Trial@home for children: novel non-invasive methodology for the pediatric clinical trial of the future

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CHAPTER 14

Saliva as sampling matrix for therapeutic drug monitoring of gentamicin in neonates: A prospective population pharmacokinetic- and simulation study

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Abstract

INTRODUCTION Therapeutic drug monitoring (TDM) of gentamicin in neonates is recommended for safe and effective doses and is currently performed by blood sampling, which is an invasive and painful procedure. In this study, feasibility of a non-invasive gentamicin TDM strategy using saliva was investigated.

METHODS This was a multicenter, observational cohort study including 54 neonates. Any neonate treated with gentamicin was eligible for the study. Up to 8 saliva samples were collected per patient at different time-points. Gentamicin levels in saliva were determined with liquid chromatography coupled with tandem mass-spectrometry. A population PK model was developed using Nonlinear Mixed-Effects Modeling (NONMEM) to describe the relation between gentamicin concentrations in saliva and blood. Simulations were performed to evaluate the efficacy of gentamicin TDM using saliva versus blood.

RESULTS Blood PK was described with an earlier published model. Time profiles of salivary concentrations were quantified using a one-compartment saliva model with first-order input (k^{13} 0.023 h^{-1}) and first-order elimination (k^{30} 0.169 h^{-1}). Inter-individual variability of k^{30} was 38%. Post menstrual age (PMA) correlated negatively with both k^{13} and k^{30}. Simulations demonstrated that TDM with 4 saliva samples was effective in 81% of the simulated cases, versus 94% when performed with 2 blood samples.

CONCLUSION TDM of gentamicin using saliva is feasible, though TDM with 2 blood samples seems to perform better.

Introduction

Neonates admitted to the neonatal intensive care unit (NICU) have a high risk for bacteremia or sepsis due to premature birth, low birth weight and indwelling central venous lines.[1] Intravenous treatment with the aminoglycoside gentamicin provides good gram-negative coverage and is part of the first line antibiotic treatment protocols in many NICU's.

Gentamicin has a narrow therapeutic index, with oto- and nephrotoxicity as its possible concentration-dependent adverse drug events. Neonates are especially vulnerable for adverse events and adequate dosing is complicated by the continuous changes in body composition and clearance caused by a changing kidney function and maturation. Gentamicin concentrations can therefore be unpredictable and therapeutic drug monitoring (TDM) is necessary to ensure adequate plasma concentrations. TDM requires repeated blood sampling, which is invasive, painful and may contribute to clinical anemia or infection.[2] As a result, TDM by blood sampling is complicated in neonates[3], possibly leading to suboptimal individual gentamicin doses and thereby causing a decrease in therapeutic efficacy and/or an increased risk of adverse events.

Therefore, there is a clinical need for non-invasive TDM methods in neonates which would allow for an increased sampling frequency and for safer and more efficacious dosing, while simultaneously decreasing the burden of blood collection. Previous studies have shown that the use of saliva as a matrix for TDM is feasible for several anti-epileptic drugs and caffeine.[4,5] Analyses of salivary gentamicin concentrations and other aminoglycosides during intravenous treatment of children and adults have been published with varying results. Some studies reported a good correlation between gentamicin saliva and blood concentrations, while others reported undetectable aminoglycoside concentrations in saliva.[6-9] So far, no such studies have been performed in a neonatal population.

The aim of this study was to prospectively measure salivary gentamicin concentrations and to compare these to the concentrations in routinely drawn blood samples in neonates.

Materials and methods

Study design

This was a multi-center, prospective, observational pharmacokinetic study conducted in the Emma children's hospital (Amsterdam UMC, Amsterdam, the Netherlands) and the

Juliana children's hospital (Haga Hospital, den Haag, the Netherlands). Gentamicin concentrations were prospectively measured in saliva and compared with blood concentrations, obtained as part of routine TDM. The local ethics committee of the Amsterdam UMC approved this study (number 2018_193). Local feasibility was tested and approved for the Haga hospital. The study was registered in the Dutch Trial Registry (NTR, NL7211).

Subjects

Inclusion of subjects took place between October 8TH 2018 and March 4TH 2020. Any neonate that was treated with gentamicin according to local clinical guidelines was eligible for the study. Patients were included in this study after signed informed consent of both parents was obtained. For the analysis, three distinct subgroups based on gestational age (GA) were pre-specified and treated with intravenous gentamicin according to local dosing protocols: 1) Neonates with GA < 32 weeks (5 mg/kg/48 hours); 2) neonates with GA ≥ 32 weeks - 37 weeks (5 mg/kg/36 hours); and 3) neonates with GA ≥ 37 weeks (4 mg/kg/24 hours at Emma Children's hospital and 5 mg/kg/36 hours at Juliana Children's Hospital). Clinical data were obtained from the digital medical files of the patients (sex, GA, postnatal age (PNA), postmenstrual age (PMA), birth weight (BW), current body weight (WT), perinatal asphyxia, therapeutic hypothermia, and concomitant medication).

