A clinical prediction rule for acute bilirubin encephalopathy in neonates with extreme hyperbilirubinemia

A retrospective cohort study

Fanhui Zhang, MD, Lihua Chen, MD, Shiqiang Shang, MD, PhD, Kewen Jiang, MD, PhD,*

Abstract
To establish a clinical prediction rule for acute bilirubin encephalopathy (ABE) in term/near-term neonates with extreme hyperbilirubinemia.

A retrospective cohort study was conducted between January 2015 and December 2018. Six hundred seventy-three out of 26,369 consecutive neonates with extreme hyperbilirubinemia were enrolled in this study. Data included demographic characteristics, total serum bilirubin (TSB), albumin, bilirubin/albumin ratio (B/A), direct antiglobulin test, glucose-6-phosphate deficiency, asphyxia, sepsis, acidosis. ABE was defined as a bilirubin induced neurological dysfunction score of 4 to 9. We used stepwise logistic regression to select predictors of ABE and devised a prediction score.

Of the 673 eligible infants, 10.8% suffered from ABE. Our prediction score consisted of 3 variables: TSB (as a continuous variable; odds ratio [OR] 1.16; 95% confidence interval [CI], 1.02–1.31; logistic coefficient 0.15), B/A (as a continuous variable; OR 1.88; 95% CI, 1.19–2.97; logistic coefficient 0.67), and sepsis (OR 3.78; 95% CI, 1.40–10.21; logistic coefficient 1.19). Multiplying the logistic coefficients by 10 and subtracting 75, resulted in the following equation for the score: Score = 12 × (if sepsis) + 1.5 × (TSB) – 7 × (B/A) – 75. The model performed well with an area under the curve of 0.871.

The risk of ABE can be quantified according to TSB, B/A, and sepsis in term/near-term neonates with extreme hyperbilirubinemia.

Abbreviations: AAP = American Academy of Pediatrics, ABE = acute bilirubin encephalopathy, AUC = area under the curve, B/A = bilirubin/albumin ratio, BIND = bilirubin induced neurological dysfunction, CI = confidence interval, DAT = direct antiglobulin test, G6PD = glucose-6-phosphate dehydrogenase, OR = odds ratio, TSB = total serum bilirubin, ZUCH = Zhejiang University Children’s Hospital.

Keywords: acute bilirubin encephalopathy, hyperbilirubinemia, neonate, predictor

1. Introduction
Acute bilirubin encephalopathy (ABE) is a severe complication of extreme hyperbilirubinemia. Infants who had intermediate or advanced phases of ABE are at high risk of developing death or chronic bilirubin encephalopathy (kernicterus). The American Academy of Pediatrics (AAP) guidelines for jaundiced term/near-term infants to prevent kernicterus are based on total serum bilirubin (TSB) levels.[1] However, TSB is poor predictor of bilirubin neurotoxicity.[2–5] Moreover, recent research reporting that kernicterus occurred only very rarely, and only at very high (>35 mg/dL) TSB levels,[6,7] suggests that previously recommended phototherapy and exchange transfusion treatment thresholds may be unnecessarily aggressive. Therefore, it is necessary to investigate clinical predictors of ABE for precise intervention. To establish a clinical prediction rule for ABE, we conducted a retrospective cohort study in term/near-term neonates with extreme hyperbilirubinemia.

2. Methods
2.1. Design and subjects
We selected subjects for this retrospective cohort study from the population of 26,369 neonates admitted to 1 hospital in
southeast area of China between January 1, 2015 and December 31, 2018. We enrolled subjects who had TSB $\geq$25 mg/dL (428 µ mol/L), gestational age $\geq$35 weeks, and admission age $\leq$14 days (n=673). We excluded infants (n=5) who had a conjugated bilirubin level $\geq$20% TSB and infants with chromosomal disorder and infants with encephalitis. After these exclusions, the study cohort had 673 infants. Infants were treated by exchange transfusion and/or phototherapy according to the guidelines of AAP subcommittee on hyperbilirubinemia.[11]

The institutional review board of the Zhejiang University Children’s Hospital (ZUCH) approved the study and waived the requirement for obtaining informed consent due to the retrospective nature of this study (2019-IRB-014).

