Measurement of Circulating Unbound Bilirubin: Will It Ever Be a Part of Routine Neonatal Care?

Total serum bilirubin (TSB) is the intravascular concentration of albumin bound bilirubin whereas the small circulating bilirubin fraction not bound to albumin or other serum proteins is the unbound (or "free") bilirubin (UB) level. The ability to measure circulating UB, although not widely available, remains of keen interest because circulating UB is in dynamic equilibrium with the extravascular tissues, including the central nervous system (CNS) and thus holds the potential to be a more reliable index of bilirubin neurotoxicity risk than the TSB.[1] This issue of *The Journal* features a report by Amin et al that adds to a growing literature on the use of circulating UB in predicting bilirubin neurotoxicity, including auditory neuropathy as manifest by abnormalities in the auditory brainstem evoked response (ABR).[2]

See related article, p 84

Amin et al highlight the vulnerability of the central auditory pathways to bilirubin-induced injury and the importance of the ABR in assessing for bilirubin neurotoxicity. Absent or abnormal ABRs coupled with normal measures of cochlear function (cochlear microphonics and otoacoustic emissions) observed acutely are consistent with bilirubin-induced auditory injury in a hyperbilirubinemic neonate, abnormalities that may be reversible with treatment.[3-5] Persistent ABR abnormalities may be a harbinger of permanent damage to the central auditory system and resultant auditory neuropathy spectrum disorder.[3] Auditory neuropathy spectrum disorder can be the predominant neurologic sequelae of bilirubin neurotoxicity or part of the constellation of disabilities that typify classic kernicterus.[3]

Most notably, Amin et al confirm that circulating UB is a more sensitive and specific predictor of bilirubin induced neurotoxicity than either TSB or the bilirubin/albumin (B/A) ratio.[2] In addition, the current study spotlights a subset of neonates whose neurotoxicity risk was only evident by measuring circulating UB (subjects 1 and 5), a finding of previous reports as well.[6,7] Subject 5 merits special comment in this regard. This $35^{1/7}$ week late preterm gestation neonate with hemolytic disease had an abnormal ABR following a peak TSB of 15.1 mg/dL (258 umol/L), a B/A ratio below double volume exchange transfusion criteria,[8,9] and only modest hypoalbuminemia (serum albumin of 2.7 g/dL derived from the reported peak TSB and B/A ratio). The circulating UB level, however, was quite elevated. These findings demonstrate that the albumin-bilirubin binding in this infant was markedly impaired. Is it possible that severe hemolysis adversely affected albumin-bilirubin binding or did some other factor or comorbid condition lead to this result? Regardless, the elevated circulating UB in this neonate predicted bilirubin neurotoxicity whereas the TSB and B/A ratio did not, underscoring the utility of circulating UB measurements in identifying bilirubin-induced injury risk.

So why isn't circulating UB measurement routinely incorporated in the evaluation and management of neonatal hyperbilirubinemia? In part, this derives from the relative ease of measuring TSB levels and the general success of TSB treatment thresholds, modulated by gestational age and neurotoxicity risk factors, in controlling neonatal hyperbilirubinemia and preventing bilirubin-induced CNS injury.[8] Although the TSB has poor specificity (many false positives),[1] extreme (≥25 mg/dL [427 umol/L]) and hazardous (≥30 mg/dL [513 umol/L]), TSB levels remain strong predictors of acute bilirubin encephalopathy and its progression as recently confirmed in a large cohort of term and late preterm neonates in Cairo, Egypt.[10] There are also several ongoing technical challenges and limitations in the measurement of circulating UB.[1,11] Furthermore, bilirubin-induced neurotoxicity depends on a complex interaction between the level and duration of CNS UB exposure (itself difficult to gauge) and the innate cellular characteristics of the CNS that may predispose or protect against bilirubin-induced neuronal injury.[12] In addition, prematurity, hemolysis, and sepsis play key roles in enhancing bilirubin neurotoxicity risk[8] that may or may not be reflected by changes in circulating UB.

Despite these limitations, it is clear that circulating UB measurement improves the bilirubin neurotoxicity risk calculus and importantly identifies a subset of at-risk infants who would otherwise be missed. It is critical, therefore, that efforts to incorporate circulating UB measurement in neonatal care move forward,[1,11] including ongoing work to improve, standardize, and validate techniques to measure circulating UB. In addition, large clinical trials to examine definitions and thresholds for hyperbilirubinemia treatment based on levels of UB compared with TSB and the B/A ratio are needed.[12] In some subjects, the

ABR	Auditory brainstem evoked response
B/A	Bilirubin/albumin
CNS	Central nervous system
TSB	Total serum bilirubin
UB	Unbound (or "free") bilirubin

The author serves as a consultant in legal cases related to neonatal jaundice.

0022-3476/$ - see front matter.
http://dx.doi.org/10.1016/j.jpeds.2016.03.044

TSB, B/A ratio, and circulating UB measurement will align in predicting risk, in others with marked hypoalbuminemia it is possible only the B/A ratio and circulating UB will prove predictive, whereas in those where impaired albumin-bilirubin binding is operative it is likely only circulating UB will suffice.

