)	0.0004	1.1
$C_{trough72h}$ (mg/L)	0.11 (0.04-0.19)	1.12 (1.04 – 1.21)	0.004	2.5
Z-score for birthweight	-0.59 (-0.98 - -0.23)	0.55 (0.38 – 0.79)	0.002	2.4

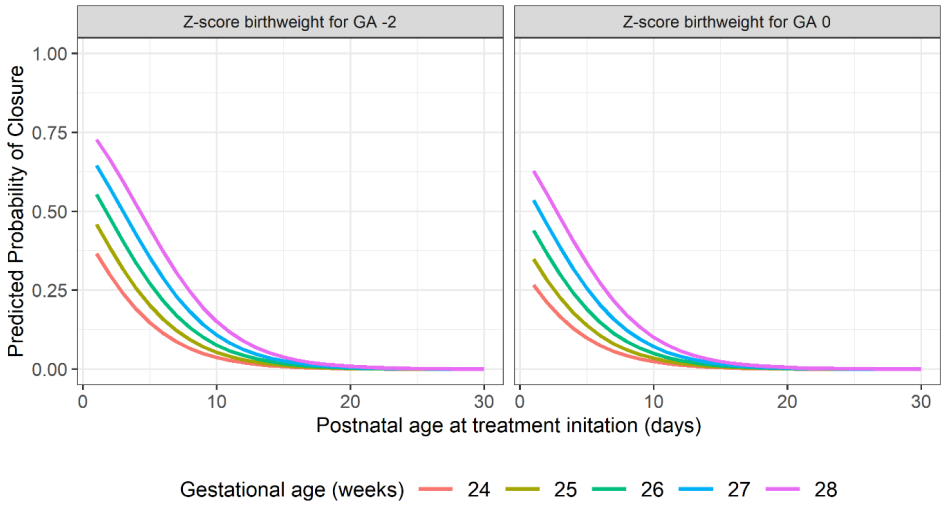


Figure 3. *Predicted probability of closure of the patent ductus arteriosus versus postnatal age at treatment initiation based on the logistic regression analysis on the complete study population, for infants with different gestational ages.*

Subgroup analysis – first week of life

In a multivariate analysis on a subset of the dataset in which the maximal PNA_{start} was 7 days, $C_{trough72h}$ was found to be more predictive for closure than PNA_{start}. With GA, PNA_{start}, $Z_{Birthweight\text{-}for\text{-}GA}$, and $C_{trough72h}$ included as predictors, PNA_{start} was the least significant predictor (p=0.16). Upon removal of PNA_{start}, all remaining predictors (GA, $Z_{Birthweight\text{-}for\text{-}GA}$, and $C_{trough72h}$) were significant predictors for closure, without a suggestion of misfit (Hosmer-Lemeshow test p-value of 0.6). Based on these parameter estimates, the odds of closure are expected to increase with GA (OR 1.77 (1.31 – 2.46 95% CI)) and increased $C_{trough72h}$ (OR 1.12 (1.04 – 1.21, 95% CI)), and to decrease with $Z_{Birthweight\text{-}for\text{-}GA}$ (OR 0.55 (0.38 - 0.79, 95% CI)). Parameter estimates are presented in Table 2. The probability of closure during the first week of life can be calculated with Equation 2.

$$(Eq.\,2)\ Probability\ of\ closure\ during\ first\ week\ of\ life = \frac{\exp\left(-17.53 + 0.11 * C_{trough72h} + 0.57 * GA - 0.59 * Z_{\text{Birthweight-for-GA}}\right)}{1 + \exp\left(-17.53 + 0.11 * C_{trough72h} + 0.57 * GA - 0.59 * Z_{\text{Birthweight-for-GA}}\right)}$$