Sample sizes could not be accurately calculated, due to absence of data on expected effect-size and variability of estimated saliva PK-parameters. A total of 60 patients (20 patients per group) were scheduled to be enrolled into the study, since 20 patients per subgroup are deemed sufficient for NONMEM analysis as a rule of thumb.[10]

Sample collection

Saliva samples were collected using SalivaBio Infant's Swabs (Salimetrics, Carlsbad, CA, USA). Swabs were placed in the cheek pouch of the neonates for approximately 90 seconds, according to the manufacturer's instructions.[11] After collection, swabs were centrifuged at 4,000 RPM for 5 minutes and extracted saliva was stored at -80° C. Up to 8 saliva samples were collected per patient using an opportunistic sampling schedule. Saliva samples were collected up to 48 hours after the last gentamicin dose. Adsorption of gentamicin to the swab was found to be less than 3.1% at the low concentration level and 8.2% at the high concentration level and therefore below the predetermined acceptable percentage of 15%. Gentamicin concentrations in blood were collected from routine peak and trough TDM measurements (0.5h post infusion (infusion duration 0.5h) and/or between 6-24h post-dose). Additional blood levels were determined in residual material, when available.

Bio-analytical assay

The major components of gentamicin (C1, C1a and C2) were quantified in saliva samples using a previously published LC-MS/MS method.[12] In short, the accuracy and within run precision at the lowest level of quantification (LLOQ) were 118% and 10.2%, respectively. The accuracy and precision were 98.4% and 3.3%, respectively, at the middle level of quantification (MLQ). At the upper limit of quantification (ULOQ), accuracy was 98.7% and precision was 3.2%. Accuracy and precision were within the predetermined acceptable ranges (LLOQ: ±20%, MLQ: ±15%, ULOQ: ±15%). The LLOQ was 0.056 mg/L and minimal sample volume was 10 µl.

Pharmacokinetic analysis

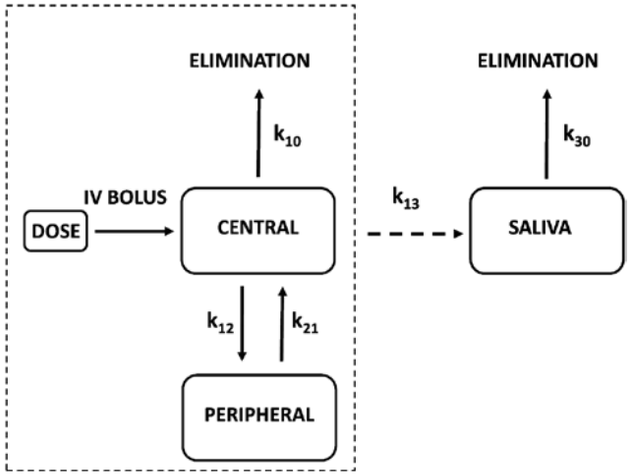
Data handling, data visualization and descriptive statistics were performed using R statistics version 4.0.2. A population PK (POP-PK) model was developed using nonlinear mixed-effects modeling (NONMEM), as implemented in NONMEM version 7.4.0 (ICON Development Solutions, Dublin, Ireland). Gentamicin concentrations in blood and saliva were logarithmically transformed.

An integrated model describing gentamicin in blood and saliva was developed using a stepwise modeling approach. First, blood PK data was described using a previously published model by Fuchs *et al*.[13], fixing the published PK parameters. The control stream for this model was provided by the original authors. This was a 2-compartment model with inter-individual variability (IIV) on clearance (CL) and central volume of distribution (V_c). Model performance was evaluated through the assessment of goodness-of-fit (GOF) plots and visual predictive checks (VPCS).

Following estimation of the individual blood PK parameters, an additional compartment describing the salivary gentamicin concentrations was appended to the model, effectively developing a 3-compartment model. The conceptual model for gentamicin in blood and saliva has been depicted in *Figure 1*. The first-order transport rate from the central

(blood) compartment to the saliva compartment was expressed as k^{13}, whilst the first-order rate of gentamicin elimination from the saliva compartment was expressed as k^{30}. No transport from the saliva compartment to the central and peripheral compartments was modeled, since the oral bioavailability of gentamicin is negligible.[14] Central gentamicin mass decrease due to transport from the central compartment to the saliva compartment was assumed to be negligible as well, as this was expected to be proportionally diminutive compared to the total amount of gentamicin in the central compartment, similar to a hypothetical effect compartment model.[15] Both fixed and random effects of rate constants k^{13} and k^{30} were estimated using the ADVAN6 subroutine in NONMEM. Model parameters were evaluated by assessing changes in the objective function value (OFV) and diagnostic plots. A δOFV of -3.81 corresponds with p = 0.05, which was the significance level for inclusion of any parameter. Gentamicin concentrations in saliva below LOQ were included in the model using the M3-method.[16] First, the structural model was estimated, describing the relations between parameters. Thereafter, the error model was developed, describing the residual error structure in the model. Finally, the covariate model explains part of the variability based on covariates.

