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Unbound Bilirubin and Auditory Neuropathy Spectrum Disorder in Late Preterm and Term Infants with Severe Jaundice

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Abstract

Objective—To evaluate if unbound bilirubin is a better predictor of auditory neuropathy spectrum disorder (ANSD) than total serum bilirubin (TSB) or the bilirubin albumin molar ratio (BAMR) in late preterm and term neonates with severe jaundice (TSB ≥ 20 mg/dL or TSB that met exchange transfusion criteria).

Study design—Infants ≥ 34 weeks gestational age with severe jaundice during the first two weeks of life were eligible for the prospective observational study. A comprehensive auditory evaluation was performed within 72 hours of peak TSB. ANSD was defined as absent or abnormal auditory brainstem evoked response waveform morphology at 80 decibel click intensity in the presence of normal outer hair cell function. TSB, serum albumin, and unbound bilirubin were measured using the colorimetric, bromocresol green, and modified peroxidase method, respectively.

Results—Five of 44 infants developed ANSD. By logistic regression, peak unbound bilirubin but not peak TSB or peak BAMR was associated with ANSD (odds ratio 4.6, 95% CI: 1.6–13.5, $p = 0.002$). On comparing receiver operating characteristic curves, the area under the curve (AUC) for unbound bilirubin (0.92) was significantly greater ($p = 0.04$) compared with the AUC for TSB (0.50) or BAMR (0.62).

Conclusions—Unbound bilirubin is a more sensitive and specific predictor of ANSD than TSB or BAMR in late preterm and term infants with severe jaundice.

Keywords

Total serum bilirubin; bilirubin:albumin molar ratio; sensitivity; specificity; bilirubin-induced neurotoxicity

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Chronic bilirubin encephalopathy (CBE), a preventable brain injury resulting from severe jaundice, is common in developing countries and has re-emerged in the US and other developed countries. (1-4) (5-9) Currently, total serum bilirubin (TSB) is primarily used for the evaluation and management of severe jaundice in neonates; although, previous studies have shown that TSB poorly predicts CBE in infants.(10-13) The sensitivity of TSB using a cut-off 20 mg/dl for CBE is high, but its specificity is poor. (14) This poor specificity of TSB results in unnecessary costly treatment of late preterm and term infants with severe jaundice to prevent bilirubin-induced neurotoxicity. Therefore, there is a need to identify a biochemical measure with better predictability than TSB for bilirubin-induced neurotoxicity.

The auditory system is highly sensitive to overt bilirubin-induced neurotoxicity, and therefore auditory evaluation is one of the best non-invasive objective means to evaluate bilirubin-induced neurotoxicity.(15) During the early phase of infant neurodevelopment, when neurological findings of CBE such as choreoathetoid movements and upward gaze palsy may not be evident, auditory brainstem evoked response (ABR) has been used to evaluate early subtle and transient changes in ABR latencies as a function of hyperbilirubinemia.(16, 17) More recently in late preterm and term infants, severe jaundice was concomitantly associated with acute auditory neuropathy spectrum disorder, an auditory disorder characterized by normal otoacoustic emission test (OAE), but abnormal or absent ABR.(18, 19) OAEs are low level sounds generated by outer hair cells. (20, 21) The ABR is a series of electrical potentials with early waves I and II represent activity of the auditory nerve, and waves III, IV and V represent activity of brainstem auditory structures. (22, 23) In auditory neuropathy spectrum disorder, the ABR is absent or may show a wave V, but with decreased amplitude and increased latency.(21, 24) However, peak TSB concentrations have failed to discriminate infants who develop acute auditory neuropathy spectrum disorder following severe jaundice.(18)

Emerging evidence suggests that free or unbound bilirubin (bilirubin not bound to albumin) may be a better predictor of bilirubin-induced neurotoxicity than TSB in premature and term infants.(15-17, 25-29) However, the usefulness of unbound bilirubin as a predictor of bilirubin-induced auditory neuropathy spectrum disorder has not been investigated using an appropriate comprehensive auditory evaluation soon after the occurrence of severe jaundice in late preterm and term infants. We hypothesized that unbound bilirubin is a more specific and sensitive predictor of bilirubin-induced auditory neuropathy spectrum disorder than TSB or bilirubin:albumin molar ratio (BAMR). Our objective was to compare TSB, unbound bilirubin, and BAMR as predictor of auditory neuropathy spectrum in late preterm and term infants with severe jaundice.