2.2. Laboratory analysis

TSB and albumin levels, glucose-6-phosphate dehydrogenase (G6PD) quantification and direct antiglobulin test (DAT) were determined in the clinical laboratory at ZUCH. TSB was measured by the spectrophotometric method using the ABL800 FLEX analyzer (Radiometer Medical Aps, Denmark, Bronshoj). Albumin was measured by the bromocresol purple method using Chemistry Analyzer AU5800 (Beckman Coulter, Brea, CA). Quantitative G6PD assay was performed by fluorometric method using a G6PDH kit (PerkinElmer, Finland, Turku). The DAT (also known as direct Coombs test) was performed using the LISS/Coombs method (DiaMed GmbH, Switzerland, Fra Rond).

2.3. Definitions

ABE is defined as a bilirubin induced neurological dysfunction (BIND) score of 4 to 9.[8] The BIND score was based on clinical changes in mental state, muscle tone, and cry; a score of 0 to 3 was assigned to each category, yielding a total score ranging from 0 to 9. The BIND score describes the progression of ABE. Scores of 4 to 6 represent moderate ABE, and scores of 7 to 9 indicate severe ABE that is highly associated with kernicterus or death. Infants with BIND scores of 1 to 3 are referred to as having mild neurotoxicity that is likely to be reversible without sequelae.

G6PD deficiency was defined as G6PD activity $<$2.6 U/g hemoglobin.[19] Sepsis was defined as clinical deterioration in the presence of leukocytosis, leucopenia, a positive C-reactive protein ($>$8 mg/L) or positive blood culture.[19] As per AAP and American College of Obstetrics and Gynecology, all the following must be present for designation of asphyxia such as, profound metabolic or mixed academia (pH $<$7) in cord, persistence of Apgar scores 0 to 3 for longer than 5 minutes, neonatal neurological sequel and multiple organ involvement.[11]

2.4. Statistical analyses

All these variables were compared between the non-ABE and ABE group. t test was used to compare continuous variables consistent with normal distribution. Skew distribution data was tested by Kruskal–Wallis test. Categorical data was tested by Chi-squared test and Fisher exact test was conducted if any theoretical frequency was expected less than 10.

We obtained multivariate odds ratios (ORs) by logistic regression. To generate a parsimonious rule, we included variables if they were significant (P $<$0.05) by using entering logistic regression. To formulate the score, we summed the 3 highest ranked predictor variables, each multiplied by 10 times its logistic regression coefficient (to avoid decimals), and subtracted 7.5 to the total (to avoid negative scores). Because the logistic coefficients are equal to the logarithms of the ORs, summing them is equivalent to multiplying their ORs. We assessed goodness of fit by using the Hosmer–Lemeshow test and discrimination by using area under the curve (AUC). We performed analyses by using SPSS (version 20) program (IBM, SPSS Statistics, IBM Corporation, Chicago, IL).

3. Results

3.1. Cohort characteristics

Characteristics of the study cohort are shown in Table 1. The median gestational age was 38.3 weeks (38–39 weeks) weeks, 409 (60.7%) infants were male. The mean birth weight was 3286 $\pm$432 grams. 162 (24.1%) infants were born by cesarean section. 55 (8.2%) infants’ weight were lost over 12%. 431 (64.0%) infants got breast milk feeding. The mean TSB was 29.0 $\pm$4.1 mg/dL. The mean age at admission was 6 (4–8) days. The mean serum albumin was 3.85 $\pm$0.46 g/dL. The mean bilirubin/albumin ratio (B/A) was 7.51 $\pm$1.23 mg/g. 149 (22.1%) infants had positive DAT. 60 (8.9%) infants had G6PD deficiency. No infants had asphyxia. Of the infants with sepsis, 15 had positive blood culture and 21 were proven sepsis compared to clinical sepsis with negative cultures. One infant had acidosis. 195 (29.0%) infants were given exchange transfusion.

