Pharmacokinetics of penicillin G in preterm and term neonates

Helgi Padari[1], Tuuli Metsvaht[1], Eva Germovsek[2], Charlotte I Barker[2,3], Karin Kipper[3,4], Koit Herodes[4], Joseph F Standing[2], Kersti Oselin[5], Tõnis Tasa[6], Hiie Soeorg[7]#, Irja Lutsar[7]

[1]Pediatric Intensive Care Unit, Tartu University Hospital, Tartu, Estonia

[2]UCL Great Ormond Street Institute of Child Health, University College London, London, UK

[3]Paediatric Infectious Diseases Research Group, Institute for Infection and Immunity, St George's University of London, London, UK

[4]Institute of Chemistry, University of Tartu, Estonia

[5]Clinic of Haematology and Oncology, North Estonia Medical Centre, Tallinn, Estonia

[6]Institute of Computer Science, University of Tartu, Estonia

[7]Department of Microbiology, University of Tartu, Tartu, Estonia

#**Corresponding author**: Hiie Soeorg; e-mail address: hiie.soeorg@ut.ee

Running title: Pharmacokinetics of penicillin G in neonates

Abstract

Group B streptococci are common causative agents of early-onset neonatal sepsis (EOS). Pharmacokinetic (PK) data for penicillin G have been described for extremely preterm neonates but poorly for late-preterm and term neonates. Thus, evidence-based dosing recommendations are lacking. We described PK of penicillin G in neonates with gestational age (GA) ≥32 weeks and postnatal age <72 h. Penicillin G was administered intravenously at a dose of 25,000 or 50,000 IU/kg/q12h. At steady state, PK blood samples were collected prior to and at 5 min, 1 h, 3 h, 8 h, 12 h after injection. Non-compartmental PK analysis was performed with WinNonlin. In combination with data from neonates with GA ≤28 weeks we developed a population PK model using NONMEM software and performed probability of target attainment (PTA) simulations. In total, 16 neonates with GA ≥32 weeks were included in non-compartmental analysis. The median (interquartile range) volume of distribution (VD) was 0.50 (0.42-0.57) L/kg, clearance (CL) 0.21 (0.16-0.29) L/h and half-life 3.6 (3.2-4.3) h. In population PK analysis that included 35 neonates, a two-compartment model best described the data. The final parameter estimates were 10.3 L/70kg and 29.8 L/70kg for VD of the central and peripheral compartment, respectively, and 13.2 L/h/70kg for CL. Considering fraction of unbound penicillin G of 40%, PTA of time when the unbound drug exceeds MIC of 40% was >90% for MICs ≤2 mg/L with doses of 25,000 IU/kg/q12h. In neonates, regardless of GA, PK parameters of penicillin G are similar. The dose of 25,000 IU/kg/q12h is suggested for treatment of group B streptococcal EOS diagnosed within the first 72 hours of life.

Introduction

Group B streptococci (GBS) are the most common causative agent of early-onset sepsis (EOS) in neonates (1, 2). Furthermore, the incidence of EOS caused by GBS is increasing despite the implementation of intrapartum antibacterial prophylaxis (3, 4). GBS has remained universally susceptible to penicillin G with the minimum inhibitory concentration (MIC) that inhibits 90% of isolates (MIC_{90}) being 0.06 mg/L (5). Guidelines recommend penicillin G in combination with an aminoglycoside for empiric antibacterial treatment of EOS (6). Although some units use ampicillin instead of penicillin G (1), penicillin G could be preferable due to its narrow antibacterial spectrum. The use of penicillin G is also supported by its equivalence to ampicillin-containing regimens (1, 7).

Penicillin G is one of the most frequently prescribed antibiotics in neonatal intensive care units in Europe, but the administered doses vary nearly fifteen-fold (8). The variations arise probably in part due to insufficient pharmacokinetic (PK) data and consequently few evidence-based dosing recommendations for very preterm neonates (9, 10), known to be at highest risk of development of EOS (1, 2). In neonates with a gestational age (GA) ≤28 weeks and <32 weeks, the doses of 25,000 IU/kg and 50,000 IU/kg, respectively, twice a day have been suggested for empiric treatment of EOS in previous PK studies (9, 10). Although the majority of EOS cases occur in term neonates (2), PK of penicillin G has been described in only few term neonates and no dosing recommendations were made (11). As penicillin G is primarily eliminated by kidneys and renal function is reduced in neonates with smaller GA (12), we hypothesized that doses needed to achieve sufficient serum concentrations could be higher in late-preterm and term compared with very preterm neonates, similar to other beta-lactams, for example ampicillin (13).

