Cerebrospinal fluid compartmental pharmacokinetics of amikacin in neonates

K Allegaert, I Scheers, E Adams, G Brajanoski, V Cossey, BJ Anderson

1 Neonatal Intensive Care Unit, University Hospitals Leuven, Belgium
2 Laboratorium voor Farmaceutische Chemie en Analyse van Geneesmiddelen, Faculteit Farmaceutische Wetenschappen, Leuven, Belgium
3 Department of Anaesthesiology, University of Auckland, New Zealand

Correspondence to
Karel Allegaert, MD PhD
Neonatal Intensive Care Unit
Division of Woman and Child
University Hospital, Gasthuisberg,
Herestraat 49, 3000 Leuven, Belgium
Tel : 00-32-16-343210
Fax : 00-32-16-343209
E-mail: karel.allegaert@uz.kuleuven.ac.be

Key words
Amikacin – developmental pharmacology – ontogeny – allometry – cerebrospinal fluid kinetics

Running title: CSF kinetics of amikacin in neonates

Word counts : abstract 247, full text 2911, 2 tables, 7 figures

Acknowledgments
The clinical research of K Allegaert is supported by the Clinical Research Fund of the University Hospitals Leuven, Belgium. E Adams is a post-doctoral fellow of the Fund for Scientific Research - Flanders (Belgium)
Abstract

To describe and investigate covariate effects of cerebrospinal fluid (CSF) amikacin pharmacokinetics in neonates, CSF samples were prospectively collected from neonates in whom amikacin had been initiated before a diagnostic lumbar puncture was performed. CSF analysis (amikacin concentration, white blood count, glucose content, protein concentration) and amikacin therapeutic drug monitoring results (peak and trough concentrations) in serum were recorded. Correlations (Spearman rank) between CSF amikacin concentration and CSF white blood count (WBC), glucose and protein concentration were investigated.

There were 44 CSF amikacin concentrations and 83 serum samples available from 43 neonates (mean postmenstrual age 36 weeks, range 26-41 weeks, mean weight 2.43 kg, range 0.87-3.86 kg). Median time interval between initiation of amikacin administration and CSF sampling was 25 h (range 2.5 – 93.7 h). Median amikacin concentration in the CSF was 1.08 (0.34 – 2.65) mg/L, mean trough and peak amikacin concentrations in serum were 3.8 (SD 2.5) and 35.7 (SD 5.9) mg/L respectively. A correlation between CSF amikacin and CSF protein content (p < 0.01, r = 0.41, 95% CI 0.13, 0.63), but not with CSF WBC or CSF glucose were documented. A two compartment (central and CSF) linear disposition model was used to estimate population pharmacokinetics. The half-time for equilibration (Teq) between serum and CSF compartments was used as a measure of blood brain barrier permeability. The Teq was 7.58 h (CV 49.1%) with a partition coefficient of 0.103 (CV 26.4%). There was no relationship between Teq and CSF WBC, CSF glucose or CSF protein.
Introduction

The incidence of early-onset bacterial infection in the neonate varies from 0.5% to 1% of the total number of deliveries, but is significantly higher in preterm neonates where infection is causally linked with premature delivery in about 50% of cases (14). Late-onset, nosocomial bacterial infections occur in a significant number of preterm and term neonates during their stay in the neonatal intensive care unit. Bacteraemia, pneumonia, necrotizing enterocolitis and meningitis are the most frequent loci of infection in neonates. Empiric treatment for suspected infection in early neonatal life consists of combination therapy based on a time-dependent (beta-lactamases) and a concentration dependent (aminoglycoside) antibiotic coverage (e.g. *Streptococcus agalactiae*, *Escherichia coli*) while in late neonatal life *coagulase negative Staphylococcus* should also be covered.

The bactericidal effectiveness of amikacin is linked to intermittent, discontinuous peak concentrations, while renal side effects and ototoxicity relate to the average serum concentration that contributes to saturation of renal and cochlear cell binding sites. The combination of bactericidal effect and toxicity data has resulted in the concept of administration of relative larger doses with extended dosing intervals between consecutive doses. The safety and effectiveness of extended interval dosing of aminoglycosides in neonates has been reviewed (19). After the first days of postnatal life, size, postmenstrual age (PMA), renal function and ventilation contribute to renal drug clearance variability in neonates and subsequent serum concentration (1,2,3).