Figure 1. Conceptual model for gentamicin PK in blood and saliva. Within dashed lines: Gentamicin in blood. Dose is administered as an iv bolus to the central compartment. k^{12}: Transport rate from central to peripheral compartment. k^{21}: Transport rate from peripheral compartment to central compartment. k^{10}: Elimination rate from the central compartment. Outside dashed lines: gentamicin PK in saliva. k^{13}: Transport rate from central compartment to saliva compartment. The dashed arrow signifies that gentamicin loss from the central compartment is assumed to be negligible. k^{30}: : Elimination rate from saliva.



GA, PNA, PMA, BW, WT, sex, perinatal asphyxia, therapeutic hypothermia, and concomitant drugs were evaluated as covariates for this model. Covariate analysis was performed with stepwise forward inclusion (α=0.05) and backwards elimination (α=0.01). Continuous covariates were included in the model as a power equation function (*Eq.1:* $p = \theta_p * (cov/median)\theta_{cov}$).

Parameter p was calculated from typical parameter θ_p, multiplied with the fractional deviation from the median value of the covariate. The magnitude of the covariate effect was estimated as θ_{cov}. Dichotomous covariates were coded in NONMEM as shown in *Eq.2* (*Eq.2*: $p = \theta_p + cov * \theta_{cov}$).

Dichotomous covariates could take the value of either 0 or 1. Reference parameter value θ_p was estimated, and the parameter difference between covariate parameters was estimated as θ_{cov} to calculate parameter p. Assessments of diagnostic tools, such as GOF plots, parameter residual standard error (RSE), η-shrinkage and ε-shrinkage were used for model evaluation during all steps. Bootstrap analyses (n=1000), as well as the simulation based prediction-corrected VPCS (PCVPC) were employed for assessment of the model robustness and internal validation of the final model.[17]

TDM performance simulation

R version 4.02 and the mrgsolve[18] package were used for Monte Carlo simulations. A simulation cohort (n = 3000) with a uniform distribution of GA and corresponding WT[19] was prepared, and a single administration of 5 mg/kg/48h (GA < 32 weeks), 5 mg/kg/36h (GA ≥ 32-37 weeks) or 4 mg/kg/24h (GA ≥ 37 weeks) was simulated for each subject in accordance with Dutch dosing guidelines.

For blood and saliva TDM, different sampling schedules were simulated with measurements at different time-points after the first dose. First, a schedule with a single intermediate (14h post-dose) sample was simulated and the performance of this schedule in the context of TDM was appraised. Second, a two-sample schedule was evaluated with a peak- (1.5h for blood and 3h for saliva samples) and trough (0.5h before next dose) sample. Next, the combination of peak-, intermediate- and trough samples was evaluated. Finally, schedules were evaluated in which samples were added (at 7 h post-dose; at 7 - 18 h post-dose; at 1 h pre-dose and 7 - 18 h post-dose) were evaluated. Bayesian maximum a posteriori (MAP) optimization was used to estimate the empirical Bayes estimates of the individual CL, VC and k^{30} for each subject based on the simulated samples.[20] Then,

based on the estimated CL and VC, the peak- and trough concentrations were estimated for each subject, who then entered a basic decision rule optimizing the dose to reach a targeted peak concentration between 9-11 mg/L and trough concentration < 0.8 mg/L after the third dose. Target ranges were deliberately set stricter compared to clinical guidelines (peak 8-12 mg/L and trough < 1 mg/L) to account for residual error in the estimations. For each subject, two additional dose intervals of gentamicin were simulated after dose adjustment. Finally, the proportions of subjects with true peak- and trough concentrations within clinical guideline reference ranges (target attainment) after the third dose were calculated. Simulation runs were performed for blood TDM, saliva TDM, model-based dose optimization (using the blood model of Fuchs *et al.*) and 'no TDM' (standard dosing regimen during the complete simulation period). The proportion of subjects with target attainment after each simulated scenario was calculated and compared in order to appraise the added value of saliva and blood TDM.

Table 1. Demographic characteristics of the study population

Demographic	Value
Enrolled patients - n	54
Males - n (%)	31 (57.4)
GA in weeks - median (range)	34.8 (24.3 - 41.7)
< 32 weeks - n (%)	21 (38.9)
32 - 37 weeks - n (%)	13 (24.1)
≥ 37 weeks - n (%)	20 (38.9)
PMA in days - median (range)	244.2 (170.5 - 294.2)
PNA in days - median (range)	1.5 (0.3 - 6.8)
Birth weight in kg - median (range)	2.4 (0.7 - 4.5)
Actual weight in kg - median (range)	2.4 (0.7 - 4.3)
Total saliva samples - n (%)	267 (100)
Analyzed - n (%)	194 (72.7)
Failed - n (%)	73 (27.3)
Analyzed saliva samples per patient - median (range)	3 (1 - 8)
Plasma samples - n	99
Plasma samples per patient - median (range)	2 (1 - 4)
Oro-esophageal congenital anomalies - n	1
Controlled hypothermia - n	3
Perinatal asphyxia - n	3

GA: Gestational age. PNA: Postnatal age. PMA: Postmenstrual age.

