Regular Article - Ms 2019-2373 R1

Hearing in Schoolchildren after Neonatal Exposure to a High-Dose Gentamicin Regimen

Dagny Hemmingsen, MD [a,b], Camilla Mikalsen [a], Alexander Rydland Hansen [a], Jon Widding Fjalstad, MD, PhD [b], Niels Christian Stenklev, MD, PhD [c] Claus Klingenberg, MD, PhD [b,d]

Affiliations (all in Norway):
[a] Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital of North Norway; [b] Paediatric Research Group, Faculty of Health Sciences, University of Tromsø-Arctic University of Norway, Tromsø; [c] Ear-Nose-Throat Unit, Ishavsklinikken, Tromsø; [d] Department of Paediatrics and Adolescence Medicine, University Hospital of North Norway, Tromsø

Address correspondence to: Claus Klingenberg. Dept. of Paediatrics, University Hospital of North Norway, N-9038 Tromsø, Norway. Phone +47 77669845. Fax: +47 77626369
Email: claus.klingenberg@unn.no

Short title: Hearing after Exposure to High-Dose Gentamicin

Funding Source: All phases of this study were supported by Northern Norway Regional Health Authority and by the Research Department at University Hospital of North-Norway. A grant from Eckbo's legat supported presentation of preliminary data.

Financial Disclosure: The authors have no financial relationships relevant to this article to disclose.

Conflict of Interest: The authors have no conflicts of interest to disclose.

Clinical Trial Registration: NCT03253614

Data sharing statement: The raw data supporting the conclusion of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Abbreviations: NICU: Neonatal Intensive Care Unit; OAE, Otoacoustic Emissions; GA, Gestational age; TPC, Trough Plasma Concentration; EHF, Extended High Frequency; PTA, Pure tone average; EHFA, Extended High Frequency Average

Table of Contents Summary: We performed pure tone audiometry, including the extended high-frequency range, in schoolchildren exposed to a high-dose gentamicin regimen in the neonatal period to assess ototoxicity.

What's known on this subject: Evidence for ototoxic hearing loss after gentamicin exposure is mainly from studies in adults and older children. Neonatal studies report low rates of ototoxicity, but have commonly used only moderate sensitive hearing tests.

What this study adds: We performed pure tone audiometry, including the extended high-frequency range, in 219 schoolchildren (median age 9 years) exposed to a high-dose gentamicin regimen in the neonatal period. We found no association between exposure to gentamicin and hearing levels.

Contributors' Statement Page

Dagny Hemmingsen conceptualized and designed the study, carried out the initial analysis and wrote the first draft of the manuscript.

Camilla Mikalsen collected data and reviewed and revised the manuscript.

Alexander Rydland Hansen collected data, carried out initial analysis and reviewed and revised the manuscript.

Jon Widding Fjalstad reviewed all gentamicin data and established the cohort from the neonatal period. He also contributed to statistical analyses and revised the manuscript.

Niels Christian Stenklev provided substantial contribution to study design and interpretation of the data, and reviewed and revised the manuscript.

Claus Klingenberg conceptualized and designed the study, coordinated and supervised data collection, directed all phases of the study, and revised the final manuscript.

Hemmingsen and Klingenberg had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ABSTRACT

OBJECTIVES: To assess the association between gentamicin exposure in the neonatal period and hearing in school age.

METHODS: This study included children exposed to a high-dose (6 mg/kg) gentamicin regimen as neonates (2004-2012), invited for follow-up in school age, and a healthy age-matched control group. We assessed hearing with pure tone audiometry including the extended high-frequency range. Outcomes were average hearing thresholds in the mid-frequencies (0.5-4 kHz) and the extended high-frequencies (9-16 kHz). The measures of gentamicin exposure were cumulative dose and highest trough plasma concentration (TPC). We used linear regression models to assess the impact of gentamicin exposure, and other peri- and postnatal morbidities, on hearing thresholds.

RESULTS: A total of 219 gentamicin-exposed and 33 healthy control children were included in the audiological analysis. In the gentamicin cohort, 39 (17%) had a birth weight < 1500 g. Median (interquartile range) cumulative dose and TPC were 30 (24-42) mg/kg and 1.0 (0.7-1.2) mg/L, respectively. Median hearing thresholds (decibel hearing level) for the mid- and extended high-frequencies were 2.5 (0 - 6.3) and -1.7 (-5.0 - 5.0), both within normal range. In adjusted analysis, increasing hearing thresholds were associated with lower birth weight and postnatal middle ear disease, but not with level of gentamicin exposure. After adjusting for birth weight there was no difference in hearing thresholds between the gentamicin-exposed cohort and healthy controls.

CONCLUSIONS: Exposure to a gentamicin high-dose regimen in the neonatal period was not associated with an increase in hearing thresholds in schoolchildren being able to complete audiometry.

INTRODUCTION

Gentamicin is widely used for treatment of neonatal sepsis.[1,2] Extended-interval dosing regimens are currently recommended.[3] To ensure effective therapy it is necessary to attain a high circulating dose and some experts suggest that each dose should be as high as 7.5 mg/kg due to the large distribution volume in neonates.[4] There is still uncertainty about the optimal dosing regimen and safety, in particular regarding potential ototoxicity.

Ototoxic hearing loss typically first affects the high frequencies (> 8 kHz), may then progress to involve lower frequencies and is usually bilateral and irreversible.[5,6] Neonates admitted to neonatal intensive care units (NICUs) have up to 10-fold increase in prevalence of hearing loss.[7,8] Prolonged gentamicin treatment and high trough plasma concentrations (TPC) have been suggested to increase the risk of ototoxicity.[3,9,10] Prematurity and low birth weight, severe perinatal morbidities, other ototoxic drugs and environmental noise are also risk factors for hearing loss.[8,11–13] These factors will often co-exist with gentamicin treatment making it difficult to delineate which risk factor is of greatest clinical importance.

Current evidence indicates a low risk of hearing loss after gentamicin treatment in neonates.[5,14,15] However, data are limited by several factors. The objective testing methods used in newborn hearing screening (otoacoustic emissions (OAE) or automated brain stem audiometry) evaluate hearing at frequencies between 2-6 kHz and do not detect mild hearing loss or early signs of ototoxicity. Moreover, most studies have evaluated hearing shortly after exposure to gentamicin and could not identify late-onset or progressive hearing loss.

