Journal of Perinatology
https://doi.org/10.1038/s41372-019-0365-2

ARTICLE

A multi-center evaluation of a device for measurement of bilirubin binding capacity in neonates: the effects of gestational age, Intralipid exposure and illness severity

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Received: 23 December 2018 / Revised: 15 February 2019 / Accepted: 11 March 2019

Abstract

Objective Measure daily bilirubin-binding capacity (BBC) variation using an automated, not as-yet FDA approved, Point-of-Care hematofluorometer. Measure the effects of prematurity, clinical instability and exposure to Intralipid on BBC.

Subjects Convenience sample of 109 infants from well-baby and intensive care nurseries. Gestational ages 28–41 weeks. 261 specimens obtained from postnatal ages 1–4 days. Unstable neonates were defined by need for at least noninvasive respiratory support and FiO2 ≥ 0.25.

Results Median interday variation was 2.9 ± 5.1 mg/dL. BBC (0.254 mg/dL/wk) and albumin (0.037 g/dL/wk) increased for each week of gestation. BBC was lower in unstable compared to well infants (26.1 ± 7.6 mg/dL v 28.6 ± 6.3 mg/dL). BBC was not significantly different in infants receiving or not receiving IL.

Conclusions BBC measurements using the device had acceptable intraspecimen reproducibility and interday variability. BBC may be helpful in guiding the assessment of aggressive versus conservative management decisions in preterm and sick infants with hyperbilirubinemia.

Introduction

The management of neonatal hyperbilirubinemia has typically been based on using nomograms that relate an infant's total serum/plasma bilirubin level (TB) to age in hours. For infants ≥ 35 weeks' gestational age (GA), the 2004 American Academy of Pediatrics (AAP) guideline [1] also recommends considering various risk factors in determining management. The algorithm that suggests treatment thresholds for infants < 35 weeks' GA [2] groups infants into five separate GA groups, and states ranges of bilirubin levels to initiate phototherapy or exchange transfusion irrespective of hours of life. Bilirubin-induced neurological dysfunction (BIND) is caused by unbound bilirubin (UB) crossing the blood–brain barrier, which can disrupt neurological activity [3]. It has been well established that UB levels and bilirubin binding capacity (BBC) may better correlate with the risk of BIND, but until recently, obtaining these values has only been available in the research setting [4]. The Aviv Bili-4 Hematofluorometer (Aviv Biomedical Inc., Lakewood, NJ) is a point-of-care (POC) device, not yet FDA approved, that is capable of measuring BBC using only 50 µL of whole blood. The hematofluorometer takes advantage of the fact that when the bilirubin-albumin complex is excited by light of 430 nm wavelength, it fluoresces at 530 nm with the degree of fluorescence proportional to the amount of the bilirubin-albumin complex. In practice a drop of infant's blood is placed on one slide while a second drop of infant's blood is mixed with exogenous bilirubin to saturate the infant's albumin. These two specimens are automatically drawn into the device where the fluorescence is measured, and the total binding capacity and the infant's degree of albumin saturation is determined.

The primary objective of this study was to determine the intraspecimen and interpatient variabilities of the device in a

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Published online: 27 March 2019

group of neonates at three different hospitals. A secondary objective was to investigate the variability of BBC among infants of different GAs, as well as the effect of albumin (Alb) level, intralipid (IL) infusions and illness severity on BBC.

Design and methods

A convenience sample of 109 infants was enrolled from the well-baby and intensive care nurseries of three different institutions. 261 individual specimens were obtained on days one to four of life. Specimens were obtained concurrently with other clinically indicated lab studies as ordered by the attending physicians. The devices and reagents were loaned to the participating institutions by the manufacturer, who had no other interaction with the study. This study was approved by the Institutional Review Boards of all participating institutions and informed consent was obtained for all participants.

BBC of each specimen was measured by trained clinical personnel or research personnel one to three times using the Bili 4-Hematofluorometer as described previously [5]. Briefly, a 15-µL aliquot of whole blood was first mixed with bilirubin to saturate albumin. This aliquot and another separate 15-µL aliquot of whole blood were placed on 2 separate glass slides and inserted into the device. Fluorescence of the two samples was then measured and the BBC calculated. 163 of the 261 specimens (62.5%) were tested in an office adjacent to the NICU with results available within 15 min of being drawn. The other 98 specimens were measured in laboratories distant from the units.