Methods

This was a prospective observational study involving late preterm and term infants admitted to the University of Rochester Medical Center with severe jaundice. Parental consent was obtained for each subject enrolled. The study was approved by the Institutional Research Review Board.

Infants 34 weeks gestational age (GA) who had severe jaundice or unconjugated hyperbilirubinemia (TSB ≥ 20 mg/dL or TSB concentration that met exchange transfusion criteria according to American Academy of Pediatrics [AAP] guidelines) during the first two weeks of life were eligible for the study.(10, 19, 30) Our exclusion criteria included conditions often associated with sensori-neural hearing loss (31): a) Cranio-facial malformations; b) Chromosomal disorders; c) Family history of congenital deafness or auditory neuropathy unrelated to prematurity or hyperbilirubinemia; d) TORCH (toxoplasmosis, other infections, rubella, cytomegalovirus infection and herpes simplex) infections; and e) Infants requiring surgical interventions at the time of severe jaundice. In addition, infants with failed newborn hearing screening test prior to readmission for severe jaundice were excluded. For 34^{0/7}-34^{6/7} weeks' GA infants, TSB concentration that met exchange transfusion criteria was considered the same as that for 35^{0/7}-37^{6/7} GA infants. GA was assessed by obstetric history, or if obstetric history was unreliable, by Ballard examination.

Bilirubin-Albumin Binding Variables

Blood samples for the measurement of TSB for individual subjects were drawn as clinically indicated at the discretion of the attending neonatologist in amber colored serum separator tubes to protect from light. TSB concentration was measured (mg/dL, multiply by 17.1 to convert to $\mu\text{mol/L}$) immediately (in < 2 hours) by the clinical chemistry laboratory using the standard colorimetric method. The same aliquot of blood used to measure TSB was used to measure unbound bilirubin concentration ($\mu\text{g/dL}$, multiply by 17.1 to convert to nmol/L). Unbound bilirubin was measured by the modified peroxidase method at two enzyme concentrations (1:25 and 1:12.5 dilutions) of pre-calibrated peroxidase (Arrows Co, Ltd; Osaka Japan) using an FDA approved Arrows unbound bilirubin analyzer UA-1 (Arrows Company, Ltd; Osaka Japan). Serum albumin level (g/dL, multiply by 151 to convert to $\mu\text{mol/L}$) was measured for each individual subject using the bromocresol green method. The peak BAMR was calculated for each subject using the peak TSB concentration and the concurrent serum albumin concentration. The peak TSB, peak unbound bilirubin, and peak BAMR were determined for each subject.

Comprehensive Auditory Evaluation

Each subject had comprehensive auditory evaluation (tympanometry, OAE test, and ABR) performed in both ears within 72 hours of peak TSB concentration and after resolution of severe jaundice by an audiologist unaware of the degree of jaundice. ABR tests were recorded with a Biological Navigator Evoked Response System (Bio-logic, Mundelein, IL) using 80 decibel (dB) broadband stimulus with the subjects lying supine in the crib and a skin temperature $> 35.5^{\circ}\text{C}$. The clicks were presented at a repetition rate of 29.9/sec and 3 runs of 2000 repetitions were recorded for each ear. The response was amplified ($\times 200\,000$), and band-pass filtered (100-3000 Hz), and averaged over a 20-msec time epoch. The 2 most reproducible runs for each ear were averaged and used for analysis. ABRs were also performed using reverse polarity (condensation and rarefaction) to evaluate for cochlear microphonics. The OAE test was administered using DP Echoport ILO 292 (Otodynamics, London, England) on all subjects using an 80 dB click stimulus. A normal OAE was defined as a replicable response (3 dB Signal to Noise ratio) in the 3 highest frequency bands (2000,

3000 and 4000 Hz). Tympanometry was performed in each ear to exclude middle ear disease on all infants. Infants with abnormal ABR morphology or absent ABR waveform but normal OAE or presence of cochlear microphonics were diagnosed to have acute auditory neuropathy spectrum disorder.