Pure tone audiometry in the extended high-frequency (EHF) range is the most sensitive subjective testing method to detect ototoxic hearing loss, even before it becomes evident in the conventional hearing range.[16,17] For this method children must be able to cooperate.[18,19] In this study we performed hearing assessment of schoolchildren exposed to a high-dose gentamicin regimen in the neonatal period in order to assess the long-term safety.

PATIENTS AND METHODS

Setting, study design and participants

Children included in this study had been admitted to the NICU at the University Hospital of North Norway (UNN) and received gentamicin therapy between 2004 and 2012. This NICU is the only unit offering care for infants born before 32 weeks gestation, and for all other newborn infants (≥ 32 weeks) in need for mechanical ventilation or intensive care, in the two northernmost counties in Norway. We previously validated our extended-interval, high-dose (6 mg/kg) gentamicin dosing regimen in 440 neonates who were exposed to at least three doses of gentamicin between 2004 and 2012.[20] The vast majority of TPCs (94%) were within the normal range, there was a low rate of prescription errors and we found no evidence of early-onset ototoxicity using a transient evoked OAE screening test before hospital discharge.[20]

For the current study (Figure 1), 357 children from the original cohort were invited for a detailed hearing assessment at age 6-14 years. We also, from public primary schools, recruited a control group of 33 healthy children with no history of previous use of aminoglycosides, and no prior hearing problems or tympanostomy tubes. Parents of all children filled out a questionnaire including any history of middle ear infections, treatment with tympanostomy tubes and use of intravenous antibiotics after the neonatal period.

Neonatal characteristics

For the gentamicin-exposed cohort, we collected data on birth weight, gestational age (GA), Apgar scores, neurological abnormalities, mechanical ventilation and any phototherapy for jaundice. Preterm neonates are more susceptible to bilirubin-induced neurologic damage, suffer adverse effects at lower total serum bilirubin (TSB) levels and receive more phototherapy than term infants.[21,22] We recorded the peak TSB level within the first 2 weeks of life and divided this value by GA in weeks; creating an age-adjusted variable of possible

bilirubin toxicity instead of using crude peak TSB levels. To assess level of gentamicin exposure during hospitalization we recorded two variables; the highest measured gentamicin TPC (mg/L) and the cumulative gentamicin dose (mg/kg). For the healthy control group we collected data on birth weight, admission to a NICU for other reasons than infection and any phototherapy for jaundice.

Base line investigations

Participants attended one study visit between September 2017 and September 2018. We did otoscopy and tympanometry at 226 Hz (Otometrics, Zodiac, Taastrup, Denmark) prior to pure tone audiometry. Tympanogram results were classified as Type A (normal), B (flat) and C (negative pressure). We collected a urine sample for analysis of the mitochondrial 1555A>G gene mutation in all gentamicin-exposed children. DNA was extracted using the Quick-DNA Urine Kit (Zymo Research, Irvine, CA). The m.1555A>G gene mutation was analyzed using PCR amplification and melting curve analysis (LightCycler 480, Roche, Basel, Switzerland).

Audiometric data acquisition

Pure tone audiometry thresholds were measured with the Equinox 2.0 clinical audiometer using Equinox suite version 2.9.0 software (Interacoustics A/S, Middelfart, Denmark). The audiometer was calibrated according to the manufacturer's specifications and in accordance to ISO references.[23,24] We used the DD45 supra-aural earphones (Radioear Co, Midelfart, Denmark) for the conventional frequencies (0.125-8 kHz) and Sennheiser HDA200 closed circum-aural earphones (Sennheiser electronics, Wedemark, Germany) for the EHF's (9-16 kHz). Testing was done first in the conventional frequency range prior to the EHF range. We used the ascending method to acquire thresholds.[25] Special care was taken for each child to avoid fatigue and loss of concentration. The first ear tested (left or right) was randomized by the survey management software (REDCap®). Audiometry testing was done by a trained

audiologist or an audiology trained ear-nose-throat physician. The hearing thresholds are expressed as decibel (dB) hearing levels (HL).

Audiological outcomes

The main audiological outcomes were average hearing thresholds in the conventional frequencies and the EHF range. We calculated the established pure tone average (PTA), representing the mean of the conventional mid-frequencies 0.5, 1, 2 and 4 kHz, according to an established reference method.[26] There is no established equivalent to PTA in the EHF range. We chose to use the average of all six EHFs (9, 10, 11.2, 12.5, 14 and 16 kHz), hereafter coined EHFA. Middle ear problems can be unilateral, but ototoxic hearing losses are most often bilateral. Thus, we used the PTA and EHFA for the best ear in the final analysis. A relevant clinical hearing loss was defined as PTA and/or EHFA threshold > 20 dB in the best ear. We report tympanogram results corresponding to the best ear result.

Ethics and trial registration

The study was approved by the committee for human medical research ethics, Region North in Norway. All parents signed a written informed consent and all participating children received age-appropriate written information about the study. The study was in August 2017 registered with ClinicalTrials.gov, number NCT03253614.

Sample size and power calculation

Based on previous studies [27,28] we estimated that mean EHFA threshold would be around 5-10 dB in the healthy control group. We realistically hoped to include 60-70% of the 357 invited gentamicin-exposed children. We considered that a 10 dB difference in the EHFA hearing threshold would represent a clinically relevant difference between healthy controls and the gentamicin-exposed group. By including around 30 healthy controls and around 250 gentamicin-exposed children we would have 80% power, with a two-sided 5% level of significance to detect a differences of 4-5 dB between the groups. Moreover, within the group

of gentamicin-exposed children we knew that around half of them had a gentamicin TPC ≥ 1.0 mg/l and the rest < 1.0 mg/l. With 125 children in each group we would have 80% power, with a two-sided 5% level of significance to detect a difference of 3-4 dB between the groups.

Data analysis and statistics

All clinical data were first entered into REDCap®, a secure, web-based software platform designed to support data capture for research studies (Vanderbilt University, Nashville, USA). Clinical and audiometry data were analyzed using IBM-SPSS statistical software version 23 (IBM, New York, USA). Descriptive results are expressed as median (interquartile range-IQR). We used a univariable linear regression model to analyze level of gentamicin exposure and other predictors that may affect hearing thresholds.[29] We then plotted all predictors in a directed acyclic graph, and based on clinical and biological knowledge we identified birth weight being the central confounder of both the outcome and other predictor variables. Finally, we therefore adjusted each predictor separately for birth weight. Results from univariable and adjusted analysis are presented as regression coefficients with 95% confidence intervals. We defined p values < 0.05 as significant.