Based on this model the typical infant in our population with a GA of 26.1 weeks, a $Z_{Birthweight\text{-}for\text{-}GA}$ of -0.4 and a $C_{trough72h}$ of 4.6 mg/mL would have a probability of ductus closure of 13%. Increasing $C_{trough72h}$ with 1 mg/mL to 5.6 mg/L increases the probability of closure to 15% and the maximum $C_{trough72h}$ of 25.9 mg/mL results in a probability of closure of 63%. The estimated probability of closure upon different treatment strategies based on $C_{trough72h}$ when ibuprofen is given in the first week of life is presented in Figure 4. The figure illustrates that the regimen of 20-10-10 mg/kg resulted in the highest values for $C_{trough72h}$ (Supplementary Figure 1) with the highest probability of closure compared to 10-5-5 mg/kg and PNA adjusted dosing[8]. Because the minimum PNA_{start} at which 20-10-10 mg/kg was administered in our population was 4 days, probabilities of closure below this age are extrapolated (dashed lines). Interindividual variability in pharmacokinetics and the resulting variability in $C_{trough72h}$ and predicted probability are is presented in Supplementary Figure 2), and variability in predicted exposure arising from the logistic regression model coefficient variation is presented in Supplementary Figure 3.

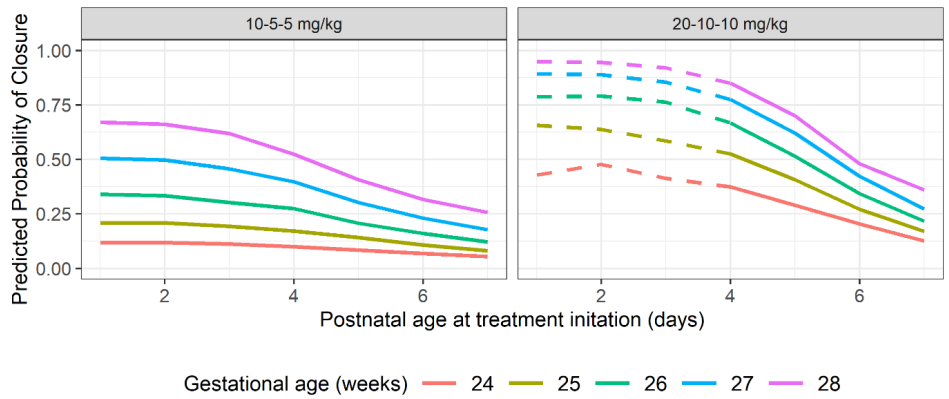


Figure 4. *Probability of closure of the ductus arteriosus versus postnatal age at treatment initiation during the first week of life, predicted by the logistic regression model based on the subset of the dataset with a maximal postnatal age at treatment initiation of 7 days. Dashed lines represent extrapolations of the study population, since the 20- 10- 10 mg/kg regimen was not administered to infants below a PNA of 4 days. PNA, postnatal age.*

Sensitivity analyses on the assumptions on PDA status after 3 doses for infants without an ultrasound, and on the availability of PK samples for a subset of the population are presented in Supplementary Table 1, 2 and 3. Estimated probability and observed closure versus identified predictors by the subgroup analysis are presented in Supplementary Figure 4. Weighted residuals of the subgroup model are presented in Supplementary Figure 5. These analyses show little sensitivity to the assumptions and adequate fit of the data.

DISCUSSION

In this study we included a large cohort of preterm infants with a PDA that was treated with ibuprofen initiated at a wide range of postnatal ages. In the overall population, the odds of closure decrease with PNA_{start} and increase with GA, and lower birthweight for gestational age , and with these predictors no additional influence of ibuprofen exposure could be identified in this population that all received ibuprofen in varying dosing regimens. If treatment is initiated during the first week of life however, concentrations of ibuprofen were predictive for closure where PNA_{start} was not. Importantly, a very low closure rate was observed in the study population, especially after the first week of life. Spontaneous closure is most likely to occur in the first days after birth [7,18], which might explain these results. In our study population, very low exposure to ibuprofen was observed when treatment was initiated beyond the first week of life (Figure 2), even if high ibuprofen dosages were used as suggested by the meta-analysis of Mitra *et al*.[5] Therefore, we cannot exclude that the low closure rates are explained by the low ibuprofen concentrations in these neonates with a PNA beyond the first week of life. Lower effectiveness of pharmacological treatment upon later treatment initiation was also found by Relangi *et al*.[19], however, low-quality evidence suggests no important differences in mortality or PDA ligation upon treatment initiation during or beyond the first week of life[6].