TB and Alb were determined in the clinical laboratories of each institution using the diazo [6] and bromcresol green [7] methods, respectively. The infusion rate of 20% Intralipid (IL) was recorded daily. Severe illness was defined as the need for support with at least noninvasive respiratory support and >0.25 FiO_2. Statistics were performed using R statistical package v3.2.5. Intraspecimen variability (test-retest variability) was determined where multiple measurements of BBC had been performed on a single specimen using the intraclass correlation coefficient. The relationship of GA to illness severity was measured by the interaction coefficient. Continuous variables were measured by Student's t-tests with significance being <0.05.

Results

The 109 infants in the study reflected a wide diversity of GAs and races/ethnicities (Table 1). 18 (16.5%) of the infants were deemed to be clinically unstable since they required noninvasive respiratory support and >0.25 FiO_2.

Table 1 Demographics

	N (109)	%
Gestational age (weeks)		
28 and 29	8	7.3
30 and 31	13	11.9
32–34	52	47.7
≥35	36	33.1
Race		
White	31	28.5
African American	37	33.9
Asian	16	14.7
Other	25	22.9
Ethnicity		
Hispanic	28	25.7
Clinically unstable	18	16.5

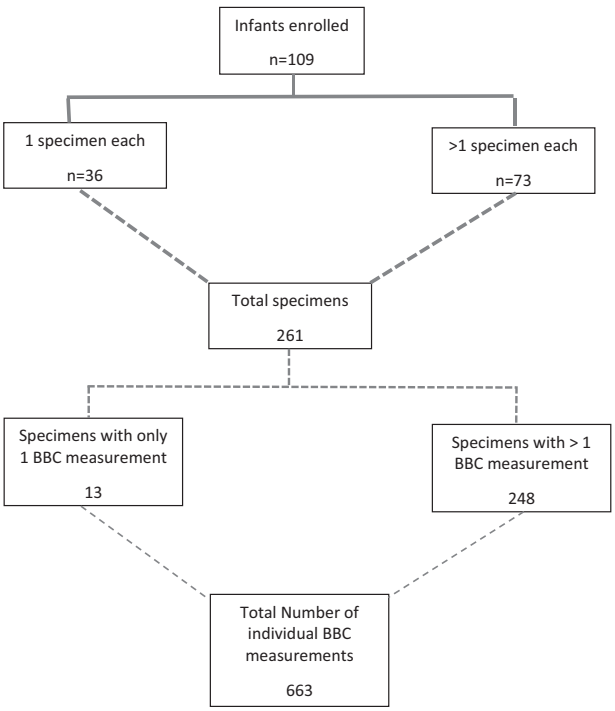

Fig. 1 Frequency of multiple specimens and BBC measurements

A total of 261 individual specimens were drawn with a total of 676 individual BBC measurements performed (Fig. 1). There was no significant difference between BBCs tested adjacent to the NICU (28.21 ± 6.97 mg/dL) and those tested at a distance (29.15 ± 7.34 mg/dL, $p = 0.30$). BBC, Alb and TB ranged from 10.5 to 52.9 mg/dL, 1.5 to 3.3 g/dL and 1.7 to 16.1 mg/dL, respectively. Intraspecimen measurements had good test-retest reliability with correlations among the 3 repeated measurements ranging from an r of 0.797 to 0.876 (Table 2). 169 specimens were each measured in

Table 2 Intraspecimen reliability

BBC repeated measurements on individual specimens			
	Measurement 1	Measurement 2	Measurement 3
Measurement 1	1		
Measurement 2	0.797	1	
Measuerment 3	0.799	0.876	1