RESULTS

After parental consent, 226/357 (63%) of gentamicin-exposed children were included. Eight children had a relevant hearing loss (Table 1). Five of these had known etiology (3 with ongoing middle ear disease and 2 with developmental delay and genetic hearing loss), and were therefore not included in the main audiological analysis. The 3 remaining children had hearing loss of uncertain etiology and were included in the main audiological analysis. Two more children were excluded from the main audiological analysis due to obvious lack of concentration during testing with uncertain validity of audiometry results.

High-quality audiometry results were obtained for 219 children exposed to gentamicin in the neonatal period and for 33 healthy controls (Table 2). In the gentamicin cohort, thirty-nine (17%) had a very low birth weight (VLBW, < 1500 g birth weight) and forty-six (20%) had been treated with mechanical ventilation. One child was diagnosed with a m.1555A>G gene mutation. This child had a culture-confirmed group B streptococcal early-onset sepsis, received gentamicin for 12 days, but had normal audiometry results (best ear thresholds PTA 6 dB and EHFA 8 dB). Three term born children who underwent therapeutic hypothermia due to severe perinatal asphyxia also received gentamicin; all three had later a normal psychomotor development and no hearing loss.

Overall, the gentamicin-exposed cohort and the control group had normal hearing thresholds for the whole frequency range (Table 2, Figure 2). Unadjusted statistical analysis showed a 2.5 dB absolute difference in median EHF hearing thresholds between the gentamicin-exposed and the healthy controls, which is not of clinical significance. After adjusting for birth weight the statistical difference was lost (Table 2). No international ISO-references exist for the EHF range in children. We compared our results with data from the hitherto largest published reference study, including 90 healthy children and adolescents aged 5-19 years.[30] EHF hearing thresholds between groups from the current study and the reference study were comparable (Figure 2).

Table 3 displays the linear regression analysis of predictors for hearing thresholds in the conventional mid-frequencies and the EHFs. In the conventional mid-frequencies, we found that birth weight, mechanical ventilation and tympanometry results were all significant predictors in the unadjusted analysis. After adjusting each predictor for the birth weight, only birth weight and tympanometry result remained significant predictors. In the EHFs, we found that cumulative gentamicin dose, birth weight, phototherapy, being small for gestational age, mechanical ventilation and tympanostomy tubes were significant predictors in the unadjusted

analysis. After adjusting each predictor for the birth weight, only birth weight and tympanostomy tubes remained significant predictors.

We compared data from the population-based original study cohort, including all gentamicin-exposed neonates during the 8-year study period (n=440), with data from the follow-up cohort (n=226), in order to assess representativeness of the follow-up cohort. There were no differences in birth weight, the proportion of VLBW infants, the cumulative gentamicin doses, the highest median gentamicin TPCs and the proportion of children with gentamicin TPC > 2.0 mg/L between the two cohorts (Online Table 1).

DISCUSSION

The main objective of this study was to perform a detailed hearing assessment of schoolchildren exposed to a high-dose gentamicin regimen in the neonatal period in order to assess potential clinical or subclinical signs of ototoxic hearing loss as markers of long-term harm or safety. We tested hearing in both the conventional frequencies and the EHFs, we adjusted findings for other potential peri- and postnatal risk factors for hearing loss, and we compared audiological data with a healthy control cohort. We found no association between level of gentamicin exposure in the neonatal period and hearing thresholds after 9 years median follow-up time.

Previous studies and reviews indicate a low risk of gentamicin-induced ototoxicity in the newborn period, regardless of dosing regimen. However, there is a paucity of long-term, detailed follow-up studies. One recent case-control study compared level of gentamicin exposure in 25 VLBW infants who presented with hearing loss during first 5 years of life and a matched control group without hearing loss, and found no differences in gentamicin exposure between groups.[31] One study from the 1970s reported 4 year follow-up hearing results after newborn aminoglycoside therapy, using play audiometry (0.5-4 kHz). Only 25%

of their original cohort were assessed at 4 years, but the authors did not identify any substantial aminoglycoside-attributable hearing loss.[32] Our study is the first long-term follow-up study performing high quality pure tone audiometry, including the EHFs, of children exposed to gentamicin in the newborn period. A delay between exposure and hearing loss is well known from platinum-induced hearing loss in children.[33,34] This has also been suggested in sporadic cases after neonatal treatment with gentamicin.[35,36] We found no indication of late-onset gentamicin-induced ototoxicity in our study.

The mechanisms behind gentamicin-induced ototoxicity are not fully understood.[37] Currently, there is stronger evidence for aminoglycoside ototoxicity in older children than in neonates.[6,18,19] A possible explanation is that older children, e.g. with cystic fibrosis or cancer, receive larger cumulative doses than those commonly administered in neonates.[6,18,19] Alternatively, the newborn inner ear is less vulnerable to ototoxicity or gentamicin-induced ototoxicity may be partly reversible. Indeed, reversible ototoxic effects from aminoglycosides have been demonstrated in animal models.[38] Moreover, transient hearing loss in neonates is reported and could be explained by a transient cochlear dysfunction due to inflammation [39] or a delayed maturation of the auditory system.[40] However, in our study cohort there were neither signs of ototoxicity at NICU discharge nor at follow-up in children exposed to gentamicin.

Hearing loss in infants admitted to NICUs has a prevalence of around 2-4 % compared to 0.1-0.3 % in the general newborn population.[7,29,41,42] Low gestational age, VLBW, mechanical ventilation, perinatal infections, hyperbilirubinemia and severe asphyxia are all identified as risk factors for hearing loss.[8,11,13] In line with others, we found a strong association between decreasing birth weight and increasing hearing thresholds.[14] Some authors argue that low birth weight itself does not cause hearing loss,[43] but is rather associated with other perinatal factors that more directly affects hearing. We evaluated other possible

predictors for hearing such as Apgar scores, hyperbilirubinemia/phototherapy and mechanical ventilation, but none of these were associated with increasing hearing thresholds after adjusting for birth weight.