Clinical Risk Factors

Clinical risk factors such as perinatal asphyxia (Apgar score < 3 at 5 minutes and/or cord pH < 7.0), congenital sepsis (culture proven or clinical sepsis requiring at least 7 days of intravenous antibiotics), and hemolytic disorders (Rh incompatibility, ABO incompatibility, G6PD deficiency, hereditary spherocytosis, etc.) were prospectively collected. All subjects were evaluated by the physician for clinical signs and symptoms of acute bilirubin encephalopathy on admission as recommended by the AAP for consideration of immediate exchange transfusion.(30) Phototherapy and exchange transfusion were used as per AAP guidelines.(30) Intravenous gamma globulin was used as per the institutional policy for hemolytic jaundice secondary to ABO or Rh incompatibility.

Sample Size Calculation

Sample size calculation was based on McNemar test statistic testing the difference in specificity between the unbound bilirubin and TSB levels, and sensitivity was fixed near 100%. From published data, when the empirical sensitivity is fixed at 100%, the specificities for TSB and unbound bilirubin are 0.025 and 0.275 respectively.(27, 28) A prevalence of acute auditory neuropathy spectrum disorder was expected to be at least 10% based on previous reported studies.(18, 19, 32) A sample size calculation for specificity with sensitivity >.98, a sample size of 40 was required for an 80% power to detect the difference in specificity of 0.25 between TSB and unbound bilirubin at two-sided significance level.

Statistical Analyses

All statistical analyses were conducted using STATA 10. Infants' characteristics were summarized with descriptive statistics and compared between those with auditory neuropathy spectrum disorder and those without auditory neuropathy spectrum disorder. The Fisher exact test or the chi-square test was used for categorical variables, and t-test or Wilcoxon Rank Sum test was used for continuous variables. All analyses were 2-sided at the 0.05 level of significance. Logistic regression was used to evaluate the independent association between each of the bilirubin albumin binding variables (peak TSB, peak unbound bilirubin, and peak BAMR) and acute bilirubin-induced auditory neuropathy spectrum disorder. Variables with significant association ($p \leq 0.2$) to outcome or exposure variables were included in the regression model. Co-linearity diagnostics was performed for continuous variables before being included in the regression model. Final model building was performed using a backward selection method. Likelihood ratio tests were performed to evaluate the inclusion of potential confounders in the final logistic regression model. Goodness-of-fit was evaluated using the Hosmer-Lemeshow test. Potential confounding factors were controlled in each of the final logistic regression models. The strength of associations between peak TSB, peak unbound bilirubin, and peak BAMR and auditory neuropathy spectrum disorder was evaluated using odds ratio and 95% confidence interval (CI)

Receiver operating characteristic (ROC) curves were plotted for each of the bilirubin-albumin binding variables predicting acute bilirubin-induced auditory neuropathy spectrum disorder and area under the curves (AUC) were compared using the non-parametric test.(33)

Results

A total of 55 infants were admitted with severe jaundice over a 6 year period and all infants met study criteria. Of 55 infants, 44 consented and participated in the study. The mean birth weight and GA for the infants were 3287g (standard deviation [SD] 473) and 37.9 weeks (SD 1.8), respectively. There were 23 males (52%). The population was mostly Caucasian (n = 30, 68%) and non-Hispanic (n = 39, 89%).

