1   **Pharmacokinetics of penicillin G in preterm and term neonates**

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17   **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).

3

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).

4

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$^{\text{o}}$ C and transferred to -80$^{\text{o}}$

100 C within 24 h. The samples were stored at -80$^{\text{o}}$ 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.

112   Transitions of m/z 335 [M+H]$^+$ to m/z 160, 176 and m/z 518 [M+H]$^+$ to m/z 143, 160 were

113   used for the quantification and qualification of penicillin G and I.S., respectively.

114   A matrix matched calibration was used for validation of the described methodology according

115   to the European Medicines Agency guidelines (21). The calibration curves were linear in

116   concentration range 0.15-150 μg/mL in serum and had $r^2 > 0.9996$. The limit of quantification

117   (LoQ) for serum samples was 0.147 μg/mL and the limit of detection was 0.05 μg/mL. The

118   within-day accuracy ranged from 2% to 9% for the serum calibration curve. The between-day

119   precision for serum samples was <6%.

120   **Monitoring of study patients**. Vital parameters were continuously monitored and recorded at

121   screening, immediately prior to PK sampling and within 72 hours after completion of the

122   penicillin G treatment. All concomitant medications, respiratory support, vasoactive treatment

123   and laboratory parameters from blood samples drawn for clinical indications were also

124   recorded. Serum creatinine was measured by the compensated Jaffe kinetic method

125   standardized against isotope dilution mass spectrometry.

126   **PK analyses**. Non-compartmental analysis (NCA) of concentration-time data was performed

127   in Phoenix WinNonlin software (version 6.5.1; Pharsight Corporation, CA, USA). The area

128   under the concentration-time curve over the dosing interval of 0 to 12 h (AUC_{0-12}) was

129   calculated by use of the log-linear trapezoidal rule. The AUC_{0-12} was used to calculate the

130   total body clearance. The apparent volume of distribution (VD) was determined by calculating

131   the mean residence time extrapolated to infinity.

132   PopPK analysis was performed in NONMEM software (version 7.3; ICON plc, Dublin,

133   Ireland). Concentration-time data from the current and our previous study (9) were pooled and

134   analyzed simultaneously. One-, two- and three-compartment structural models were compared

135   in which, due to the *a priori* assumption of the dependence of renal maturation on

136   postmenstrual age (PMA), clearance was scaled as recommended by Germovsek *et al*. (22),

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137   by adding allometric weight scaling and a sigmoid renal maturation model that includes PMA

138   (23). After choosing the model that provided the best fit, the influence of the following

139   covariates on clearance and VD was tested: birth weight (BW), GA, serum creatinine

140   concentration and need for continuous positive airway pressure or mechanical ventilation. A

141   covariate was retained in the model if it caused a significant decrease in the objective function

142   value, corresponding to $p<0.01$.

143   **Probability of target attainment (PTA)**. The final popPK model was used in 5000-patient

144   Monte Carlo simulation generating concentration-time curves at steady state for penicillin G

145   doses of 25,000, 50,000 and 100,000 IU/kg administered at 12-hour intervals as a 3-min

146   infusion if protein binding was not considered and with the fraction of unbound penicillin G

147   of 40% according to penicillin binding data from adult studies (24). PMA were simulated by

148   sampling from a uniform distribution (range: 24-42 weeks), and the corresponding body

149   weights were obtained using the model by Sumpter & Holford (25). Pharmacodynamic targets

150   fT>MIC and fAUC/MIC ratio were calculated for MIC values 0.006, 0.125, 0.25, 0.5, 1 and 2

151   mg/L applicable for GBS and enterococci (26). PTA was calculated for fT>MIC of 40% and

152   100% and fAUC/MIC >100. All simulations were performed in R software (version 3.2.2;

153   The R Foundation, Vienna, Austria).

154   The protocol was approved by the Ethics Committee of the University of Tartu. A parent

155   signed an informed consent prior to the inclusion of neonate in the study. The study was

156   registered with the EU Clinical Trials Register (EudraCT Number: 2012-002836-97).

157

158   **Results**

159   **Patients**. For the current study, a total of 25 neonates with GA ≥32 weeks were screened, of

160   whom 17 were enrolled. Reasons for exclusion were lack of informed consent (n=3), absence

161   of arterial or central venous catheter (n=3), participation in another study (n=1) and change in

7

162 antimicrobial therapy (n=1). The demographic and clinical characteristics are shown in Table

163 1. Penicillin G was administered for treatment of EOS (n=4), congenital pneumonia (n=1),

164 other/suspected congenital infection (n=9) and meconium aspiration syndrome (n=3). All

165 patients received concomitant therapy with gentamicin (4 mg/kg/q24h), but none received

166 other potentially nephrotoxic drugs on the PK sampling day. None of the neonates had a

167 positive blood culture.

