

1 **Development and evaluation of a gentamicin pharmacokinetic model that facilitates**
2 **opportunistic gentamicin therapeutic drug monitoring in neonates and infants.**

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22 **A short running title:** Gentamicin PK model for TDM in neonates and infants

23
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25

26 **Abstract**

27 Trough gentamicin therapeutic drug monitoring (TDM) is time-consuming, disruptive to neonatal
28 clinical care and a patient safety issue. Bayesian models could allow TDM to be performed
29 opportunistically at the time of routine blood tests. This study aimed to develop and prospectively
30 evaluate a new gentamicin model and a novel Bayesian computer tool (neoGent) for TDM use in
31 neonatal intensive care. We also evaluated model performance for predicting peak concentrations and
32 AUC(0-t). A pharmacokinetic meta-analysis was performed on pooled data from three studies (1325
33 concentrations from 205 patients). A 3-compartment model was used with covariates being:
34 allometric weight scaling, postmenstrual and postnatal age, and serum creatinine. Final parameter
35 estimates (standard error) were: clearance: 6.2 (0.3) L/h/70kg; central volume (V) 26.5 (0.6) L/70kg;
36 inter-compartmental disposition: $Q=2.2$ (0.3) L/h/70kg, $V_2=21.2$ (1.5) L/70kg, $Q_2=0.3$ (0.05)
37 L/h/70kg, $V_3=148$ (52.0) L/70kg. The model's ability to predict trough concentrations from an
38 opportunistic sample was evaluated in a prospective observational cohort study that included data
39 from 163 patients with 483 concentrations collected in five hospitals. Unbiased trough predictions
40 were obtained: median (95% confidence interval (CI)) prediction error was 0.0004 (-1.07, 0.84) mg/L.
41 Results also showed peaks and AUC(0-t) could be predicted (from one randomly selected sample)
42 with little bias but relative imprecision with median (95% CI) prediction error being 0.16 (-4.76, 5.01)
43 mg/L and 10.8 (-24.9, 62.2) mg h/L, respectively. NeoGent was implemented in R/NONMEM, and in
44 the freely available TDMx software.

45

46 Introduction

47 The aminoglycoside antibiotic gentamicin is the most commonly used antimicrobial on neonatal
48 units(1, 2) and is effective against Gram negative bacteria. Gentamicin use is limited by its narrow
49 therapeutic index and risk of toxicity, specifically nephro- and ototoxicity(3). It is not metabolized in
50 the liver(4) and is almost entirely eliminated by the kidneys; clearance therefore depends on renal
51 function. During the first two weeks of life, renal and intra-renal blood flow increase rapidly, causing
52 a steep rise in glomerular filtration rate (GFR)(5, 6).

53 Therapeutic drug monitoring (TDM) is required to ensure maximal efficacy and especially minimal
54 toxicity, particularly in the neonatal population where variability in pharmacokinetic (PK) parameters
55 is large. Dose individualization approaches focus on toxicity(7, 8) and include single-level methods
56 and nomograms(9, 10), area under the curve (AUC) methods(11), and Bayesian methods(12). The use
57 of nomograms is limited as they cannot readily incorporate covariates affecting PK parameters. AUC
58 methods use a simplified 1-compartment PK model and require at least two gentamicin
59 measurements, which is not appropriate in neonates with limited blood volumes. These drawbacks
60 make Bayesian approaches the most attractive for newborn infants.

61 Deriving a Bayesian prior for TDM requires a non-linear mixed-effect PK model, and several such
62 studies of neonatal gentamicin have been published(13-24). However, these studies are limited by
63 their heterogeneity and use of sparse data (often identifying only a 1-compartment model when
64 gentamicin follows multi-compartment kinetics(25, 26)) and fail to account for age-related differences
65 in creatinine during the immediate newborn period. Although gentamicin is not a new drug, its dosing
66 and monitoring is still a current issue as identified in the UK National Patient Safety alert
67 (<http://www.nrls.npsa.nhs.uk/alerts/?entryid45=66271>) and a recent publication by Valitalo *et al*(27),
68 who used simulations to define dosing guidelines.

69 We aimed to investigate whether opportunistic sampling can predict trough gentamicin concentrations
70 so that standard TDM could be performed from a blood sample taken for other purposes (e.g. routine
71 blood gases). As a secondary aim, we evaluated the model's ability to predict peak gentamicin
72 concentrations and AUC(0-t) using one randomly selected sample.