The effectiveness of amikacin for meningitis depends on concentrations in the central nervous system and this compartment is separated from the blood by the blood-brain barrier (BBB). Access of aminoglycosides to the central nervous compartment is limited in healthy children and adults (8,26,28), but CSF aminoglycoside exposure after systemic administration is more extended in children with meningitis than those without meningitis (24). Correlations between antibiotic concentration and CSF glucose, CSF leukocytosis or protein concentrations have been investigated (12,13,15,26,27). Ototoxicity relates to the mean aminoglycoside concentration in the central nervous system compartment but genetic predisposition to develop aminoglycoside-related ototoxicity has also been documented (4,10,17,23).
Observations on maturational amikacin CSF disposition in neonates are therefore of relevance (17,19). However, data on amikacin CSF concentrations are limited in neonates and amikacin CSF time-concentration profiles in preterm neonates have not been reported (9). In the current paper, time-concentration profiles of amikacin in serum and single CSF samples were collected to describe the relationship between serum and CSF concentration and to explore the impact of CSF inflammatory markers (leuko-, gluco- and protidorhachia) on this relationship during the first month of postnatal life in a cohort of (pre)term neonates.
Methods

Clinical characteristics and data reporting

From June 2005 until May 2007, CSF samples were prospectively collected from neonates in whom amikacin had been initiated before a diagnostic lumbar puncture was performed. Although this approach is debatable, lumbar puncture is often not performed as part of the initial neonatal sepsis evaluation (14). A CSF sample for amikacin concentration assay was taken when a lumbar puncture was performed for clinical indications, i.e. to exclude or document meningitis as judged by the attending neonatologist. Informed consent of the parents for the additional amikacin concentration assay of the CSF sample and for the study-related registration of clinical data was obtained.

The exact time between the start of administration of amikacin and CSF sampling was recorded. Clinical characteristics (weight, PMA, postnatal age (PNA), creatinaemia and ibuprofen administration, blood culture, hearing assessment), CSF analysis (white blood count, glucose content, protein concentration, culture) and amikacin therapeutic drug monitoring (TDM) results in serum were collected. Macroscopic blood during CSF sampling was an exclusion criterion. Data were reported by median and range or incidence. Correlations (spearman rank) between the amikacin CSF concentration and the CSF-WBC, CSF-glucose and CSF-protein concentration respectively were investigated.

Amikacin: drug administration and therapeutic drug monitoring

A PMA-based dosing chart was implemented in 2002 based on the suggestions of Langhendries et al. (16) (PMA < 28 wks: 20 mg/kg/42 hours, PMA 28-30 wks: 20 mg/kg/36 hours, PMA 31-33 wks: 18.5 mg/kg/30 hours, PMA 34-37 wks: 17 mg/kg/24 hours, PMA > 37 wks: 15.5 mg/kg/24 hours) with an additional dosing interval increase of 6 hours if ibuprofen was co-administered or if neonates had suffered asphyxia or hypoxia (1). Amikacin (Amukin® 50 mg/ml paediatric vial, Bristol Myers Squibb Belgium) was given as an intravenous infusion over 20 minutes by syringe driver (SIMS Graseby®, Watford, United Kingdom). Blood samples for TDM were collected by arterial line or venous puncture just before (“trough”) and one hour after initiation of administration (“near peak”) of the second dose of amikacin, approximately 40 minutes after the 20 minutes intravenous infusion (1,2).
Amikacin assay in serum and cerebrospinal fluid

Amikacin serum concentration measurements were performed using fluorescence polarization immunoassay (TDx – Abbott) in the hours following sample collection and were reported in mg/L. Drug recovery from extraction was 100% (SD 2.6%) over the tested concentration range 3-35 mg/L. Precision was assessed at 5, 15 and 30 mg/L. These yielded a within run CV 1.37-2.09%, between day CV 0-1.74% and a total CV of 2.6-3.2%. The minimal quantifiable concentration was 0.8 mg/L defined by a CV of less than 20% (Abbott information). Coefficient of variation (CV) was typically <5% based on internal quality assessment covering the concentration range up to 50 mg/L (1,2).