The mean TSB and unbound bilirubin for the infants were 23.2 mg/dL (SD 4.8) and 2.0 µg/dL (SD 1.2), respectively. The mean postnatal day of peak TSB was 3.8 day (SD 2.2). Two infants had hypoalbuminemia (< 3 g/dL). None of the infants had history of *in-utero* drug exposure, perinatal asphyxia or culture proven sepsis. All infants received intensive phototherapy. There were 14 infants with hemolytic disorders (7 with ABO incompatibility, 6 with Rh incompatibility, 1 with hereditary spherocytosis). Nine infants received exchange transfusion as per the AAP guidelines. None of the infants required respiratory support during the study period. Blood gases were measured in 7 infants who underwent exchange transfusion. None of these 7 infants had hypoxia ($\text{PaO}_2 < 45$ mm of Hg) or acidosis ($\text{pH} < 7.25$). There were 4 infants with polycythemia (hematocrit > 65%), 1 infant with cephalhematoma, and 1 infant with extensive bruising. The majority of infants (91%) received breast milk feeding prior to the occurrence of severe jaundice. None of the infants received intravenous lipid, ceftriaxone, ibuprofen or indomethacin during the study period.

Five out of 44 infants (11%) had auditory neuropathy spectrum disorder (4 with absent ABR waveform and 1 with abnormal ABR morphology [absent wave I and III with small wave V]). Of these five infants, two infants also had clinical signs of acute bilirubin encephalopathy. The demographic and clinical characteristics of infants as a function of auditory neuropathy spectrum disorder are shown in Table I. There was no significant difference in GA, birth weight, race, ethnicity, and sex between infants who developed auditory neuropathy spectrum disorder and infants who did not develop auditory neuropathy spectrum disorder. There was no significant difference in maternal chorioamnionitis, mode of delivery, Apgar score at 5 minutes, clinical sepsis, and type of enteral feeding (breast milk or formula) between the 2 groups. There was also no significant difference in the incidence of hemolytic disorders and polycythemia between the 2 groups. The proportion of infants who had exchange transfusion for severe jaundice was not significantly different between the 2 groups.

There was a significant difference in peak unbound bilirubin concentration but not in peak TSB concentration and peak BAMR between infants who developed auditory neuropathy spectrum disorder and infants who did not develop auditory neuropathy spectrum disorder (Table II). The mean unbound bilirubin concentrations of infants with auditory neuropathy spectrum disorder were significantly greater than those for infants without auditory neuropathy spectrum disorder. Three of the five infants with auditory neuropathy spectrum

disorder had very low calculated bilirubin albumin binding affinity ($< 45 \text{ L}/\mu\text{mol}$), a measure of the avidity with which albumin binds bilirubin. The clinical characteristic features of five infants with auditory neuropathy spectrum disorder are shown in Table III.

Because TSB, BAMR, and unbound bilirubin correlated with each other (TSB and BAMR $r = 0.90$, $p = 0.001$; TSB and unbound bilirubin, $r = 0.38$, $p = 0.01$; and unbound bilirubin and BAMR, $r = 0.39$, $p = 0.009$), these bilirubin exposure variables were not included in the same regression model. To account for this, three separate regression models were built for each bilirubin biochemical measure, specifically TSB, BAMR, and unbound bilirubin. Among the covariates (Table I), only sex and Apgar score at 5 minutes were initially included in the regression analyses. However, neither sex nor Apgar score at 5 minutes were identified as a confounder by log likelihood ratio tests. Therefore, these variables were not included in the final logistic regression models. There was a strong association between peak unbound bilirubin concentration and auditory neuropathy spectrum disorder (Odds Ratio: 4.6, 95% CI: 1.6-13.5) as shown in Table II. There was no significant association between peak TSB and auditory neuropathy spectrum disorder as well as between peak BAMR and auditory neuropathy spectrum disorder as shown in Table II.