73 **Methods**

74 Study population

75 This study used two datasets: a model-building dataset and a prospectively collected evaluation
76 dataset.

77 To collect data for model development, the electronic bibliographic database PubMed was searched in
78 January 2015 without time limitations. The search strategy included: (neonat* OR newborn*) AND
79 (gentamicin) AND (pharmacokinetic* OR PK); gentamicin samples had to be prospectively collected
80 and covariates (weight, gestational age (GA), postnatal age (PNA), serum creatinine measurements),
81 also had to be reported. Additionally, we also searched the reference lists in identified papers. The
82 authors of the publications that met the inclusion criteria (n=8) (11, 15, 21, 22, 28-31) were then
83 invited to contribute their data.

84 Data for the evaluation of the PK model were collected as a prospective observational cohort study
85 from five UK hospitals (St George's University Hospitals NHS Foundation Trust, Liverpool Women's
86 NHS Foundation Trust, Oxford University Hospitals, Portsmouth Hospitals NHS Trust and Coventry
87 & Warwickshire University Hospitals NHS Trust) from July 2012 to November 2013. Infants were
88 eligible for inclusion if the following criteria were met: more than 36 hours gentamicin therapy
89 anticipated, postnatal age of less than 90 days, not receiving extracorporeal membrane oxygenation,
90 peritoneal dialysis or hemofiltration, and expected to survive the study period (as judged by the
91 clinical team). Each patient provided a minimum of two gentamicin concentrations – a trough sample
92 from routine TDM (i.e. a pre-dose sample taken before a non-initial dose) and an additional study
93 sample (taken opportunistically during a course of gentamicin when the infant required blood
94 sampling for clinical care). These samples will be referred to as routine (trough) and (opportunistic)
95 study samples in this manuscript. Exact times of gentamicin dosing and sampling were recorded,
96 along with the patient's weight, age and serum creatinine (Table 1). Written informed consent was
97 obtained from parents and the study was approved by the London Central Ethics committee (reference
98 12/LO/0455).

99

100 Gentamicin dosing and sampling procedure in the prospective evaluation dataset

101 Gentamicin treatment was initiated at the discretion of the clinical team for possible infection and
102 dosed and monitored using trough concentrations according to the standard practice at each hospital.
103 Gentamicin was administered as a slow (<2 min) bolus via intravenous cannula, percutaneous long
104 line, or umbilical venous catheter.

105

106 Bioanalytical techniques

107 An enzyme immunoassay (EMIT, Syva)(15), a fluorescence polarization immunoassay (TDx,
108 Abbot)(15, 21), and high performance liquid chromatography coupled to tandem mass spectrometry
109 (UHPLC-MS/MS) (32) were used to determine gentamicin concentration in the model-building
110 dataset; and the Jaffe reaction (33) was used to determine serum creatinine concentrations. In the
111 prospective evaluation dataset, gentamicin serum concentrations were analyzed using immunoassay
112 techniques (Table S1); and creatinine concentrations were determined by either a Jaffe-based or an
113 enzymatic method (137 neonates and 26 neonates, respectively).

114

115 Pharmacokinetic analysis

116 The observed concentration-time data from only the model-building studies were pooled and
117 simultaneously analyzed with non-linear mixed-effects software NONMEM version 7.3(34). The first
118 order conditional estimation method with interaction was used.

119

120 *Basic model*

121 One-, 2-, and 3-compartment structural models were considered when defining the basic structural
122 population PK model. The inter-individual variability (IIV) was assumed to follow a log-normal
123 distribution and tested on all parameters. An additive, a proportional, and a combination of both
124 (Equation 1) residual error models were tested.

$$125 y_{ij} = f(t_{ij}; \phi_i) + f(t_{ij}; \phi_i) \cdot \varepsilon_{ij(\text{proportional})} + \varepsilon_{ij(\text{additive})}, \quad (\text{Equation 1})$$

126 where y_{ij} is an observed gentamicin concentration at time t_{ij} , f is the function that represents the
127 gentamicin model, ϕ_i is a vector of parameters, ε_{ij} is a residual error term.

128 Inter-occasion variability (IOV) was also assumed to be log-normally distributed and it was tested for
129 all parameters with an occasion defined as a single dosing interval.