In lieu of such comparative data, an elevated B/A ratio itself may be a useful UB proxy as it correlates with circulating UB[9] and is a sensitive measure of bilirubin-induced injury risk,[10] particularly when hypoalbuminemia is present.[13,14] The B/A ratio has been advanced as a factor in determining the need for exchange transfusion,[8,9] an approach endorsed by the American Academy of Pediatrics.[8] However, the findings of the current study coupled with improved bedside applicable measurement of circulating UB and bilirubin binding capacity in development,[1,11] should be a powerful impetus to conduct large comparative studies of circulating UB, TSB, and the B/A ratio in predicting bilirubin neurotoxicity. As suggested by the authors of the current paper, abnormalities in the ABR are likely to be the most sensitive marker of acute bilirubin-induced injury and, thereby, the most relevant bilirubin-specific primary outcome for clinical study. Undertaking this study will be no small task but such evidence is necessary to potentially modify current clinical practice and further decrease the risk for bilirubin-induced CNS injury. Now is the time to act. ■

Jon F. Watchko, MD
Division of Newborn Medicine
Department of Pediatrics
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania

Reprint requests: Jon F. Watchko, MD, Division of Newborn Medicine, Department of Pediatrics, Magee-Women's Hospital, 300 Halket Street, Pittsburgh, PA 15213. E-mail: jwatchko@mail.magee.edu

References

1. Ahlfors CE, Wennberg RP, Ostrow JD, Tiribelli C. Unbound (free) bilirubin: improving the paradigm for evaluating neonatal jaundice. Clin Chem 2009;55:1288–99.
2. Amin SB, Wang H, Laroia N, Orlando M. Unbound bilirubin and auditory neuropathy spectrum disorder in late preterm and term infants with severe jaundice. J Pediatr 2016;173:84–9.
3. Shapiro SM. Kernicterus. In: Stevenson DK, Maisels MJ, Watchko JF, editors. Care of the jaundiced neonate. New York: McGraw Hill; 2012. p. 229–42.
4. Wennberg RP, Ahlfors CE, Bickers R, McMurtry CA, Shetter JL. Abnormal auditory brainstem response in a newborn infant with hyperbilirubinemia: improvement with exchange transfusion. J Pediatr 1982;100:624–6.
5. Nwaesei CG, Van Aerde J, Boyden M, Perlman M. Changes in auditory brainstem responses in hyperbilirubinemic infants before and after exchange transfusion. Pediatrics 1984;74:800–3.
6. Cashore WJ, Oh W. Unbound bilirubin and kernicterus in low-birthweight infants. Pediatrics 1982;69:481–5.
7. Ahlfors CE, Parker AE. Unbound bilirubin concentration is associated with abnormal automated auditory brainstem response for jaundiced newborns. Pediatrics 2008;121:976–8.
8. 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 [Erratum, Pediatrics 2004;114:1138.].
9. Ahlfors CE. Criteria for exchange transfusion in jaundiced newborns. Pediatrics 1994;93:488–94.
10. Iskander I, Gamaleldin R, El Houchi S, El Shenawy A, Seoud I, El Gharbawi N, et al. Serum bilirubin and bilirubin/albumin ratio as predictors of bilirubin encephalopathy. Pediatrics 2014;134:e1330–9.
11. Amin SB, Lamoloa AA. Newborn jaundice technologies: unbound bilirubin and bilirubin binding capacity in neonates. Semin Perinatol 2011;35:134–40.
12. Watchko JF, Tiribelli C. Bilirubin-induced neurologic damage—mechanisms and management approaches. N Engl J Med 2013;369:2012–30.
13. Govaert P, Lequin M, Swarte R, Robben S, De Coo R, Weisglas-Kuperus N, et al. Changes in globus pallidus with (pre) term kernicterus. Pediatrics 2003;112(6 Pt 1):1256–63.
14. Watchko JF, Maisels MJ. The enigma of low bilirubin kernicterus in premature infants: why does it still occur, and is it preventable? Semin Perinatol 2014;38:397–406.

Executive Function in Patients with Congenital Heart Disease: Only the Tip of the Iceberg?

There is an increasing recognition of a common neurodevelopmental phenotype in pediatric survivors of complex congenital heart disease (CHD).[1,2] One of the major features of this phenotype is the relative preservation of lower level cognitive skills and substantial difficulty integrating or coordinating these skills to achieve higher order goals.[1-5] Impairments of executive function are of particular importance to the population with CHD because they are likely to have widespread repercussions on a myriad of cognitive and behavioral domains throughout the developmental course.[3-9] In recent years, the number of studies reporting impairments of executive function in children and adolescents with CHD, corrected or palliated with infant open heart surgery, has increased rapidly.[2-14] However, studies investigating whether and how executive function deficits impact children's everyday life are scarce.

See related article, p 154

CHD	Congenital heart disease

J.C. is supported by a Thrasher Early Career Award. The author declares no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2016.02.066