The ROC curves for TSB, BAMR and unbound bilirubin as predictors of auditory neuropathy spectrum disorder are shown in the Figure. There was a significant difference ($p = 0.04$) in the AUC's between the unbound bilirubin (0.92, 95% CI: 0.80-1.00) and TSB (0.50, 95% CI: 0.14-0.85) as well as between the unbound bilirubin and BAMR (0.62, 95% CI: 0.24-0.88) with unbound bilirubin as the best predictor of auditory neuropathy spectrum disorder among all three exposure variables. Although the AUC for BAMR was greater than the AUC for TSB, the difference was not significant. The sensitivity and specificity of TSB

20 mg/dL, a cut-off used to define severe jaundice, was 0.80 and 0.15, respectively for bilirubin-induced auditory neuropathy spectrum disorder. The unbound bilirubin concentration of $2.4 \mu\text{g}/\text{dL}$ was associated with 0.80 sensitivity (95% CI: 0.29 - 0.99) and 0.80 specificity (95% CI: 0.64 - 0.91) for bilirubin-induced auditory neuropathy spectrum disorder in late preterm and term infants. The odds ratio using this cut-off of unbound bilirubin is 15.5 (95% CI: 1.52-158.52). Four out of five infants with acute auditory neuropathy spectrum disorder had follow-up audiology evaluation at two to three months after the initial evaluation. Of these four infants, two infants showed improvement and two infants had persistent abnormal auditory findings.

Discussion

Severe jaundice in late preterm and term infants may be associated with bilirubin-induced neurotoxicity. However, TSB, the primary biochemical measure used for the evaluation and management of severe jaundice, is a poor predictor of bilirubin-induced neurotoxicity, including auditory neuropathy spectrum disorder. Our findings suggest that unbound bilirubin is a better predictor of auditory neuropathy spectrum disorder than TSB or BAMR in late preterm and term infants with severe jaundice. This prospective study demonstrates the usefulness of unbound bilirubin as a predictor of acute bilirubin-induced auditory neuropathy spectrum disorder in late preterm and term infants with severe jaundice.

CBE, characterized by choreoathetoid cerebral palsy, auditory disorders, gaze paresis, and enamel hypoplasia, is a life-long debilitating disorder caused by severe jaundice, therefore, early identification of at-risk infants during the neonatal period is paramount to facilitate intervention and improve the long-term outcome. There is ample evidence that severe jaundice may be associated with auditory neuropathy spectrum disorder during the neonatal period. (18, 19, 32, 34) The prospective studies have demonstrated usefulness of comprehensive auditory evaluation during the neonatal period for identification of bilirubin-induced auditory toxicity, specifically auditory neuropathy spectrum disorder in late preterm and term infants with severe jaundice.(18, 19) However, these studies used only TSB as a biochemical measure for its association with auditory neuropathy spectrum disorder which failed to discriminate infants at risk for bilirubin-induced auditory toxicity.(18, 19)

Our findings of the usefulness of unbound bilirubin as a predictor of bilirubin-induced neurotoxicity are in agreement with most studies in term and preterm infants that evaluated more subtle ABR changes as a function of bilirubin albumin binding variables.(16, 17, 27-29) In term infants, unbound bilirubin was reported to be a more sensitive and specific predictor of prolonged ABR wave latencies than TSB.(16, 17) This was corroborated by Ahlfors et al who reported that unbound bilirubin was a better predictor than TSB of failed hearing screening tests, as evaluated by automated ABR in a retrospective case-control study.(28) However, compared with our study, Ahlfors et al used proximate TSB and unbound bilirubin within 4 hours of automated ABR and failed to evaluate usefulness of BAMR. The automated ABR typically involves 35 dB and a failed screening test does not necessarily imply an auditory disorder, specifically auditory neuropathy spectrum disorder. Compared with previous studies, we used 80 dB ABR as recommended for the evaluation of auditory neuropathy spectrum disorder. Similar to findings in term infants, in a prospective study involving premature infants, unbound bilirubin was found to be a more sensitive and specific predictor than TSB or the BAMR of acute abnormal changes in ABR morphology. (29) More recently, unbound bilirubin was more strongly associated than TSB or BAMR with other acute neurological manifestations such as central apnea in premature infants.(25)