In the first week of life the odds of ductus closure were found to increase with $C_{trough72h}$, and not by PNA_{start}. To our knowledge only one exposure-response study on ibuprofen for the treatment of PDA was performed before, namely by Hirt *et al*.[9] In this study, with a maximal PNA_{start} of 11 days, a target $AUC_{0\text{-}72h}$ of 900 mg*h/L was identified. In our population this target was achieved in more than 50% of the population while the closure rate was much lower (21% vs. 86% observed by Hirt *et al*.[9]). Based on the PK model used to determine ibuprofen exposure, $C_{trough72h}$ was more variable than $AUC_{0\text{-}72h}$, especially at lower $C_{trough72h}$'s, which might explain why $C_{trough72h}$ was found to be more predictive than $AUC_{0\text{-}72h}$ in the subgroup analysis. Better prediction of closure by $C_{trough72h}$ was suggested before[15] and can be explained by a required minimal inhibition of COX.

However, while a dosing regimen of 20-10-10 mg/kg may result in higher levels of $C_{trough72h}$, higher peak levels and the higher risk for toxicity should be considered as well[15]. Especially during the first days of life where the risk of intraventricular haemorrhage is high due to hyporeactivity of platelets[20], additional risks of high ibuprofen doses should be carefully outweighed against expected benefits. In order to achieve higher $C_{trough72h}$ values, twice-daily dosing as previously suggested by Flint *et al*.[15] would be a good alternative. Continuous administration of ibuprofen has also been shown to result in higher efficacy with fewer complications[21]. PNA adapted dosing is necessary to obtain comparable $C_{trough72h}$ for all preterm infants.

As expected, GA was predictive for closure. PDA is most common in the most extreme preterm infants, who unfortunately also might be most vulnerable for the side effects of ibuprofen. It remains a challenge to balance the expected benefit of ibuprofen treatment early in life with increased risk for adverse events. Even though clearance is estimated to increase with GA, similar exposure across the range in GA was observed. At lower gestational ages the ductus is most sensitive for vasodilatory signals and less to vasoconstricting signals[2], which might be translated into a need for higher ibuprofen exposure if GA is low. Based on the available dataset, it was not possible to identify a target $C_{trough72h}$, or to identify whether such a target $C_{trough72h}$ is dependent on GA or PNA_{start}. In order to examine the need for an individualized target, further studies are necessary in which doses are adapted such that $C_{trough72h}$ across the range in PNA_{start}. Safety of higher exposure with potentially increased risks for side-effects including kidney failure and intraventricular haemorrhages should also be carefully evaluated.

Infants with a low $Z_{Birthweight\text{-}for\text{-}GA}$ had an increased probability of ductus closure in our analyses. This is an interesting finding that is in line with a previous study that found a similar increased effect in small for gestational age infants[22]. According to the population PK model used to calculate exposure, small for GA (SGA) infants ($Z_{Birthweight\ -for\text{-}GA} < -2$) had an increased clearance of ibuprofen[8], resulting in lower $C_{trough72h}$ compared to their weight appropriate counterparts (Figure 2). Therefore, counterintuitively the relatively smallest infants are expected to have a higher probability of closure with lower $C_{trough72h}$. Neither the effect of being SGA on ibuprofen clearance, nor the effect of $Z_{Birthweight\text{-}for\text{-}GA}$ on the probability of closure can be explained physiologically and would need further validation to adjust clinical practice to, but both findings can be interpreted as a sign that these infants require further studies, and should be examined as a separate group of patients that differ from AGA neonates.

A strength of this study is the combination of pharmacokinetic and pharmacodynamics analyses in a cohort of patients with a wide range in PNA at ibuprofen treatment initiation. Pharmacokinetics are rapidly changing in the neonatal population, resulting in

up to two-fold differences in $Cmin_{72h}$ and $AUC_{0\text{-}72h}$ between treatment initiation upon the same dose in mg/kg on PNA day 1 and 7. To account for these changes, it is essential to examine exposure, and not just the dose per kg bodyweight. This is illustrated by the fact that the cumulative first 3 dose amounts was not a significant predictor for closure of the PDA in the univariate analysis, but $C_{trough72h}$ was.

