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Unbound bilirubin predicts abnormal automated auditory brainstem response in a diverse newborn population

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Abstract

Objective—The objective of this study was to determine if plasma unbound or 'free' bilirubin concentration (B_f) measured during the first 30 days of life is associated with subsequent abnormal hearing screening testing by automated auditory brainstem response (AABR) in a diverse population of newborns.

Study Design—An observational study of newborns receiving AABR, plasma total bilirubin concentration (TBC) and B_f measurements and without underlying conditions known to affect hearing was conducted. Logistic regression was used to determine associations between abnormal AABR and B_f or TBC. The impacts of a variety of clinical factors on the regression model were also assessed.

Result—A total of 191 patients with birth weights and gestations ranging from 406 to 4727 g and 24 to 42 weeks, respectively, were studied. Among them, 175 (92%) had normal (bilateral PASS) AABR and 16 had abnormal AABR (6 had unilateral REFER AABR, and 10 had bilateral REFER AABR). Mean TBC was not significantly different in babies with normal or abnormal AABR, but mean B_f was greater in the latter group (1.76 versus 0.93 μg per 100 ml, respectively, $P = 0.012$). B_f, but not TBC, was associated with an abnormal AABR (B_f adjusted odds ratio 3.3, 95% CI 1.8 to 6.1). Comparing receiver-operating characteristics curves, the B_f/TBC ratio was a better predictor of an abnormal AABR than B_f alone. Intraventricular hemorrhage was the only confounding clinical variable.

Conclusion—An abnormal AABR is associated with an elevated B_f or B_f/TBC ratio, but not the TBC alone. The prevalence of bilirubin neurotoxicity as a cause of audiological dysfunction may be underestimated if the TBC alone is used to assess the severity of newborn jaundice.

Keywords

hyperbilirubinemia; unbound bilirubin; automated auditory brainstem response

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Introduction

The automated auditory brainstem response (AABR) is widely used as a hearing screening test during the first few days of life in term newborns and before discharge from hospital in premature newborns. A recent study reported that the likelihood of a proximate abnormal (bilateral ear REFER) AABR in jaundiced term newborns increases with increasing plasma unbound or 'free' bilirubin concentration (B_f) but not with increasing total bilirubin concentration (TBC).[1] There have, however, been several reports of auditory deficits in both premature and term newborns associated with peak TBC levels that are generally below those at which exchange transfusion is recommended.[2–5] We undertook this retrospective case–control study to determine whether B_f or TBC is associated with an abnormal AABR, irrespective of the clinical circumstances and the proximity in time of the TBC, B_f and AABR measurements within the time framework of the newborn period.

Methods

Study population

All neonates who were admitted to the neonatal intensive care unit (NICU) of the California Pacific Medical Center between 1998 and 2003 and who had B_f and AABR performed for clinical care were eligible for the study. The review of their medical records for the purposes of this study was approved by the institutional review board of California Pacific Medical Center. Infants with congenital anomalies, genetic disorders, TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes, syphilis, and human immunodeficiency viral infections) were excluded. In addition, infants who died or were transferred to other hospitals before the AABR test was carried out were excluded.

Plasma bilirubin exposure variables

TBC and B_f measurements—TBC (mg per 100 ml) and B_f (μg per 100 ml) were measured by the peroxidase method in a Clinical Laboratory Improvement Amendments certified laboratory using an FDA-approved Arrows UB Analyzer (Arrows Co. Ltd., Osaka, Japan).[6] B_f was also measured at half the recommended peroxidase concentration to obtain the equilibrium B_f as described elsewhere.[7]

Because the peroxidase test was carried out in the NICU and required a dedicated technician, it was used at the discretion of the attending physician. A total of 256 newborns underwent the test between 1998 and 2003. B_f was typically measured when TBC exceeded 20 mg per 100 ml in term babies or when conditions associated with increased risk of bilirubin toxicity were present, such as hemolysis or prematurity.