Results

Demographic characteristics

Table 1 depicts the demographic characteristics of the included patients. In total 54 of the planned 60 neonates were enrolled in this study due to its early termination during the SARS-COV-2 pandemic, which posed restrictions for clinical research. A total of 267 saliva samples were collected during the study, though 79 (29.6%) saliva samples could not be analyzed because of low sample volume or contamination of the saliva with blood. The demographic characteristics were representative for the population of neonates treated with gentamicin.

Gentamicin pharmacokinetics in blood

Model diagnostic figures indicated that the model provided by Fuchs *et al.* could adequately describe the blood PK data of the study population (*Figure 2*). The model was used to estimate individual blood PK and served as a basis for the construction of the saliva model.

Gentamicin pharmacokinetics in saliva

The salivary PK of gentamicin was described by adding a saliva compartment to the blood model (*Figure 1*). For the structural model, a k^{13} of 0.036 h^{-1} and k^{30} of 0.267 h^{-1} were estimated, as well as IIV on k^{30} of 63.6% (*Table 2*). The estimate of IIV on k^{30} had a η-shrinkage of 17%. Residual error was described with a logarithmic proportional error model, which was estimated as 58.4%. Since 14% of all analyzed saliva samples were found to be below the LLOQ, these measurements were accounted for with the M3 method.[16] Inclusion of additional transit compartments to account for lag in saliva uptake did not improve the model fit; neither did 1^{ST} order transport from the peripheral to the salivary compartment. Though it was also possible to successfully fit a model with estimations for both IIV on k^{30} and k^{13}, η-shrinkage on these parameters was respectively 56% and 34%. These levels of η-shrinkage were unacceptable and therefore that model was rejected.[21]

Stepwise forward inclusion of PMA as a power function covariate on k^{13} led to the largest decrease in OFV (δOFV = -61.33). PMA was also included as a covariate on k^{30} as a

Figure 2. Model diagnostic plots blood PK. A: Population predictions versus observed concentrations in blood. B: Individual predictions versus observed concentrations in blood. C: Population predictions versus conditional weighted residuals (CWRES). D: Time versus CWRES. E: prediction corrected VPC

