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Unconjugated free bilirubin in preterm infants

Lori W.E. van der Schoor [a,*], Peter H. Dijk [b], Henkjan J. Verkade [a], Anna C.J. Kamsma [b], Andrea B. Schreuder [b], Henk Groen [c], Christian V. Hulzebos [b]

[a] Division of Pediatric Gastroenterology and Hepatology, Department of Pediatrics, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands
[b] Division of Neonatology, Department of Pediatrics, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands
[c] Department of Epidemiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

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ABSTRACT

Background: Hyperbilirubinemia guidelines are based on total serum bilirubin (TSB), in combination with either gestational age (GA) or birth weight (BW), postnatal age and specific risk factors. However, TSB is a poor predictor of bilirubin-induced neurotoxicity (BIND). Free unconjugated bilirubin (UCBfree) and the UCBfree/TSB ratio are more directly related to BIND, but data on their postnatal courses are unknown.
Aims: To characterize the postnatal courses of UCBfree and UCBfree/TSB ratio, and assess their relationships with clinical characteristics.
Subjects: 72 preterm infants ≤ 32 weeks GA, admitted to the University Medical Center Groningen, The Netherlands.
Study design: During the first postnatal week, bilirubin plasma parameters were analyzed and their relationship with clinical parameters was analyzed. Postnatal changes were analyzed using Generalized Estimating Equations. Data are expressed as medians [ranges].
Results: Less than 10% of the cohort (GA: 29 [26–31] weeks; BW: 1165 [600–1975] g) showed hyperbilirubinemic risk factors. We observed a large variation in UCBfree (27 [1–197] nmol/L), that could partly be explained by postnatal age and gender, but not by other risk factors. Maximal UCBfree levels of 50 [13–197] nmol/L occurred at day 4 and were higher in males. In contrast to TSB, UCBfree/TSB ratios (0.19 [0.01–1.04]) were higher in infants with low GA/BW.
Conclusion: UCBfree levels vary considerably in preterm infants, despite a low incidence of hyperbilirubinemic risk factors and similar TSB-based phototherapy treatment. UCBfree could not be predicted by GA or BW, but UCBfree/TSB ratios are highest in the smallest preterms, while they have the lowest TSB levels.

1. Introduction

In preterm infants below 35 weeks of gestational age (GA), current management guidelines of unconjugated hyperbilirubinemia are mainly consensus based [1]. The treatment thresholds are based on total serum bilirubin (TSB) and mainly depend on either GA or birth weight (BW) [1–3], in combination with risk factors, such as hemolysis, hypoalbuminemia, sepsis, acidosis and respiratory problems [1,4]. The threshold TSB levels at which phototherapy is started are based on studies in which TSB levels were associated with (impaired) neurodevelopmental outcome. Consequently, TSB treatment thresholds increase with postnatal age and higher bilirubin levels are tolerated at older age. Yet, studies on bilirubin-induced neurological damage only provide limited evidence on harmful TSB levels, because most factors that increase the risk of neurodevelopmental delay (e.g. asphyxia, intracranial hemorrhage, prematurity) also increase TSB levels and bilirubin neurotoxicity susceptibility. In essence, the peak TSB level is a poor predictor of the likelihood of bilirubin-induced neurotoxicity (BIND) [1], especially in preterm infants. To illustrate this, kernicterus can occur in extremely low birth weight infants with only modestly elevated TSB levels [5,6].

A more appropriate parameter to base management guidelines on could be unconjugated non-albumin-bound bilirubin (UCBfree). UCBfree can translocate across the blood-brain barrier [7,8], where it may induce apoptosis and necrosis in specific brain areas [8,9]. Preterm infants, especially when ill, may have high UCBfree levels, partly attributable to a low bilirubin-albumin binding affinity (Ka) compared to term infants [10,11]. The UCBfree/TSB ratio, which represents the combination of magnitude (represented by TSB) and distribution (represented by UCBfree) of the accumulated bilirubin load, has been proved

Abbreviations: UCBfree, free bilirubin; TSB, total serum bilirubin; UCBfree/TSB ratio, free bilirubin/total serum bilirubin ratio; Ka, bilirubin-albumin binding affinity; GA, gestational age; BW, birth weight; HRP, horseradish peroxidase; GEE, Generalized Estimating Equations.