A limitation of the available dataset was the observational nature. In our cohort, a selection bias applies as all included patients had a PDA at a relatively late time point in life. The closure rate of the overall population was relatively low compared to other ibuprofen cohorts[9], but also compared to recorded spontaneous closure[7]. Physicians had freedom to deviate from treatment protocols, which make these results a good reflection of real practice, but also dependent on the NICU and corresponding treatment practices, and increased the number of assumptions that had to be made. For example, the status of the PDA was assumed to be open after 3 doses if the ultrasound was postponed. This assumption was deemed appropriate for the majority of affected infants and was therefore preferred over excluding all patients with a postponed ultrasound since these were not selected randomly but based on clinical sings, which therefore would introduce more bias. Additionally, because this study was not placebo-controlled the results should be interpreted with caution. For further optimization of the treatment of PDA, in future studies with ibuprofen both spontaneous closure and changing pharmacokinetics should be considered during the study design. Another limitation was that plasma samples were not available for all patients. Despite the inclusion of covariates on ibuprofen clearance, interindividual variability remained high (40%) in the PK study meaning that true exposure might deviate from the population predicted exposure.[8] Plasma samples for all patients could therefore have resulted in more precise exposure calculations, combined with more knowledge on bioavailability of ibuprofen which was now assumed to be 100%. The assumption that CL did not further increase beyond a postnatal age of 18 days might have resulted in slightly overestimated exposure for infants above PNA_{start} of 18 days. However, due to the limited number of infants and the already very low predicted exposure for these infants we expect little impact of this assumption.

In conclusion, in our cohort of relatively late treated preterm born infants, we showed a very low closure rate, especially after the first week of life. There is a window of opportunity for ductus closure within the first days of life, with a probability of closure that depends on GA, birthweight-for-GA, and ibuprofen exposure. Increased, yet unstudied dosages might be needed to increase the exposure and effect of ibuprofen beyond the first week.

REFERENCES

1 Hamrick SEG, Hansmann G. Patent Ductus Arteriosus of the Preterm Infant. *Pediatrics* 2010;125:1020–30. doi:10.1542/peds.2009-3506

2 Hundscheid T, van den Broek M, van der Lee R, et al. Understanding the pathobiology in patent ductus arteriosus in prematurity—beyond prostaglandins and oxygen. *Pediatr Res* 2019;86:28–38. doi:10.1038/s41390-019-0387-7

3 Ferguson JM. Pharmacotherapy for patent ductus arteriosus closure. *Congenit Heart Dis* 2018;14:52–6. doi:10.1111/chd.12715

4 Sridharan K, Ansari E Al, Mulubwa M, et al. Population pharmacokinetic-pharmacodynamic modelling of acetaminophen in preterm neonates with hemodynamically significant patent ductus arteriosus. *Eur J Pharm Sci* 2021;167:106023. doi:10.1016/j.ejps.2021.106023

5 Mitra S, Florez ID, Tamayo ME, et al. Association of placebo, indomethacin, ibuprofen, and acetaminophen with closure of hemodynamically significant patent ductus arteriosus in preterm infants a systematic review and meta-analysis. *JAMA - J Am Med Assoc* 2018;319:1221–38. doi:10.1001/jama.2018.1896

6 Mitra S, Scrivens A, von Kursell AM, et al. Early treatment versus expectant management of hemodynamically significant patent ductus arteriosus for preterm infants. *Cochrane Database Syst Rev* 2020;2020. doi:10.1002/14651858.CD013278.pub2

7 Klerk JCA De, Engbers AGJ, Beek F Van, et al. Spontaneous Closure of the Ductus Arteriosus in Preterm Infants : A Systematic Review. *Front Pediatr* 2020;8:1–12. doi:10.3389/fped.2020.00541

8 Engbers AGJ, Klerk JCA De, Flint RB, et al. Enantiomer specific pharmacokinetics of ibuprofen in preterm neonates with patent ductus arteriosus. *Br J Clin Pharmacol* 2020;:1–12. doi:10.1111/bcp.14298

9 Hirt D, Van Overmeire B, Treluyer JM, et al. An optimized ibuprofen dosing scheme for preterm neonates with patent ductus arteriosus, based on a population pharmacokinetic and pharmacodynamic study. *Br J Clin Pharmacol* 2008;65:629–36. doi:10.1111/j.1365-2125.2008.03118.x

10 Hoftiezer L, Hof MHP, Dijs-Elsinga J, et al. From population reference to national standard: new and improved birthweight charts. *Am J Obstet Gynecol* 2019;220:383.E1-383.E17. doi:10.1016/j.ajog.2018.12.023

11 Klerk JCA De, Paassen N Van, Beynum IM Van, et al. Ibuprofen treatment after the first days of life in preterm neonates with patent ductus arteriosus. *J Matern Neonatal Med* 2019;0:1–7. doi:10.1080/14767058.2019.1667323

12 Sangtawesin V, Sangtawesin C, Raksasinborisut C, et al. Oral ibuprofen prophylaxis for symptomatic patent ductus arteriosus of prematurity. *J Med Assoc Thail* 2006;89:314–21.