168 **Non-compartmental PK analysis**. NCA was performed on data from 16 patients. One

169 neonate with GA >35 weeks was excluded due to insufficient number of PK samples (n=2).

170 The median values of the CL, VD and half-life were similar regardless of GA (largest relative

171 difference in VD – mean 0.54 L/kg and 0.46 L/kg (p=0.25) in neonates with GA 32-34 weeks

172 and ≥35 weeks, respectively) and thus the values of the PK parameters are presented only

173 based on the dose of penicillin G (Table 2). As expected, the dose of 50,000 IU/kg resulted in

174 higher values of C_{max}, C_{min} and fAUC than 25,000 IU/kg (Table 2).

175 **PopPK analysis.** In total 35 neonates (17 from the current study and 18 from the previous

176 study (9)) were included in the popPK analysis. A two-compartment model with allometric

177 weight scaling and a renal maturation function provided the best fit to the concentration-time

178 data. None of the covariates tested significantly improved the model fit and were thus not

179 retained in the final model. The PK parameter estimates of the final model are shown in Table

180 3. Parameters for median values for the population used in the popPK modelling (current

181 weight 1.28 kg, PMA 32.3 weeks) were as follows: clearance 0.15 L/h, central VD 0.19 L,

182 intercompartmental clearance 2.76 L/h, peripheral VD 0.54 L.

183 Overall, goodness-of-fit plots (Figure 1) and the visual predictive check (Figure 2) showed

184 good prediction of data by the model.

8

185 **PTA analysis**. The PTA for fT>MIC of 40% was >90% for all tested MIC values and doses

186 of 25,000, 50,000 and 100,000 IU/kg if protein binding was not considered and if the fraction

187 of unbound penicillin G was 40% (data not shown).

188 The PTA of fT>MIC of 100% was >90% for all doses for MIC values of ≤0.5 mg/L only if

189 protein binding was not considered (Figure 3A). If the fraction of unbound penicillin G was

190 40%, the same target was achieved with all doses only if MIC was ≤0.125 mg/L (Figure 3B).

191 If protein binding was not considered, the PTA of fAUC/MIC >100 was >90% for all tested

192 MIC values with doses of 50,000 and 100,000 IU/kg and remained >90% with dose of 25,000

193 IU/kg for MIC values ≤1 mg/L (Figure 4A). If the fraction of unbound penicillin G was 40%,

194 the PTA >90% was achieved with doses 25,000, 50,000 and 100,000 IU/kg only if MIC was

195 ≤0.5, ≤1 and ≤2 mg/L, respectively (Figure 4B).

196

197 **Discussion**

198 This study reports, to our best knowledge, the largest neonatal penicillin G population PK

199 analysis to date. We demonstrated that in neonates PK parameters of intravenously

200 administered penicillin G during the first week of life are similar regardless of GA. According

201 to popPK model the dose of 25,000 IU/kg every 12 hours could be suggested for treatment of

202 EOS caused by GBS regardless of pharmacodynamic target (fT>MIC 40%, fT>MIC 100% or

203 fAUC/MIC >100) and the GA (ranging from 24 to 40 weeks).

204

205 Contrary to our hypothesis, the values of half-life and volume of distribution of penicillin G in

206 late-preterm and term neonates were comparable to those in very and extremely preterm

207 neonates that vary in the ranges of 3.8-4.6 h and 0.41-0.64 L/kg, respectively (9, 10). This

208 corroborates previous findings that half-life of penicillin G in serum in neonates does not

209 depend on BW or GA (10, 27). Similarity in VD could result in part from relatively larger

210   weight loss after birth in more premature neonates compared with more mature ones (28) and

211   several factors could contribute to similarity in clearance throughout neonatal period. First,

212   tubular secretion that is the main elimination mechanism of penicillin G in adults (29) is

213   equally reduced in preterm and term neonates as a result of decreased renal blood flow to

214   peritubular areas (30). Second, glomerular filtration has been suggested to be relatively more

215   important than tubular secretion in elimination of penicillin G in neonates (27). Although

216   clearance depends on GA (31), the difference between preterm and term neonates in

217   glomerular filtration rate is less pronounced within the first days of life, increasing only by

218   0.0205 mL/min/kg per each week of postconceptional age (32, 33). Finally, the fraction of

219   beta-lactams bound to proteins is reduced in more premature neonates that may also

220   contribute to higher clearance (34). Notably, for other beta-lactams, such as doripenem and

221   cefepime, clearance was similar regardless of GA within the first week of life (35, 36). Even

222   for amikacin that is almost entirely eliminated by glomerular filtration the difference in

223   clearance was only slightly higher in the first postnatal day in neonates with larger BW

224   compared with those with smaller BW (37). However, contribution of elimination

225   mechanisms other than kidneys cannot be excluded in neonates, as the fraction of penicillin G

226   dose excreted into urine is considerably lower in neonates (26-37%) (9, 27), compared to

227   adults (58%) (29).