Bilirubin is mainly bound to albumin in blood, therefore, BAMR, an index of bilirubin binding capacity, is often used in conjunction with TSB for the evaluation and management of severe jaundice.(30) However, BAMR failed to discriminate infants with ANSD and our findings of ROC curves suggest that BAMR does not significantly improve prediction over TSB. This is in agreement with a recent study that reported that BAMR does not improve prediction of bilirubin-induced neurotoxicity compared with TSB in term infants with severe jaundice.(35) Our findings of decreased calculated bilirubin albumin binding affinity explain higher unbound bilirubin in three out of five infants with auditory neuropathy spectrum disorder and possibly explain failure of the BAMR to better predict auditory neuropathy spectrum disorder. We did not identify acidosis, hypoxia, or sepsis among these three infants that may explain the decrease in bilirubin binding affinity. We speculate the presence of unknown factors influencing bilirubin albumin binding affinity in these three infants who developed auditory neuropathy spectrum disorder. Nonetheless, until the availability of the technology of unbound bilirubin measurement for clinical use, BAMR should be used in conjunction with TSB for the evaluation and management of severe jaundice as recommended by the AAP.

Despite adherence to the AAP guidelines, 5 infants developed auditory neuropathy spectrum disorder. The AAP guidelines which are based on limited evidence recommend that for readmitted infants with TSB level above the exchange level, TSB measurement should be repeated every 2 to 3 hours and exchange transfusion should be considered if the TSB remains above the levels indicated after administering intensive phototherapy for 6 hours. (30) Three out of 5 infants who met exchange transfusion criteria did not receive exchange transfusion as they responded to intensive phototherapy within 6 hours as recommended by the consensus based AAP guidelines. Our findings suggest that adherence to AAP guidelines which are based on TSB may not completely prevent bilirubin-induced brain injury. Larger studies involving infants with severe jaundice are required to corroborate our findings and inform evidence-based guidelines.

Because the use of hearing screening tests, such as OAE or automated-ABR, alone or in combination, has limitations in identifying cases of auditory neuropathy spectrum disorder (24, 36), all late preterm and term infants with severe jaundice should have comprehensive auditory evaluation performed to identify infants with auditory neuropathy spectrum disorder. Identification of children with auditory neuropathy spectrum disorder is important because their clinical characteristics and treatment differ from other cases of hearing loss in children.(36) A typical child with auditory neuropathy spectrum disorder presents with difficulty understanding speech that is out of proportion to the audiometric threshold impairment. These children with auditory neuropathy spectrum disorder are at increased risk for abnormal language development.(37)

The strengths of the study are prospective measurement of biochemical measures, including unbound bilirubin measurement using the modified peroxidase method, and timely and adequate auditory evaluation of infants with severe jaundice. The findings of auditory neuropathy spectrum disorder were observed despite immediate and appropriate treatment of severe jaundice. Our findings therefore represent more persistent and significant bilirubin-induced neurotoxicity. The weakness of the study is that very few infants had co-morbid conditions other than hemolytic disorders that are known to be associated with increased risk of bilirubin-induced neurotoxicity at a lower level of TSB levels. The study also could not evaluate the influence of the duration of severe jaundice as all infants responded with rapid resolution of jaundice with aggressive treatment.

In summary, jaundice is associated with auditory neuropathy spectrum disorder, which is predicted by unbound bilirubin and not TSB or BAMR in late preterm and term infants with severe jaundice. Our findings add to the growing evidence for the usefulness of unbound bilirubin in the evaluation of bilirubin-induced neurotoxicity. Future larger studies are needed to evaluate the incidence and natural course of jaundice-associated auditory neuropathy spectrum disorder in late preterm and term infants. A larger study may also help to evaluate the usefulness of unbound bilirubin as a predictor of bilirubin-induced neurotoxicity in the presence of clinical risk factors such as asphyxia, sepsis, etc. Future studies are also warranted to evaluate the long-term prognostic significance of identifying infants with bilirubin-induced acute auditory neuropathy spectrum disorder during the neonatal period.