130

131 *Covariate model*

132 Allometric scaling was used *a priori* to standardize all PK parameters to 70 kg (35), and a maturation
133 function, describing the maturation of the GFR with postmenstrual age (PMA) (Equation 2) with fixed
134 parameters from a previous study (5), was used to scale clearance. Allometric exponents were fixed to
135 0.632 for central clearance and 0.75 for inter-compartmental clearances. Different exponents were
136 used because these values were shown best for describing the maturation of renal elimination(5) and
137 tissue blood flows(36), respectively. Allometric exponents for volumes of distribution were fixed to 1.
138 The combination of allometric weight scaling and sigmoidal maturation function was suggested as a
139 standard method for scaling clearance in the pediatric population in a recent comparison of different
140 approaches(37).

$$141 \text{ maturation function} = \frac{PMA^{Hill}}{PMA_{50}^{Hill} + PMA^{Hill}}, \quad (\text{Equation 2})$$

142 where *Hill* is the sigmoidicity coefficient and PMA_{50} is PMA when maturation of GFR reaches 50%
143 of adult values.

144 As it is known that PNA and serum creatinine are important indicators of gentamicin clearance and
145 also based on the posthoc estimates of η versus covariates plots, they were tested on clearance.
146 These time-varying covariates were considered to significantly improve the fit and therefore included
147 in the model if the difference in objective function value (Δ OFV) after their inclusion was >3.84
148 ($p < 0.05$). Additionally, linear extrapolations between observations were made. To account for
149 endogenous creatinine, maternal creatinine and also the change in renal function with age, a typical
150 value of serum creatinine (TSCr) for a specific PMA was determined using data from Cuzzolin *et*
151 *al*(38) for preterm (GA < 37 weeks) newborns and Rudd *et al*(39) for term newborns. A linear decline
152 in TSCr with increasing PMA was found according to Equation 3:

$$153 TSCr = -2.849 \cdot PMA (\text{weeks}) + 166.48. \quad (\text{Equation 3})$$

154 A possible influence of serum creatinine on clearance was tested according to the following Equation
155 4, where measured serum creatinine (MSCr) was standardized by TSCr for PMA and departures from
156 it estimated as follows:

$$157 \left(\frac{MSCr}{TSCr} \right)^\theta. \quad (\text{Equation 4})$$

158 The effect of PNA was investigated with a logistic function (Equation 5) to account for the rapid
159 changes in gentamicin clearance in the first hours of life. The first day of life was defined as day 1.

$$160 \textit{postnatal age function} = \frac{PNA}{PNA_{50} + PNA}, \quad (\text{Equation 5})$$

161 where PNA_{50} is the PNA when clearance has reached 50% of typical adult's clearance.

162 After the forward selection ($\Delta OFV > 3.84$) of all covariates (full model), backward elimination was
163 performed, with a p -value retention cut-off of 0.001 ($\Delta OFV < 10.83$).

164

165 Evaluation

166 *Internal model evaluation*

167 Basic goodness-of-fit plots for observations *versus* population and individual predictions, conditional
168 weighted residuals *versus* population predictions and *versus* time after dose were produced using
169 statistical software R version 3.1.0 (R Core Team (2014). R: A language and environment for
170 statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from:
171 <http://www.R-project.org/>) and visually examined. The assumptions of normality and homogeneity of
172 the residuals errors were investigated by inspecting a histogram and a qq-plot.

173 Standard errors from NONMEM covariance step and non-parametric bootstrap analysis with 1,000
174 replicates were used to determine the precision of the final PK parameter estimates.

175 Additionally, we simulated 1,000 datasets using parameter estimates from the final model, and plotted
176 95% confidence intervals (CI) around the 2.5th, 50th, and 97.5th prediction percentiles of the simulated
177 data. Then, the observations were overlaid on the plot, also called the visual predictive check (VPC).

178 Perl-speaks-NONMEM (PsN) software(40) was used for the bootstrap analysis and to produce the
179 VPC, which was visualized using R-package Xpose4(41).

180

181 *External model evaluation*

182 The prospective evaluation dataset was used to evaluate the predictive performance of the model. No
183 additional fitting was done, and the diagnostic plots and the VPC were generated as described above.

184 Bayesian model-predicted trough concentrations were computed using the model as a prior and
185 information from only the opportunistic study samples. These predictions were compared with the
186 observed trough concentrations by calculating the prediction error (PE) (42), and also the mean PE
187 (MPE) (i.e. a measure of bias), and root-mean-square error (RMSE), a measure of precision(43)
188 (Equations 6).

