Predicting bilirubin neurotoxicity in jaundiced newborns

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Purpose of review

The management of jaundice in the newborn infant is an area of clinical practice sorely lacking an evidence-based foundation, and neonatal bilirubin neurotoxicity (kernicterus) continues to occur worldwide.

Recent findings

Studies suggest that measuring serum or plasma bilirubin binding, in particular the nonalbumin-bound or unbound bilirubin concentration (B_f), would improve jaundice management as it better predicts bilirubin neurotoxicity than the conventionally used total bilirubin concentration (B_T). However, many misconceptions persist regarding the relationships between B_T, B_f, the magnitude and distribution of the neonatal bilirubin load, and the risk of bilirubin neurotoxicity.

Summary

Overcoming these misconceptions and integrating B_f and B_T into the management of neonatal jaundice may help move clinical practice from its tradition-based approach centered primarily on B_T toward an evidence-based approach that will substantially improve our ability to predict bilirubin neurotoxicity and improve the clinical management of this generally benign, but potentially catastrophic, newborn condition.

Keywords

bilirubin encephalopathy, bilirubin neurotoxicity, kernicterus, unbound or free bilirubin

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Introduction

Transient neonatal jaundice is the most commonly encountered and arguably one of the most poorly managed conditions in the immediate postnatal period [1,2]. Bilirubin neurotoxicity (acute bilirubin encephalopathy, kernicterus) is preventable, yet sporadic cases still occur in developed countries [3–6] and in many undeveloped countries it is endemic [7]. The spectrum of acute and chronic neurological damage caused by bilirubin is still poorly understood, especially in the premature or ill newborn, and the obstacles impeding further clarification of the role of bilirubin toxicity in neurodevelopmental impairment are considerable [8,9•].

Little evidence-based data is available for managing neonatal jaundice despite decades of basic research and clinical studies aimed at understanding the neurotoxicity of unconjugated bilirubin-IXα, the primary bilirubin isomer that accumulates during newborn jaundice [10,11•]. A major reason has been the continued use of the total bilirubin concentration (B_T) in serum, plasma, or in cell or tissue culture medium as the primary intervention or outcome measure in most in-vivo and in-vitro studies [10,11•,12••]. Though unconjugated hyperbilirubinemia is a necessary condition for bilirubin neurotoxicity, peak B_T *per se* has been repeatedly shown to be poorly associated with bilirubin neurotoxicity [10,11•,12••].

Nonetheless, the unfounded premise that bilirubin neurotoxicity and the magnitude of B_T are intimately linked remains so entrenched in mainstream clinical thinking that misstatements confusing causation and association such as 'At high levels, total serum bilirubin causes kernicterus' [13] or 'It is controversial whether modest elevations of total serum bilirubin (hereafter referred to simply as bilirubin) cause brain damage in preterm infants' [9•] are glossed over by reviewers, editors, and readers alike. Even more subtle has been the use of the hour-specific B_T to predict significant hyperbilirubinemia rather than an increased risk of bilirubin neurotoxicity [14]. If preventing bilirubin neurotoxicity were simply a matter of minimizing B_T, administering sulfisoxazole, or some other bilirubin binding competitor, to drive bilirubin from the vascular compartment into the tissues would be the treatment of choice rather than contraindicated in newborn jaundice [15].

Improving our ability to predict bilirubin neurotoxicity may be as much about having the courage to move past our reliance on B_T alone as the cornerstone of jaundice management as it is about opening our minds to new approaches or reconsidering old ones that were perhaps

too hastily discarded [12••,16]. This includes recognizing the potential error inherent in studies dismissing bilirubin as the possible cause of neurological injuries, for example, deafness, which is a known complication of bilirubin neurotoxicity, simply because B_T did not reach an arbitrary value [17].

Recent studies demonstrate that the plasma unbound or 'free' bilirubin concentration (B_f) is more closely associated with [18••,19] and predictive of [20••] abnormal hearing tests than B_T. In addition, imaging techniques can now demonstrate the presence of bilirubin in areas of the central nervous system known to be damaged by bilirubin [21,22]. These advances provide a foundation for generating evidence-based data that may substantially improve our approach to the jaundiced newborn, and, paradoxically, B_T will remain a major component of that foundation.

Predicting bilirubin neurotoxicity from vascular bilirubin measurements

Improving the prediction of bilirubin neurotoxicity is fundamental to improving the management of jaundice in the newborn infant, which results from the normal, transient postnatal imbalance between bilirubin production and elimination. The likelihood of bilirubin neurotoxicity depends on the magnitude and distribution of the accumulated bilirubin load, also referred to as the miscible bilirubin pool because the accumulating bilirubin moves readily between the vascular and extravascular compartments [12••,23,24]. The distribution of the load between compartments is determined in part by the plasma binding of bilirubin, mostly by albumin, but the range of extravascular fractions encountered clinically in human newborns has not been rigorously studied. The extravascular fraction was 0.5 at a B_T of about 25 mg/dl in a child with Crigler–Najjar syndrome [23], and in healthy adults and in jaundiced (jj) Gunn rats the extravascular fraction is about 0.8 [23,24].

