REVIEW ARTICLE

Toward Understanding Kernicterus: A Challenge to Improve the Management of Jaundiced Newborns

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ABSTRACT

PURPOSE. We sought to evaluate the sensitivity and specificity of total serum bilirubin concentration (TSB) and free (unbound) bilirubin concentration (B_f) as predictors of risk for bilirubin toxicity and kernicterus and to examine consistency between these findings and proposed mechanisms of bilirubin transport and brain uptake.

METHODS. A review of literature was undertaken to define basic principles of bilirubin transport and brain uptake leading to neurotoxicity. We then reviewed experimental and clinical evidence that relate TSB or B_f to risk for bilirubin toxicity and kernicterus.

RESULTS. There are insufficient published data to precisely define sensitivity and specificity of either TSB or B_f in determining risk for acute bilirubin neurotoxicity or chronic sequelae (kernicterus). However, available laboratory and clinical evidence indicate that B_f is better than TSB in discriminating risk for bilirubin toxicity in patients with severe hyperbilirubinemia. These findings are consistent with basic pharmacokinetic principles involved in bilirubin transport and tissue uptake.

CONCLUSIONS. Experimental and clinical data strongly suggest that measurement of B_f in newborns with hyperbilirubinemia will improve risk assessment for neurotoxicity, which emphasizes the need for additional clinical evaluation relating B_f and TSB to acute bilirubin toxicity and long-term outcome. We speculate that establishing risk thresholds for neurotoxicity by using newer methods for measuring B_f in minimally diluted serum samples will improve the sensitivity and specificity of serum indicators for treating hyperbilirubinemia, thus reducing unnecessary aggressive intervention and associated cost and morbidity.

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Key Words
bilirubin–albumin binding, brainstem auditory evoked potentials, bilirubin, hyperbilirubinemia, kernicterus

Abbreviations
TSB—total serum bilirubin concentration
AAP—American Academy of Pediatrics
BBB—blood-brain barrier
B_f—free bilirubin concentration
AB—albumin-bound bilirubin concentration
A—serum albumin concentration
ABR—brainstem auditory evoked response
AN—auditory neuropathy
AD—auditory dyssynchrony
G6PD—glucose-6-phosphate dehydrogenase

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THERE IS SUBSTANTIAL evidence that unconjugated bilirubin is neurotoxic and that high serum levels in newborns can produce brain damage (kernicterus).[1,2] In the absence of obstructive jaundice, the serum concentration of unconjugated bilirubin is best estimated by measuring the total serum bilirubin concentration (TSB). Calculating the "indirect fraction" (TSB minus the "direct reacting" bilirubin concentration) can be misleading in most newborns, because high levels of unconjugated bilirubin can produce an elevated direct fraction (~10% of TSB), which does not represent nontoxic conjugated bilirubin.

Recently published management guidelines for jaundiced term and near-term infants by the American Academy of Pediatrics (AAP) are based on the premise that TSB is the best available predictor of risk for kernicterus.[3] However, clinical evidence indicates that TSB, beyond a threshold value of ~20 mg/dL (342 μmol/L), is a poor discriminator of individual risk for bilirubin toxicity. Kernicterus may rarely occur in healthy term infants with a TSB of <25 mg/dL (428 μmol/L),[4–7] the level currently recommended by the AAP for aggressive intervention, and most infants with a TSB of >25 mg/dL escape without recognized permanent sequelae.[7–10] How can this be? Can the very poor sensitivity and specificity of TSB in predicting bilirubin toxicity be attributed solely to variations in the blood-brain barrier (BBB) or neuronal susceptibility?

In addressing this problem, this review examines (1) determinants of TSB, (2) mechanisms involved in bilirubin movement into the brain, (3) predictability of bilirubin toxicity using TSB and/or the free-bilirubin (not bound to serum proteins) concentration (B_f), and (4) the challenge of developing a more rational management protocol for infants with severe jaundice. To avoid semantic confusion, the term "kernicterus" is restricted to patients with autopsy-proven kernicterus and patients with permanent bilirubin-induced brain injury. "Early bilirubin toxicity" refers to patients with transient or reversible clinical evidence of toxicity in the neonatal period.