189
$$PE = \text{observed} - \text{predicted}$$

190
$$MPE = \frac{1}{N} \cdot \sum_{i=1}^N \cdot PE_i \quad (\text{Equations 6})$$

$$RMSE = \sqrt{\frac{1}{N} \cdot \sum_{i=1}^N \cdot PE_i^2}$$

191 Also, we counted the number of “correct” predictions that were below or above the currently
192 recommended gentamicin trough concentration thresholds of 1 mg/L or 2 mg/L (the National Institute
193 for Health and Care Excellence (NICE) ([http://www.nice.org.uk/guidance/CG149/chapter/1-](http://www.nice.org.uk/guidance/CG149/chapter/1-Guidance#therapeutic-drug-monitoring-for-gentamicin)
194 [Guidance#therapeutic-drug-monitoring-for-gentamicin](http://www.nice.org.uk/guidance/CG149/chapter/1-Guidance#therapeutic-drug-monitoring-for-gentamicin)) and British National Formulary for Children
195 (BNFc) ([http://www.evidence.nhs.uk/formulary/bnfc/current/5-infections/51-antibacterial-drugs/514-](http://www.evidence.nhs.uk/formulary/bnfc/current/5-infections/51-antibacterial-drugs/514-aminoglycosides/gentamicin)
196 [aminoglycosides/gentamicin](http://www.evidence.nhs.uk/formulary/bnfc/current/5-infections/51-antibacterial-drugs/514-aminoglycosides/gentamicin))).

197 Further analysis of paired samples (that is both study and routine samples taken in the same dosing
198 interval) was undertaken for the following scenarios: study samples ≥ 1 , ≥ 2 , and ≥ 3 mg/L, compared
199 with only unpaired samples.

200

201 *Cross-validation*

202 The subset with the study sample above 3 mg/L provided the most important comparison, since in this
203 case the study sample was still above the pre-specified trough threshold. As there were only 18 pairs
204 with opportunistic study concentration ≥ 3 mg/L in the evaluation dataset, these pairs were merged
205 with paired samples of the same characteristics from the model-building dataset. The pooled dataset

206 was then randomly split into five subsets, and cross-validation was performed; meaning that in each
207 subset 20% of the pairs were randomly removed and the model was re-estimated. The re-estimated
208 model was then used as a prior to predict the troughs, and compared to the observed trough
209 concentrations as previously described.

210 Whether the model is able to predict peak concentrations from one randomly selected non-peak
211 sample was tested similarly as described above, using paired samples from both the model-building
212 and the evaluation dataset, and performing cross-validations. Additionally, as a possible
213 pharmacokinetic-pharmacodynamic target for aminoglycosides can also be $AUC(0-24)/MIC$ (44), the
214 model was also evaluated on how it predicts $AUC(0-t)$. Only a subset of the data where five or more
215 samples were collected after the same dose was used for defining $AUC(0-t)$, and the model-predicted
216 *versus* observed (non-compartmental) $AUC(0-t)$ was compared.

217

218 *Comparison with other models*

219 To compare our mechanistic model which scales for size, age and expected renal function with
220 previously published models using empirical covariate analysis, predictions for the measured trough
221 from the routine opportunistic samples in our prospective dataset were generated.

222

223 neoGent software

224 The model was implemented using R and NONMEM (see Supplementary material). It works by
225 reading an individual's data into R, then Bayesian estimates generated in NONMEM are used to
226 predict outcomes of interest (e.g. the time when the concentration falls below 2 mg/L).

227

228

229 **Results**230 Patients

231 Out of eight contacted authors identified in the literature search we obtained two large neonatal
 232 gentamicin datasets (15, 21). We received no response from four authors (11, 28-30); and although an
 233 initial response was received from two authors (22, 31) no data were actually shared. Additionally, we
 234 obtained some previously unpublished data taken during a PK study of ampicillin and penicillin (32).
 235 The data were pooled and comprised 1325 gentamicin concentrations from 205 neonates (Table 1).
 236 This dataset was used to derive the model.

237 For the model evaluation, gentamicin serum concentrations were prospectively collected from a total
 238 of 194 neonates. Of the enrolled patients, 163 were included in the PK analysis (Table 1). Reasons for
 239 exclusion (31 patients) included inexact sampling times, insufficient samples, or the gentamicin
 240 opportunistic study concentration being below the limit of quantification (n=12). The final evaluation
 241 dataset comprised 483 gentamicin serum measurements, with 229 study and 254 routinely taken
 242 trough concentrations. Median (range) time after dose was 13.3 (0.08-53.3) h and 31.1 (8.0-79.7) h for
 243 study and routine concentrations, respectively. Patients were on treatment for up to 20 days.

244

245 Pharmacokinetic analysis

246 Initially, a 2-compartment model provided a better fit to the data ($\Delta\text{OFV}=7.4$ with a 3-compartment
 247 model) and was therefore chosen as the basic structural model. But, after the addition of the fixed
 248 allometric and renal function parameters, covariates and IOV, a 3-compartment model described the
 249 data better (47-unit drop in OFV). The IIV was described with an exponential error structure, and the
 250 best residual error model was a combination of a proportional and additive error.