63 In adults, penicillin G is considered to achieve sufficient efficacy if time when the unbound
64 drug exceeds MIC (fT>MIC) is at least 40% of the dosing interval (14). However, dosing
65 regimens that provide continuous concentrations above MIC are potentially more effective
66 (15) and target fT>MIC of 100% has been recommended for immunocompromised patients,
67 including neonates (16). A recent study, however, demonstrated that in neonates the ratio of
68 the 24-hour area under the unbound drug concentration-time curve to the MIC (fAUC/MIC)
69 was better correlated with bactericidal effect than fT>MIC (17), so fAUC/MIC >100 was
70 proposed as a target (17).
71
72 Therefore, first, we aimed to describe the PK of intravenously administered penicillin G in
73 neonates with GA ≥32 weeks requiring treatment for confirmed or suspected EOS. Second, in
74 combination with the PK data from our previous study on neonates with GA ≤28 weeks (9),
75 we aimed to develop a population PK (popPK) model to define an evidence-based dosing
76 regimen for neonates.
77
78 **Methods**
79 **Study patients**. A prospective study was carried out from December 21, 2012 to November
80 24, 2013 in the tertiary pediatric intensive care unit of Tartu University Hospital. Neonates
81 with GA of ≥32 weeks were eligible if they (i) required penicillin G for treatment of
82 suspected or confirmed EOS or pneumonia with clinical and laboratory criteria described
83 elsewhere (18) and (ii) had an arterial or central venous catheter inserted for clinical
84 indications. Neonates who were likely to be infected with microorganisms resistant to
85 penicillin G or participated in any other study (apart from observational studies involving only
86 data registration) were excluded. The neonates were stratified into two groups based on GA
87 (32≤ to <35 weeks and ≥35 weeks).

88 **Study drug administration**. Penicillin G (Sandoz GmbH, Kundl, Austria) was reconstituted
89 in 0.9% sodium chloride to a final concentration of 60 mg/mL no more than 10 minutes prior
90 to administration. A dose of 25,000 IU (15 mg)/kg or 50,000 IU (30 mg)/kg, chosen by the
91 treating physician (50,000 IU/kg if meningitis was suspected, i.e. disturbances of
92 consciousness, lethargy, worsening apnoea, seizures or suspicion of seizures, bulging
93 fontanelle), was based on the current body weight and administered every 12 hours as a 3-
94 minute infusion into a central or peripheral venous catheter.
95 **Sampling and sample handling**. PK samples were collected at steady state (after at least 36
96 h of therapy), mostly after the fifth dose of penicillin G. Blood was drawn from the arterial or
97 central venous catheter prior to and at 5 min, 1 h, 3 h, 8 h and 12 h after the dose. As
98 penicillin G is stable at room temperature for at least 1 hour (19), samples were immediately
99 centrifuged at 3,500 rpm for 10 minutes and thereafter frozen at -20^{o} C and transferred to -80^{o}
100 C within 24 h. The samples were stored at -80^{o} C for maximum of 12 months during which
101 penicillin G remains stable (19, 20) until concentrations were measured.
102 **Penicillin G assay.** Samples were thawed at room temperature. For protein precipitation, 50
103 µL of serum was mixed with 50 µL of acetonitrile containing piperacillin as an internal
104 standard (I.S.) at a concentration of 10 µg/mL. Supernatant obtained after centrifugation of
105 serum were filtered and transferred into the autosampler vials.
106 From each prepared sample 3 µL was injected into an Agilent 1290 Infinity UHPLC system.
107 Gradient elution with methanol and 5 mM 1,1,1,3,3,3-hexafluoro-2-methyl-2-propanol in
108 water (pH adjusted to 10.5 using ammonium hydroxide) at a flow rate of 0.3 mL/min was
109 used for chromatographic separation on Waters Acquity UPLC BEH C18 column (2.1 × 100
110 mm, 1.7 µm) with pre-column. For detection Agilent Series 1100 LC/MSD Trap XCT was
111 used with electrospray interface in positive mode using multiple reaction monitoring.

Transitions of m/z 335 $[M+H]^+$ to m/z 160, 176 and m/z 518 $[M+H]^+$ to m/z 143, 160 were used for the quantification and qualification of penicillin G and I.S., respectively.

A matrix matched calibration was used for validation of the described methodology according to the European Medicines Agency guidelines (21). The calibration curves were linear in concentration range 0.15-150 µg/mL in serum and had $r^2 > 0.9996$. The limit of quantification (LoQ) for serum samples was 0.147 µg/mL and the limit of detection was 0.05 µg/mL. The within-day accuracy ranged from 2% to 9% for the serum calibration curve. The between-day precision for serum samples was <6%.

Monitoring of study patients. Vital parameters were continuously monitored and recorded at screening, immediately prior to PK sampling and within 72 hours after completion of the penicillin G treatment. All concomitant medications, respiratory support, vasoactive treatment and laboratory parameters from blood samples drawn for clinical indications were also recorded. Serum creatinine was measured by the compensated Jaffe kinetic method standardized against isotope dilution mass spectrometry.

PK analyses. Non-compartmental analysis (NCA) of concentration-time data was performed in Phoenix WinNonlin software (version 6.5.1; Pharsight Corporation, CA, USA). The area under the concentration-time curve over the dosing interval of 0 to 12 h (AUC_{0-12}) was calculated by use of the log-linear trapezoidal rule. The AUC_{0-12} was used to calculate the total body clearance. The apparent volume of distribution (VD) was determined by calculating the mean residence time extrapolated to infinity.