Although the risk of bilirubin neurotoxicity is more closely linked with the extravascular fraction of the load [15], in clinical practice vascular bilirubin measurements, historically the magnitude of B_T, have been use to assess the severity of jaundice and guide management. Theoretical arguments [25] and several human studies [18••,19,20••,26–29] suggest B_f rather than B_T would be the vascular bilirubin measurement more closely associated with bilirubin neurotoxicity because the extravascular fraction of the load varies directly with B_f but unpredictably with B_T [12••]. This does not mean that B_f and B_T should be viewed as competing, independent determinants of bilirubin neurotoxicity. B_T is needed to gauge the size of the load and B_f its distribution, and both are therefore required when using vascular bilirubin measurements to assess the risk of bilirubin neurotoxicity [12••].

The vascular bilirubin fraction and the corresponding B_T at a specified bilirubin load will increase as the strength of plasma bilirubin binding increases. At identical bilirubin loads (all else being equal) a baby with stronger plasma binding will have a higher B_T (i.e., higher intravascular fraction) but paradoxically lower risk of bilirubin neurotoxicity (i.e., lower extravascular fraction) than a baby with weaker binding. Binding strength or avidity is quantified using the mass action bilirubin–albumin association binding constant (K) as shown in the equation below in which B_T is substituted for the albumin-bound bilirubin ($B_T - B_f$), because B_T is orders of magnitude greater than B_f (i.e., $B_T - B_f \approx B_T$).

$$K = \frac{B_T - B_f}{B_f([\text{albumin}] - B_T + B_f)} \cong \frac{B_T}{B_f([\text{albumin}] - B_T)}$$

B_T (i.e., albumin-bound bilirubin) will vary directly with K whereas B_f (the concentration of bilirubin not bound to albumin) and the extravascular fraction of the load will vary inversely with K.

As K varies considerably in newborns [30,31], a wide range of B_f and extravascular bilirubin levels are likely at any given B_T. B_f, therefore, intrinsically provides a better vascular indicator of the size of the extravascular bilirubin fraction than B_T. This is often misinterpreted as meaning that B_f causes bilirubin neurotoxicity (i.e., B_f is the 'toxic' bilirubin fraction). It is the bilirubin bound to cell membranes and organelles, not B_f, that likely disrupts cell function and ultimately causes injury [32], and without a sufficiently large load of bilirubin there is little risk of bilirubin neurotoxicity regardless of the magnitude of B_f. B_f and B_T are therefore co-dependent determinants of bilirubin neurotoxicity; together they provide information as to whether the size and distribution of the bilirubin load are typical or atypical at a given B_T. For example, a baby with a B_T of 20 mg/dl but weak binding (e.g., $B_f > 2$ SD above average) is likely to have a much larger bilirubin load and greater risk of bilirubin neurotoxicity (greater extravascular fraction) than the average baby with a B_T of 20 mg/dl. B_f is best viewed as an adjunct in the interpretation of B_T and vice versa.

Introducing B_f into the clinical setting requires suitable clinical laboratory techniques for measuring B_f, normative B_f population data, and reference values for intervention that utilize B_f and B_T. Bilirubin binding can be measured by a number of methods, but the most frequent method is the peroxidase test for measuring B_f [12••]. The US Food and Drug Administration has approved a peroxidase method, but it is currently available only in Japan [33]. Nonetheless, the many misconceptions,

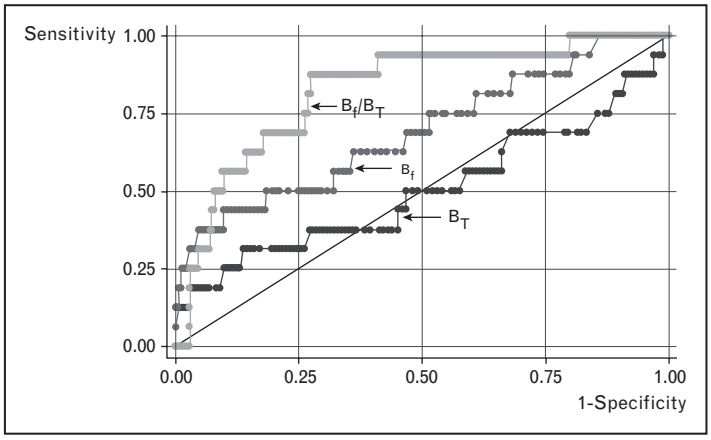
misunderstandings, and malaise surrounding bilirubin binding have contributed far more to the absence of binding measurements in the clinical arena than an inability to measure B_f [1,12••,16].