TBC and B_f intervention levels—Standard TBC exchange transfusion guidelines modified for albumin concentration were used.[8] Phototherapy was administered at the discretion of the attending physician. In many babies, the B_f was measured only once and often early in the clinical course to help identify babies with weaker binding that might be at risk for bilirubin neurotoxicity at TBC or TBC/albumin ratios below the standard exchange transfusion guidelines. Weaker binding is associated with higher bilirubin–albumin

equilibrium dissociation constants (K_d), and because at clinically relevant TBC and albumin concentrations TBC–$B_f \approx$ TBC,

$$K_d = \frac{B_f}{TBC}(Albumin - TBC)$$

Because many TBC and B_f were not the peak values of the variables, we also included B_f/TBC ratio, which is proportional to K_d and therefore a measure of relative binding weakness, as an exposure variable in addition to TBC and B_f in the various statistical models.

Outcome variables: AABR

AABR in premature newborns was routinely measured a few days before discharge (ALGO hearing screening system; Natus Medical, San Carlos, CA, USA) by the nurses or attending staff trained in the procedure. In addition to routine AABR measurements, term infants often had additional AABR measurements when the TBC exceeded 20 mg per 100 ml. For term babies with repeat AABR, the AABR result most proximate in time to the bilirubin-binding measurements was used for the study. The instrument's algorithm assumes an infant is deaf until the acquired data fits with 99.96% likelihood, a template composed of auditory brainstem responses obtained at 35 dB from normal hearing newborns. Failure of an ear to attain this likelihood is reported as a REFER (versus a PASS), which indicates the baby needs follow-up hearing testing. For this study, we considered a bilateral ear PASS AABR (PP AABR, bilateral ear PASS (normal) result in an AABR hearing screening test) as normal and the babies with PP AABR as the control group. The case group comprised babies with abnormal AABR results, indicated by unilateral or bilateral ear REFER (PR AABR or RR AABR, bilateral ear REFER (abnormal) result in an AABR hearing screening test). All abnormal results were confirmed by at least one repeat AABR test.

Clinical variables—The medical records of all eligible subjects were reviewed to collect information on possible confounders (Table 1). Hemolysis was defined as a positive direct antiglobulin test (DAT) with increased reticulocyte count (reticulocyte count >5% during the first week after birth irrespective of gestational age). Chronic lung disease was defined as oxygen requirement with appropriate chest X-ray changes at 28 days of life. Sepsis was defined as a condition associated with positive blood culture warranting antibiotic therapy. Pneumonia was defined as changes in chest X-ray, clinical symptoms and organisms in the tracheal aspirate warranting antibiotic therapy. Intraventricular hemorrhage (IVH) was diagnosed by neuro-ultrasound and necrotizing enterocolitis by pneumatosis on abdominal X-ray with clinical symptoms. Retinopathy of prematurity was diagnosed by a pediatric ophthalmologist.

Statistical analyses

Statistical analyses were carried out using Stata 8 (Stata Corporation, College Station, TX, USA). Bivariate analyses were carried out with Student's *t*-test for continuous variables and χ^2- analyses or Fisher's exact test for categorical variables, to evaluate association between each clinical variable (covariates) and AABR (main outcome). Stratified analyses with

Breslow–Day homogeneity tests and Mantel–Haenszel tests were used to evaluate for possible effect modifiers and confounders. Variables with significant association ($P<0.2$) to abnormal AABR were considered as possible confounders and were included in the regression model. Gestational age was considered *a priori* a confounder based on the literature. Colinearity diagnostics were carried out using variance inflation factor and tolerance before being included in the regression model.