PopPK analysis was performed in NONMEM software (version 7.3; ICON plc, Dublin, Ireland). Concentration-time data from the current and our previous study (9) were pooled and analyzed simultaneously. One-, two- and three-compartment structural models were compared in which, due to the *a priori* assumption of the dependence of renal maturation on postmenstrual age (PMA), clearance was scaled as recommended by Germovsek *et al*. (22),

by adding allometric weight scaling and a sigmoid renal maturation model that includes PMA (23). After choosing the model that provided the best fit, the influence of the following covariates on clearance and VD was tested: birth weight (BW), GA, serum creatinine concentration and need for continuous positive airway pressure or mechanical ventilation. A covariate was retained in the model if it caused a significant decrease in the objective function value, corresponding to $p<0.01$.

Probability of target attainment (PTA). The final popPK model was used in 5000-patient Monte Carlo simulation generating concentration-time curves at steady state for penicillin G doses of 25,000, 50,000 and 100,000 IU/kg administered at 12-hour intervals as a 3-min infusion if protein binding was not considered and with the fraction of unbound penicillin G of 40% according to penicillin binding data from adult studies (24). PMA were simulated by sampling from a uniform distribution (range: 24-42 weeks), and the corresponding body weights were obtained using the model by Sumpter & Holford (25). Pharmacodynamic targets fT>MIC and fAUC/MIC ratio were calculated for MIC values 0.006, 0.125, 0.25, 0.5, 1 and 2 mg/L applicable for GBS and enterococci (26). PTA was calculated for fT>MIC of 40% and 100% and fAUC/MIC >100. All simulations were performed in R software (version 3.2.2; The R Foundation, Vienna, Austria).

The protocol was approved by the Ethics Committee of the University of Tartu. A parent signed an informed consent prior to the inclusion of neonate in the study. The study was registered with the EU Clinical Trials Register (EudraCT Number: 2012-002836-97).

Results

Patients. For the current study, a total of 25 neonates with GA ≥32 weeks were screened, of whom 17 were enrolled. Reasons for exclusion were lack of informed consent (n=3), absence of arterial or central venous catheter (n=3), participation in another study (n=1) and change in

antimicrobial therapy (n=1). The demographic and clinical characteristics are shown in Table 1. Penicillin G was administered for treatment of EOS (n=4), congenital pneumonia (n=1), other/suspected congenital infection (n=9) and meconium aspiration syndrome (n=3). All patients received concomitant therapy with gentamicin (4 mg/kg/q24h), but none received other potentially nephrotoxic drugs on the PK sampling day. None of the neonates had a positive blood culture.

Non-compartmental PK analysis. NCA was performed on data from 16 patients. One neonate with GA >35 weeks was excluded due to insufficient number of PK samples (n=2). The median values of the CL, VD and half-life were similar regardless of GA (largest relative difference in VD – mean 0.54 L/kg and 0.46 L/kg (p=0.25) in neonates with GA 32-34 weeks and ≥35 weeks, respectively) and thus the values of the PK parameters are presented only based on the dose of penicillin G (Table 2). As expected, the dose of 50,000 IU/kg resulted in higher values of C_{max}, C_{min} and fAUC than 25,000 IU/kg (Table 2).

PopPK analysis. In total 35 neonates (17 from the current study and 18 from the previous study (9)) were included in the popPK analysis. A two-compartment model with allometric weight scaling and a renal maturation function provided the best fit to the concentration-time data. None of the covariates tested significantly improved the model fit and were thus not retained in the final model. The PK parameter estimates of the final model are shown in Table 3. Parameters for median values for the population used in the popPK modelling (current weight 1.28 kg, PMA 32.3 weeks) were as follows: clearance 0.15 L/h, central VD 0.19 L, intercompartmental clearance 2.76 L/h, peripheral VD 0.54 L.

Overall, goodness-of-fit plots (Figure 1) and the visual predictive check (Figure 2) showed good prediction of data by the model.

PTA analysis. The PTA for fT>MIC of 40% was >90% for all tested MIC values and doses of 25,000, 50,000 and 100,000 IU/kg if protein binding was not considered and if the fraction of unbound penicillin G was 40% (data not shown).

The PTA of fT>MIC of 100% was >90% for all doses for MIC values of ≤0.5 mg/L only if protein binding was not considered (Figure 3A). If the fraction of unbound penicillin G was 40%, the same target was achieved with all doses only if MIC was ≤0.125 mg/L (Figure 3B). If protein binding was not considered, the PTA of fAUC/MIC >100 was >90% for all tested MIC values with doses of 50,000 and 100,000 IU/kg and remained >90% with dose of 25,000 IU/kg for MIC values ≤1 mg/L (Figure 4A). If the fraction of unbound penicillin G was 40%, the PTA >90% was achieved with doses 25,000, 50,000 and 100,000 IU/kg only if MIC was ≤0.5, ≤1 and ≤2 mg/L, respectively (Figure 4B).