Bilirubin binding measurements, as with all laboratory tests used clinically, require normative population data [34]. As B_f will vary with B_T and the albumin concentration (see equation above), the avidity of binding (e.g., average, below, or above average) might be quantified using K, which assumes a single bilirubin–albumin binding site per albumin molecule. However, bilirubin binds at multiple sites [35] and, as the B_T/albumin ratio increases, K increasingly becomes a composite of several constants when calculated by the equation above. B_f/B_T, which expresses binding as the fraction of unbound bilirubin present and which would vary inversely with the strength of binding, may provide a more practical quantification of binding that includes both variables [20••]. Regardless, collecting normative binding data is simply a matter of measuring binding variables (B_T, B_f, albumin concentration) in large numbers of babies from various populations (e.g., well term, ill term, preterm, with hemolysis present, etc). It should be noted that this can be facilitated by titrating samples with bilirubin to obtain B_f at several B_T in individual newborn or umbilical cord samples [36].

The most difficult, but not insurmountable, task is obtaining reference-binding levels for intervention with phototherapy or exchange transfusion [12••]. Even without reference levels, however, once normative binding data are available, measuring binding early in the clinical course (e.g., screening for jaundice) allows early identification of babies with weak binding [12••]. These babies could then be monitored more closely and treated to prevent B_T levels of concern, which might be lower than the conventional B_T currently guiding intervention [12••,30].

Reference levels for a test are obtained by first determining the specificity (true negative/all negative tests) and sensitivity (true positive/all positive tests) of the test in detecting the presence of a given condition or outcome. Receiver operating characteristics curves (ROC, Fig. 1), which plot the sensitivity versus 1 – specificity, are then used to determine whether the test has more than a random chance of predicting the condition or outcome [i.e., the area under the ROC (AUC) is significantly greater than 0.5] and to compare tests (i.e., the AUC of one test is significantly greater than the AUC of another test). The test result providing the highest fraction of true negatives (specificity) and lowest fraction of false positives can be used as a reference value for the test. Nakamura *et al.* [27] used ROC to demonstrate that B_f predicted bilirubin neurotoxicity better than B_T in premature newborns. It is important to note that binding of bilirubin and other ligands by albumin is impaired by illness [37], and illness also increases the likelihood of poor neurological outcome. Broad outcome measures such as impaired neurodevelopmental outcome [9•] may therefore be associated with poor bilirubin binding (higher relative B_f or lower K) but not be caused by bilirubin *per se*. Only outcomes closely associated with bilirubin neurotoxicity [19,20••] or that are documented in proximity to jaundice [18••,28,29,38] will ultimately provide the most helpful estimations of bilirubin binding reference values. Advances in assessing newborn hearing have incidentally provided a robust outcome measure for evaluating bilirubin binding measurements.

Figure 1 Receiver operating characteristics curves showing 'free' bilirubin concentration (B_f)/total bilirubin concentration (B_T), B_f, and B_T as predictors of an abnormal (REFER) automated auditory brainstem response



Automated auditory brainstem response hearing screening test results are PASS (ear passes test) and REFER (ear does not pass and patient should be referred for further testing). A test that has no correlation with the outcome would have an AUC = 0.5 (straight line in graph). The AUC of 0.83 for B_f/B_T is significantly greater than that for B_f (0.69), and both AUC are significantly greater than the AUC of 0.50 for B_T, which indicates no correlation between B_T and the outcome. Adapted from [20••].

The auditory brainstem response (ABR) in recent years has been shown to be a very sensitive indicator of bilirubin interference with neurological function [28,29,38–43]. In term newborns, bilirubin-induced ABR changes begin at B_T around 10 mg/dl [41,42], and studies in humans and animals show that changes in ABR wave latency amplitude worsen until the signal is ultimately lost as the bilirubin load increases or is redistributed by administering a bilirubin binding competitor [39,42,44,45]. Ahlfors and Parker [18••] reported that, in term jaundiced babies with B_T more than 14.4 mg/dl, abnormal newborn hearing screening tests (automated ABR) were associated with elevated B_f but not B_T. In a diverse newborn population (Fig. 1), ROC revealed that B_f, and in particular B_f/B_T, but not B_T alone, predicted abnormal automated ABR regardless of the proximity of the binding test to the automated ABR measurement [20••].

Future studies are needed correlating binding measurements with acute and chronic clinical findings suggesting bilirubin-induced neurological dysfunction, such as auditory dyssynchrony/dysfunction [46], ABR and automated ABR changes, imaging techniques demonstrating bilirubin dispersed in critical brain nuclei [21,22], and clinical findings suggestive of bilirubin toxicity [47], to provide the reference bilirubin binding values needed to better predict bilirubin neurotoxicity. Such studies may also add substantially to our understanding of the spectrum of injuries caused by exposure of newborns to unconjugated bilirubin [9•].

Conclusion

Improving our ability to predict bilirubin neurotoxicity requires recognizing the limitations of measuring B_T alone when assessing newborn jaundice. Adjunct vascular measurements such as the albumin concentration, B_f, B_f/B_T, and K may provide much more insight into the likelihood of bilirubin neurotoxicity at any given B_T, and studies evaluating them may eventually provide the much needed evidence-based approach to newborn jaundice. The potential benefits include reducing or eliminating bilirubin neurotoxicity as well as the extensive unnecessary treatment that contributes to the extremely high financial and emotional costs of newborn jaundice.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 246).

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