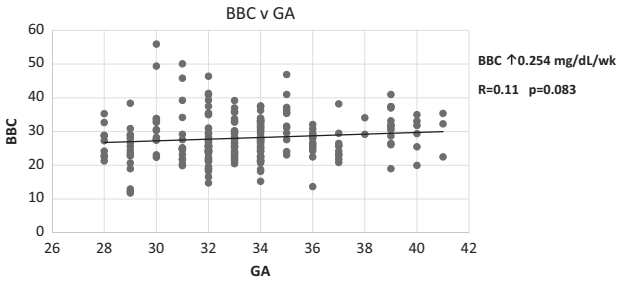

Fig. 2 BBC v. GA

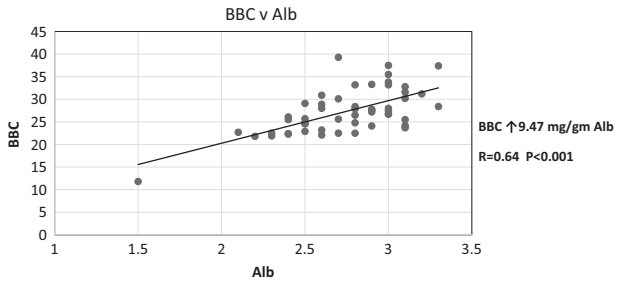

Fig. 3 BBC v. Albumin

triplicate and showed an excellent intraclass correlation coefficient of 0.931.

For each week of GA, BBC (Fig. 2) and Alb levels (data not shown) increased 0.254 mg/dL/wk and 0.037 g/dL/wk, respectively. BBC as a function of Alb level is noted in Fig. 3. Specimens drawn from infants on more than one day (at 2–4 days of life), revealed a median interday BBC variation of 2.9 ± 5.1 mg/dL (10.1 ± 15.6%, Table 3). Daily measurements of BBC did not vary significantly and were not affected by exposure to IL (Table 4). Overall, there were 76 episodes of IL exposure on two or more days among 33 infants. IL infusion rates varied from 0.1–1.2 mL/hr. No infant received more than 3 g/kg/day of IL. In 38 instances, the rates of infusion increased daily and in 12 instances it decreased. There was no significant difference in BBC in either instance (Table 4). The overall variability of BBC values stratified by GA is shown in Table 5.

Severely-ill infants ($n = 18$) had significantly lower BBCs than well infants (26.1 ± 6.3 vs. 28.6 ± 7.6 mg/dL, respectively, $p = 0.02$). BBCs of ill infants also decreased by 2.1 mg/dL/wk. However, the effect of GA on BBC was independent of illness severity (interaction coefficient = −0.304; $R = 0.291$; $p = 0.599$).

Table 3 BBC Daily variation

	All samples	No IL exposure	*p* value
N	151	70	
ΔBBC (mg/dL)	4.4 ± 5.1	4.8 ± 4.8	0.58
ΔBBC (%)	14.4 ± 15.6	15.5 ± 15.4	0.62

Table 4 Effect of Changing IL infusion rate on daily BBC

	All IL cases	IL increasing	IL decreasing	*p* value
N	76	38	12	
ΔIL (mL/h)	0.08 ± 0.4	0.36 ± 0.18	−0.69 ± 0.48	< 0.0001
ΔBBC (mg/dL)	0.3 ± 9.0	−0.33 ± 10.8	3.3 ± 10.2	0.31
ΔBBC (%)	4.1 ± 18.9	4.5 ± 20.9	6.9 ± 23.5	0.74

Table 5 Range of BBC values by GA

	Range of BBC values (mg/dL)		
GA (weeks)	Schutzman	Lamola [5]	Morioka [12]
28–30	11–49	8–37	15–28
31–33	15–50	9–42	18–34
34–37	14–47	11–42	20–35
> 37	19–41	9–49	20–38