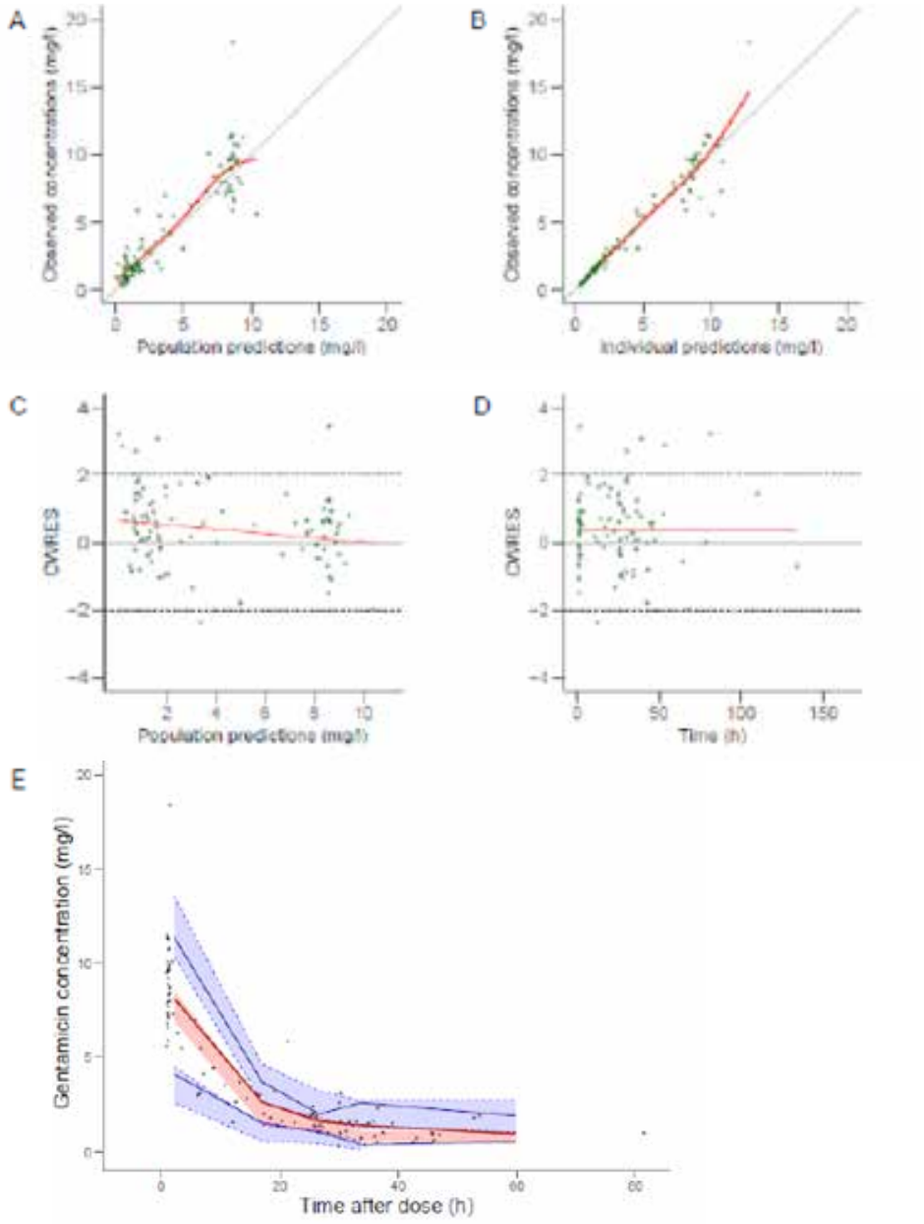


Figure 3. Goodness of fit plots of the final model. A: Population predictions versus observed concentrations in saliva. B: Individual predictions versus observed concentrations. C: Population predictions versus conditional weighted residuals (CWRES). D: Time versus CWRES.

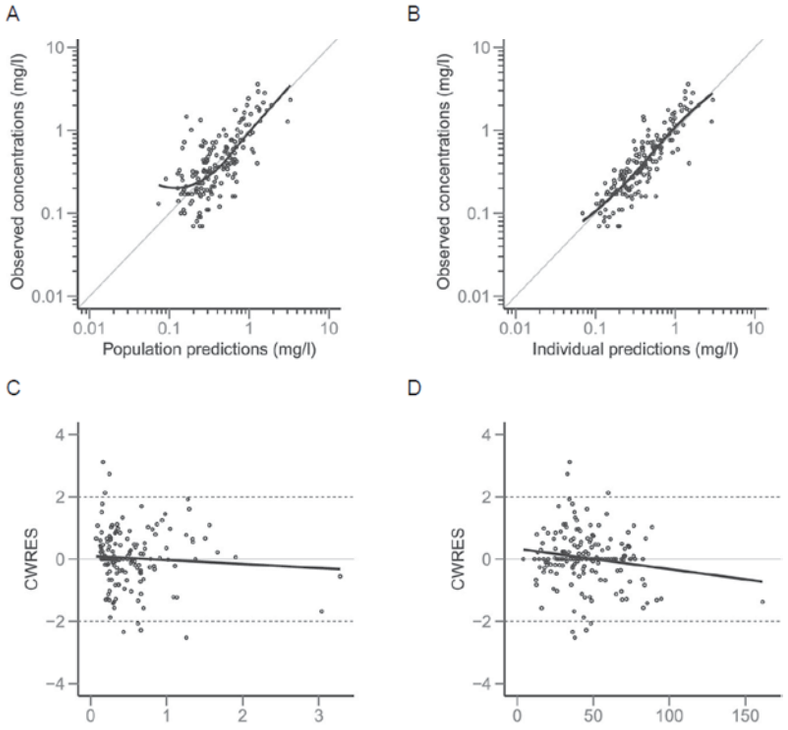
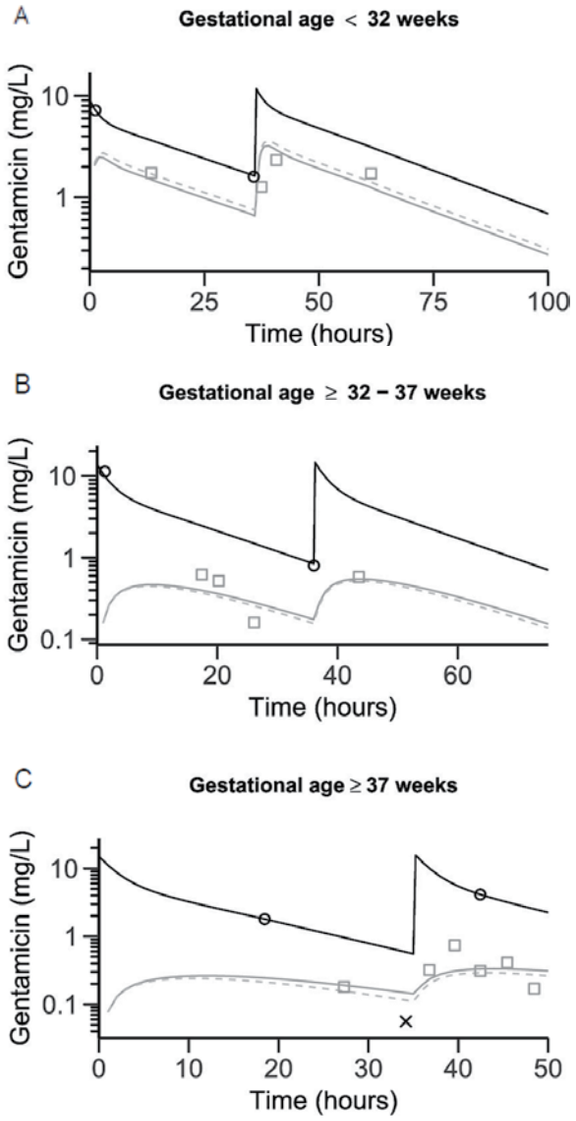