Discussion

This study reports, to our best knowledge, the largest neonatal penicillin G population PK analysis to date. We demonstrated that in neonates PK parameters of intravenously administered penicillin G during the first week of life are similar regardless of GA. According to popPK model the dose of 25,000 IU/kg every 12 hours could be suggested for treatment of EOS caused by GBS regardless of pharmacodynamic target (fT>MIC 40%, fT>MIC 100% or fAUC/MIC >100) and the GA (ranging from 24 to 40 weeks).

Contrary to our hypothesis, the values of half-life and volume of distribution of penicillin G in late-preterm and term neonates were comparable to those in very and extremely preterm neonates that vary in the ranges of 3.8-4.6 h and 0.41-0.64 L/kg, respectively (9, 10). This corroborates previous findings that half-life of penicillin G in serum in neonates does not depend on BW or GA (10, 27). Similarity in VD could result in part from relatively larger

weight loss after birth in more premature neonates compared with more mature ones (28) and several factors could contribute to similarity in clearance throughout neonatal period. First, tubular secretion that is the main elimination mechanism of penicillin G in adults (29) is equally reduced in preterm and term neonates as a result of decreased renal blood flow to peritubular areas (30). Second, glomerular filtration has been suggested to be relatively more important than tubular secretion in elimination of penicillin G in neonates (27). Although clearance depends on GA (31), the difference between preterm and term neonates in glomerular filtration rate is less pronounced within the first days of life, increasing only by 0.0205 mL/min/kg per each week of postconceptional age (32, 33). Finally, the fraction of beta-lactams bound to proteins is reduced in more premature neonates that may also contribute to higher clearance (34). Notably, for other beta-lactams, such as doripenem and cefepime, clearance was similar regardless of GA within the first week of life (35, 36). Even for amikacin that is almost entirely eliminated by glomerular filtration the difference in clearance was only slightly higher in the first postnatal day in neonates with larger BW compared with those with smaller BW (37). However, contribution of elimination mechanisms other than kidneys cannot be excluded in neonates, as the fraction of penicillin G dose excreted into urine is considerably lower in neonates (26-37%) (9, 27), compared to adults (58%) (29).

We found that a two-compartment model described data best. This is in agreement with the only published study describing population pharmacokinetics of penicillin G in neonates conducted by Muller *et al*. (10), who analyzed data from neonates with GA <32 weeks. However, while in the model by Muller *et al*., GA was not included in the final model (possibly due to the small range of GA), in our study GA was included indirectly, i.e. incorporated in the PMA-dependent renal maturation function. The use of PMA rather than

GA is supported by a recent comparison of models for scaling clearance in children by accounting for size and maturation (22).

Our study showed that in neonates, regardless of GA, the target of fT>MIC of 40% was achieved with PTA >90% when the fraction of unbound penicillin G of 40% was assumed using a dose of 25,000 IU/kg twice a day for all MIC values tested (up to 2 mg/L). This dose is within the range recommended by Neofax (15-30 mg/kg every 12 hours) (38), but is less than that suggested by the British National Formulary for Children (25 mg/kg every 12 hours) (39). Still, according to evidence statements in NICE Clinical Guidelines, a dose of 25,000 IU/kg is effective in preterm neonates, although no evidence was identified for dosing in term neonates (6). Although the previous popPK study of penicillin G in neonates with GA <32 weeks suggested dosing regimen of 50,000 IU/kg twice daily (10), our proposed dosing regimen proposed should be adequate for treatment of EOS, due to several reasons. a) GBS is susceptible to penicillin G with MIC_{90} as low as 0.06 mg/L (5) and viridans-group streptococci that may cause up to 19% of EOS cases (2) have MIC_{90} 0.5 mg/L (40). b) Although we did not measure the fraction of unbound penicillin G and no prior data are available in neonates within the first days of life, the fraction unbound is known to be reduced immediately after birth compared with adults (24). Thus, the unbound drug fraction of 40% that is based on values in adults (24) should be a conservative estimate and the actual unbound concentrations in neonates are most likely higher than estimated in this study. c) While penicillin G bactericidal activity requires fT>MIC 38% in adults, the same study showed that in neonates fT>MIC 32% was bactericidal (17). Therefore, for neonates with immature immune systems the somewhat higher target of fT>MIC of 40% should be appropriate (42, 43). d) Even the target of fT>MIC as high as 100% that is more likely associated with clinical cure (15, 16) was achieved with PTA >90% for MIC values ≤0.125

mg/L and with PTA approximately 80% for MIC values ≤0.5 mg/L with the dose of 25,000 IU/kg twice daily. Moreover, PTA of fAUC/MIC >100 that was shown to be better correlated with bactericidal activity in neonates (17) remained >90% for MIC values ≤0.5 mg/L. Therefore, 25,000 IU/kg should be appropriate and avoids unnecessarily high doses of penicillin G that may counteract treatment by evoking the so-called Eagle effect, which results in a reduced killing rate of GBS by penicillin G concentrations above the optimal level (44). Moreover, excessively high doses of penicillin G may cause toxicity including encephalopathy (46) or coagulation disorders (47). The dose of 25,000 IU/kg was well tolerated in a clinical study that included 142 neonates with suspected EOS (7) and no drug-related adverse events were observed in our study.