Discussion

Management of hyperbilirubinemia is one of the key components of neonatal care in the well-baby and intensive care nurseries. It can be difficult at times to identify infants at significant risk for BIND, and a failure to do so can lead to devastating and lifelong neurological sequelae for the affected individual [3]. Current recommendations for management of infants > 35 weeks gestation [1] involve the determination of an infant's risk for severe hyperbilirubinemia by plotting TB versus age in hours on the Bhutani [8] nomogram as well as accounting for various risk factors as described in the 2004 AAP guideline [1]. Risk factors for BIND include hemolysis, prematurity, decreased Alb levels, race/ethnicity, illness/sepsis and acidosis. A more direct and accurate way to determine an infant's risk for BIND would be to measure their BBC or UB levels [5]. This would also allow us to determine the effect of exposure to medications which might displace bilirubin from Alb. Until recently, measurements of BBC and UB have only been available in the research setting. By using a prototype POC

hematofluorometer, we were able to measure the BBC at multiple time points in a diverse group of infants in both the well- baby and intensive care nurseries of three different institutions. The results of 62.5% of the specimens were available within 15 min of the sample being drawn. However, in no instance was BBC used to guide clinical management.

We found that the measurements of BBC were reproducible. 248 of the 261 specimens had BBC measured either two or three times with good test-retest reliability (Table 2). Using a device of the same generation, Chetta et al. [9] found a SD within sample test-retest of 0.73 which was better than our SD of 3.31. 73 of the 109 infants in our study had specimens drawn on more than one day for a total of 151 specimens. Our median daily change in BBC was 2.9 ± 5.1 mg/dL (10.1 ± 15.6%) which agrees satisfactorily with that reported by Chetta, et al. [9] of 2.6 mg/dL. In another study using a similar hematofluorometer [10], the authors reported that BBC varied by approximately 5% among the 11 babies with serial measurements. These findings are expected, since an infant's BBC (particularly those who are premature) along with other physiologic parameters should appear to increase daily during the first few days of life due to postnatal physiologic diuresis. Although the absolute number of bilirubin molecules able to be bound to Alb is the same, since the baby's blood volume has decreased and since BBC is measured in mg/dL, as the infant's blood volume decreases, the BBC will appear to increase. A similar finding has been demonstrated for Alb by Watchko, et al. [11]

In our cohort we observed that both BBC (0.254 mg/dL/wk; $p = 0.083$) and Alb (0.037 g/dL per week; $p = 0.018$) increased for each additional week of gestation (Fig. 2). These increases were less than those reported by Lamola, et al. [5] and Morioka, et al. [12] in separate studies, who found increases in BBC of 0.93 and 0.38 mg/dL/wk and Alb of 0.11 and 0.043 g/dL per week., respectively, using the same generation of hematofluorometer. One possible reason for the difference in rate of rise of BBC may be a different proportion of infants at term or near-term among the different studies. Morioka, et al. [12] noted that the slope of the increase of BBC was much less beginning at 35 weeks' GA. This intuitively makes sense as Alb (and hence BBC) levels do not continue to increase indefinitely and reach a plateau at approximately 3 months of age. Similarly, it is also unlikely that the initial production of Alb by the fetus is linear, nor does production start at conception. The slope of the regression line from Lamola, et al. [5], when extended backwards, intercepts the x-axis at −8.9 weeks' GA, which is clearly not reasonable. Had the regression line in Lamola, et al. [5] been calculated to begin at 4 weeks' GA (approximately when Alb first appears in the body), the slope would have only been 0.63 mg/dL/wk. Our rate of rise of Alb, and consequently BBC, was almost identical to that of Morioka et al. [12]. The difference in Alb is unlikely to have been caused by laboratory methodologies. All Alb levels were measured by the clinical laboratories of all institutions with Lamola, et al. [5] and us using the bromcresol green method [7] while Morioka, et al. [12] used the bromcresol purple [13] method. The generally recognized relationship between BBC and Alb (before accounting for the effects of prematurity, sepsis, and other factors potentially affecting BBC) is Alb x 8.8 [12]. Thus, the difference in rate of rise of BBC as a function of GA between us and Lamola, et al. [5] can almost completely be accounted for by the difference of rate of rise of Alb.