251 Postnatal age and standardized serum creatinine had a significant effect on clearance ($\Delta\text{OFV}=134.1$
 252 and $\Delta\text{OFV}=17.2$, respectively) and were thus included in the final model. Backward elimination
 253 ($p=0.001$) confirmed that these covariates remained significant with the 3-compartment model. The
 254 final gentamicin population PK model is summarized with Equations 7.

$$255 \quad CL = \theta_{CL} \cdot \left(\frac{WT}{70}\right)^{0.632} \cdot \frac{PMA^{3.33}}{55.4^{3.33} + PMA^{3.33}} \cdot \left(\frac{MSCr}{TSCr}\right)^{\theta_{SCr}} \cdot \frac{PNA}{\theta_{P50} + PNA} \cdot e^{(\eta_{CL} + \kappa_{CL})},$$

$$256 \quad V = \theta_V \cdot \left(\frac{WT}{70}\right) \cdot e^{\eta_V}, \quad (\text{Equations 7})$$

$$257 \quad Q = \theta_Q \cdot \left(\frac{WT}{70}\right)^{0.75} \cdot e^{\eta_Q},$$

258

259 where CL is gentamicin clearance, V is gentamicin volume of distribution, Q is inter-compartmental260 gentamicin clearance, WT is body weight in kilograms, η is IIV, κ is IOV.261 There was only a small improvement in fit ($\Delta\text{OFV}=7.6$) when the model was parameterized for time-

262 varying covariates (linear extrapolation between observed covariate values), but as this model is more

263 biologically plausible, it was chosen as the final model.

264 The OFV reduced from 2305.0 to 1217.5 between the basic and the final model. The inclusion of the

265 covariates resulted in a reduction of the IIV on PK parameters: with the basic model the IIV on CL 266 and V was 71.1% and 62.5%, respectively, and with the final model, 41.8% and 33.5%, respectively.

267 The final PK parameter estimates with uncertainty are reported in Table 2.

268

269 Evaluation270 *Internal model evaluation*

271 Figure 1 shows plots assessing goodness-of-fit by comparing observations and predictions. A VPC of

272 the final model is shown in Figure 2.

273

274 *External model evaluation*

275 The basic diagnostic plots are presented in Figure 1, and the VPC performed using the evaluation

276 dataset and the final parameters from the PK model without additional fitting in Figure 2.

277 Table 3 shows the number of correct predictions (for five different datasets from the evaluation data

278 and pooled results from the cross-validation) for gentamicin trough thresholds of 1 and 2 mg/L

279 together with prediction errors. In the total dataset, containing both paired and unpaired samples, the

280 median (95% CI) PE was 0.0004 (-1.1, 0.8) mg/L. The MPEs when predicting trough and peak

281 concentrations (using cross-validations) were 0.03 and 0.19 mg/L; and the RMSE 1.28 and 2.55 mg/L,

282 respectively (Table 3). When AUC(0-t) prediction (from one random sample) was evaluated, MPE

283 was 14.5 mg h/L, and RMSE 30.2 mg h/L.

284 Figure 3 shows the median and the range of PE for this model and previously published gentamicin
285 population PK models.

286

287 *NeoGent*

288 Figure S1 shows an example of output from neoGent.

289

290

291 **Discussion**

292 A PK model for gentamicin in neonates was developed and evaluated with prospectively collected
293 data. Through its use of mechanistic covariates the model gave unbiased predictions of trough
294 concentration from an opportunistic sample. Using this model, concentrations from samples taken at
295 any time can be used to generate informative TDM, potentially eliminating the need for specifically
296 timed trough gentamicin samples and the safety concerns and inconvenience associated with them. An
297 exploratory analysis to evaluate whether such an approach could be used for predicting individual
298 peak concentration and AUC(0-t) showed that while predictions were unbiased, they were relatively
299 imprecise (Table 3).

300

301 The small median PE (0.0004 mg/L) for trough concentrations suggests that the model implemented
302 in neoGent performs well, although some outliers were not captured (range: -2.4 – 1.6 mg/L). The
303 median prediction errors were in most cases negative (Table 3), indicating that the model slightly
304 over-predicts the trough concentrations (i.e. predicts them to be higher than they are), which might be
305 (from a safety perspective) preferable to under-predicting. Cross-validations confirmed that samples
306 do not need to be taken at a specific time when using this model for TDM, as predictions of trough
307 concentrations (using an opportunistic sample) were unbiased, with median PE of -0.04 mg/L (Table
308 3). Although we did not test the effect of the sampling time on model predictions; the samples were
309 collected from a wide range of times (0.1-53.3 h after the dose), as they would be in routine hospital
310 tests.