Some limitations of the study should be noted. First, we cannot exclude the effect of unrecorded clinical characteristics on the PK parameter estimates. For example, in our previous study all except one mother of neonates with GA of ≤28 weeks received steroid prophylaxis before birth, but betamethasone increases glomerular filtration rate (48). However, the small number of neonates studied did not allow analysis of this covariate (49). Moreover, covariates other than those reflecting size, age and renal function are only occasionally incorporated in the final models describing PK of primarily renally eliminated antibiotics (50). Second, although clearance depends on renal function in addition to growth and maturation (50), a covariate reflecting renal function was not included in our model. However, the lack of effect of creatinine on the model fit was expected, as in the first days of life neonatal serum creatinine values reflect maternal concentrations (51) and less than half of models describing PK of primarily renally eliminated antibiotics incorporate serum creatinine (50). Finally, we did not measure penicillin G concentrations in cerebrospinal fluid, which could also be considered given that concomitant meningitis occurs in 2-6% of EOS cases (2,

52), whereas in culture-positive cases the proportion is as high as 26% (52). Although 25,000 IU/kg twice a day has been suggested to be adequate (9), the PK of penicillin G in cerebrospinal fluid warrants further studies to provide evidence for dosing regimens for meningitis.

In conclusion, our results show that the current dosing regimen of 25,000 IU/kg every 12h for EOS results in sufficient serum concentrations of penicillin G. The dosing regimen is appropriate against GBS as the commonest causative agents of EOS regardless of pharmacodynamic target (fT>MIC 40% or 100% or fAUC/MIC >100) and GA due to the similarity of PK parameters of penicillin G within the first days of life in preterm and term neonates.

Acknowledgements

This study was supported by Archimedes Foundation (Project No. 3.2.1001.11-0032). EG was supported by an IMPACT PhD studentship from University College London (UCL), and received funding from the NeoMero study, part of the European Union Seventh Framework Programme for research, technological development and demonstration (Grant Agreement number 242146), and also from Action Medical Research (grant code SP4650, GN1834). CIB was funded as a Clinical Research Fellow by the Global Research in Paediatrics Network of Excellence (GRiP), part of the European Union's Seventh Framework Programme for research, technological development and demonstration (FP7/2007–2013, Grant Agreement number 261060). JFS has received funding from United Kingdom Medical Research Council Fellowships (grants G1002305 and M008665). EG, CIB and JFS have been supported by the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London.

References

1. Fjalstad JW, Stensvold HJ, Bergseng H, Simonsen GS, Salvesen B, Rønnestad AE, Klingenberg C. 2016. Early-onset Sepsis and Antibiotic Exposure in Term Infants: A Nationwide Population-based Study in Norway. Pediatr Infect Dis J 35:1-6.
2. Schrag SJ, Farley MM, Petit S, Reingold A, Weston EJ, Pondo T, Hudson Jain J, Lynfield R. 2016. Epidemiology of Invasive Early-Onset Neonatal Sepsis, 2005 to 2014. Pediatrics 138:e20162013.
3. Lamagni TL, Keshishian C, Efstratiou A, Guy R, Henderson KL, Broughton K, Sheridan E. 2013. Emerging trends in the epidemiology of invasive group B streptococcal disease in England and Wales, 1991-2010. Clin Infect Dis 57:682-8.
4. Bekker V, Bijlsma MW, van de Beek D, Kuijpers TW, van der Ende A. 2014. Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study. Lancet Infect Dis 14:1083-1089.
5. Karlowsky JA, Adam HJ, Baxter MR, Lagacé-Wiens PR, Walkty AJ, Hoban DJ, Zhanel GG. 2013. In vitro activity of ceftaroline-avibactam against gram-negative and gram-positive pathogens isolated from patients in Canadian hospitals from 2010 to 2012: results from the CANWARD surveillance study. Antimicrob Agents Chemother 57:5600-11.
6. National Collaborating Centre for Women's and Children's Health. 2012. Antibiotics for Early-onset Neonatal Infection: Antibiotics for the Prevention and Treatment of Early-onset Neonatal Infection. RCOG Press at the Royal College of Obstetricians and Gynaecologists, London.

7. Metsvaht T, Ilmoja ML, Parm U, Maipuu L, Merila M, Lutsar I. 2010. Comparison of ampicillin plus gentamicin vs. penicillin plus gentamicin in empiric treatment of neonates at risk of early onset sepsis. Acta Paediatr 99:665-72.

8. Metsvaht T, Nellis G, Varendi H, Nunn AJ, Graham S, Rieutord A, Storme T, McElnay J, Mulla H, Turner MA, Lutsar I. 2015. High variability in the dosing of commonly used antibiotics revealed by a Europe-wide point prevalence study: implications for research and dissemination. BMC Pediatr 15:41.