* Corresponding author at: University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
E-mail address: l.w.e.van.der.schoor@umcg.nl (L.W.E. van der Schoor).

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to correlate better with automated auditory brain stem response than either UCBfree or TSB alone and is suggested to be the best BIND predictor [12].

UCBfree has been frequently measured using a kinetic peroxidase methodology [13], and has even resulted in UCBfree-based treatment thresholds in one country (i.e. Japan). Its accuracy, however, has been debated [13–16]. Since then, an adapted peroxidase method has been developed [16,17]. Nevertheless, an FDA approved technology to routinely measure UCBfree in the clinic is not universally available.

As a first step towards eventual application of UCBfree levels for treatment guidelines of neonatal hyperbilirubinemia, we aimed to describe the physiological postnatal course of UCBfree levels in preterm infants. Therefore, we assessed the effect of postnatal age on UCBfree and the UCBfree/TSB ratio. Furthermore, since both GA and BW are used in TSB-based treatment guidelines [1–3], we analyzed the relation between UCBfree and the UCBfree/TSB ratio with GA and BW. In addition, we analyzed the relationship between UCBfree and UCBfree/TSB ratio and gender, since gender differences in TSB levels have been previously reported in low BW infants [18]. We also determined the effects of several hyperbilirubinemic and BIND risk factors.

2. Subjects and methods

2.1. Patients

This retrospective study was carried out in 72 preterm infants ≤32 weeks GA, treated in the neonatal intensive care unit of the Beatrix Children's Hospital, University Medical Center Groningen, between April 2007 and April 2008. The patients had been included in a multicenter randomized controlled trial investigating the additional use of the Bilirubin/Albumin ratio in the treatment of preterms with hyperbilirubinemia (BARTrial; ISRCTN 74465643I). The BARTrial included 615 children, from which 72 were randomly selected for UCBfree measurements, if remaining blood sample was available. Infants were included after parental consent within 24 h after birth. Exclusion criteria were major congenital malformations, clinical syndromes, or chromosomal abnormalities. Infants were treated with phototherapy, according to the Dutch guideline (19), which is TSB-based and depends on postnatal age and BW, in combination with risk factors; asphyxia (Apgar score < 3 at 5 min), hypoxemia (PaO_2 < 5.3 kPa for >2 h), acidosis (pH < 7.15 for > 1 h), hemolysis (with positive Coombs reaction), clinical sepsis (with need for vasopressors) and intraventricular hemorrhage (>grade 2, according to Papile) [19]. No measurements could be performed in 24 patients on day 1; 7 on day 2; 6 on day 3 and 4; 2 on day 5; 5 on day 6 and 4 on day 7. Neonatal hearing was screened by measuring the automated auditory brainstem response with an ALGO® neonatal hearing screener (Natus Medical Inc., San Carlos, CA, USA) at discharge.

2.2. Bilirubin measurements

Blood samples were collected daily and plasma was stored at −80 °C under argon protected from light. TSB and UCBfree levels were determined with a Zone Fluidics system (Global Flopro, Global Fia Inc., WA), which measures UCBfree using the horseradish peroxidase (HRP) reaction [16,17]. HRP oxidizes UCB to a colorless compound, but albumin-bound UCB is protected from oxidation [17]. 3 mg HRP and 3 mg glucose oxidase were diluted in 3000 μL 0.1 M phosphate buffer and 4 and 8 μL of this solution were used for analysis. Albumin levels were determined by routine spectrophotometry on a P800 unit from Roche Diagnostics Ltd. (Basel, Switzerland) in the same week as TSB and UCBfree measurements. Phototherapy was started when indicated based on TSB measurements from the same spectrophotometer, according to the national guidelines. Ka was calculated, using the following formula: Ka = (TSB − UCBfree) / (UCBfree × (Albumin − TSB + UCBfree)) [20].