The back selection method was used to construct the final model. Likelihood ratio tests were carried out to evaluate the inclusion of potential confounders in the final logistic regression model. Covariates that did not make a statistically significant contribution to the model as evaluated were removed from the model. Goodness of fit was evaluated using the Hosmer–Lemeshow test.[9] Potential confounding factors were controlled in the final logistic regression model. An α of 0.05 was considered statistically significant. The strength of associations between TBC, B_f, B_f/TBC and AABR was evaluated using odds ratio (OR) and 95% confidence interval. The sensitivity and specificity of TBC, B_f and TBC/B_f as predictors of abnormal AABR were evaluated and compared using receiver-operating characteristic curves (ROC).

To determine strength of associations between abnormal AABR and B_f or TBC as a function of hemolysis, we stratified infants into two groups: (1) infants with hemolysis and (2) infants without hemolysis. Stratified analyses (unadjusted OR) and logistic regression analyses (adjusted OR) were then carried out for each group.

Results

About 600 babies per year were admitted to the NICU at California Pacific Medical Center between 1998 and 2003. A total of 256 babies received TBC and B_f measurements during the study period. Two babies with trisomy 21 (both with RR AABR) and four with congenital cytomegalus virus (all with PP AABR) were excluded. Of the remaining 250 babies, 11 died and 14 were transferred to other hospitals before receiving the AABR test. Of the remaining 225 babies, 191 had AABR tests carried out and met study criteria. The mean birth weight and gestational age for the study population was 2246 g (s.d. 1104, range 406 to 4727) and 34 weeks (s.d. 5, range 24 to 41), respectively.

The mean birth weight and gestational age for the 34 babies not receiving AABR tests were not significantly different (2220 g and 33 weeks). There were 110 males (58%), and 60 babies (31%) were delivered by cesarean section. The population is mostly Caucasian (n = 78, 41%), Asian (n = 45, 24%) and Hispanic (n = 11, 6%).

For the 21 babies with more than one TBC and B_f measurement, the highest level of each is used. The mean TBC and B_f for the study population was 14.0 mg per 100 ml (s.d. 7.2, range 3.0 to 36.0) and 1.00 μg per 100 ml (s.d. 0.80, range 0.04 to 4.41), respectively. The mean B_f/TBC ratio for the study population was 0.066 μg mg^{-1} (s.d. 0.037, range 0.009 to 0.200). Out of 191 infants, 33 (16%) were DAT positive (27 ABO, 6 Rh) with reticulocyte counts >5% and were considered to have hemolysis. Out of 191 infants, 10 (5%) met the

TBC or TBC/albumin ratio exchange transfusion criteria and received one or more exchange transfusions.

AABR test was carried out at a mean of 38 weeks corrected gestation (33 to 55 weeks) and measured after resolution of jaundice in 131 patients (69%). Forty-four babies (23%) had AABR measured within 4 h of binding measurements as reported previously,[1] and in the remaining 16 babies that were jaundiced at the time of AABR measurement (8%), there was a mean of 24 h between the bilirubin-binding measurements and AABR. There were 175 PP AABR (92%), 6 PR AABR (3%) and 10 RR AABR (5%) giving a total of 16 patients with abnormal AABR (8%). There were no significant differences in the mean birth weights, gestational ages or other clinical variables for the babies with normal and abnormal AABR (Table 1). The proportion of infants who had exchange transfusion performed (2%) among those with normal AABR was significantly less than the proportion of infants who had exchange transfusion performed (37%) among infants with abnormal AABR ($P<0.05$).

Table 2 shows the relationships between the bilirubin variables and AABR. The mean TBC for the babies with normal AABR was not significantly different from that for the babies with abnormal AABR. However, the mean B_f and B_f/TBC of the babies with abnormal AABR were significantly greater than those for the babies with normal AABR.

Because TBC and B_f were found to correlate with each other, the exposure variables were not included in the same regression model. To account for this, three separate regression models were built for each exposure variable, specifically B_f, TBC and B_f/TBC. Among the covariates (Table 1), only IVH was identified as a confounder by log-likelihood ratio tests, and it was therefore included in the final logistic regression models. The three models therefore included gestational age, IVH and the exposure variable. The Hosmer– Lemeshow test suggested that each of the three models were good fits ($P>0.9$).