More important than the absolute rate of rise of BBC, is the variation of BBC at any given GA. The primary cause of variation of BBC at a given GA is the Alb level. For instance, the Alb level in our infants of 29 weeks gestation varied from 1.5–3 g/dL. As noted in Fig. 3, the Alb level is responsible for at least 41% of the variability in BBC. Other minor causes include exposure to medication that displaces bilirubin from Alb as well as illness. All three groups found large variations in BBC values at each GA (Table 5), and our range of values was similar to that reported by Lamola, et al. [5] The narrower range found by Morioka, et al. [12] may reflect the more homogeneous Japanese population. This large variability of BBC at any GA highlights the need for individualized management of a given TB at a given GA. The current AAP guideline [1] recommends action thresholds at specific TB levels for a given GA. However, given the wide range of BBCs at any GA, it may be potentially safer to use BBC in combination with TB, for example, the ratio of TB/BBC, regardless of GA to determine treatment thresholds. This is particularly pertinent for infants <35 weeks' GA where current approach [2] is based primarily on expert opinion and groups infants of different GAs together. Lamola, et al. [5] overlaid these consensus recommendations for threshold values for phototherapy and exchange transfusion over the BBC vs. GA scatterplot for their population, and found corresponding TB/BBC thresholds of 45 and 67%, respectively. The potential utility of these action thresholds relative to BBC can be seen in our 29-week infants. BBC significantly varied in this age group in our population from 8.2 to 38.4 mg/dL. By applying the AAP recommendations [2], a 29-week-GA infant would have phototherapy initiated at a TB of 6 to 8 mg/dL and an exchange transfusion level at 12 to 14 mg/dL. However, if the BBC was 38.4 mg/dL, a reasonable phototherapy threshold would now be at 17.3 mg/dL. In contrast, if the infant's BBC was only 8.2 mg/dL, an exchange threshold at 5.5 mg/dL would be considered. An alternative strategy to identifying infants at significant risk for BIND has recently been suggested by Ahlfors, et al. [14]. They focus on determining population parameters of free bilirubin (B_f) at current total bilirubin thresholds for treatment, as the B_f

would better identify infants at risk for BIND than simply the total bilirubin.

One of the difficulties with using TB, or even Alb levels as proxies for BBC is the fact that multiple drugs and clinical conditions can affect the binding of bilirubin to Alb and thus decrease BBC with a concomitant increase in UB [15]. Perhaps the most well-known competitor is sulfisoxazole [16]. This drug was widely used in the 1950s for neonatal sepsis, but was then found to cause an epidemic of kernicterus by displacing bilirubin from Alb. A similar concern today may be the potential bilirubin displacing ability of free fatty acids (FFAs). Virtually, all premature infants and many late preterm and term infants are given FFAs as part of their parenteral nutrition as IL during the first few days of life [17]. Hegyi, et al. [18] showed that UB levels increase with increasing doses of IL over the first several days of life in preterm infants, with even greater increases in UB among infants ≤ 28 weeks' GA. In our study we found essentially no effect of IL on BBC (Table 4). In neither the 38 cases where IL infusion increased from one day to the next, nor in the 12 cases where IL infusion decreased from one day to the next, were there significant changes in BBC. This is similar to the results reported by Pham, et al. [19] who found no clinically significant decreases in BBC with varying infusion rates of IL. Regarding the findings of increasing UB levels by Hegyi, et al. [18], it can be assumed the TB was also increasing at this time (days 2 to 4 of life), and therefore BBC was not affected. Additionally, in their infants who underwent phototherapy, UB and TB decreased following phototherapy, despite a large increase in FFAs observed pre- and post-phototherapy. Finally, there is some suggestion that IL may actually bind bilirubin and thus enhance BBC in the blood [20, 21].

A well-known risk factor for BIND is illness/sepsis in the newborn [22–24] with an increased incidence of kernicterus in sick infants [25, 26]. However, in many of these cases it is unclear whether illness is directly related to the lower BBC normally observed in lower GA infants and not related to the presence of illness alone. Lamola and Fanaroff in 1984 [10] showed that BBC was lower in infants with mild respiratory distress as compared with those without respiratory distress and even lower in those infants with severe respiratory distress. However, this observation may have been more related to an effect of GA rather than to the degree of illness severity on BBC. In our infants BBC was significantly lower in infants who were ill (26.1 ± 6.3 mg/dL) compared with well infants (28.6 ± 7.6 mg/dL; $p = 0.02$). This effect was not impacted by the normal decrease in BBC seen with decreasing GA (interaction coefficient = −0.304; $R = 0.29$; $p = 0.599$). Although this finding is statistically significant, given the relatively small difference between ill and well infants, it is likely not of great clinical significance. However, it does confirm that illness is one of the risk factors for BIND.