Amikacin concentration in CSF was determined by high performance liquid chromatography (HPLC) with pulsed electrochemical detection, based on adaptations from methods described in literature (29). The lower limit of quantification for amikacin was 0.06 mg/L and good linearity was obtained with correlation coefficient greater than 0.99 in the concentration range from 0.06 mg/L to 4 mg/L. Recovery of amikacin reference solutions (0.2 and 2 mg/L) was 96.6 and 99.8 % respectively.

Population pharmacokinetics

A two-compartment (central and CSF) linear model was used to fit PK data. Population parameter estimates were obtained using non-linear mixed effects modelling (NONMEM) (21). This model accounts for population parameter variability (between and within subjects) and residual variability (random effects) as well as parameter differences predicted by covariates (fixed effects). The population parameter variability in model parameters was modelled by a proportional variance model. A proportional term was used to characterise the residual unknown variability. The population mean parameters, between subject variance and residual variance were estimated using the first order conditional interaction estimate method differential equations using ADVAN6 TOL5 of NONMEM V. Convergence criterion was 3 significant digits. A Compaq Digital Fortran Version 6.6A compiler with Intel Celeron 333 MHz CPU (Intel Corp., Santa Clara, CA) under MS Windows XP (Microsoft Corp., Seattle, WA) was used to compile NONMEM.

Differential equations were used to determine parameter estimates.

$$\frac{dC_{serum}}{dt} = ratein - \left( CL \cdot C_{serum} \right) / V$$
Where *ratein* is the rate of amikacin administration (mg/h), *CL* is total body clearance (L/h), *V* is the volume of distribution (L) and *Cserum* is the concentration in the serum. The second compartment was used to model CSF concentration (*Ccsf*). These two compartments were linked using an equilibration rate constant (*Keq*). A partition coefficient (*PC*) was used to describe the ratio between CSF and serum concentration at steady-state concentration

\[
\frac{dC_{csf}}{dt} = Keq \cdot (C_{serum} \cdot PC - C_{csf})
\]

The rate constant *Keq* can be described using an equilibration half time (*Teq*)

\[
Teq = \frac{\ln(2)}{Keq}
\]

The population parameter variability is modelled in terms of random effect (*η*) variables. Each of these variables is assumed to have mean 0 and a variance denoted by *ω*², which is estimated. We report the estimate of *ω* for each variability component. The covariance between two elements of *η* (e.g., *CL* and *V*) is a measure of statistical association between these two variables. Their covariance is related to their correlation i.e.

\[
R = \frac{covariance}{\sqrt{\omega_{CL}^2 \cdot \omega_{V}^2}}
\]

The covariance of clearance, distribution volume, partition coefficient and equilibration half-time variability was estimated.

The relationship between *Teq* and CSF WBC, CSF glucose and CSF protein concentration was explored with both linear and exponential functions

\[
F_x = slope_x \cdot (Obs_x - Obs_{average})
\]

\[
F_x = EXP(slope_x \cdot Obs_x)
\]

Where *F*ₙ is the function applied to *Teq* for the covariates CSF WBC, CSF glucose and CSF protein concentration (*x*), *slope*ₙ describes the gradient of this relationship, *Obsₙ* is the observation for that covariate and *Obs_{average}* is the average observation.
The quality of fit of the pharmacokinetic model to the data was judged by NONMEM’s objective function and by visual examination of plots of observed versus predicted concentrations. Models were nested and an improvement in the objective function was referred to the Chi-squared distribution to assess significance e.g. an objective function change (OBJ) of 3.84 is significant at $\alpha=0.05$ with one additional parameter in the model.
Results

There were 44 CSF and 83 TDM serum observations available from 43 neonates. Demographic data are presented in Table 1. Median time interval between initiation of amikacin administration and CSF sampling was 25 h (range 2.5 – 93.7 h). The median amikacin concentration in the CSF was 1.08 (0.34 – 2.65) mg/L, mean trough and peak amikacin concentrations in serum were 3.8 (SD 2.5) and 35.7 (SD 5.9) mg/L respectively. A significant correlation between the amikacin CSF concentration and the CSF protein content (p <0.01, r = 0.41, 95 % CI 0.13 to 0.63, Figure 1), but not with CSF-glucose (p = 0.57, r = -0.08, 95 % CI –0.37 to 0.22) or CSF-WBC (p = 0.37 , r = 0.14 , 95 % CI –0.17 to 0.41).