3.2. Predictors of ABE

Of the 673 eligible infants, 73 (10.8%) met our definition of ABE (Table 1). Thirty-one infants (4.6%) were scored 4 to 6 and 42 infants (6.2%) were scored 7 to 9 according to the BIND score.

| Table 1 Cohort characteristics (N=673). |
| Variables | n | % |
|----------------|-----|---|
| Gestational age, wk, median (P25–75) | 38 (38–39) | – |
| Male sex | 409 | 60.7 |
| Birth wt, g, mean (SD) | 3286 (432) | – |
| Cesarean section | 162 | 24.1 |
| Weight loss >12% | 55 | 8.2 |
| Breast milk feeding | 431 | 64.0 |
| TSB at admission, mg/dL, mean (SD) | 29.0 (4.1) | – |
| Age at admission, d, median (P25–75) | 6 (4–8) | – |
| Serum albumin, g/dL, mean (SD) | 3.85 (0.46) | – |
| Bilirubin/Albumin, mg/g, mean (SD) | 7.51 (1.23) | – |
| Positive DAT | 149 | 22.1 |
| G6PD deficiency | 60 | 8.9 |
| Asphyxia | 0 | 0.0 |
| Sepsis | 36 | 5.3 |
| Acidosis | 1 | 0.1 |
| BIND score | – | – |
| 0 | 532 | 79.0 |
| 1–3 | 68 | 10.1 |
| 4–6 | 31 | 4.6 |
| 7–9 | 42 | 6.2 |
| Exchange transfusion | 195 | 29.0 |
Table 2
Univariate predictors of ABE.

| Variables                   | Non-ABE n=600 | ABE n=73  | P   |
|-----------------------------|---------------|-----------|-----|
| Gestational age, wk.        | 39 (38–39.4)  | 38 (37–39) | .014|
| median (P25–75)             |               |           |     |
| Male sex, n (%)             | 362 (60.3)    | 47 (64.4) | .503|
| Birth wt. g, mean (SD)      | 3301 (428)    | 3165 (449)| .899|
| Cesarean section, n (%)     | 136 (22.7)    | 26 (35.6)| .015|
| Weight loss >12%, n (%)     | 51 (8.5)      | 4 (5.5)  | .374|
| Breast milk feeding, n (%)  | 379 (63.2)    | 52 (71.2)| .175|
| TSB at admission, mg/dL, mean (SD) | 28.2 (3.1) | 35.3 (5.9) | <.001|
| Age at admission, d         | 6 (4–8)       | 7 (4–8)  | .702|

Table 2 shows the univariate predictors and Table 3 shows the multivariate predictors of ABE. In univariate analysis, 7 predictors were captured. Compared to the non-ABE group, the ABE group had lower gestational age (38.4 ± 3.9 wk), more cesarean section (26.6 ± 35.6%) vs 136 (22.7%), P = .015, higher TSB (35.3 ± 5.9 vs 28.2 ± 3.1 mg/dL, P < .001), higher B/A (9.34 ± 1.59 vs 7.29 ± 0.97 mg/g, P < .001), more positive DAT (24 [32.9%] vs 125 [20.8%], P = .019), more G6PD deficiency (19 [26.0%] vs 41 [6.8%], P < .001), and more sepsis (23 [3.8%] vs 13 [17.8%], P < .001) (Table 2). In adjusted analysis, three independent risk factors for ABE were identified, which were TSB, B/A, and sepsis (Table 3). TSB was associated with higher odds of ABE, especially infants whose TSB ≥ 35 mg/dL, for whom the OR was 9.52 compared with infants whose TSB ≤ 30 mg/dL. Furthermore, infants whose B/A > 8.5 mg/g had higher odds of ABE, as did those whose B/A < 7.5 mg/g (OR 5.66; 95% confidence interval [CI], 1.83–17.48) (Fig. 2).

In addition, the odds of ABE were higher for infants with sepsis (OR 3.73; 95% CI, 1.46–9.55).

3.3. Prediction rule and score
After stepwise selection, the prediction rule consisted of 3 predictors: TSB (as a continuous variable; OR 1.16; 95% CI, 1.02–1.31; logistic coefficient 0.15), B/A (as a continuous variable; OR 1.88; 95% CI, 1.19–2.97; logistic coefficient 0.67), and sepsis (OR 3.78; 95% CI, 1.40–10.21; logistic coefficient 1.19). Multiplying the logistic coefficients by 10 and subtracting 75, as previously described, resulted in the following equation for the score:

Score = 12 × (if sepsis) + 1.5 × (TSB) + 7 × (B/A) – 75.

For example, an infant with sepsis whose TSB was 35 mg/dL and B/A was 8.5 mg/g would have a score of 12 + (1.5 × 35) + (7 × 8.5) – 75 = 44, whereas a score for the same baby without sepsis would be 12 fewer, or 32.