9. Metsvaht T, Oselin K, Ilmoja ML, Anier K, Lutsar I. 2007. Pharmacokinetics of penicillin g in very-low-birth-weight neonates. Antimicrob Agents Chemother 51:1995-2000.

10. Muller AE, DeJongh J, Bult Y, Goessens WH, Mouton JW, Danhof M, van den Anker JN. 2007. Pharmacokinetics of penicillin G in infants with a gestational age of less than 32 weeks. Antimicrob Agents Chemother 51:3720-5.

11. Mulhall A. 1985. Antibiotic treatment of neonates--does route of administration matter? Dev Pharmacol Ther 8:1-8.

12. Schreuder MF, Bueters RR, Allegaert K. 2014. The interplay between drugs and the kidney in premature neonates. Pediatr Nephrol 29:2083-91.

13. Tremoulet A, Le J, Poindexter B, Sullivan JE, Laughon M, Delmore P, Salgado A, Ian-U Chong S, Melloni C, Gao J, Benjamin DK, Capparelli EV, Cohen-Wolkowiez M, Administrative Core Committee of the Best Pharmaceuticals for Children Act-Pediatric Trials Network. 2014. Characterization of the population pharmacokinetics of ampicillin in neonates using an opportunistic study design. Antimicrob Agents Chemother 58:3013-20.

14. Craig WA. 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis 26:1-10; quiz 11-2.

15. Eagle H, Fleischman R, Musselman AD. 1950. Effect of schedule of administration on the therapeutic efficacy of penicillin; importance of the aggregate time penicillin remains at effectively bactericidal levels. Am J Med 9:280-99.

16. McKinnon PS, Paladino JA, Schentag JJ. 2008. Evaluation of area under the inhibitory curve (AUIC) and time above the minimum inhibitory concentration (T>MIC) as predictors of outcome for cefepime and ceftazidime in serious bacterial infections. Int J Antimicrob Agents 31:345-51.

17. Nielsen EI, Cars O, Friberg LE. 2011. Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: a step toward model-based dose optimization. Antimicrob Agents Chemother 55:4619-30.

18. European Medicines Agency. 2010. Report on the Expert Meeting on Neonatal and Paediatric Sepsis, London.

19. Kipper K, Barker CIS, Standing JF, Sharland M, Johnston A. 2017. Development of a novel multi-penicillin assay and assessment of the impact of analyte degradation: lessons for scavenged sampling in antimicrobial pharmacokinetic study design. Antimicrob Agents Chemother.

20. Shelver WL, Chakrabarty S, Smith DJ. 2017. Comparison of Lateral Flow Assay, Kidney Inhibition Swab, and Liquid Chromatography-Tandem Mass Spectrometry for the Detection of Penicillin G Residues in Sow Urine. J Agric Food Chem 65:1778-1783.

21. European Medicines Agency. 2011. Guideline on bioanalytical method validation.

22. Germovsek E, Barker CI, Sharland M, Standing JF. 2017. Scaling clearance in paediatric pharmacokinetics: All models are wrong, which are useful? Br J Clin Pharmacol 83:777-790.

23. Rhodin MM, Anderson BJ, Peters AM, Coulthard MG, Wilkins B, Cole M, Chatelut E, Grubb A, Veal GJ, Keir MJ, Holford NH. 2009. Human renal function maturation: a quantitative description using weight and postmenstrual age. Pediatr Nephrol 24:67-76.

24. Ehrnebo M, Agurell S, Jalling B, Boréus LO. 1971. Age differences in drug binding by plasma proteins: studies on human foetuses, neonates and adults. Eur J Clin Pharmacol 3:189-93.

25. Sumpter AL, Holford NH. 2011. Predicting weight using postmenstrual age--neonates to adults. Paediatr Anaesth 21:309-15.

26. European Committee on Antimicrobial Susceptibility Testing. 2014. Breakpoint tables for interpretation of MICs and zone diameters.

27. McCracken GH, Ginsberg C, Chrane DF, Thomas ML, Horton LJ. 1973. Clinical pharmacology of penicillin in newborn infants. J Pediatr 82:692-8.

28. Anchieta LM, Xavier CC, Colosimo EA, Souza MF. 2003. Weight of preterm newborns during the first twelve weeks of life. Braz J Med Biol Res 36:761-70.

29. Rammelkamp CH, Keefer CS. 1943. The absorption, excretion, and distribution of penicillin. J Clin Invest 22:425-37.

30. Koren G. 1997. Therapeutic drug monitoring principles in the neonate. National Academy of CLinical Biochemistry. Clin Chem 43:222-7.

31. Bueva A, Guignard JP. 1994. Renal function in preterm neonates. Pediatr Res 36:572-7.

32. Aperia A, Broberger O, Elinder G, Herin P, Zetterström R. 1981. Postnatal development of renal function in pre-term and full-term infants. Acta Paediatr Scand 70:183-7.

33. Wilkins BH. 1992. Renal function in sick very low birthweight infants: 1. Glomerular filtration rate. Arch Dis Child 67:1140-5.