Table 2. Population PK parameters and bootstrap results

Parameter	Structural model		Final model		Bootstrap results		
	OFV = 877.3		OFV = 738.7		(N=1000)		
	Estimate	RSE (%)	Estimate	RSE (%)	Median	2.5th %	97.5th %
θ_{K13} (h^{-1})	0.036	79	0.023	16	0.023	0.016	0.033
θ_{K30} (h^{-1})	0.267	70	0.169	15	0.171	0.123	0.239
θPMA_{K13}	-	-	-8.8	16	-8.7	-11.7	-5.7
θPMA_{K30}	-	-	-5.1	28	-4.9	-8.1	-2.0
σ_{PROP} (%)	58.4	9	49.7	7	49.0	40.8	56.4
IIV_{K30} (%)	63.6	12	38.0	17	37.3	30.5	43.8

θ_{K13}: 1ST order rate constant from central plasma compartment to saliva compartment. θ_{K30}: 1ST order elimination rate constant from saliva compartment. θ_{PMAK13}: Power equation exponent PMA on k_{13}. θ_{PMAK30}: power equation exponent PMA on k_{30}. σ_{PROP}: Proportional error. IIV_{K30}: Inter-individual variability of k_{30}.
$K_{13} = \theta_{K13} * (PMA/244.2)^{\theta PMAK13}$, $K_{30} = \theta_{K30} * (PMA/244.2)^{\theta PMAK30}$

Figure 4. Individual pharmacokinetic profiles of gentamicin in blood and saliva for typical patients of each GA group. A: Individual patient of GA < 32 weeks; B: Individual patient of GA ≥ 32–37 weeks; C: Individual patient of GA ≥ 37 weeks. black circles: observed blood concentrations; gray squares: observed saliva concentrations; solid black line: individual predicted blood concentrations; solid gray line: individual predicted saliva concentrations; dashed gray line: population predicted saliva concentrations; black crosses: observed saliva concentrations < LLOQ.

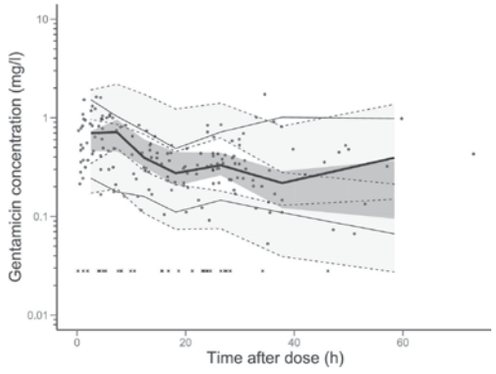


power function (δOFV = -17.25). None of the other tested covariates improved the model. The parameter estimates of the final model are shown in *Table 2*. Final estimates for k^{13} and k^{30} were 0.023 h^{-1} and 0.169 h^{-1}, respectively. IIV of k^{30} was 38% in the final model, whereas proportional residual error was 49.7%. The exponents of PMA as a covariate on k^{13} and k^{30} respectively were -8.8 and -5.1. This describes a negative correlation between PMA and both the transport and elimination rate of gentamicin in saliva, indicating that gentamicin is more readily available in the saliva of patients of low PMA, such as premature neonates. Evaluation of the GOF plots of the final model demonstrated a good description of the observed gentamicin concentrations in saliva (*Figure 3*). For demonstrative purposes, observations and model predictions have been plotted for 1 typical patient per GA group (*Figure 4*).

Bootstrap and internal model validation

The robustness of the final model was evaluated using a bootstrap procedure (n=1000). The median estimates and 95%CI for all parameters are summarized in *Table 2*. In total, 98.3% of the bootstrap runs were successful. For internal validation, a PCVPC (n=1000 samples) of the final model was evaluated (*Figure 5*). The majority of the 10TH, 50TH and 90TH percentiles of the observed values lie within the 95% confidence intervals of the 10TH, 50TH and 90TH percentiles of the simulated values for all bins.