The discrimination and fit of the predictive model using the generated score were excellent. The Hosmer–Lemeshow x² (8 degrees of freedom) was 4.7 (P = .79) and the AUC was 0.871 (95% CI, 0.824–0.919) (Fig. 1). The probability of ABE was < 12% with a prediction score of < 30 and < 5% with a prediction score of < 20 (Table 4 and Fig. 2).

4. Discussion
Our study showed that TSB, B/A, and sepsis have strong association with ABE in term/near-term neonates with extreme hyperbilirubinemia. Based on these 3 predictors, we devised a prediction score to quantify the probability of ABE. Clinical implementation of this prediction rule via a Web-based calculator...
or integration into electronic medical records could help guide management of neonatal hyperbilirubinemia.

TSB is a well-established risk factor for bilirubin neurotoxicity. In northern California, 4 children had ABE all had TSB indicated that B/A was a better predictor of ABE than TSB. In northern California, 4 children had ABE all had TSB indicated that B/A was a better predictor of ABE than TSB. In limitation of sole reliance on TSB as a predictor of neurotoxicity. The combination of TSB with other clinical predictors for predicting ABE has not been investigated in previous studies.

The use of B/A as a surrogate for plasma free bilirubin has been suggested because it contains 2 of the 3 components for deriving free bilirubin. Amin et al. found that free bilirubin was a more sensitive and specific predictor of auditory neuropathy spectrum disorder than TSB. Moriooka et al. reported that free bilirubin may be helpful for identifying extremely low birth weight infants at risk for developing kernicterus. 1 g albumin can bind to 8.5 mg bilirubin, which varies with body environment. Our study found that when B/A > 8.5 mg/g, the risk of ABE was significantly increased. This was in line with previous theory. Iskander et al. found that B/A was a strong predictor of neurotoxicity, but B/A did not improve prediction over TSB alone. Our study indicated that B/A was a better predictor of ABE than TSB. In Iskander’s study, they encountered uncertainties in assigning risk factors for ABE and therefore they did not adjust the risk factors. This might be 1 reason of the contrary result.

Sepsis is thought to be one of the risk factors for bilirubin neurotoxicity, but data ranked the risk factors are limited. Our study identified sepsis as an independent predictor of ABE. The strength of the association between other risk factors (eg, hemolytic disease, G6PD deficiency) and ABE was attenuated after controlling other variables. Consistent with our study, Gamaleldin et al. reported that ABO compatibility and G6PD deficiency created minimal if any additional risk compared with idiopathic jaundice. One possible explanation to the attenuation is that hemolytic disease or G6PD deficiency may produce brain damage by influencing the bilirubin level. Increase in pro-inflammatory cytokines such as TNF-α, interleukin-1β, and interleukin-6 are frequently observed in infants suffering from neonatal sepsis. These events might damage the endothelial integrity and increase blood brain barrier permeability, which could facilitate the entry of free bilirubin to the brain. This may be 1 possible explanation of the association between sepsis and ABE.

Our prediction score quantifies the probability of ABE to help physicians and parents evaluate need for phototherapy or exchange transfusion. For example, consider an infant who has sepsis. The infant whose TSB is 25 mg/dL and B/A is 6.5 mg/g gives a score of 20 and an estimated 4.6% probability of ABE. In comparison, the probability of ABE would increase to 17.3% if the infant’s TSB is 35 mg/dL and 16.0% if the infant’s B/A is 8.5 mg/g.

Our study has some limitations. First, this is a single-center retrospective study. Additionally, we did not evaluate auditory pathway toxicity. Alterations in brainstem auditory evoked potentials are common and may be the only manifestation of bilirubin-induced brain injury. Finally, the sample size did not allow us to validate our prediction rule.

More prospective observational studies from multiple centers are needed in the future to provide more clinical data about ABE in neonates with extreme hyperbilirubinemia. A more precise prediction rule with better generalization capacity would be developed.

**Author contributions**

Dr. Zhang conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted. Dr. Chen and Dr. Shang carried out the initial analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted. Dr. Jiang designed the data collection instruments, and coordinated and supervised data collection, critically reviewed the manuscript, and approved the final manuscript as submitted.

Fanhui Zhang orcid: 0000-0002-1870-0668.

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