A possible advantage of using BBC measurements is to individualize patient management for phototherapy. While phototherapy has been able to prevent the need for most exchange transfusions over the past sixty years, there has recently been concern that phototherapy is overprescribed in some infants, particularly those born prematurely, and this may be a cause of increased mortality in the smallest prematures [27, 28]. Phototherapy has been shown to cause breakage of DNA [29], and it has also recently been shown that there may be a small increase in the incidence of childhood seizures among those who were exposed to phototherapy [30]. 71 of our 109 infants were < 35-weeks' GA. If future studies show that applying the BBC threshold for phototherapy at 45%, as proposed by Lamola, et al. [5] is safe, then only 13 (18.3%) of our infants would have received phototherapy as opposed to the 59 (83.1%) who were treated.

We have shown that the Aviv Bili-4 Hematofluorometer has acceptable inter- and intraspecimen variabilities, can provide timely results in the clinical setting, and can be integrated into the daily care of the newborn. Additionally, we have shown that while IL infusions do not appear to significantly affect BBC, the severity of an infant's illness could potentially decrease their BBC. Further studies, particularly focused on infants <28 weeks' GA, the effects of IL on BBC and the ability for clinical personnel to use the device in a true POC manner are warranted to determine if BBC will enable a more individualized approach to managing hyperbilirubinemia.

Acknowledgements We would like to acknowledge Andrew Paoletti for his assistance with statistics.

Funding: This work was funded by: (1) Bilirubin-binding capacity to assess bilirubin load in preterm infants 1R21HD0823319-1A (NIH/NICHD) (Bhutani). (2) Point-of-care system for determination of bilirubin capacity in neonates SBIR 121769 (Aviv/Bhutani). (3) Louis I. Weisberg, MD Memorial Neonatology Research Fund