The m.1555A>G mutation is associated with hearing loss, in particular after exposure to aminoglycoside antibiotics.[44] In our cohort only one patient (0.44%) had this mitochondrial mutation, and this patient had normal hearing despite a cumulative gentamicin dose of 72 mg/kg. In another cohort of infants treated with gentamicin, 4/436 (0.9%) had a mitochondrial 12sRNA mutation, but only one showed evidence of possible hearing loss.[45] Some authors suggest testing for mitochondrial mutations prior to neonatal aminoglycoside treatment.[46] A clinical study is planning to assess rapid pharmacogenetic testing of the m.1555A>G mutation in order to avoid aminoglycoside therapy in "at risk" neonates.[47] However, given the low and variable prevalence of this mutation in different ethnic populations combined with a variable penetrance, this approach may not be justified or cost-effective in all settings.[44,48,49]

Middle ear disease in childhood may cause mechanical hearing loss because of permanent inflammatory damage and/or sensorineural hearing loss secondary to toxic effects to the inner ear.[50,51] Isolated sensorineural hearing loss in the EHFs after otitis media is also reported in children.[52] We found a significant association between previous tympanostomy tubes, a marker for more severe middle ear disease, and EHF hearing thresholds. We also found increased hearing thresholds in the conventional mid-frequencies in children with negative middle ear pressure. The latter may reflect a subtle mechanical hearing loss caused by ongoing middle ear pathology.

The strength of our study is the unique long-term audiological data sensitive enough to detect subtle and subclinical hearing loss. We also present data on different levels of gentamicin exposure, with cumulative dose being the most important proxy for exposure, but found only a weak correlation between cumulative dose and EHF-thresholds, which was not

significant after adjusting for birth weight (Table 3A and Supplementary Figure 1). It is a paradox that most neonatal gentamicin dosing regimens recommend lower gentamicin doses (4-5 mg/kg) than in older children (7 mg/kg), despite a proportionally higher distribution volume in neonates.[4] We have since 2004 used a dosing regimen with a fixed gentamicin dose (6 mg/kg) for all neonates, and a variable dosing interval (24-48 h) depending on GA and postnatal age.[20] This dosing regimen has a low risk of prescription errors.[20] Our study also has limitations. Children from the original cohort with the most severe comorbidities were not included in our follow-up, due to clinical conditions that made them unable to complete audiometric testing. Some of these may have hearing problems in addition to other disabilities. However, we are only aware of one child from the original cohort, diagnosed with a congenital cytomegalovirus infection, who has a cochlea implant. Since 2009, our unit has avoided routine use of gentamicin in children with severe asphyxia who undergo therapeutic hypothermia. Only 3 children with this condition were therefore included in the follow-up cohort, all three with normal hearing. There are conflicting results on a possible association between gentamicin exposure and hearing loss in children with severe perinatal asphyxia who have undergone therapeutic hypothermia.[53,54] Only 10% of the children in our study received more than 10 doses (> 60 mg/kg) gentamicin, and we cannot exclude that very long courses of gentamicin have a greater ototoxic potential, also in the neonatal period. Finally, a response rate of 63% adds a potential selection bias. Still, the gentamicin exposure data and the proportion of VLBW infants were similar in the original and the follow-up cohort.

Conclusion

In schoolchildren who were not severely disabled and therefore able to complete a detailed hearing assessment, we found no association between neonatal exposure to a gentamicin high-dose, extended-interval regimen and increased risk of hearing loss in the conventional mid-frequencies and the EHFs. Increasing hearing thresholds were associated with lower birth

weight and middle ear disease in childhood, but the vast majority of children had normal hearing. Potential damage to hearing early in life is of great concern because childhood hearing loss, and prelingual hearing loss in particular, may affect both language and general development.[55] It is therefore important to provide high-quality, long-term follow up data on hearing after gentamicin exposure in neonates, since this drug is widely used in neonates and safety therefore is of paramount importance.

ACKNOWLEDGMENTS

We greatly appreciate the professional work of the staff at the clinical research department, University Hospital of North Norway, Tromsø. We are also grateful to Marthe Larsen, at the clinical research department, University Hospital of North-Norway, Tromsø for statistical advice and to Bo Engdahl, Norwegian Institute of Public Health, Oslo for advice on audiological methods and analyses. Finally, we thank all children and parents for participating in the study, without their voluntarily contribution this study would not have been possible.