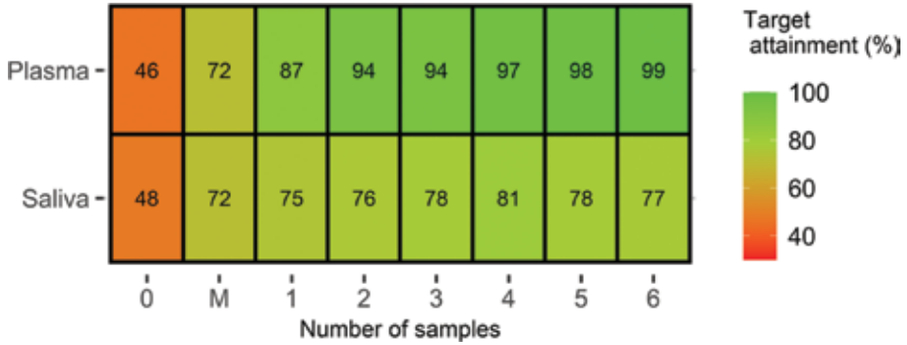
Figure 5. Prediction-corrected visual predictive check of the saliva model. Black circles: Observed gentamicin concentrations; thick black line: median observed concentrations; thin black lines: 80% interval of the observed concentrations; dark gray field: 95% confidence interval of the median prediction; light gray fields with dashed border: 95% confidence intervals of the 10TH and 90TH percentiles of the predictions; red crosses: observations below LLOQ.



Simulations

The simulated proportion of subjects with peak- and trough levels within the target range are displayed in *Figure 6*. Applying TDM using saliva led to a higher percentage of subjects reaching target attainment compared to no TDM (>75% vs 48%, respectively). However, saliva TDM led to a lower percentage of target attainment compared to blood TDM. Obtaining more than four samples for saliva TDM did not result in increased TDM performance. On the contrary, obtaining additional samples at 18h and 1h pre-dose led to a slightly decreased performance (-3% and -4%, respectively) compared to the strategy using four samples.

Figure 6. Heat map displaying the simulated proportion of subjects who reach target attainment of gentamicin after blood- and saliva TDM using an increasing number of samples. Time-points where samples were simulated: 0: standard dosing according to guidelines; M: no samples-dosing optimized according to population model and individual covariates; 1: sample (14h); 2: peak sample (3h for saliva or 1h for blood) and trough sample (0.5h pre-dose); 3: samples at peak, 14h and trough; 4: samples at peak, 7h, 14h and trough; 5: samples at peak, 7h, 14h, 18h and trough; 6: samples at peak, 7h, 14h, 18h, 1h pre-dose and trough.



Discussion

In this study, we have demonstrated the feasibility of monitoring gentamicin concentrations in saliva of neonates. Concentration-time profiles in both blood and saliva were described with an integrated PK model. The potential use of salivary concentrations in the context of TDM was assessed through Monte Carlo simulations. Simulations predicted a target attainment of up to 81% for TDM with 4 saliva samples versus 94% when performed with 2 blood samples.

In the past, several investigators have assessed the use of saliva for TDM of several drugs with varying results.[5-9] Berkovitch *et al.* reported a good correlation between blood and saliva concentrations for a once daily dosing regimen in children.[6] Other investigators reported that aminoglycosides did not penetrate into saliva of children with cystic fibrosis or tuberculosis.[8,9] Work regarding saliva TDM in neonates has covered a wide range of drugs, including caffeine, morphine and antiepileptic drugs.[5] Interestingly, all studies focused on linear correlations. Incorporating saliva concentrations in nonlinear mixed effect models may allow for more flexibility to account for delayed penetration, delayed elimination, and variability in saliva/blood ratio (S/B). To date, this is the first such model to have been developed for gentamicin, and there are only few published models which incorporate this methodology to describe saliva concentrations for other drugs.[22,23]

The model developed during this study was constructed by appending a blood PK model for gentamicin with a saliva compartment. The model by Fuchs *et al.* described the blood PK of the study population.[13] The model of Bijleveld *et al*. did not result in an improved description of the blood PK.[24] This was also true when constructing a new blood PK model with the study data. Gentamicin concentrations in saliva could best be described with drug transport from the central compartment (*Figure 1*). Models incorporating drug transport from the peripheral compartment to saliva were evaluated but did not accurately describe the data. Two separate rate constants were estimated for the saliva model. A 1ST order rate constant k^{13} of 0.023h^{-1} was estimated, whereas an elimination rate k^{30} of 0.169 h^{-1} was estimated. In this case, k^{13} was estimated to be much lower than k^{30}, indicating that transport from the central blood compartment to the saliva compartment is the rate-limiting step determining the concentration-time profile in saliva.[25] When predicting gentamicin concentrations in blood and saliva in typical patients (*Figure 4*), it seems that the S/B ratio stabilizes hours after the last dose is administered. During this phase, the concentration-time curve of saliva is perpendicular to blood, indicating that the salivary gentamicin elimination rate is linear to the blood concentration and therefore is dependent on k^{13}.

Considerable IIV was detected. Part of this was accounted for by including PMA as covariate. It was estimated that IIV on k^{30} was 38% in the final model. Post-menstrual age had a large influence on the salivary PK profile of gentamicin. Inclusion of PMA as a covariate on both k^{30} and k^{13} significantly improved the model. The exponents of the power equation functions were -5.5 and -8.8 for k^{30} and k^{13} respectively, suggesting a very strong age dependency of gentamicin disposition in saliva. With increasing PMA, k^{13} and k^{30}

decrease by a large margin. Indeed, it was observed that salivary gentamicin levels were generally much lower in term neonates, compared to premature neonates. Furthermore, 75% of samples below the LLOQ were from term neonates (PMA>260 days). Though the model did not contain a parameter describing the IIV in k^{13}, inclusion of PMA as a covariate on k^{13} significantly improved model fit, decreased RSE on all parameters and decreased residual error. It was quite notable that gentamicin was more freely distributed in saliva of premature neonates. However, no biological explanation for this phenomenon could be found in literature. Nonetheless, this finding may be indicative that salivary TDM could be more efficacious and possibly more accurate in premature neonates.