Blood-culture proven infections were documented in 26/44 cases [Escherichia coli (n = 10), Streptococcus agalactiae (n = 7), Staphylococcus epidermidis (n = 5), Streptococcus viridans (n = 1), alfa hemolytic Streptococcus (n = 1), Micrococcus (n = 1), Sphingomonas paucimobilis (n = 1)]. CSF culture proven infections were documented 6/44 cases [enteroviral infections (n = 2), Streptococcus agalactiae (n = 3), Escherichia coli (n = 1)].

Hearing evaluation, based on brainstem evoked response audiometry (BERA) was performed before discharge in 39 of 55 neonates, 4 additional neonates were evaluated with automated auditory brainstem response (ALGO test) at home, one baby died (Escherichia coli infection) before hearing evaluation. Of the 54 neonates tested, one neonate had a unilateral hearing loss of 40-50 dB.

Population parameter estimates and their variability are shown in Table 2a. The correlation of between subject variability for CL, V, Teq and PC is shown in Table 2b. Figures 2 and 3 demonstrate the quality of fit of the pharmacokinetic data. Individual concentration predictions are based on values of maximum a posteriori (MAP) Bayesian estimates of the parameters while predicted population concentrations are based on population parameters and covariate information. The population estimate for Teq was 7.58 h (CV 49.1%, SE 29.9%). There was no relationship between Teq and white blood count, glucose content or protein concentration. CSF markers and their relationship to Teq are shown figuratively for CSF-WBC (Figure 4), CSF-Glucose (Figure 5) and CSF-protein (Figure 6). The temporal relationship between amikacin concentration in serum and in CSF for a typical 2.5 kg 36 week PMA neonate given amikacin 17 mg/kg is shown in Figure 7.
Discussion

A parameter describing the amikacin half time between serum and CSF compartments (Teq) in neonates was estimated (7.58 h, CV 49.1 %) and subsequently used to investigate the impact of covariates on CSF permeability. We hypothesized to find two distinct Teq group: short Teq implying rapid movement of drug across the BBB and a long Teq suggesting limited permeability, dependent on the meningeal inflammation. This hypothesis was not confirmed since we were unable to link this Teq parameter to any biochemical indicator of meningeal inflammation or meningitis.

A single intramuscular administration of amikacin 75 mg resulted in CSF concentrations below 0.5 mg/L for up to 8.5 hours in healthy adults (7) while Yow et al. documented that amikacin concentrations were between < 0.2 and 2.7 mg/L in 10 neonates following intravenous administration of 10 mg/kg amikacin (28). In an attempt to reduce the number of inconclusive assay results below the lower limit of quantification, a specific HPLC method with a lower limit of quantification for amikacin of 0.06 mg/L was used in the current study (29). Such an approach allowed investigation of amikacin disposition at the anticipated low concentrations. Based on this HPLC method, a median amikacin CSF concentration 1.08 mg/L (range 0.34-2.65 mg/L) was documented in neonates, but the absence of any correlation between markers of CSF inflammation and Teq are in contrast to other reports in children and adults where CSF amikacin concentrations appear increased in the presence of meningitis (7,8,13,24,26,28).

We did note a correlation between the amikacin CSF concentration and the CSF protein content (Figure 1), but are uncertain about the significance of this finding since CSF protein concentration also displays age-dependent differences. Mean CSF protein concentration is 900 mg/L in term and 1150 mg/L in healthy preterm neonates, with a PMA dependent decrease in mean CSF protein concentration in former preterm neonates from 1770 mg/L at 26-28 to 1170 mg/L at 38-40 weeks PMA (25) while amikacin clearance increases with increasing PMA (1,2,9). In the current cohort of preterm and term neonates, we also were able to document a significant inverse correlation between CSF protein and PMA (r = - 0.36, 95 % CI - 0.59 to - 0.07, p < 0.05). Consequently, we might expect higher concentrations in younger infants because more amikacin is available in serum to cross the BBB independent of maturational or disease related aspects of this barrier.
CSF WBC higher than 21 cells per mm$^3$ had sensitivity at 79% and specificity at 81% for the diagnosis of neonatal meningitis in a recently published cohort of 9111 neonates (14). Using this WBC threshold (21 cells per mm$^3$) as a dichotomous variable, we were unable to show any significant difference in amikacin CSF concentration in this cohort of neonates (1.16 versus 1.15, p = 0.95). Gaillard et al. documented amikacin CSF concentrations in a cohort of 16 children (range 4 months to 8 years) with community acquired bacterial meningitis given intravenous amikacin (7.5 mg/kg twice daily) (13). An inverse correlation with CSF glucose level but not with the CSF protein concentration or leukocyte count was demonstrated. These findings are similar to observations described by Trujillo et al. in paediatric patients with meningitis (24).