DETERMINANTS OF TSB

Textbooks state that the TSB is determined by the rates of bilirubin production, excretion, and intestinal reabsorption (enterohepatic shunt). This statement is not quite accurate. These 3 factors determine the net bilirubin load that accumulates over time and is distributed in multiple compartments within the body. Plasma is but one of the many compartments (including liver, skin, red blood cells, brain, phospholipid membranes, etc) that compete for binding the miscible (exchangeable) bilirubin load. Plasma is a unique compartment in that it serves as a "mixer" for the miscible pool of bilirubin. The "force" that drives this distribution toward equilibrium is the B_f.

The distribution of the bilirubin pool between the various compartments depends on the number of binding sites in each compartment and their affinity for binding bilirubin. The bilirubin-binding affinity is quantified by a binding constant, *K*, which equals the ratio of association and dissociation-rate constants (k_1/k_{-1}) of any specific receptor.

$$\text{free bilirubin} + \text{receptor} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{receptor} \cdot \text{B}$$

In plasma, albumin is the main (but not the only) carrier for bilirubin with a high *K* approaching 10^7 L/mol (similar to *K* in $H_2O \leftrightarrow H^+ + OH^-$).[11] Despite the ability of bilirubin to accumulate in rather high concentrations in both plasma and tissue, the B_f in the body (and plasma) is very low, rarely exceeding 3 to 4 μg/dL even in cases of marked hyperbilirubinemia. The low B_f is mandated in part by the very low solubility of bilirubin, which has been estimated to be 7 nmol/L[12] on the basis of dissolution of bilirubin crystals in water or 70 nmol/L (4.1 μg/dL) on the basis of partitioning between water and chloroform.[13] The latter value is more consistent with B_f reported in infant sera. Higher metastable concentrations can exist but would tend to aggregate and precipitate in tissue.

The relationship between B_f, albumin-bound bilirubin concentration (AB), and serum albumin concentrations (A) at clinically relevant TSB and A can be expressed as

$$B_f \approx \frac{AB}{(A - B) \times K}.$$

Because TSB = AB + B_f, and the concentration of B_f is extremely low relative to TSB, AB ≈ TSB, and the equation can be expressed as

$$B_f = \frac{TSB}{(A - TSB) \times K}.$$

At a given TSB, B_f is inversely proportional to A and its intrinsic ability to bind bilirubin (*K*). Given the same B_f and *K*, an infant with an albumin level of 4 g/dL will have a TSB that is twice as high as a newborn with 2 g/dL albumin. However, the "driving force" (B_f) that sends bilirubin to tissue sites will be nearly identical. In other words, given identical *K* values, an infant with a TSB of 15 mg/dL and A of 2 g/dL will have nearly the same risk for bilirubin toxicity as an infant with a TSB of 30 mg/dL and albumin of 4 g/dL.

Unfortunately, the albumin-bilirubin binding constant, *K*, is not "constant" but varies considerably among newborns. It is also lower in sick infants and may increase with postnatal age. Furthermore, the effective concentration of albumin, A, can be reduced by the presence of drugs or compounds that bind to the same

locus as bilirubin such as sulfonamides or benzoate, the metabolite of benzyl alcohol. Both drugs have been associated with clusters of kernicterus in premature infants.[14,15]

The equation shown above considers only 1 binding site on albumin and assumes that 1 molecule of albumin binds a single bilirubin molecule. However, albumin has 1 to 2 additional bilirubin-binding sites with lower *K* values, and apolipoprotein D also binds bilirubin in plasma.[16] Thus, the measured B_f in plasma reflects the equilibrium of bilirubin with multiple binding loci, and TSB is distributed among the various plasma protein-binding sites according to their relative concentrations and binding constants. Interindividual variations in *K*, effective albumin concentration, and alternative binding sites in plasma limit the usefulness of estimating B_f by measuring the TSB/A ratio. Even in cases of kernicterus, TSB does not exceed the "binding capacity" of plasma, although the B_f (B_f/TSB) increases more rapidly as the TSB/A molar ratio approaches unity (~9 mg of bilirubin per g of albumin).