311

312 Comparison of the developed model with the existing published models showed that the predicted
313 trough concentrations were the least biased (i.e. the median prediction error was the smallest) when
314 our model was used (Figure 3). However, due to unavailability of some covariates in our dataset, three
315 models were used without all of the covariates (APGAR score(15, 19), sepsis(19), co-medication with
316 dopamine(23)) included, which could explain their worse predictive performance.

317

318 The rich data in our model-building dataset (6.5 samples per patient) supported a 3-compartment
319 model, where the final estimates for the third compartment were: inter-compartmental clearance 0.3
320 L/h/70kg and peripheral volume of distribution of 148 L/70kg. Additionally, the terminal half-life for
321 a typical subject from the prospective evaluation dataset (weight 2.0 kg, PMA 34.9 weeks, PNA 6
322 days, MSCr 47.0 $\mu\text{mol/L}$, TSCr 66.4 $\mu\text{mol/L}$) was 189.7 hours. This could indicate uptake of
323 gentamicin into the renal cortex, and slow excretion from it (45); and is in agreement with previously
324 found evidence of deep tissue accumulation of gentamicin (26, 46).

325

326 Unfortunately many authors were unwilling or unable to share their data and we only managed to
327 obtain data from two (15, 21) out of eight identified studies for our model building dataset. We did
328 obtain one further subsequent dataset where assays from another pharmacokinetic study in neonates
329 also receiving gentamicin were used (32). Due to differences in model structure and parameterization,
330 it was not possible to extract relevant information for model building from the published reports.
331 However, in part because data from Nielsen *et al*(21) was of such high quality with multiple samples
332 per patient, our final model described both model building and the evaluation datasets well, as shown
333 in Figures 1 and 2. The histogram and the qq-plot of the conditional weighted residuals (data not
334 shown) confirmed that they follow a normal distribution. The final estimates for clearance (CL) and
335 volume of distribution (V) were (mean (standard error)) 6.21 (0.30) L/h/70 kg and 26.5 (1.11) L/70kg,
336 respectively (Table 2). The values of the PK parameters for a typical infant from the model-building
337 dataset (weight 2.12 kg, PMA 33.0 weeks, PNA 5.4 days, MSCr 78 $\mu\text{mol/L}$, TSCr 71.4 $\mu\text{mol/L}$) were
338 0.077 L/h and 0.80 L (and 0.10 L/h and 0.78 L for a neonate from the evaluation dataset) for CL and
339 V, respectively. These values are in agreement with estimates for clearance from previous neonatal
340 studies of gentamicin pharmacokinetics(13, 14, 18, 22-24). The reported value for CL from Nielsen *et*
341 *al*(21) may appear to be lower (0.026 L/h), but when our median demographic values were used in
342 their model, the CL became similar to our estimates (0.095 L/h). The final estimate for volume of
343 distribution is consistent with the estimate from Fuchs *et al*(23) and Botha *et al*(24), but it is not in
344 accordance with what was found by Garcia *et al* (20) (0.252 L). The probable reason for this is a

345 different studied population, as when the median weight from our dataset was used in their model, the
346 resulting V was 0.968 L, in agreement with our estimate.

347

348 We did not attempt to estimate the allometric power exponents and constants of the maturation
349 function as the PMA in the studied neonates (23.3-43.8 weeks) was insufficient to capture the age
350 when maturation is complete ($PMA_{50}=55.4$ weeks(5)); instead, these constants were fixed to the
351 values from another study in which the main focus was renal maturation(5). This type of scaling was
352 used to improve the model usefulness by allowing it to be extrapolated to different subpopulations
353 (for example, neonates with a different weight, or PMA). In addition to changes in clearance due to
354 long-term maturation that extends throughout gestation and into the first two years of life, we
355 attempted to capture the short-term changes in clearance that occur after birth regardless of gestational
356 age. A benefit of fixing the long-term maturation based on known relationships between PMA and
357 renal function was that this short-term maturation was apparent with our estimate of PNA_{50} of 40.8
358 hours, indicating that clearance rapidly increases over the first few days of life. In the first day of life
359 the clearance was at 37% of the value for a typical adult, and it reached 95% by the end of the first
360 month of age.