2.3. Statistics

UCBfree, TSB and albumin levels were measured, and the UCBfree/TSB ratio, Ka, and the TSB/albumin (B/A ratio) were calculated. In addition, the ranges of these parameters were assessed for every individual and for the entire cohort. Changes in UCBfree, TSB, UCBfree/TSB ratio, Ka, albumin, and the B/A ratio over time were analyzed using Generalized Estimating Equations (GEE), a technique used to assess the capacity of independent variables to explain the intra- and interindividual variation of a certain dependent variable [21]. It does not depend on complete data but includes data for all individual time points and therefore maximizes the use of available data. Demographic factors and clinical risk factors were entered in a GEE model to assess their influence on the variation in bilirubin parameters. For univariate analysis, all demographic and clinical risk factors described in Table 1 were individually entered in different GEE models to explain variation in UCBfree, TSB, UCBfree/TSB ratio, Ka, albumin, and B/A ratio, respectively. Based on univariate analysis, further GEE analyses were performed using GA, BW, gender, albumin and postnatal age. In addition to individual parameters, interaction variables (GA*postnatal day, BW*postnatal day and gender*postnatal day) were analyzed to assess differences in bilirubin course depending on GA, BW or gender. For all GEE analyses, an exchangeable working correlation matrix was used, assuming a fixed correlation between measurements within the same subject.

The relationship between bilirubin parameters, and GA and BW, was assessed at the time of maximal UCBfree levels. For this analysis, infants were divided in BW and GA cohorts. BW cohorts were based on the

Table 1
Characteristics of 72 preterm infants < 32 weeks (total X (%) infants).

Clinical characteristics	Infants (N = 72)
Gestational age in weeks, median [range]	29.1 [26.1–31.9]
Birth weight in g, median [range]	1165 [600–1975]
Male/female	38/34
Antenatal steroids (%)	
Yes	38/72 (53%)
Not completed	23/72 (32%)
No	6/72 (8%)
Unknown	5/72 (7%)
Birth trauma, total (%)	6/72 (8%)
Caput succedaneum, cephalic hematoma	1/72 (1%)
Other hematoma	5/72 (7%)
Other bruising	0/72 (0%)
Agar score < 3 at 5 min (%)	0/72 (0%)
Coombs (%)	
Positive reaction	0/72 (0%)
Negative reaction	42/72 (58%)
Unknown	30/72 (42%)
Irregular antibodies child (%)	
Positive	0/72 (0%)
Negative	61/72 (85%)
Unknown	11/72 (15%)
Irregular antibodies mother (%)	
Negative	68/68 (100%)
Sepsis (%) requiring volume expansion or vasopressants	3/72 (4%)
Hypoxemia (%)	2/72 (3%)
Acidosis (%)	0/72 (0%)
Meningitis, positive liquor culture (%)	1/72 (1%)
Intracerebral hemorrhage > grade 2 (%)	3/72 (4%)
Abnormal auditory brain stem response at discharge	3/72 (4%)
Bilirubin parameters	**Median [range]**
UCBfree (nmol/L)	27 [1–197]
TSB (μmol/L)	152 [38–304]
UCBfree/TSB ratio	0.19 [0.01–1.04]
UCBfree maximum (nmol/L)	50 [13–197]
Ka (L/μmol)	109 [23–399]
Ka minimum (L/μmol)	60 [14–279]
Albumin (g/L)	32 [17–44]
B/A ratio	4.9 [1.0–11.0]

Data are displayed as n/N (%), except for gestational age, birth weight and male/female ratio and bilirubin parameters, which are displayed as median [range].