34. Kimura T, Sunakawa K, Matsuura N, Kubo H, Shimada S, Yago K. 2004. Population pharmacokinetics of arbekacin, vancomycin, and panipenem in neonates. Antimicrob Agents Chemother 48:1159-67.

35. Cirillo I, Vaccaro N, Castaneda-Ruiz B, Redman R, Cossey V, Bradley JS, Allegaert K. 2015. Open-Label Study To Evaluate the Single-Dose Pharmacokinetics, Safety, and Tolerability of Doripenem in Infants Less than 12 Weeks in Chronological Age. Antimicrob Agents Chemother 59:4742-9.

36. Capparelli E, Hochwald C, Rasmussen M, Parham A, Bradley J, Moya F. 2005. Population pharmacokinetics of cefepime in the neonate. Antimicrob Agents Chemother 49:2760-6.

37. De Cock RF, Allegaert K, Schreuder MF, Sherwin CM, de Hoog M, van den Anker JN, Danhof M, Knibbe CA. 2012. Maturation of the glomerular filtration rate in neonates, as reflected by amikacin clearance. Clin Pharmacokinet 51:105-17.

38. Young T, Mangum B. 2010. Neofax. Thomson Reuters, Montvale, NJ.

39. Paediatric Formulary Committee. 2017. BNF for Children. BMJ Group and Pharmaceutical Press, London.

40. Prabhu RM, Piper KE, Baddour LM, Steckelberg JM, Wilson WR, Patel R. 2004. Antimicrobial susceptibility patterns among viridans group streptococcal isolates from infective endocarditis patients from 1971 to 1986 and 1994 to 2002. Antimicrob Agents Chemother 48:4463-5.

41. Bins JW, Mattie H. 1988. Saturation of the tubular excretion of beta-lactam antibiotics. Br J Clin Pharmacol 25:41-50.

42. Shoji K, Bradley JS, Reed MD, van den Anker JN, Domonoske C, Capparelli EV. 2016. Population Pharmacokinetic Assessment and Pharmacodynamic Implications of Pediatric Cefepime Dosing for Susceptible-Dose-Dependent Organisms. Antimicrob Agents Chemother 60:2150-6.

43. Bradley JS, Sauberan JB, Ambrose PG, Bhavnani SM, Rasmussen MR, Capparelli EV. 2008. Meropenem pharmacokinetics, pharmacodynamics, and Monte Carlo simulation in the neonate. Pediatr Infect Dis J 27:794-9.

44. Eagle H, Musselman AD. 1948. The rate of bactericidal action of penicillin in vitro as a function of its concentration, and its paradoxically reduced activity at high concentrations against certain organisms. J Exp Med 88:99-131.

45. Jokipii L, Brander P, Jokipii AM. 1985. Reverse inoculum effect in bactericidal activity and other variables affecting killing of group B streptococci by penicillin. Antimicrob Agents Chemother 27:948-52.

46. Raichle ME, Kutt H, Louis S, McDowell F. 1971. Neurotoxicity of intravenously administered penicillin G. Arch Neurol 25:232-9.

47. Andrassy K, Ritz E, Hasper B, Scherz M, Walter E, Storch H. 1976. Penicillin-induced coagulation disorder. Lancet 2:1039-41.

48. van den Anker JN, Hop WC, de Groot R, van der Heijden BJ, Broerse HM, Lindemans J, Sauer PJ. 1994. Effects of prenatal exposure to betamethasone and indomethacin on the glomerular filtration rate in the preterm infant. Pediatr Res 36:578-81.

49. Ribbing J, Jonsson EN. 2004. Power, selection bias and predictive performance of the Population Pharmacokinetic Covariate Model. J Pharmacokinet Pharmacodyn 31:109-34.

455 50. Wilbaux M, Fuchs A, Samardzic J, Rodieux F, Csajka C, Allegaert K, van den Anker
456 JN, Pfister M. 2016. Pharmacometric Approaches to Personalize Use of Primarily
457 Renally Eliminated Antibiotics in Preterm and Term Neonates. J Clin Pharmacol
458 56:909-35.
459 51. Guignard JP, Drukker A. 1999. Why do newborn infants have a high plasma
460 creatinine? Pediatrics 103:e49.
461 52. Drageset M, Fjalstad JW, Mortensen S, Klingenberg C. 2017. Management of early-
462 onset neonatal sepsis differs in the north and south of Scandinavia. Acta Paediatr
463 106:375-381.
464

465 **Table 1**. The demographic and clinical characteristics of the two study groups[a]

	Study group based on gestational age	
	32–34 weeks (n = 7)	≥35 weeks (n = 10)
Male sex (no. of subjects)	4	7
Birth weight (kg)	2.1 (2.0-2.5)	3.3 (3.0-3.9)
Body weight on PK sampling day (kg)	2.0 (1.8-2.3)	3.1 (2.9-3.8)
Postnatal age on PK sampling day (days)	3.0 (2.0-3.5)	2.5 (2.0-3.0)
Number of penicillin G doses before PK sampling	6.0 (5.0-8.5)	5.0 (5.0-6.5)
Duration of treatment with penicillin G (days)	6.5 (5.8-7.3)	6.0 (3.9-7.6)
Vasoactive support[b] (no. of subjects)	2	3
Respiratory support[c] (no. of subjects)	5	3
Serum creatinine[d] (μmol/L)	52.0 (41.5-66.0)	61.0 (50.8-68.3)
Albumin[d] (g/L)	31.0 (27.0-34.0)	32.0 (31.0-32.3)
C-reactive protein[d] (mg/L)	2.0 (1.0-11.0)	15.0 (5.3-62.5)
Bilirubin[d] (μmol/L)	156.0 (129.0-227.0)	131.0 (116.5-137.0)

466 [a]Data are presented as median (interquartile range) unless otherwise specified.