13 Anderson BJ, Hannam JA. A target concentration strategy to determine ibuprofen dosing in children. *Paediatr Anaesth* 2019;29:1107–13. doi:10.1111/pan.13731

14 Prusakiewicz JJ, Duggan KC, Rouzer CA, et al. Differential sensitivity and mechanism of inhibition of COX-2 oxygenation of arachidonic acid and 2-arachidonoylglycerol by ibuprofen and mefenamic acid. *Biochemistry* 2009;48:7353–5. doi:10.1021/bi900999z

15 Flint RB, ter Heine R, Spaans E, et al. Simulation-based suggestions to improve ibuprofen dosing for patent ductus arteriosus in preterm newborns. *Eur J Clin Pharmacol* 2018;74:1585–91. doi:10.1007/s00228-018-2529-y

16 Ohlsson A, Walia R, Shah SS. Ibuprofen for the treatment of patent ductus arteriosus in preterm or low birth weight (or both) infants. *Cochrane Database Syst Rev* 2020;2020. doi:10.1002/14651858.CD003481.pub8

17 Harrell FEJ. *Regression Modeling Strategies*. Second. Springer International Publishing doi:10.1007/978-3-319-19425-7

18 Semberova J, Sirc J, Miletin J, et al. Spontaneous closure of patent ductus arteriosus in infants ≤1500 g. *Pediatrics* 2017;140:e20164258. doi:10.1542/peds.2016-4258

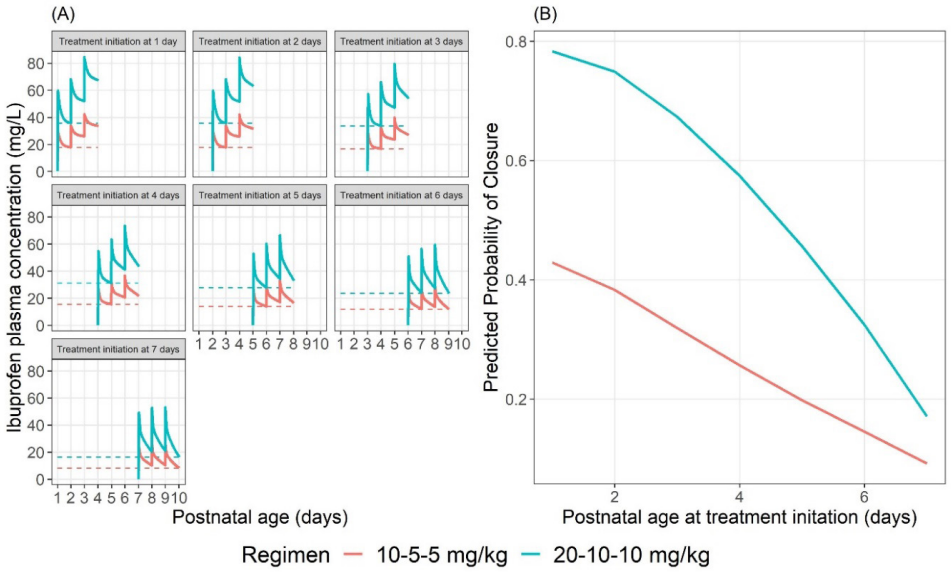
19 Relangi D, Somashekar S, Jain D, et al. Changes in Patent Ductus Arteriosus Treatment Strategy and Respiratory Outcomes in Premature Infants. *J Pediatr* 2021;235:58–62. doi:10.1016/j.jpeds.2021.04.030

20 Bednarek FJ, Bean S, Barnard MR, et al. The platelet hyporeactivity of extremely low birth weight neonates is age-dependent. *Thromb Res* 2009;124:42–5. doi:10.1016/j.thromres.2008.10.004