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Abbreviations

ANSD	Auditory Neuropathy Spectrum Disorder
TSB	Total Serum Bilirubin
BAMR	Bilirubin Albumin Molar Ratio
CBE	Chronic Bilirubin Encephalopathy
OAE	Otoacoustic Emission Test
ABR	Auditory Brainstem Evoked Response
dB	Decibel
GA	Gestational Age
AAP	American Academy of Pediatrics
SD	Standard Deviation
ROC	Receiver Operating Characteristic
CI	Confidence Interval
AUC	area under the curve
TORCH	Toxoplasmosis, Other infections, Rubella, Cytomegalovirus, and Herpes simplex

Figure.

Receiver operating characteristics (ROC) curves of the peak TSB, peak unbound bilirubin, and peak BAMR as predictors of Auditory Neuropathy Spectrum Disorder are shown. The straight line is the expected curve (unity) if the variable has no predictive value (area under unity curve 0.5). The areas under the curves are unbound bilirubin 0.92, TSB 0.50, and BAMR 0.62. The area under the unbound bilirubin curve is significantly greater than the area under the TSB and BAMR curves.

Table 1
Demographic and Clinical Characteristics as a Function of Auditory Neuropathy Spectrum Disorder (ANSD)

	Infants without ANSD (n = 39)	Infants with ANSD (n = 5)	P
Gestational Age (weeks) [#]	37.9 ± 1.7	37.3 ± 2.6	0.69 [*]
Birth Weight (grams) [#]	3295 ± 472	3224 ± 531	0.78 [*]
Sex, n (% Male)	19 (48)	4 (80)	0.16 [∞]
Race, n (% White)	26 (67)	4 (80)	0.6 [∞]
Ethnicity, n (% Hispanic)	5 (13)	0 (0)	1 [∞]
Chorioamnionitis, n (%)	1 (2)	0 (0)	1 [∞]
Mode of Delivery, n (% cesarean)	7 (18)	1 (20)	1 [∞]
Clinical Sepsis, n (%)	2 (5)	1 (20)	0.31 [∞]
Apgar Score at 5 min, median (IQR) ^{**}	9 (9-9)	9 (7-9)	0.2 [*]
Hemolytic disorders, n (%)	12 (31)	2 (40)	0.64 [∞]
Polycythemia, n (%)	3 (7)	1 (20)	0.4 [∞]
Breast Milk Feeding, n (%)	35 (90)	5 (100)	0.8 [∞]
Exchange Transfusion n (%)	7 (18)	2 (40)	0.27

[#] Mean ± standard deviation;

^{*} Mann-Whitney U test;

[∞] Fisher exact test;

^{**} IQR denotes Interquartile range

Table 2
Bilirubin Albumin Binding Variables and Auditory Neuropathy Spectrum Disorder (ANSD) in Late Preterm and Term Infants with Severe Jaundice

	Infants without ANSD (n = 39)	Infants with ANSD (n = 5)	Odds Ratio (95% CI)	<i>P</i>
Peak Total Serum Bilirubin (mg/dL) [#]	23.1 (20.9 - 24.9)	22.3 (20.4 - 28)	1.01 (0.8-1.2)	0.93
Peak Bilirubin Albumin Molar Ratio [#]	0.72 (0.64 - 0.78)	0.72 (0.68 - 0.83)	10.7 (0.02-39)	0.43
Peak Unbound Bilirubin (µg/dL)	1.66 (1.22 - 2.27)	4.97 (2.74 - 5.21)	4.6 (1.6-13.5)	0.002

[#] denotes median (interquartile range); P-values were based on logistic regression analyses predicting ANSD.

Table 3
Characteristics of Individual Subjects with Auditory Neuropathy Spectrum Disorder

Subject	Gestational Age (weeks)	Hemolytic Jaundice	Peak Total Serum Bilirubin (mg/dL)	Peak Bilirubin Albumin Molar Ratio	Peak Unbound Bilirubin (µg/dL)
1	38	No	22.3	0.68	2.74
2	39.5	No	28	0.83	2.1
3	34	No	20.4	0.72	4.97
4	40	Yes	31.4	0.98	5.21
5	35.1	Yes	15.1	0.65	6.07