References

1. Fjalstad JW, Stensvold H, Bergseng H, et al. Early-onset Sepsis and Antibiotic Exposure in Term Infants: A Nationwide Population-based Study in Norway. *Pediatr Infect Dis J*. 2016;35(1):1-6. doi:10.1097/inf.0000000000000906
2. Hsieh EM, Hornik CP, Clark RH, Laughon MM, Benjamin DK, Smith PB. Medication use in the neonatal intensive care unit. *Am J Perinatol*. 2014;31(9):811-821. doi:10.1055/s-0033-1361933
3. Rao SC, Srinivasjois R, Moon K. One dose per day compared to multiple doses per day of gentamicin for treatment of suspected or proven sepsis in neonates. *Cochrane Database Syst Rev*. 2016;(12). doi:10.1016/j.jog.2012.04.008
4. Van Donge T, Pfister M, Bielicki J, et al. Quantitative Analysis of Gentamicin Exposure in Neonates and Infants Calls into Questions Its Current Dosing Recommendations. *Antimicrob Agents Chemother*. 2018;62(4):1-12.
5. Kent A, Turner MA, Sharland M, Heath PT. Aminoglycoside toxicity in neonates: something to worry about? *Expert Rev Anti Infect Ther*. 2014;12(3):319-331. doi:10.1586/14787210.2014.878648
6. Chen KS, Bach A, Shoup A, Winick NJ. Hearing Loss and Vestibular Dysfunction Among Children With Cancer After Receiving Aminoglycosides. *Pediatr Blood Cancer*. 2013;60:1772-1777. doi:10.1002/pbc
7. Hille ETM, Van Straaten HLM, Verkerk PH, et al. Prevalence and independent risk factors for hearing loss in NICU infants. *Acta Paediatr Int J Paediatr*. 2007;96(8):1155-1158. doi:10.1111/j.1651-2227.2007.00398.x
8. Year 2007 position statement: Principles and guidelines for early hearing detection and intervention programs. *Pediatrics*. 2007;120(4):898-921. doi:10.1542/peds.2007-2333
9. De Hoog M, Van Zanten BA, Hop WC, Overbosh E, Weisglas-Kuperus N, Van Den Anker JN. Newborn hearing screening: Tobramycin and vancomycin are not risk factors for hearing loss. *J Pediatr*. 2003;142(1):41-46. doi:10.1067/mpd.2003.mpd037
10. Borradori C, Fawer CL, Buclin T, Calame A. Risk factors of sensorineural hearing loss in preterm infants. *Biol Neonate*. 1997;71(1):1-10. doi:10.1159/000244391
11. Bielecki I, Horbulewicz A, Wolan T. Risk factors associated with hearing loss in infants: An analysis of 5282 referred neonates. *Int J Pediatr Otorhinolaryngol*. 2011;75(7):925-930. doi:10.1016/j.ijporl.2011.04.007
12. Cross CP, Liao S, Urdang ZD, Srikanth P, Garinis AC, Steyger PS. Effect of sepsis and systemic inflammatory response syndrome on neonatal hearing screening outcomes following gentamicin exposure. *Int J Pediatr Otorhinolaryngol*. 2015;79(11):1915-1919. doi:10.1016/j.ijporl.2015.09.004
13. Marlow E, Hunt L, Marlow N. Sensorineural hearing loss and prematurity. *Arch Dis Child Fetal Neonatal Ed*. 2000;82:F141-F144. http://discovery.ucl.ac.uk/792081/.
14. Puia-Dumitrescu M, Bretzius OM, Brown N, et al. Evaluation of Gentamicin Exposure in the Neonatal Intensive Care Unit and Hearing Function at Discharge. *J Pediatr*. 2018:1-6. doi:10.1016/j.jpeds.2018.07.101
15. Vella-Brincat JWA, Begg EJ, Robertshawe BJ, Lynn AM, Borrie TL, Darlow BA. Are gentamicin and/or vancomycin associated with ototoxicity in the neonate? A retrospective audit. *Neonatology*. 2011;100(2):186-193. doi:10.1159/000324857
16. Fausti SA, Henry JA, Schaffer HI, Olson DJ, Frey RH, McDonald WT. High-Frequeney Audiometric Monitoring for Early Detection of Aminoglycoside Ototoxicity. *J Infect Dis*. 1992;165(6):1026-1032.
17. Fausti SA, Larson VD, Noffsinger D, Wilson RH, Phillips DS, Fowler CG. High-frequency audiometric monitoring strategies for early detection of ototoxicity. *Ear Hear*. 1994;15(3):232-239. doi:10.1097/00003446-199406000-00004

18. Al-Malky G, Dawson SJ, Sirimanna T, Bagkeris E, Suri R. High-frequency audiometry reveals high prevalence of aminoglycoside ototoxicity in children with cystic fibrosis. *J Cyst Fibros*. 2015;14(2):248-254. doi:10.1016/j.jcf.2014.07.009
19. Al-Malky G, Suri R, Dawson SJ, Sirimanna T, Kemp D. Aminoglycoside antibiotics cochleotoxicity in paediatric cystic fibrosis (CF) patients: A study using extended high-frequency audiometry and distortion product otoacoustic emissions. *Int J Audiol*. 2011;50(2):112-122. doi:10.3109/14992027.2010.524253
20. Fjalstad JW, Laukli E, Van Den Anker JN, Klingenberg C. High-dose gentamicin in newborn infants: Is it safe? *Eur J Pediatr*. 2014;173(4):489-495. doi:10.1007/s00431-013-2194-1
21. Olds C OJS. Bilirubin-Induced Audiologic Injury in Preterm Infants. *ClinPerinatol*. 2016;43(2):313-323. doi:10.1016/j.clp.2016.01.006.
22. Mreihil K, Benth JŠ, Stensvold HJ, et al. Phototherapy is commonly used for neonatal jaundice but greater control is needed to avoid toxicity in the most vulnerable infants. *Acta Paediatr*. 2018;107(4):611-619. doi:10.1111/apa.14141
23. ISO/TC 43. ISO 389-5:2006. Reference equivalent threshold sound pressure levels for pure tones in the frequency range 8 kHz to 16 kHz. https://www.iso.org/standard/40535.html.
24. ISO/TC 43. ISO 389-1:2017 Reference equivalent threshold sound pressure levels for pure tones and supra-aural earphones. https://www.iso.org/standard/69855.html.
25. ISO/TC 43. ISO 8253-1:2010 Pure-tone air and bone conduction audiometry. https://www.iso.org/standard/43601.
26. Mathers C, Smith A, Concha M. Global burden of hearing loss in the year 2000. *World Heal Organ*. 2000;(4):1-30. http://www.who.int/healthinfo/statistics/bod_hearingloss.pdf.
27. Anastasio AR, Radael RD, Cavalcante JM, Hatzopoulos S. A report of extended high frequency audiometry thresholds in school-age children with no hearing complaints. *Audiol Res*. 2012;2(1):39-42. doi:10.4081/audiores.2011.e8
28. Müller R, Fleischer G, Schneider J. Pure-tone auditory threshold in school children. *Eur Arch Oto-Rhino-Laryngology*. 2012;269(1):93-100. doi:10.1007/s00405-011-1616-9
29. Coenraad S, Goedegebure A, van Goudoever JB, Hoeve LJ. Risk factors for sensorineural hearing loss in NICU infants compared to normal hearing NICU controls. *Int J Pediatr Otorhinolaryngol*. 2010;74(9):999-1002. doi:10.1016/j.ijporl.2010.05.024
30. Rodríguez Valiente A, Trinidad A, García Berrocal JR, Górriz C, Ramírez Camacho R. Extended high-frequency (9-20 kHz) audiometry reference thresholds in 645 healthy subjects. *Int J Audiol*. 2014;53(8):531-545. doi:10.3109/14992027.2014.893375
31. Fuchs A, Zimmermann L, Bickle Graz M, et al. Gentamicin Exposure and Sensorineural Hearing Loss in Preterm Infants. *PLoS One*. 2016;11(7):1-11. doi:10.1371/journal.pone.0158806
32. Finitzo-Hieber T, McCracken GH, Roeser RJ, Allen DA, Chrane DF, Morrow J. Ototoxicity in neonates treated with gentamicin and kanamycin: Results of a four-year controlled follow-up study. *Pediatrics*. 1979;63(3):443-450. http://www.ncbi.nlm.nih.gov/pubmed/312486.
33. Bertolini P, Lassalle M, Mercier G, et al. Platinum compound-related ototoxicity in children: Long-term follow-up reveals continuous worsening of hearing loss. *J Pediatr Hematol Oncol*. 2004;26(10):649-655. doi:10.1097/01.mph.0000141348.62532.73
34. Al-Khatib T, Cohen N, Carret AS, Daniel S. Cisplatinum ototoxicity in children, long-term follow up. *Int J Pediatr Otorhinolaryngol*. 2010;74(8):913-919. doi:10.1016/j.ijporl.2010.05.011