361

362 The typical serum creatinine (used in the model) was determined using SCr concentrations,
363 determined by the Jaffe assay, because the same method was used to determine SCr in the model-
364 building dataset. But to determine SCr in the evaluation dataset, assays, based on both the Jaffe and
365 the enzymatic methods, were used. However, the goodness-of-fit to the evaluation dataset and the
366 predictive performance of the model were good, therefore no correction factor was included. Also, the
367 enzymatic assay was only used in 16% of patients. Due to the range of the data that was used to
368 determine typical-for-PMA SCr the model can be used for a neonate with PMA <44 weeks or a term
369 neonate of <4weeks of age. The power exponent on the creatinine function was estimated to be -0.13,
370 meaning that if observed SCr and typical SCr were $70 \mu\text{mol/L}$ and $60 \mu\text{mol/L}$, respectively, clearance
371 would be 2% lower.

372

373 Large η -shrinkage indicates that the data do not contain enough information to make a reliable
374 individual estimation. And whilst the shrinkage was large on the peripheral volumes of distribution
375 (V2 and V3), it was relatively small on clearance (6.9%) (Table 2), which is important for making
376 predictions of trough gentamicin concentrations and AUC(0-t). The η -shrinkage was also relatively
377 small (15%) on the central volume of distribution (Table 2).

378

379 Although the main aim was to evaluate whether the model can predict trough concentrations, the
380 ability of the model to predict peak gentamicin concentration (from a randomly-selected non-peak
381 sample) was also examined. Cross-validations showed that the median prediction error (95% CI)
382 when predicting peaks was 0.16 (-4.76, 5.01) mg/L, indicating unbiased, but not very precise
383 predictions. This is perhaps not surprising, given that concentrations collected at a median time after
384 dose of 19.3 hours were used to predict concentrations at median 1h post dose. The prediction of
385 AUC(0-t) (also from one sample) was similarly unbiased (median prediction error 10.8 mg h/L), but
386 imprecise (95% CI: -24.9, 62.2 mg h/L) (Table 3). However, normalized RMSEs (by the range of
387 observed data) for peak and AUC(0-t) prediction were 7.0% and 17.6%, respectively; indicating that
388 considering the range of possible values, the precision is perhaps more acceptable. Target AUC(0-24)
389 or peak values have not been defined in neonates, and slow clearance and a narrow therapeutic index
390 mean that adjusting doses to target efficacy in this population may not be realistic. However, our
391 model does now give unbiased predictions of both metrics from an opportunistically collected single
392 sample, which should prove useful in future clinical research to define efficacy targets in this age
393 group. At present, due to their imprecision, these predictions (for peak concentration and AUC(0-t))
394 should currently only be used for research purposes, and not for dose adjustment.

395

396 Conclusion

397 A new gentamicin model has been developed and evaluated with prospectively collected data. We
398 used mechanistic covariate parameterization informed by principles of allometric size scaling, known
399 scaling of glomerular filtration maturation, and standardization for age-expected creatinine. This
400 “biological prior” information gave a model with better predictive performance on prospectively

401 collected external data than any previously published gentamicin model. Using this we developed a
402 software tool neoGent (see Supplementary material for provisional stand-alone version, and
403 implemented in the web TDM application TDMx (<http://www.tdmx.eu/>) (47)), which can be used to
404 predict when the trough concentration will fall below 2 mg/L and so guide the dosing interval.
405 Furthermore, peak concentration or AUC(0-24) from any post-dose sample can also be predicted with
406 little bias.

407

408

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424

425 **Transparency declarations**

426 None to declare.

427 **References**

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544 clinical routine. *Int J Antimicrob Agents* **45**:442-444.
- 545
- 546

547 **Tables and figures**

548

549 Table 1: A summary of demographics and dosing

	Model-building dataset	Evaluation dataset
n	205	163
weight (g) ^a	2.12 (0.53-5.05)	2.03 (0.48-5.05)
gestational age (weeks) ^a	34.0 (23.3-42.1)	34.3 (23.9-42.3)
postnatal age (days) ^a	5.4 (1-66)	6 (1-78)
postmenstrual age (weeks) ^a	33.0 (23.3-43.8)	34.9 (24-43.3)
females (%)	89 (43%)	68 (41.7%)
gentamicin samples per patient ^b	6.5	3.0
gentamicin concentration (mg/L) ^a	3.4 (0.3-37.6)	1.0 (0.1-13.2)
time after the dose (h) ^a	8.0 (0.02-54.1)	23.5 (0.08-79.7)
occasion ^a	2 (1-22)	2 (1-7)

550 Weight and gestational age are values at treatment initiation, the rest are values at time of gentamicin

551 sampling/dosing; an occasion was defined as a dose with subsequent gentamicin samples taken; day