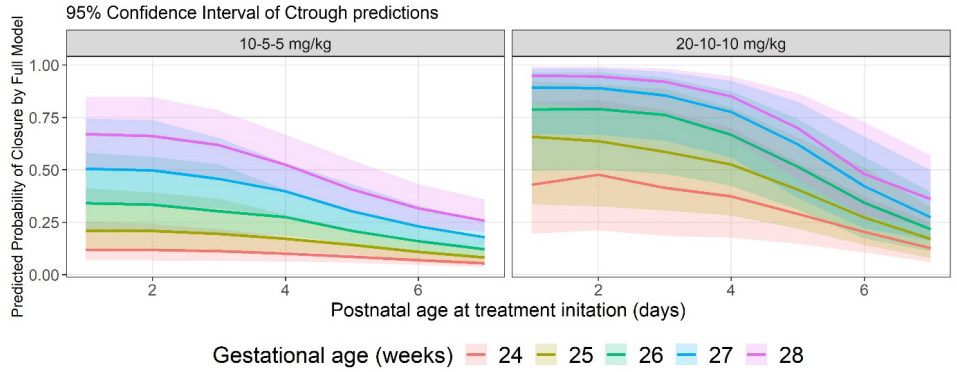
21 Lago P, Salvadori S, Opocher F, et al. Continuous infusion of ibuprofen for treatment of patent ductus arteriosus in very low birth weight infants. *Neonatology* 2013;105:46–54. doi:10.1159/000355679

22 Boghossian NS, Do BT, Bell EF, et al. Efficacy of pharmacologic closure of patent ductus arteriosus in small-for-gestational-age extremely preterm infants. *Early Hum Dev* 2017;113:10–7. doi:10.1016/j.earlhumdev.2017.07.011

SUPPLEMENTAL FILES



Supplementary Figure 1A. *Concentration-time profiles for a male infant with a gestational age of 26 weeks, with varying postnatal ages at treatment initiation (different panels). B: Predicted probability for male infant with a gestational age of 26 weeks and a Z-score for birthweight of 0, with the lowest ibuprofen trough concentration ($C_{trough0\text{-}72h}$) from the respective panels in A (dashed lines).*



Supplementary Figure 2. *Probability of closure of the ductus arteriosus versus postnatal age at treatment initiation during the first week of life, predicted by the logistic regression model based on the subset of the dataset with a maximal postnatal age at treatment initiation of 7 days. 1000 hypothetical preterm infants were simulated of which the median (solid lines) and the 95% confidence intervals (shaded area) of minimal trough concentration were calculated and used for the predicted probability.*

Supplementary Figure 3. *Probability of closure of the ductus arteriosus versus postnatal age at treatment initiation during the first week of life, predicted by the logistic regression model based on the subset of the dataset with a maximal postnatal age at treatment initiation of 7 days. Shaded areas represent the 95% confidence interval of the coefficient of the effect of minimal trough concentration.*

Supplementary Table 1. *Sensitivity analysis on the assumption that for infants without an ultrasound made after 3 doses the ductus was not closed at that time point. Of the 114 patients without an ultrasound after 3 doses, 11 patients were later confirmed to have a closed ductus. The final logistic regression model was repeated on the complete study population as presented in the manuscript (left) and on a subset of the complete study population without the 11 patients who were confirmed to have a closed ductus after more than 4 doses of ibuprofen (right).*

	Complete Study Population			Subset without 11 patients without ultrasound after 3 doses but later confirmed closure		
Characteristic	**log(OR)[1]**	**95% CI[1]**	**p-value**	**log(OR)[1]**	**95% CI[1]**	**p-value**
Gestational Age (weeks)	0.38	0.12, 0.66	0.006	0.37	0.10, 0.65	0.008
Postnatal age of treatment initiation (days)	-0.30	-0.45, -0.18	<0.001	-0.29	-0.44, -0.17	<0.001
Z-score for birthweight	-0.23	-0.46, 0.00	0.049	-0.24	-0.48, -0.02	0.037