467 [b]Dobutamine (n=4), dopamine and dobutamine (n=1)

468 [c]Mechanical ventilation (n=3), continuous positive airway pressure (n=5)

469 [d]Laboratory parameters were measured on the PK sampling day ± 1 day.

470 **Table 2**. The pharmacokinetic parameters (median (interquartile range)) estimated by non-
471 compartmental analysis for the neonates in this study in comparison with the values for
472 neonates with GA ≤28 weeks in our previous study (9)

	Neonates with gestational age			
	≥32 weeks (this study)		≤28 weeks (previous study (9))	
Study group based on dose	25,000 IU/kg (n=12)	50,000 IU/kg (n=4)	25,000 IU/kg (n=9)	50,000 IU/kg (n=8)
Actual dose (IU/kg)	26,820 (25,845-27,178)	51,076 (50,594-51,980)	23,913 (22,936-24,124)	46,875 (46,440-48,143)
VD (L/kg)	0.48 (0.38-0.51)	0.63 (0.58-0.67)	0.64 (0.50-0.71)	0.41 (0.33-0.57)
CL (L/h/kg)	0.21 (0.17-0.29)	0.25 (0.19-0.35)	0.09 (0.07-0.11)	0.07 (0.07-0.08)
$t_{1/2}$ (h)	3.5 (3.0-4.2)	4.2 (3.9-5.0)	4.6 (3.8-5.6)	3.8 (3.3-7.0)
C_{max} (mg/L)	62.5 (51.1-74.8)	94.5 (87.3-98.7)	58.9 (52.9-77.5)	145.5 (108.6-157.3)
C_{min} (mg/L)	3.3 (2.3-4.9)	6.4 (5.4-7.5)	3.4 (2.9-3.6)	7.1 (5.2-12.9)
AUC_{0-12} (h*mg/L)	173.6 (127.6-205.7)	225.1 (212.0-295.0)	161.2 (136.3-169.6)	389.3 (341.3-436.2)

473 AUC_{0-12}, area under the drug concentration-time curve over the dosing interval of 0 to 12 h;
474 C_{max}, the maximum concentration in serum; C_{min}, the minimum concentration in serum; CL,
475 clearance; VD, volume of distribution; $t_{1/2}$, half-life.

476 **Table 3**. The pharmacokinetic parameters estimated by the final population pharmacokinetic
477 model

	Mean	SE	RSE (%)	CV (%)	ETA shrinkage (%)
CL (L/h/70kg)	13.2	1.04	7.9	39	2.00
V (L/70kg)	10.3	2.17	21.0	23	55.1
Q (L/h/70kg)	55.6	10.2	18.4	-	-
V2 (L/70kg)	29.8	2.56	8.6	35	23.8

478 CL, clearance; CV, coefficient of variation; Q, intercompartmental clearance; RSE, relative
479 standard error; SE, standard error; V, volume of distribution of the central compartment; V2,
480 volume of distribution of the peripheral compartment.
481 Residual error (proportional): 13%
482 Residual error (additive): 0.278

Figure 1. Goodness-of-fit plots from the final population pharmacokinetic model. DV, observed penicillin G concentration (mg/L); PRED, population-predicted concentration (mg/L); IPRED, individual-predicted concentration (mg/L); CWRES, conditional weighted residuals; TAD, time after dose in hours.

Figure 2. Visual predictive check. The points represent the observed data. The black lines (dashed and solid) represent the 2.5th, 50th, 97.5th percentiles of the observed data and the grey bands represent the 95% confidence interval around these percentiles (from n=1000 simulations).

Figure 3. Probability of target attainment of time above minimum inhibitory concentration (MIC) of 100% with doses of 25,000 (red), 50,000 (green) and 100,000 (blue) IU/kg for different MIC values if protein binding was not considered (panel A) and with the fraction of unbound penicillin G of 40% (panel B). Dotted line presents probability of target attainment of 90%.

Figure 4. Probability of target attainment of the ratio of the 24-hour area under the unbound drug concentration-time curve to the minimum inhibitory concentration (MIC) >100 with doses of 25,000 (red), 50,000 (green) and 100,000 (blue) IU/kg for different MIC values if protein binding was not considered (panel A) and with the fraction of unbound penicillin G of 40% (panel B). Dotted line presents probability of target attainment of 90%.