228

229   We found that a two-compartment model described data best. This is in agreement with the

230   only published study describing population pharmacokinetics of penicillin G in neonates

231   conducted by Muller *et al*. (10), who analyzed data from neonates with GA <32 weeks.

232   However, while in the model by Muller *et al*., GA was not included in the final model

233   (possibly due to the small range of GA), in our study GA was included indirectly, i.e.

234   incorporated in the PMA-dependent renal maturation function. The use of PMA rather than

235   GA is supported by a recent comparison of models for scaling clearance in children by

236   accounting for size and maturation (22).

237

238   Our study showed that in neonates, regardless of GA, the target of fT>MIC of 40% was

239   achieved with PTA >90% when the fraction of unbound penicillin G of 40% was assumed

240   using a dose of 25,000 IU/kg twice a day for all MIC values tested (up to 2 mg/L). This dose

241   is within the range recommended by Neofax (15-30 mg/kg every 12 hours) (38), but is less

242   than that suggested by the British National Formulary for Children (25 mg/kg every 12 hours)

243   (39). Still, according to evidence statements in NICE Clinical Guidelines, a dose of 25,000

244   IU/kg is effective in preterm neonates, although no evidence was identified for dosing in term

245   neonates (6). Although the previous popPK study of penicillin G in neonates with GA <32

246   weeks suggested dosing regimen of 50,000 IU/kg twice daily (10), our proposed dosing

247   regimen proposed should be adequate for treatment of EOS, due to several reasons. a) GBS is

248   susceptible to penicillin G with MIC_{90} as low as 0.06 mg/L (5) and viridans-group

249   streptococci that may cause up to 19% of EOS cases (2) have MIC_{90} 0.5 mg/L (40). b)

250   Although we did not measure the fraction of unbound penicillin G and no prior data are

251   available in neonates within the first days of life, the fraction unbound is known to be reduced

252   immediately after birth compared with adults (24). Thus, the unbound drug fraction of 40%

253   that is based on values in adults (24) should be a conservative estimate and the actual

254   unbound concentrations in neonates are most likely higher than estimated in this study. c)

255   While penicillin G bactericidal activity requires fT>MIC 38% in adults, the same study

256   showed that in neonates fT>MIC 32% was bactericidal (17). Therefore, for neonates with

257   immature immune systems the somewhat higher target of fT>MIC of 40% should be

258   appropriate (42, 43). d) Even the target of fT>MIC as high as 100% that is more likely

259   associated with clinical cure (15, 16) was achieved with PTA >90% for MIC values ≤0.125

260   mg/L and with PTA approximately 80% for MIC values ≤0.5 mg/L with the dose of 25,000

261   IU/kg twice daily. Moreover, PTA of fAUC/MIC >100 that was shown to be better correlated

262   with bactericidal activity in neonates (17) remained >90% for MIC values ≤0.5 mg/L.

263   Therefore, 25,000 IU/kg should be appropriate and avoids unnecessarily high doses of

264   penicillin G that may counteract treatment by evoking the so-called Eagle effect, which

265   results in a reduced killing rate of GBS by penicillin G concentrations above the optimal level

266   (44). Moreover, excessively high doses of penicillin G may cause toxicity including

267   encephalopathy (46) or coagulation disorders (47). The dose of 25,000 IU/kg was well

268   tolerated in a clinical study that included 142 neonates with suspected EOS (7) and no drug-

269   related adverse events were observed in our study.

270

271   Some limitations of the study should be noted. First, we cannot exclude the effect of

272   unrecorded clinical characteristics on the PK parameter estimates. For example, in our

273   previous study all except one mother of neonates with GA of ≤28 weeks received steroid

274   prophylaxis before birth, but betamethasone increases glomerular filtration rate (48).

275   However, the small number of neonates studied did not allow analysis of this covariate (49).

276   Moreover, covariates other than those reflecting size, age and renal function are only

277   occasionally incorporated in the final models describing PK of primarily renally eliminated

278   antibiotics (50). Second, although clearance depends on renal function in addition to growth

279   and maturation (50), a covariate reflecting renal function was not included in our model.