Similar equations govern the distribution of bilirubin in tissue; at equilibrium, B_f will be the same in all compartments. However, in a system involving multiple compartments, some of which may involve nonreversible metabolic processes (eg, bilirubin oxidase, conjugation by glucuronyl transferase), a true equilibrium will never be achieved, but the movement of bilirubin will be toward compartments with lower B_f approaching "steady-state" levels of B_f. This movement of bilirubin between compartments has sometimes been referred to as "the free-bilirubin theory." It is not a theory but a law: the law of mass action.

ENTRY OF BILIRUBIN INTO BRAIN

In contrast to other reservoirs for bilirubin binding, the brain is unique by having a BBB that slows the equilibrium between plasma and brain. If the BBB is disrupted, bilirubin–albumin moves rapidly into the extracellular space of brain,[17] and at sufficiently high B_f bilirubin will produce immediate global neurotoxicity.[18–20]

When the BBB is intact, the rate of bilirubin uptake by brain is determined by (1) the B_f, (2) the permeability and surface area of the capillary endothelium, (3) the transit time through the capillary bed (time available for the extracted bilirubin to be replenished from the albumin reservoir), (4) the dissociation rate of bilirubin–albumin, k_{-1}, (how quickly the B_f will be replaced by albumin-bound bilirubin as free bilirubin leaves the vascular space), and (5) the blood flow (number of capillaries recruited in a given region).[21] The BBB is quite permeable to free bilirubin, with single-pass uptakes estimated as high 28% in rats.[22] Bilirubin uptake may be increased by alterations in BBB permeability to bilirubin or albumin (eg, hyperosmolality, severe asphyxia), prolonged transit time (eg, increased venous pressure), an increase in blood flow (eg, hypercarbia), or an increase in the albumin-bilirubin dissociation rate (eg, altered binding in sick infants).

Because the transit time is short, ~1 second, and the bilirubin–albumin dissociation rate is slow (eg, compared with the dissociation of H_2O), there is little time to replenish the unbound bilirubin that is transported across the brain capillary. At a given B_f, brain uptake of bilirubin could theoretically be facilitated by a high TSB (ie, a high A) simply because there would be a larger plasma reservoir to replenish the extracted free bilirubin. Using published dissociation rate constants obtained in dilute albumin solutions,[11] Robinson and Rapoport[23] concluded that the slow dissociation rate precludes rapid replenishment of bilirubin removed and that brain uptake is governed largely by the TSB until the high-affinity bilirubin-binding sites on albumin are saturated. Unfortunately, their analysis did not consider that significant bilirubin is bound to secondary sites (probably with higher dissociation rates) long before this occurs. Furthermore, subsequent evidence indicates that the binding constant (ratio of association/dissociation rates) is an order of magnitude lower in undiluted serum and extremely variable between infants,[24,25] which calls into question their assumption of a low k_{-1} being rate limiting for brain uptake of bilirubin.[26]

Clinically, bilirubin neurotoxicity, which occasionally is irreversible, will occur before primary sites are saturated. Still, because of the slow transport of bilirubin across the BBB, time of exposure to a high B_f may be critical in determining the magnitude of brain load and toxicity. In primates, several hours of exposure to a very high TSB and B_f are usually required to create neurotoxicity, and even longer exposure is needed to produce nuclear staining.[27,28] Most cases of kernicterus in term infants occur at several days of age and often after the TSBs have been very high for extended periods of time. This "exposure-time factor" complicates outcome studies that examine only the peak (or a single readmission) TSB.