Table 3 demonstrates the strength of association between the exposure variables and an abnormal ABBR. Logistic regression applied to the population showed B_f to be a significantly associated with an abnormal AABR whereas TBC was not. B_f was even more strongly associated with abnormal AABR in the presence of hemolysis (unadjusted OR 14, 95% CI 3.6 to 54; adjusted OR 3.7 to 10^{50}) compared to in the absence of hemolysis (unadjusted OR 2.4, 95% CI 1.2 to 5.0; adjusted OR 2.34, 95% CI 1.1 to 4.6). TBC was not significantly associated with abnormal AABR in infants with hemolysis (unadjusted OR 1.17, 95% CI 0.94 to 1.4; adjusted OR 1.32, 95% CI 0.94 to 1.84). There was an especially strong association between B_f/TBC and abnormal AABR.

The ROC for TBC, B_f and B_f/TBC as predictors of a PR or RR AABR are shown in Figure 1. There were significant differences in the areas under the curves, with B_f/TBC the best predictor of abnormal AABR among all three exposure variables.

Subgroup analysis: babies <35 weeks gestation

As we previously demonstrated that B_f is associated with RR AABR in jaundiced term newborns >34 weeks gestation,[1] we studied as a subgroup the 97 subjects with gestations below 35 weeks. There were 89 normal AABR (92%) and 8 abnormal AABR (4 PR AABR,

and 4 RR AABR) Once again, B_f was associated with an abnormal AABR (OR 2.25, 95% CI 1.07 to 4.72) whereas TBC was not associated with an abnormal AABR (OR 0.98, 95% CI 0.84 to 1.15).

Discussion

A REFER AABR result for one or both ears occurs in perhaps 4% of newborn hearing screening tests, whereas congenital deafness occurs in about 1 to 2 babies per 1000.[10] B_f is not only associated with a proximate RR AABR during jaundice,[1] but our data indicate that B_f in general is an important and unappreciated predictor of an abnormal AABR. These findings suggest that bilirubin induced hearing dysfunction, and perhaps other neurological sequelae[11–14] may be more prevalent than is currently thought because TBC is conventionally used to gauge the severity of neonatal jaundice. For example, using our data, a TBC ≥25 mg per 100 ml[15] is not significantly associated with an abnormal AABR (OR 2.65, 95% CI 0.68 to 10.4), and without the B_f data, the relationship between bilirubin and an abnormal AABR in our study population could not have been documented.

It is interesting but perhaps not surprising that the B_f/TBC ratio proved to be the best predictor of abnormal AABR. The B_f and TBC were often not peak values, but their ratio is proportional to K_d. A higher K_d translates into a lower peak TBC at any given bilirubin production/excretion mismatch, which confounds the relationship between peak TBC and bilirubin toxicity. An acceptable (low) TBC may be providing false reassurance when K_d is high, and the B_f/ TBC ratio helps identify this situation.

Bilirubin-induced changes in the auditory brainstem response may be transient or permanent,[16–19] and these changes are more closely correlated with B_f than TBC.[20,21] Treatment with exchange transfusion can acutely reverse these changes,[18] and several of our term babies with PR or RR AABR had the AABR revert to PP following exchange transfusion. Although this and inadequate follow-up data prevented us from assessing the relationship between B_f and permanent audiological sequelae, a prospective study would seem warranted, particularly in light of the possible function of bilirubin in auditory dysfunction or auditory dyssynchrony.[5,22,23]

The major limitation of our study is its retrospective nature and the associated selection and ascertainment bias. It is therefore possible that the association between B_f and abnormal AABR may be partially explained by an unknown confounder not included in the regression. Despite these limitations, it is very unlikely that the very strong associations between B_f and B_f/TBC and abnormal AABR would become nonsignificant after including any missing confounder(s). Another limitation is that the outcome is a screening ABR and not a formal diagnostic ABR, which would have better confirmed auditory toxicity and the nature of the toxicity.