TDM performance was assessed through simulation in a fictional cohort of 3,000 neonates with a realistic distribution of covariates.[19] Applying Bayesian MAP during simulation, one can use information obtained from multiple samples to estimate the peak- and trough concentrations, which reduces the prediction error in the process. Additionally, the optimization process takes residual variability into account, and the prediction shrinks towards the population mean in the case of high residual variability. This prevents that outlier saliva observations are extrapolated to extreme estimated blood concentrations on which dose adaptions are made. During the simulations, each virtual subject was subjected to a rigid dose decision rule for dose optimization. In practice, more nuances can be applied. Moreover, results from this simulation study may be quite optimistic, since inter occasion variability (IOV) is not accounted for, such as time-dependent changes in CL. However, the simulations give a crude indication of the expected reliability of TDM with saliva samples versus blood samples, as well as the comparative performance of several sampling schedules.

Simulations indicated that a target attainment of 81% is possible with saliva TDM. Obtaining the 4 saliva samples necessary in this scenario is logistically feasible. However, target attainment following TDM with 2 blood samples was higher (94%). This difference in performance for saliva and blood TDM can be explained by the large difference in residual error between the two matrices. The uncertainty in the Bayesian optimization process introduced by these parameters was too large to achieve adequate precision with additional sampling or different sampling schedules. Moreover, assessed saliva sampling schedules were equal for all dosing regimens, therefore the evaluated additional samples may have had limited value for dosing regimens of 36 or 48 hours. Blood TDM performs better in settings where collection of 2 blood samples is protocol. However, in many clinical settings TDM protocols require a single intermediate concentration sample. In that case, blood TDM has a predicted target attainment of 87% (*Figure 6*). This difference with saliva TDM is substantially smaller. Taken together with the uncertainties of simulations, TDM with 4 saliva samples may be a suitable alternative to blood TDM with a single intermediate concentration sample. Given that gentamicin was more readily available in the saliva of premature neonates and no different sampling strategies were employed based on dose regimen during simulation, the difference in predicted target attainment may not be clinically relevant, especially for premature neonates that could benefit most from a non-invasive TDM method.

This study has several limitations. First, there was a large proportion of saliva samples with insufficient volumes for analysis. This may be due to inadequate sampling technique or insufficient saliva production by subjects, especially with premature neonates. Future studies may employ a different sampling strategy to ensure that an adequate volume of saliva is drawn, such as use of a different swab or cutting the saturated end of the swab.[26,27] Currently no standardized method for the collection of saliva from neonates exists. Nonetheless, many samples were available for model development, thus we do not expect this has influenced the parameter estimates. Second, due to the low volumes of the collected samples, it was not possible to determine pH of the collected samples. Saliva pH has been proposed to influence salivary distribution of drugs.[28] Though little has been published regarding saliva pH of neonates, we expect that fluctuations in saliva pH have little influence on the protonated fraction of gentamicin, since the strongest basic pK_A is 10.18.[29] Regardless, influence of pH on salivary gentamicin concentrations may be assessed, if possible. Finally, assumptions made during simulation, such as the underlying covariate distribution and sampling strategies, have an influence on the proportion of subjects reaching target attainment. However, considering that the goal of the simulation was to compare saliva and blood TDM, the comparative differences found in these simulation scenarios should be independent of these assumptions.

Strengths of this study are the employment of POP-PK, allowing for the description of nonlinear relations between blood and saliva gentamicin concentrations. In addition, a relatively large cohort of neonates of different GA receiving varying dosing regimens originating from both a peripheral pediatric ward and NICU, improved the generalizability of the model. Moreover, use of highly sensitive LC-MS/MS allowed for determination gentamicin concentrations in small sample volumes. The LC-MS/MS method had an LLOQ of 0.056 mg/l, which was substantially lower than earlier publications investigating gentamicin in saliva.[7-9] Population pharmacokinetic modeling allowed for opportunistic

sampling schedules and identification of covariates. The TDM simulations of a wide range of sampling strategies give an adequate overview of the expected performance of saliva TDM in different scenarios.

Conclusion

With this study, we demonstrate that TDM of gentamicin in saliva is feasible. A target attainment of 81% was found based on explorative simulations with 4 saliva samples and performance is close to blood TDM with 1 intermediate sample. In the future, the real-life performance of saliva TDM employing an improved sampling technique should be investigated prospectively in premature neonates, as gentamicin appears more readily in the saliva of premature neonates and these most fragile infants may benefit most from non-invasive TDM.

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