Net transport of bilirubin across the BBB also may be influenced by the energy-dependent multidrug-resistant transporters MDR1 and MRP1. MDR1, or P-glycoprotein, is one of several transporters that are involved in cellular efflux of xenobiotics and is expressed in capillary endothelial cells of the BBB, astrocytes, and the choroids plexus.[29] Unconjugated bilirubin is a weak substrate for MDR1.[30] In a study by Watchko et al,[31] brain uptake of bilirubin in $Mdr1a^{-/-}$ knockout mice infused with high concentrations of bilirubin was twice that of controls ($Mdr1a^{+/+}$). Inhibition of P-glycoprotein potentiates bilirubin-induced apoptosis in a human neuroblastoma line[32] and increases bilirubin content in brains of young adult rats (measured at very high TSB).[33]

The multidrug-resistance–associated protein, MRP1, performs similar functions. MRP1 is highly expressed in

choroid plexus epithelium, astrocytes, (rat) neurons, and placenta trophoblast but has minimal expression in capillary endothelium in whole brain.[34] It is upregulated in cultured cells that are exposed to unconjugated bilirubin and mediates ATP-dependent cellular export of bilirubin.[35] Inhibition of MRP1 in cultured astrocytes potentiates apoptosis induced by low concentrations of bilirubin.[36] Other potential cellular defense mechanisms include mitochondrial bilirubin oxidase,[37,38] other transporters, and antiapoptosis factors.[39]

The extent to which these cellular defense mechanisms contribute to variations in sensitivity to the toxic effects of bilirubin remains uncertain, but all of these protective mechanisms can be overwhelmed by the rapid infusion of a binding competitor. Under steady-state conditions, these endogenous metabolites and exogenous drugs effectively decrease the albumin concentration. However, peak drug levels after intravenous infusion can have a devastating effect, and like the splash from a boulder thrown into a pond, there is a transient surge in B_f, an immediate decrease in TSB, and an acute increase in brain bilirubin content, with potentially severe neurologic consequences.[27,28,40,41]

The cellular mechanism(s) of early reversible bilirubin toxicity (eg, behavioral changes and prolongation of interwave latencies in the brainstem auditory evoked response [ABR]) are unknown, but exposure of neurons to high levels of B_f will produce apoptosis[42–44] and/or necrosis associated with mitochondria dysfunction, possibly produced by bilirubin disruption of the proton gradient required for oxidative phosphorylation.[44]

In summary, the distribution of bilirubin in the body can be described by using established pharmacokinetic principles. Although much is known about the physiology of bilirubin transport, brain uptake, and toxicity, transformation of this "bench" knowledge[22,34,39,45] to clinical research and bedside strategy has been very slow.

ASSESSING RISK FOR NEUROTOXICITY AND KERNICTERUS BY USING TSB

Bilirubin can produce behavioral changes and alterations in the ABR at TSBs well below 20 mg/dL.[46] As the TSB increases, changes in the ABR progress from small increases in interwave intervals to decreased amplitude and eventual loss of waves III and V, which represents altered brainstem conduction.[47–52] Abnormalities in wave I, which reflects auditory nerve function, may be seen at higher bilirubin levels and in kernicteric infants with hearing loss.[51,52] Although severe ABR changes occur only at high TSB (usually >20 mg/dL), TSB does not differentiate newborns with and without ABR changes.[48–50]

ABR abnormalities may reverse or improve after phototherapy or exchange transfusion[47] but sometimes require months to normalize.[53] This slow recovery may be testimony to the plasticity of developing injured brain rather than truly "reversible" bilirubin toxicity. Permanent neurosensory hearing loss may be the only clinical manifestation of kernicterus. A similar sequence of ABR changes has been observed in jaundiced Gunn rat pups given an albumin-binding displacer (sulfadimethoxine).[54–56] The abnormalities could be partially reversed with rapid intervention.[54]