TBC has long been the conventional metric for assessing the severity of newborn jaundice vis-á-vis the likelihood of bilirubin-induced neurotoxicity and need for treatment. Evidence continues to accumulate that B_f is a more reliable biochemical indicator of the likelihood of bilirubin toxicity, and this may help explain in part the lack of evidence-based data

supporting TBC intervention guidelines.[24] In addition, as noted above failure to account for bilirubin–albumin binding may lead to erroneous conclusions about the relationships between bilirubin and neurological sequelae.[15] Advances in the clinical management of newborn jaundice await better indicators of bilirubin neurotoxicity, and further studies of B_f and perhaps the AABR, which is readily available in the NICU, seem warranted.

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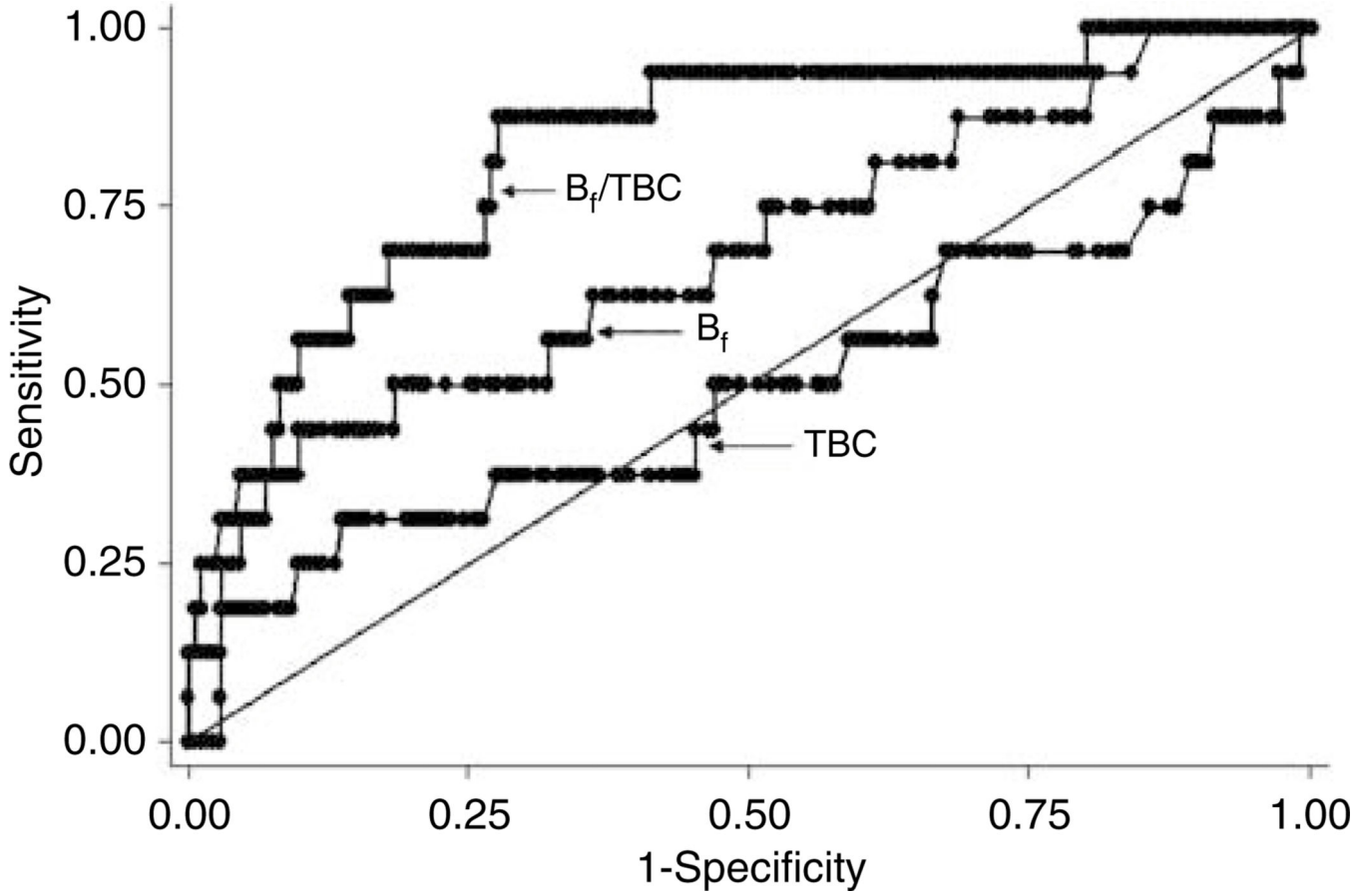