[1]OR = Odds Ratio, CI = Confidence Interval

Supplementary Table 2. *Sensitivity analysis on the assumption that for infants without an ultrasound made after 3 doses the ductus was not closed at that time point. Of the 114 patients without an ultrasound after 3 doses, 11 patients were later confirmed to have a closed ductus. The final logistic regression model was repeated on the subset of the study population with a maximal postnatal age (PNA) of 7 days as presented in the manuscript (left) and on a subset of the complete study population without the 11 patients who were confirmed to have a closed ductus after more than 4 doses of ibuprofen (right).*

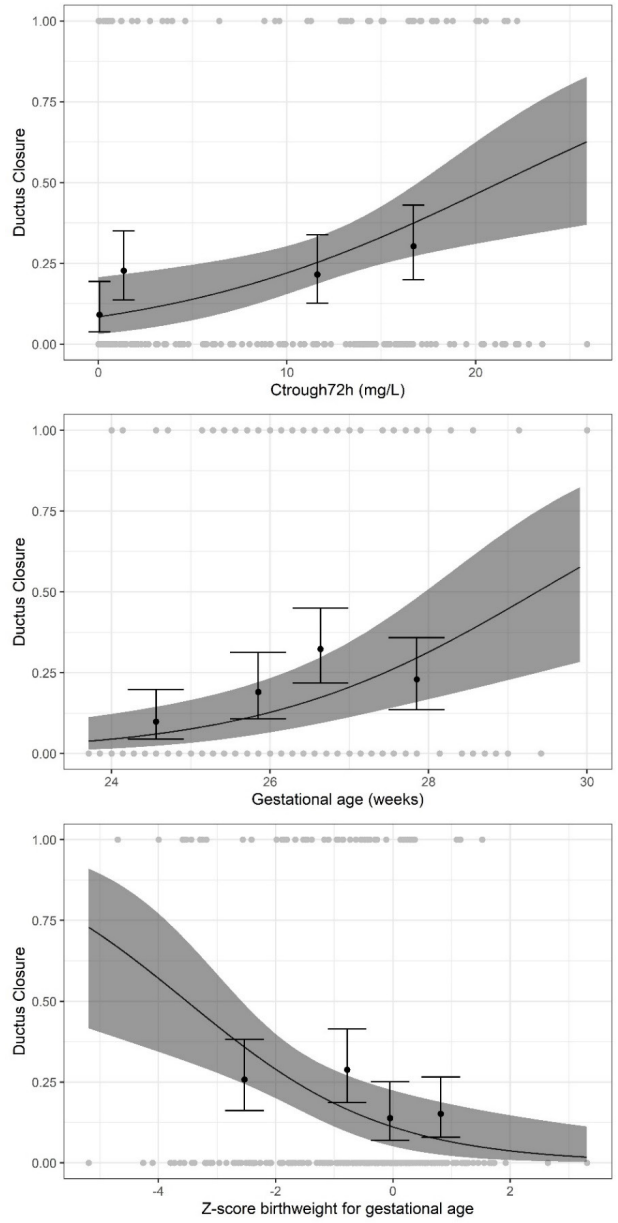
	Complete Study Subgroup max. PNA 7 days			Subset without 11 patients without ultrasound after 3 doses but later confirmed closure Subgroup max. PNA 7 days		
Characteristic	**log(OR)[1]**	**95% CI[1]**	**p-value**	**log(OR)[1]**	**95% CI[1]**	**p-value**
Z-score for birthweight	-0.59	-1.0, -0.23	0.002	-0.58	-1.0, -0.23	0.002
Ctrough72h (mg/L)	0.11	0.04, 0.19	0.004	0.11	0.03, 0.18	0.006
Gestational Age (weeks)	0.57	0.27, 0.90	<0.001	0.57	0.26, 0.90	<0.001