280   However, the lack of effect of creatinine on the model fit was expected, as in the first days of

281   life neonatal serum creatinine values reflect maternal concentrations (51) and less than half of

282   models describing PK of primarily renally eliminated antibiotics incorporate serum creatinine

283   (50). Finally, we did not measure penicillin G concentrations in cerebrospinal fluid, which

284   could also be considered given that concomitant meningitis occurs in 2-6% of EOS cases (2,

285 52), whereas in culture-positive cases the proportion is as high as 26% (52). Although 25,000

286 IU/kg twice a day has been suggested to be adequate (9), the PK of penicillin G in

287 cerebrospinal fluid warrants further studies to provide evidence for dosing regimens for

288 meningitis.

289

290 In conclusion, our results show that the current dosing regimen of 25,000 IU/kg every 12h for

291 EOS results in sufficient serum concentrations of penicillin G. The dosing regimen is

292 appropriate against GBS as the commonest causative agents of EOS regardless of

293 pharmacodynamic target (fT>MIC 40% or 100% or fAUC/MIC >100) and GA due to the

294 similarity of PK parameters of penicillin G within the first days of life in preterm and term

295 neonates.

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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.

21

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.

22

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

483 **Figure 1**. Goodness-of-fit plots from the final population pharmacokinetic model. DV,

484 observed penicillin G concentration (mg/L); PRED, population-predicted concentration

485 (mg/L); IPRED, individual-predicted concentration (mg/L); CWRES, conditional weighted

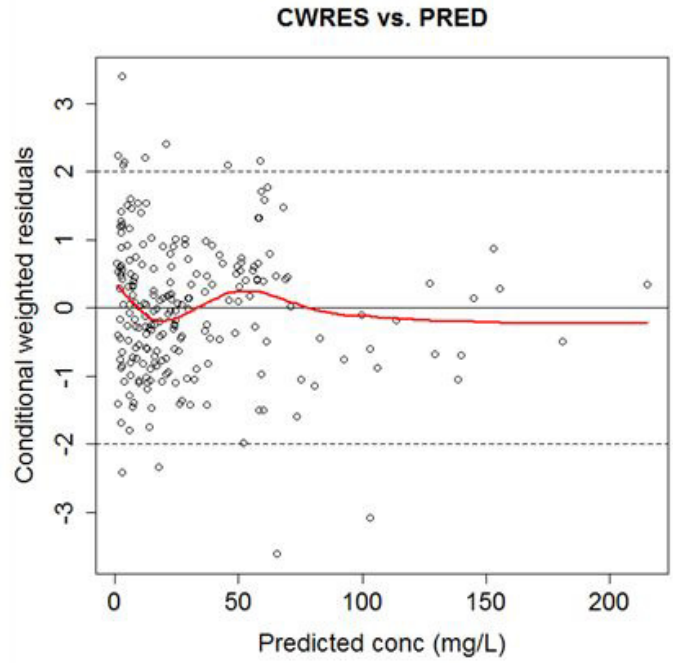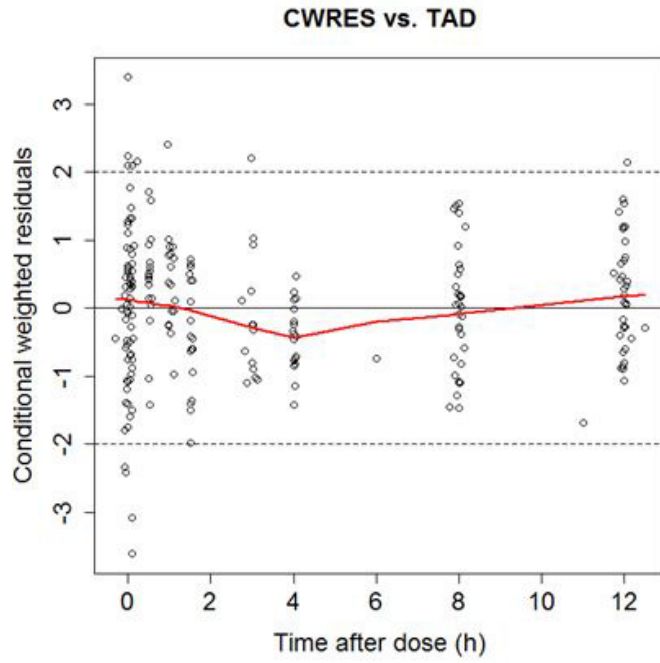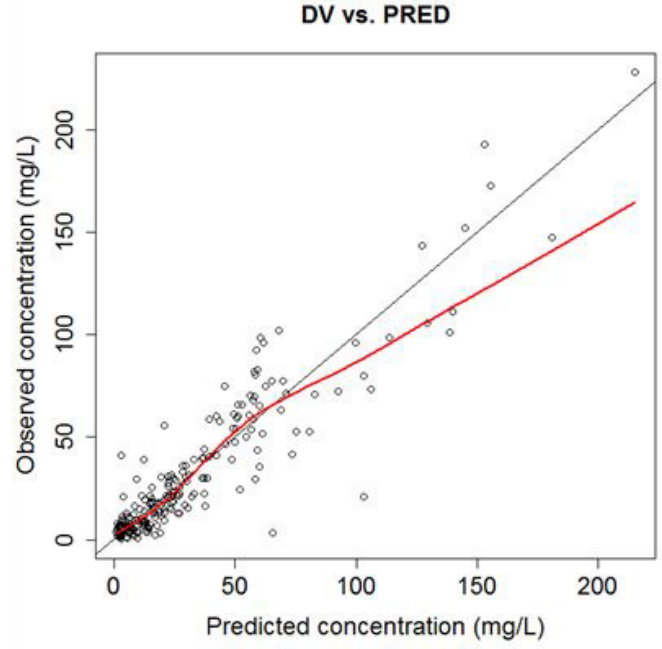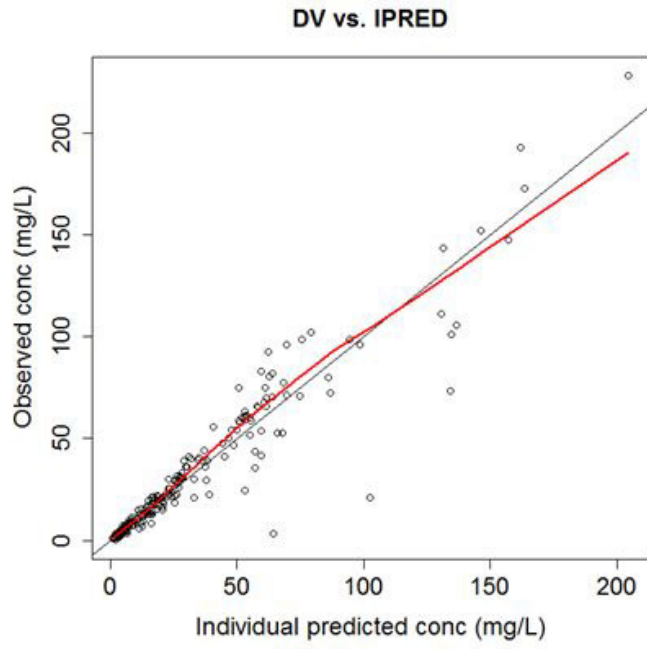486 residuals; TAD, time after dose in hours.