552 of birth was defined as day 1; ^amedian (range); ^bmean

553

554 Table 2: Final parameter estimates from NONMEM output file and from the bootstrap analysis

	Parameters from the final model				Bootstrap analysis		
	mean	SE	%CV	η -shrinkage	median	2.5%ile	97.5%ile
CL (L/h/70kg)	6.21	0.30	-	-	6.14	5.47	6.75
θ_{SCr}	-0.13	0.055	-	-	-0.13	-0.25	-0.03
PNA ₅₀ (days)	1.70	0.30	-	-	1.68	1.15	2.30
V (L/70kg)	26.5	1.11	-	-	26.3	23.6	28.4
Q (L/h/70kg)	2.15	0.32	-	-	2.19	1.68	3.25
V2 (L/70kg)	21.2	1.50	-	-	20.9	17.9	24.2
Q2 (L/h/70kg)	0.27	0.047	-	-	0.28	0.19	0.38
V3 (L/70kg)	148	52.0	-	-	152	65.2	534
IIV on CL	0.175	0.038	41.8	6.9	0.170	0.104	0.254
IIV on V	0.112	0.032	33.5	15.2	0.113	0.057	0.190
covariance CL-V	0.116	0.030	-	-	0.115	0.060	0.184
IIV on V2	0.132	0.060	36.3	57.8	0.117	0.023	0.281
IIV on V3	0.177	0.216	42.1	85.0	0.114	0.00002	4.18
inter-occasion variability	0.014	0.007	11.8	-	0.013	0.001	0.029
residual error (proportional)	0.036	0.006	19.0	-	0.036	0.025	0.049
residual error (additive)	0.016	0.007	-	-	0.015	0.000002	0.032

555 CL is clearance, V is volume of distribution, Q is inter-compartmental CL, IIV is inter-individual

556 variability, SE is standard error obtained with NONMEM 7.3 covariance step, CV is coefficient of

557 variation.

558

559

560 Table 3: Summary of external evaluation with the evaluation dataset

dataset	Limit = 1 mg/L			Limit = 2 mg/L			PE (mg/L)	MPE (mg/L)	RMSE (mg/L)
	n correct (%)	OP	UP	n correct (%)	OP	UP			
paired + unpaired	214/254 (84.3)	20	20	242/254 (95.3)	10	2	0.0004 (-1.07, 0.84)	0.007	0.45
paired: study \geq 1mg/L	53/57 (93.0)	3	1	56/57 (98.2)	1	0	-0.04 (-0.57, 0.70)	-0.03	0.32
paired: study \geq 2mg/L	31/33 (93.9)	2	0	33/33 (100)	0	0	-0.08 (-0.50, 0.74)	-0.05	0.35
paired: study \geq 3mg/L	19/20 (95.0)	0	1	20/20 (100)	0	0	-0.06 (-0.56, 0.82)	-0.02	0.42
unpaired	136/161 (84.5)	14	11	155/161 (96.3)	5	1	0.02 (-1.11, 0.70)	-0.001	0.43
XV: paired: study \geq 3mg/L	478/502 (95.2)	12	12	460/502 (91.6)	21	21	-0.04 (-1.77, 3.03)	0.03	1.28
XV: peaks ^a	-	-	-	-	-	-	0.16 (-4.76, 5.01)	0.19	2.55
AUC(0-t) ^a	-	-	-	-	-	-	10.8 (-24.9, 62.2) ^b	14.5 ^b	30.2 ^b

561 Correct indicates that the predicted trough concentration agrees with the measured concentration (is
562 above/below the limit); OP is overprediction, UP is underprediction; PE is prediction error (median
563 (95% confidence interval)), MPE is mean prediction error, RMSE is root mean square error, XV is
564 cross-validation. Except ^a all results refer to trough prediction evaluation. ^b in mg h/L.

565
566

567 Figure legends

568

569 Figure 1: Observed versus population predicted gentamicin serum concentrations (top left for the
570 model-building dataset and bottom left for the evaluation dataset) and conditional weighted residuals
571 versus time after dose (top right for the model-building dataset and bottom right for the evaluation
572 dataset).

573

574 Figure 2: Visual predictive check of 1000 simulated concentration-time datasets from the final model,
575 using the model-building dataset (left) and the evaluation dataset (right). Points are the observations,
576 black lines are the 2.5th, 50th, and 97.5th percentiles, and the shaded areas are the 95% confidence
577 intervals of the corresponding predicted gentamicin concentrations.

578

579 Figure 3: Comparison of predictive performance of the developed model (shaded box plot) and
580 previously published neonatal gentamicin PK models.

581