[1]OR = Odds Ratio, CI = Confidence Interval

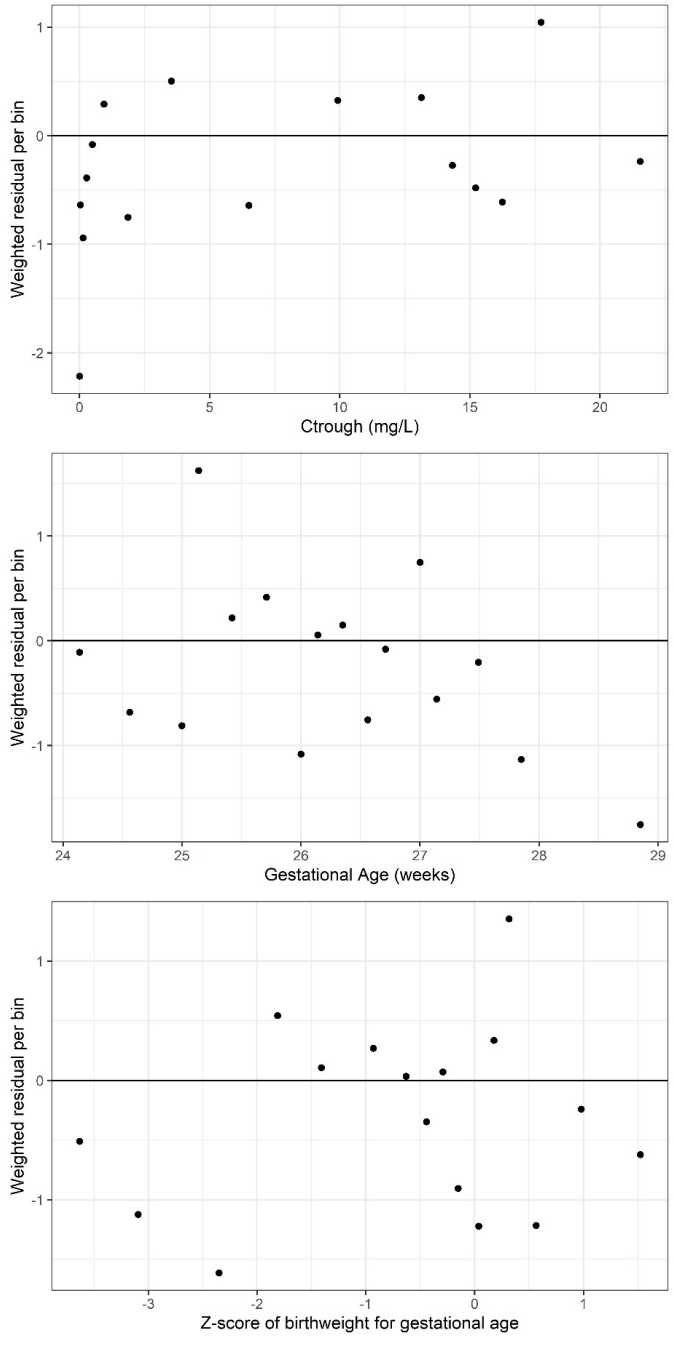
Supplementary Table 3. *Sensitivity analysis on the availability of pharmacokinetic (PK) samples for a subset of the population (n=43). The final logistic regression model was repeated on the dataset as presented in the manuscript, using individual predicted concentrations for patients with a PK sample and population predicted concentrations for patients without PK samples (left). For the right section population predicted concentrations were used for all patients, independent of the availability of a PK sample.*

	Individual Predicted Ctrough72h if PK sample available			Population Predicted Ctrough72h for all patients		
Characteristic	**log(OR)[1]**	**95% CI[1]**	**p-value**	**log(OR)[1]**	**95% CI[1]**	**p-value**
Gestational Age (weeks)	0.38	0.12, 0.66	0.006	0.38	0.12, 0.66	0.006
Postnatal age of treatment initiation (days)	-0.30	-0.45, -0.18	<0.001	-0.30	-0.45, -0.17	<0.001
Z-score for birthweight	-0.23	-0.46, 0.00	0.049	-0.23	-0.46, 0.00	0.048

[1]OR = Odds Ratio, CI = Confidence Interval



Supplementary Figure 4. *Figure 2 Predicted probability of ductus closure (black line) with 95% confidence interval based on minimal ibuprofen trough concentration during the first 3 days of treatment (top), gestational age (middle) and Z-score of birthweight for gestational age (bottom). Grey dots represent individual observed outcomes with 0 for no observed ductus closure and 1 for observed ductus closure. Black dots represent the average outcome in each quartile with error bars representing the 95% confidence intervals of the observed probability.*



Supplementary Figure 5. *Weighted residuals of the subgroup model comparing the observed and expect closure of the ductus arteriosus in each of 16 bins. Weighted residuals were calculated as the difference between the observed and predicted closure per bin, divided by the square root of the variance, defined as ∑predicted probability of closure∗(1-predicted probability of closure).*

5