35. Kawashiro N, Tsuchihashi N, Koga K, Kawano T, Itoh Y. Delayed post-neonatal intensive care unit hearing disturbance. *Int J Pediatr Otorhinolaryngol*. 1996;34(1-2):35-43. doi:10.1016/0165-5876(95)01232-X
36. Nield TA, Schrier S, Ramos AD, Platzker AC, Warburton D. Unexpected hearing loss in high-risk infants. *Pediatrics*. 1986;78(3):417-422.
37. Jiang M, Karasawa T, Steyger PS. Aminoglycoside-Induced Cochleotoxicity: A Review. *Front Cell Neurosci*. 2017;11:308. doi:10.3389/fncel.2017.00308
38. Bramhall NF, Shi F, Arnold K, Hochedlinger K, Edge ASB. Lgr5-positive supporting cells generate new hair cells in the postnatal cochlea. *Stem Cell Reports*. 2014;2(3):311-322. doi:10.1016/j.stemcr.2014.01.008
39. Zorowka P, Schmitt HJ, Eckel HE, Lippert KL, Schönberger W, Merz E. Serial measurements of transient evoked otoacoustic emissions (TEOAEs) in healthy newborns and in newborns with perinatal infection. *Int J Pediatr Otorhinolaryngol*. 1993;27(3):245-254. doi:10.1016/0165-5876(93)90230-Z
40. Finitzo-Hieber T, McCracken GH, Brown KC. Prospective controlled evaluation of auditory function in neonates given netilmicin or amikacin. *J Pediatr*. 1985;106(1):129-136. doi:10.1016/S0022-3476(85)80485-6
41. Wang CH, Yang CY, Lien R, et al. Prevalence and independent risk factors for hearing impairment among very low birth weight infants. *Int J Pediatr Otorhinolaryngol*. 2017;93:123-127. doi:10.1016/j.ijporl.2016.12.029
42. Erenberg A, Lemons J, Sia C, Tunkel D, Ziring P. Task Force on Newborn and Infant Hearing. *Pediatrics*. 1999;103(2):527-529. doi:10.1542/peds.103.2.527 Updated
43. Cristobal R, Oghalai JS. Hearing loss in children with very low birth weight: Current review of epidemiology and pathophysiology. *Arch Dis Child Fetal Neonatal Ed*. 2008;93(6):462-468. doi:10.1136/adc.2007.124214
44. Bindu LH, Reddy PP. Genetics of aminoglycocide-induced and prelingual non-syndromic mitochondrial hearing impairment : A review. *Int J Audiol*. 2008;47(11):702-707. doi:10.1080/14992020802215862
45. Johnson RF, Cohen AP, Guo Y, Schibler K, Greinwald JH. Genetic mutations and aminoglycoside-induced ototoxicity in neonates. *Otolaryngol - Head Neck Surg*. 2010;142:704-707. doi:10.1016/j.otohns.2010.01.030
46. Bitner-Glindzicz M, Pembrey M, Duncan A, et al. Prevalence of Mitochondrial 1555A→G Mutation in European Children. *N Engl J Med*. 2009;360(6):640-642. doi:10.1056/NEJMc0806396
47. Mahood R. Pharmacogenetics to avoid loss of hearing. ISRCTN Registry number 1370489. https://doi.org/10.1186/ISRCTN13704894.
48. Kullar P, Alston CL, Ball S, et al. The frequency of the m.1555A>G(MTRNR1) variant in UK patients with suspected mitochondrial deafness. *Hear Balanc Commun*. 2016;14(2):101-102. doi:10.1056/nejmc0806397
49. Jing W, Zongjie H, Denggang F, et al. Mitochondrial mutations associated with aminoglycoside ototoxicity and hearing loss susceptibility identified by meta-analysis. *J Med Genet*. 2015;52(2):95-103. doi:10.1136/jmedgenet-2014-102753
50. Yehudai N, Most T, Luntz M. Risk factors for sensorineural hearing loss in pediatric chronic otitis media. *Int J Pediatr Otorhinolaryngol*. 2015;79(1):26-30. doi:10.1016/j.ijporl.2014.10.025
51. Jensen RG, Koch A, Homøe P. The risk of hearing loss in a population with a high prevalence of chronic suppurative otitis media. *Int J Pediatr Otorhinolaryngol*. 2013;77(9):1530-1535. doi:10.1016/j.ijporl.2013.06.025
52. Hunter LL, Margolis RH, Rykken JR, Le CT, Daly KA, Giebink GS. High frequency hearing loss associated with otitis media. *Ear Hear*. 1996;17(1):1-11.

53. Smit E, Liu X, Gill H, Sabir H, Jary S, Thoresen M. Factors associated with permanent hearing impairment in infants treated with therapeutic hypothermia. *J Pediatr*. 2013;163(4):995-1000. doi:10.1016/j.jpeds.2013.06.012
54. Fitzgerald MP, Reynolds A, Garvey CM, Norman G, King MD, Hayes BC. Hearing impairment and hypoxia ischaemic encephalopathy: Incidence and associated factors. *Eur J Paediatr Neurol*. 2019;23(1):81-86. doi:10.1016/j.ejpn.2018.10.002
55. Yoshinaga-Itano C, Sedey AL, Coulter DK, Mehl AL. Language of Early- and Later-identified Children With Hearing Loss. *Pediatrics*. 1998;102(5):1161-1171. doi:10.1542/peds.102.5.1161

Table 1. Children with hearing loss defined as PTA threshold > 20 dB HL (n=3) and/or EHFA threshold > 20 dB HL (n=5)