We can only speculate why the amikacin Teq correlates poorly with CSF WBC, glucose or protein concentration in neonates in contrast to observations in children (5, 14), but the absence of robust covariates for Teq in neonates in line with the cohort of 9111 neonates in whom culture proven meningitis was neither diagnosed nor reflected accurately by CSF glucose or by CSF protein (14). Blood and CSF glucose levels are relatively lower in neonates and there is a more blunted distress-related surge in glycaemia and as mentioned earlier, the protein content of CSF in neonates is higher compared to children in the absence of inflammation (25). The additional meningitis-related increase in protein content of the CSF may be less robust in neonates when compared to children (5, 14).

In the current study, CSF samples were taken when neonates were already treated with antibiotics. This was a specific pre-requisite for inclusion in this pharmacokinetic study. It is to be anticipated that such an approach results in a more limited number of positive bacterial CSF cultures, but the clinical practice not to routinely perform lumbar puncture before initiation of antibiotics when the a priori risk for a meningitis is perceived to be low or when the clinical condition of the patient necessitates an additional delay before CSF sampling has been reported in literature (14).

The number of CSF observations (n = 44) is the largest cohort of data reported in neonates. Consequently, we were able to investigate the partition coefficient. Studies in rabbits suffering meningitis after intracisternal injection of *Escherichia coli* suggest a PC of 0.1-0.23 at 8 h, but this ratio increased with time over the study period (22). These rabbit data are consistent with our own finding of a PC of 0.103. The Teq of 7.58 h suggests the mean peak CSF concentration will rise from
1.5 mg/L after the first dose (Figure 7) to a mean peak concentration of 2.5 mg/L in the CSF after 3 doses of systemic amikacin. A higher mean amikacin serum/CSF ratio of 3:1 has been reported from one CSF sample taken up to 7 hours after dose in children with bacterial meningitis (24). This ratio, however, may change at different stages of the illness.

The current observations on amikacin CSF disposition in neonates are also of relevance to assess potential risk factors for ototoxicity related to CSF disposition of this drug (17,19). Ototoxicity relates to the average concentration that contributes to saturation of cochlear cell binding sites (4) and the antibiotic penetration of the middle ear with inflammation is increased and this increased permeability may also contribute to hearing loss after meningitis (20). Epidemiologic studies in cohorts of neonates in whom aminoglycosides were administered for a variety of infectious diseases could not document an independent impact of this drug when an extended time interval approach was used (4,6,10,17). In contrast, meningitis is a well know risk factor for ototoxicity (6,11).

The current observations with the absence of any link between the amikacin Teq and indicators of meningitis in neonates, suggest that the ototoxicity after meningitis relates more to the disease process itself than to an enhanced amikacin permeability of the blood-brain barrier. The longer Teq estimates result in progressive increase in amikacin CSF concentration during repeated administration in (pre)term neonates while the age-dependent higher concentrations in both amikacin and CSF protein (Figure 1) suggest that aminoglycoside-dependent ototoxicity is more likely to occur in preterm neonates. Based on these pharmacokinetic observations, it is to be anticipated that preterm neonates after repeated dose administration are most likely to develop aminoglycoside mediated ototoxicity.
References


Table 1

Clinical characteristics and CSF observations in 43 neonates in whom 44 CSF samples were collected.

Data are reported by median and range or incidence.