Figure 1.

Receiver-operating characteristics of the total bilirubin concentration (TBC), plasma unbound or 'free' bilirubin concentration (B_f) and the B_f/TBC ratio as predictors of an abnormal automated auditory brainstem response (AABR) 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 B_f/TBC ratio 0.83, B_f 0.69 and TBC 0.50. The areas under the B_f/TBC and B_f are significantly greater than that under the TBC curve.

Table 1

Bivariate analysis to evaluate associations between clinical factors and normal (PP AABR) or abnormal (PR or RR) AABR in 191 newborns

Clinical factor	Normal AABR (*n* = 175)	Abnormal AABR (*n* = 16)	*p*[a]
Birth weight (g)			
mean ± standard deviation	2253 ± 1098	2168 ± 1204	0.66[a]
Gestational age (week)			
mean ± standard deviation	34 ± 5	34 ± 5	0.97*
Gender (male/female)	99/76	11/5	0.43
Apgar <5 at 5 min	1/175	0/16	1.00
Hemolysis	30/175	3/16	0.74
Mechanical ventilation	75/175	7/16	0.94
Oxygen >4 h	78/175	8/16	0.67
Chronic lung disease at 28 days	39/175	3/16	0.57
Intraventricular hemorrhage	9/175	2/16	0.23
Infection (sepsis or pneumonia)	22/175	3/16	0.45
Necrotizing enterocolitis	2/175	0/16	0.83
Retinopathy of prematurity	20/175	1/16	0.52
Antibiotics	90/175	9/16	0.71

[a] Student's *t*-test; χ^2-test or Fisher's exact test for all others.

Table 2

Comparison of mean bilirubin exposure variables in babies with or without abnormal AABR

Bilirubin exposure variables	Normal AABR ($n = 75$) Mean ± s.d. (range)	Abnormal AABR ($n = 6$) Mean ± s.d. (range)	P
TBC (mg per 100 ml)	14.8 ± 6.9 (3.0–29.4)	15.8 ± 10.5 (3.2–36.0)	0.98[a]
B_f (μg per 100 ml)	0.93 ± 0.70 (0.04–3.45)	1.76 ± 1.31 (0.31–4.41)	0.01[a]
B_f/TBC (μg mg^{-1})	0.062 ± 0.034 (0.009–0.200)	0.109 ± 0.039 (0.034–0.167)	0.001[b]

[a] Mann—Whitney U-test, unequal variances.

[b] Student's t-test.

Table 3

Strength of association between plasma bilirubin exposure variable and abnormal AABR adjusted for confounders

Bilirubin exposure variables	Adjusted odds ratio* (95% confidence Interval)
TBC	1.04 (0.94–1.16)
Bf	3.3 (1.8–6.1)
B_f/TBC	3.38×10^{13} (2.43×10^{7}–4.7×10^{19})

*A separate logistics regression model was used for each bilirubin exposure variable.