487

488 **Figure 2**. Visual predictive check. The points represent the observed data. The black lines

489 (dashed and solid) represent the 2.5th, 50th, 97.5th percentiles of the observed data and the

490 grey bands represent the 95% confidence interval around these percentiles (from n=1000

491 simulations).

492

493 **Figure 3.** Probability of target attainment of time above minimum inhibitory concentration

494 (MIC) of 100% with doses of 25,000 (red), 50,000 (green) and 100,000 (blue) IU/kg for

495 different MIC values if protein binding was not considered (panel A) and with the fraction of

496 unbound penicillin G of 40% (panel B). Dotted line presents probability of target attainment

497 of 90%.

498

499 **Figure 4**. Probability of target attainment of the ratio of the 24-hour area under the unbound

500 drug concentration-time curve to the minimum inhibitory concentration (MIC) >100 with

501 doses of 25,000 (red), 50,000 (green) and 100,000 (blue) IU/kg for different MIC values if

502 protein binding was not considered (panel A) and with the fraction of unbound penicillin G of

503 40% (panel B). Dotted line presents probability of target attainment of 90%.

24

DV vs. IPRED

DV vs. PRED

CWRES vs. TAD

CWRES vs. PRED

Visual Predictive Check

(A)

(B)

(A)

Probability of target attainment (%)

MIC (mg/L)

Dose (IU/kg)
- 25000 IU/kg
- 50000 IU/kg
- 100000 IU/kg

(B)

Probability of target attainment (%)

MIC (mg/L)

Dose (IU/kg)
- 25000 IU/kg
- 50000 IU/kg
- 100000 IU/kg