Author contributions: VKB and AL were responsible for the study concept, design, data interpretation and critical review of the manuscript. IF, EO and MC were responsible for data collection and interpretation, and critical review of the manuscript. DLS was responsible for data collection and interpretation and preparation of the manuscript. RJW was responsible for study design, data interpretation and critical revision of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004;114: 297–316.
2. Maisels MJ, Watchko JF, Bhutani VK, Stevenson DK. An approach to the management of hyperbilirubinemia in the preterm infant less than 35 weeks of gestation. J Perinatol. 2012;32:660–4.
3. Watchko JF, Tiribelli C. Bilirubin-induced neurologic damage-mechanisms and management approaches. N Engl J Med. 2013; 369:2021–30.
4. Ahlfors CE, Wennberg RP, Ostrow JD, Tiribelli C. Unbound (free) bilirubin: improving the paradigm for evaluating neonatal jaundice. Clin Chem. 2009;55:1288–99.
5. Lamola AA, Bhutani VK, Du L, Cuadrado MC, Chen L, Shen Z, et al. Neonatal bilirubin binding capacity discerns risk of neurological dysfunction. Pediatr Res. 2015;77:334–9.
6. Shell BC, Lees H, Li PK. Mechanism of interference by hemoglobin in the determination of total bilirubin. II. Method of Jendrassik-Grof. Clin Chem. 1980;26:26–9.
7. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 1971;31:87–96.
8. Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischarge hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in helthy term and near term infants. Pediatrics. 1999;103:6–14.
9. Chetta KE, Pham O, Tyson JE, Pedroza C, Bhutani VK, Castillo Cuadrado ME, et al. Measuring bilirubin binding capacity in very low birthweight newborns. EPAS. 2018;2360:7.
10. Lamola AA, Fanaroff AA. Bilirubin fluorescence and prevention of kernicterus. Diagn Med. 1984;7:9–12.
11. Watchko JF, Spitzer AR, Clark RH. Prevalence of hypoalbuminemia and elevated bilirubin/albumin ratios in a large cohort of infants in the neonatal intensive care unit. J Pediatr. 2017;188:280–6.
12. Morioka I, Kurokawa D, Iwatani S, Iijima H, Lamola AA, Bhutani VK, et al. Monitoring bilirubin binding parameters in a cohort of Japanese neonates. J Clin Lab Med. 2018;3. https://doi.org/10.16966/2572-9578.119.
13. Muramoto Y, Matsushita M, Irino T. Reduction of reaction differences between human mercaptalbumin and human non-mercaptalbumin measured by the bromcresol purple method. Clin Chem Acta. 1999;289:69–78.
14. Ahlfors CE, Bhutani VK, Wong RJ, Stevenson DK. Bilirubin binding in jaundiced newborns: from bench to bedside? Pediatr Res. 2018;84:494–8.
15. Wennberg RP, Ahlfors CE, Bhutani VK, Johnson LH, Shapiro SM. Toward understanding kernicterus: a challenge to improve the management of jaundiced newborns. Pediatrics. 2006;117:474–85.
16. Harris RC, Lucey JF, Maclean JR. Kernicterus in premature infants associated with low concentrations of bilirubin in the plasma. Pediatrics. 1958;21:875–84.
17. Deshpande G, Simmer K. Lipids for parenteral nutrition in neonates. Curr Opin Clin Nutr Metab Care. 2011;14:145–50.
18. Hegyi T, Kleinfeld A, Huber A, Weinberger B, Memon N, Shih WJ, et al. Effects of soybean lipid infusion on unbound free fatty acids and unbound bilirubin in preterm infants. J Pediatr. 2017;184:45–50.
19. Pham O, Chetta KE, Tyson JE, Pedroza C, Bhutani VK, Castillo Cuadrado ME, et al. The effect of intravenous lipid dose on bilirubin binding capacity in very low birthweight newborns. E-PAS2018. 2018;2877:658.
20. Thaler MM, Wennberg RP. Influence of intravenous nutrients on bilirubin transport.II. Emulsified lipid solutions. Pediatr Res. 1977;11:167–71.
21. Burckart GJ, Whitinton PF, Helms RA. The effect of two intravenous fat emulsions and their components on bilirubin binding to albumin. Am J Clin Nutr. 1982;36:521–6.
22. Cashore WJ, Oh W, Brodersen R. Reserve albumin and bilirubin toxicity index in infant serum. Acta Pediatr Scand. 1983;72:415–9.
23. Cashore WJ. Free bilirubin concentrations and bilirubin-binding affinity in term and preterm infants. J Pediatr. 1980;96:521–7.
24. Ebbesen F, Brodersen R. Risk of bilirubin acid precipitation in perterm infants with respiratory distress syndrome: onsiderations of blood/brain bilirubin transfer equilibrium. Early Hum Dev. 1982;6:341–55.
25. Perlman JM, Rogers BB, Burns D. Kernicteric findings at autopsy in two sick near term infants. Pediatrics. 1997;99:612–5.
26. Gartner LM, Snyder RN, Chabon RS, Bernstein J. Kernicterus: high incidence in premature infants with low serum bilirubin concentrations. Pediatrics. 1970;45:906–17.
27. Morris BH, Oh W, Tyson JE, Stevenson DK, Phelps DL, O'Shea TM, et al. Aggressive versus conservative phototherapy for infants with extremely low birth weight. New Engl JMed. 2008;359:1885–96.
28. Tyson JE, Pedroza C, Langer J, Green C, Morris B, Stevenson D, et al. Does aggressive phototherapy increase mortality while decreasing profound impairment among the smallest and sickest newborns? J Perinatol. 2012;32:677–84.
29. Rosenstein BS, Ducore JM. Enhancement by bilirubin of DNA damage induced in human cells exposed to phototherapy light. Pediatr Res. 1984;18:3–6.
30. Newman TB, Wu YW, Kuzniewicz MW, Grimes BA, McCulloch CE. Childhood seizures after phototherapy. Pediatrics. 2018;142:32.