Age	PTA Best ear (dB HL)	EHFA Best ear (dB HL)	Clinical characteristics	Gentamicin TPC (mg/L)	Gentamicin cumulative dose (mg/kg)	Included main audiological analysis
12 years	14	22	GA 41 weeks Middle ear effusion	3.5	66	NO
14 years	14	38	GA 32 weeks Middle ear effusion	1.2	30	NO
7 years	21	NA	GA 39 weeks Middle ear effusion	1.8	24	NO
9 years	18	41	Twin, GA 28 weeks Mild psychomotor delay of unknown cause. Genetic hearing loss; diagnosed at school age	0.3	72	NO
9 years	58	55	Twin, GA 28 weeks Mild psychomotor delay of unknown cause. Genetic hearing loss; diagnosed at school age	0.3	54	NO
12 years	46	49	Twin, GA 24 weeks Long respiratory support. Hearing loss diagnosed at age 8 years.	0.6	72	YES
9 years	6	28	GA 26 weeks Normal middle ear No mechanical ventilation	0.7	108	YES
6 years	15	21	GA 41 weeks Admitted NICU for observation, no perinatal complications. Normal middle ear, but previous tympanostomy tubes.	0.9	18	YES

NA, not available: TPC, through plasma concentration; PTA, pure tone average; EHFA, extended high frequency average; GA, gestational age; dB HL, decibel hearing level

Table 2. Background characteristics, gentamicin exposure data and audiometry results

Variables and results	Gentamicin cohort (n=219)	Control cohort (n= 33)
Age at study visit (years)	9 (7-11)	10 (9-12)
Female	84/219 (38.4%)	17/33 (51.5%)
Birth weight (grams) • < 1500 g • 1500-2499 g • ≥ 2500 g	3360 (2154-3896) • 39/219 (17.8%) • 25/219 (11.4%) • 155/219 (70.8%)	3500 (3239-3816)
Gestational age (weeks) • ≤ 31 • 32-36 • ≥ 37	39 (33-41) • 47/219 (21.5%) • 28/219 (12.8%) • 144/219 (65.8%)	No information
Small for gestational age (< 10th centile)	19/219 (8.7%)	
Mechanical ventilation	46/219 (21%)	0/33 (0%)
Apgar score - 5 min	9 (7-10)	No information
Phototherapy	71/219 (32.4 %)	3/33 (10 %)
Neurological abnormalities as neonates • Intracranial hemorrhage • Cystic periventricular leukomalacia • Meningitis	13/219 (5.9 %) • 8/219 (3.7%) • 3/219 (1.4%) • 3/219 (1.4%)	0/33 (0%)
Gentamicin trough plasma concentration (mg/L) • Trough plasma concentration < 1 mg/L • Trough plasma concentration ≥ 1 mg/L	1.0 (0.7-1.2) • 128/219 (58.4%) • 91/219 (41.6%)	NR
Gentamicin cumulative dose (mg/kg) • Receiving ≤ 30 mg/kg (3 - 5 doses) • Receiving ≥ 36 mg/kg (6 doses or more)	30 (24-42) • 111/219 (50.7%) • 108/219 (49.3)	NR
Mitochondrial 1555 G>A mutation	1/219 (0.5%)	Not tested
Tympanostomy tubes, any	19/219 (8.7%)	0/33 (0%)
PTA threshold (dB HL) - best ear *†	2.5 (0 to 6.25)	2.5 (-0.6 to 3.8)
EHFA threshold (dB HL) - best ear **††	-1.7 (-5.0 to 5.0)	-4.2 (-5.9 to 0)

All data are median and interquartile range (IQR) or number and percentage (%).

EHFA, extended high-frequency average; PTA, pure tone average; NR, not relevant; dB HL, decibel hearing level.

Unadjusted analysis, gentamicin-exposed vs. healthy control cohort, *P=0.10 and **P<0.02.

Adjusted analysis for birth weight, gentamicin-exposed vs healthy control cohort, †P=0.33 and ††P=0.10

Table 3A. Regression analysis of gentamicin exposure and other predictors for hearing thresholds in the conventional mid-frequencies in the gentamicin-exposed cohort (n=219)

	Univariable		**Adjusted for birth weight**	
PTA threshold (dB HL) - best ear	**Beta (95% CI)**	***P* value**	**Beta (95% CI)**	***P* value**
Gentamicin - cumulative dose	0.01 (-0.01 to 0.03)	0.35	-0.002 (-0.03 to 0.02)	0.83
Gentamicin - highest TPC	-0.17 (-1.4 to 1.1)	0.78	-0.03 (-1.2 to 1.1)	0.96
Birth weight - per 500 g	-0.4 (-0.7 to -0.1)	0.004		**< 0.02***
Mechanical ventilation	2.3 (0.7 to 3.9)	0.004	1.5 (-0.4 to 3.4)	0.13
Phototherapy	1.2 (-0.2 to 2.6)	0.10	0.02 (-1.6 to 1.7)	0.98
Peak bilirubin (n=161)	0.08 (-0.3 to 0.5)	0.68	-0.07 (-0.5 to 0.3)	0.72
Apgar 5 min < 6	0.7 (-1.1 to 2.5)	0.43	-1.1 (-2.9 to 0.7)	0.22
Small for gestational age	1.2 (-1.2 to 3.5)	0.33	0.4 (-2.0 to 2.7)	0.76
Age at study visit	-0.2 (-0.5 to 0.1)	0.17	-0.3 (-0.6 to 0.02)	0.07
Tympanostomy tubes	1.6 (-0.7 to 3.9)	0.18	1.4 (-0.9 to 3.7)	0.22
Tympanometry – best ear	-4.4 (-7.1 to -1.6)	0.002	-4.1 (-6.8 to -1.4)	**0.003**

CI, confidence interval; PTA, pure tone average; TPC, trough plasma concentration.
* the *P* value for birth weight remained < 0.02 when adjusting for all predictors, except for a strong correlation between birth weight and mechanical ventilation and thus a P value of 0.13 for this adjusted analysis

Table 3B. Regression analysis of gentamicin exposure and other predictors for hearing thresholds in the extended high-frequencies in the gentamicin-exposed cohort (n=219)