**Clinical characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median</th>
<th>Range</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>2.430</td>
<td>(0.865 – 3.860)</td>
<td>kg</td>
</tr>
<tr>
<td>Gestational age</td>
<td>35</td>
<td>(25 - 41)</td>
<td>weeks</td>
</tr>
<tr>
<td>Postmenstrual age</td>
<td>36</td>
<td>(26 - 41)</td>
<td>weeks</td>
</tr>
<tr>
<td>Postnatal age</td>
<td>3</td>
<td>(1 - 29)</td>
<td>days</td>
</tr>
<tr>
<td>Creatinaemia</td>
<td>0.86</td>
<td>(0.48 – 1.26)</td>
<td>mg/L</td>
</tr>
</tbody>
</table>

**Cerebrospinal fluid analysis**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Median</th>
<th>Range</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin concentration</td>
<td>1.08</td>
<td>(0.34 – 2.65)</td>
<td>mg/L</td>
</tr>
<tr>
<td>White blood count</td>
<td>13</td>
<td>(0 – 4177)</td>
<td>/µL</td>
</tr>
<tr>
<td>Glucose</td>
<td>50</td>
<td>(10 – 90)</td>
<td>mg/dL</td>
</tr>
<tr>
<td>Protein</td>
<td>1 116</td>
<td>(10 – 4 970)</td>
<td>mg/L</td>
</tr>
</tbody>
</table>
Table 2a. Amikacin Population Pharmacokinetic Parameter Estimates

(PPV is the population parameter variability expressed as the square root of its variance, SE is the standard error of the estimate)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>PPV</th>
<th>%SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (L/h)</td>
<td>0.0901</td>
<td>0.563028</td>
<td>8.6</td>
</tr>
<tr>
<td>V (L)</td>
<td>1.07</td>
<td>0.364692</td>
<td>5.9</td>
</tr>
<tr>
<td>Residual Unidentified Variability</td>
<td>0.08%</td>
<td></td>
<td>12.7</td>
</tr>
<tr>
<td>Teq (h)</td>
<td>7.58</td>
<td>0.490918</td>
<td>16.2</td>
</tr>
<tr>
<td>PC</td>
<td>0.103</td>
<td>0.263818</td>
<td>6.7</td>
</tr>
<tr>
<td>Residual Unidentified Variability</td>
<td>0.0002%</td>
<td></td>
<td>31.7</td>
</tr>
</tbody>
</table>

Table 2b. The correlation of between subject variability for CL, V, Teq and PC

<table>
<thead>
<tr>
<th></th>
<th>CL</th>
<th>V</th>
<th>Teq</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>0.843</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teq</td>
<td>-0.327</td>
<td>0.181</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>-0.145</td>
<td>-0.132</td>
<td>-0.381</td>
<td>1</td>
</tr>
</tbody>
</table>
Legends

Figure 1. The correlation (spearman rank) between amikacin CSF concentration and the CSF protein concentration based on 44 CSF samples collected in neonates (p <0.01, r = 0.41, 95 % CI 0.13 to 0.63).

Figure 2. Amikacin serum data (A) Individual Bayesian concentration predictions based on values of the parameters for the specific individual are compared to observed. (B) Population predictions are compared to observed. The line x=y is the line of identity.

Figure 3. Amikacin CSF data (A) Individual Bayesian concentration predictions based on values of the parameters for the specific individual are compared to observed. (B) Population predictions are compared to observed. The line x=y is the line of identity.

Figure 4. Absence of any significant relation between WBC and Teq.

Figure 5. Absence of any significant relation between CSF protein concentration and Teq.

Figure 6. Absence of any significant relation between CSF glucose concentration and Teq.

Figure 7. Time-concentration profiles (with 95%CI) for serum and CSF after amikacin 17 mg/kg in a 2.5 kg 36 week PMA neonate.
Figure 1

![Graph showing the relationship between Protein (mg/L) and CSF amikacin concentration (mg/L).](image-url)
Figure 2A

![Graph showing individual Bayesian prediction vs. observation for mg/L.]

Figure 2B

![Graph showing population prediction vs. observation for mg/L.]

observation (mg/L) vs. individual Bayesian prediction (mg/L) for Figure 2A.

observation (mg/L) vs. population prediction (mg/L) for Figure 2B.
Figure 3A

![Graph showing individual Bayesian prediction vs observation](image)

Figure 3B

![Graph showing population prediction vs observation](image)
Figure 7

![Graph showing plasma and CSF concentration over time.](image-url)