	Univariable		**Adjusted for birth weight**	
EHFA threshold (dB HL) - best ear	**Beta (95% CI)**	***P* value**	**Beta (95% CI)**	***P* value**
Gentamicin - cumulative dose	0.05 (0.01 to 0.08)	0.007	0.02 (-0.01 to 0.06)	0.21
Gentamicin - highest TPC	-0.6 (-2.5 to 1.3)	0.538	-0.29 (-2.2 to 1.6)	0.76
Birth weight - per 500 g	-0.9 (-1.3 to -0.5)	<0.001		**< 0.02***
Mechanical ventilation	4.6 (2.1 to 7.2)	<0.001	0.41 (-s0.6 to 5.5)	0.12
Phototherapy	3.6 (1.3 to 5.8)	0.002	1.5 (-1.2 to 4.1)	0.28
Peak bilirubin (n=161)	0.3 (-0.3 to 0.9)	0.38	-0.02 (-1.5 to 0.6)	0.96
Apgar 5 min < 6	1.7 (-1.2 to 4.6)	0.25	-2.5 (-5.4 to 0.3)	0.08
Small for gestational age	3.8 (0.01 to 7.5)	0.049	2.1 (-1.7 to 5.9)	0.28
Age at study visit	0.4 (-0.06 to 0.9)	0.08	0.3 (-0.2 to 0.8)	0.22
Tympanostomy tubes	9.1 (5.5 to 12.7)	<0.001	8.8 (5.3 to 12.2)	**< 0.001**
Tympanometry – best ear	-3.0 (-7.9 to 1.8)	0.22	-2.1 (-6.8 to 2.7)	0.39

CI, confidence interval; EHFA, extended high-frequency average; TPC, trough plasma concentration.

* the *P* value for birth weight remained < 0.02 when adjusting for all predictors, except for a strong correlation between birth weight and mechanical ventilation and thus a P value of 0.12 for this adjusted analysis

Supplementary Table 1. Comparison of gestational age, birth weight and gentamicin exposure in the original gentamicin cohort and the follow-up cohort 6-14 years later.

	Original cohort (n= 440)	**Follow-up cohort (n=219)**
Gestational age (weeks)	39 (32-40)	39 (33-41)
Birth weight (gram)	3281 (1850 to 3815)	3360 (2154-3896)
• < 1500 g	84/440 (19%)	39/219 (18%)
Gentamicin TPC (mg/L)	1.0 (0.7 to 1.3 mg/L)	1.0 (0.7 to 1.2 mg/L)
• TPC > 2.0 mg/L	26 (6.0%)	11/219 (5.0%)
Gentamicin cumulative dose (mg/kg)	30 (24 to 36)	30 (24 to 42)

All data are median and interquartile range (IQR) or number and percentage (%).

TPC, through plasma concentration

Figure legends

Figure 1: Participant flow diagram. This figure displays the final study populations, from the original cohort through exclusions.

Figure 2: Hearing thresholds in dB HL (mean and standard deviation) in the conventional and extended high-frequency range in gentamicin-exposed cohort, healthy controls and a reference population.[30]

dB HL, decibel hearing level

Supplementary Figure 1: Scatter plot showing the correlation between cumulative gentamicin dose (mg/kg) in all infants and the hearing threshold in the extended high-frequencies (9-16 kHz). R^2 =0.033

P = 0.007 using linear regression statistics.

P = 0.15 using Spearman's non-parametric correleation.

EHFA, extended high-frequency average

dB HL, decibel hearing level

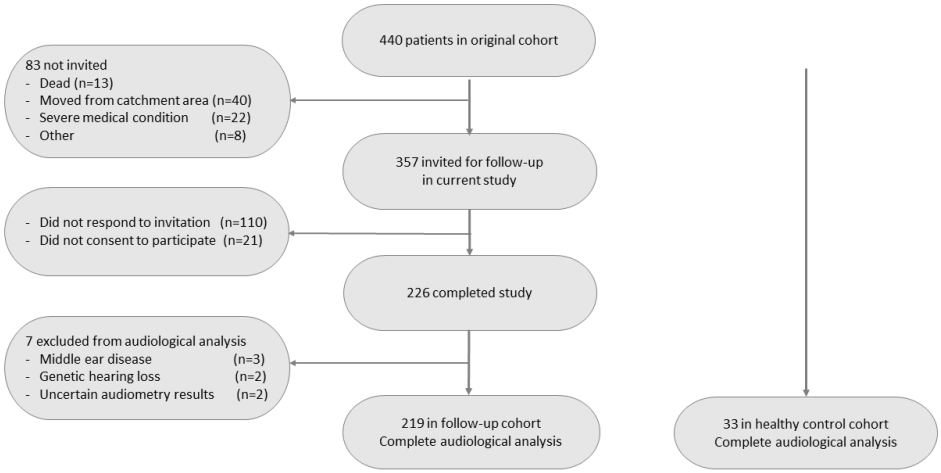


Figure 1

338x190mm (96 x 96 DPI)

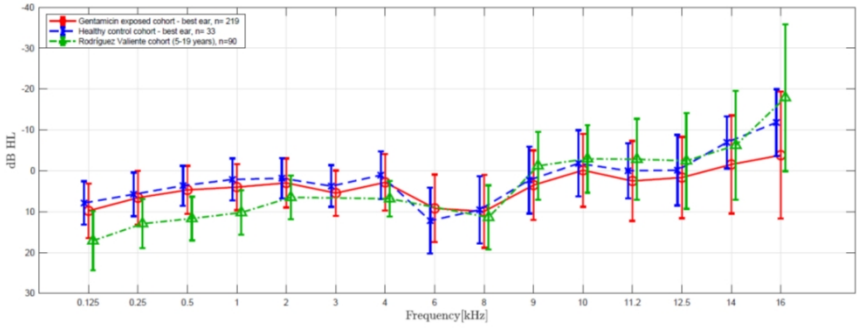
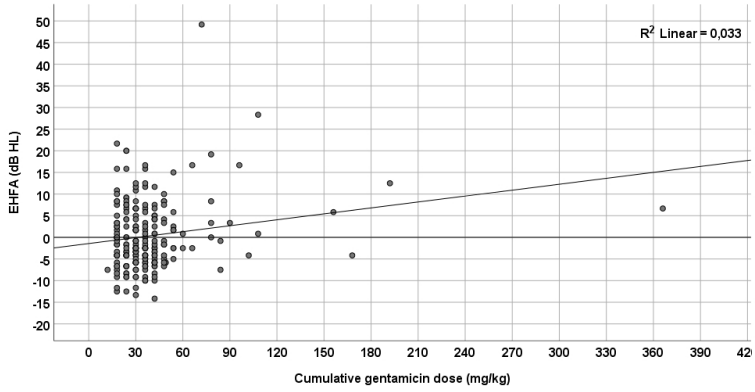


Figure 2

338x190mm (96 x 96 DPI)



338x190mm (96 x 96 DPI)