Hyperbilirubinemia can also produce changes in electrocortical activity (increased delta frequency and decreased frequency and amplitude of theta, alpha, and beta waves recorded at TSB levels ranging from 16 to 33 mg/dL),[57] delay electrocortical maturation in term newborns,[57] and delay ABR maturation in premature infants.[58] Premature and near-term infants may be particularly sensitive to bilirubin-induced ABR changes and residual hearing loss.[59,60] An association of apnea and bradycardia during the first 2 weeks of life with increased TSB, B_f, and ABR changes was reported recently,[61] suggesting that brainstem toxicity may extend beyond auditory pathways in premature infants. Apnea is a common manifestation of toxicity in premature primates that are infused with bilirubin and may precede changes in the ABR.[27]

In 1996, Starr et al[62] described a syndrome of auditory neuropathy (or auditory dyssynchrony) (AN/AD)[63–65] characterized by an absent or severely distorted ABR, normal otoacoustic emissions (ie, normal hair cells), normal cochlear microphonic responses (auditory nerve), and variable hearing impairment. The same constellation of findings had been observed previously by Chisin et al[66] in 1979 in children with hearing loss caused by hyperbilirubinemia. In sparing the inner ear and acoustic nerve, AN/AD is quite distinct from most causes of hearing loss.

Children with AN/AD have difficulty understanding speech in the absence of significant hearing loss and may have delayed speech development, behavioral problems, and learning disability.[67] AN/AD may account for 11% of children with permanent hearing deficits,[67,68] with a reported prevalence of 5.3% to 14.8% in infants discharged from NICUs.[69] Hyperbilirubinemia and prematurity are significant risk factors for AN/AD,[68,70–72] accounting for more than half of the patients with the syndrome.[73] When associated with hyperbilirubinemia, AN/AD is more likely to improve over time. Although the incidence of AN/AD in the premature population and its relationship to TSB and B_f remains to be firmly established, the syndrome seems to be an important component of the bilirubin-induced brain-injury spectrum involving auditory pathways.[61]

Among healthy term or near-term infants without isoimmune hemolytic disease, there is no evidence that a TSB concentration maintained at <20 to 21 mg/dL will produce significant permanent neurologic sequelae,[5–9] but how well does TSB discriminate infants at risk for kernicterus when the TSB exceeds 20 mg/dL? How

many patients with severe hyperbilirubinemia escape injury, and what is the TSB in patients who develop kernicterus?

Data relating TSB to kernicterus in otherwise healthy term infants are extensive, but conclusions are sparse because of the rarity of the event, logistic and ethical constraints in conducting prospective outcome research with controlled intervention, inadequate documentation of TSB before readmission with symptoms, and varying methods of assessing outcome (eg, acute bilirubin-induced neurologic dysfunction versus permanent injury; subtle deficits; focal or severe damage). Newman and Maisels[8] conducted an extensive analysis of available data in 1990 that was dominated by a reanalysis of outcome data from the Collaborative Perinatal Project, a multicenter cohort study of >54 000 pregnancies between 1959 and 1965. Their conclusion that "infants without hemolysis are not at risk of mental or physical impairment until serum bilirubin levels rise well above 20 mg/dL" was instrumental in liberalizing the standard of care in the 1994 AAP practice parameter.[8,74,75] In their analysis, Newman and Maisels assumed a linear relationship between TSB and outcome that, as described above, is unlikely to occur. In a subsequent study of outcome at 7 to 8 years of age,[9] a significant association of hyperbilirubinemia and abnormal or suspicious neurologic abnormalities was found when comparing groups who had neonatal TSBs of <10 mg/dL and those who had TSBs of >20 mg/dL. More recent studies have also reported that mild neurologic abnormalities are more frequent in infants with high bilirubin levels who are evaluated at ages ranging from 12 months to 15 years,[74,77] although the functional significance of abnormalities in all 3 studies is uncertain.

Three retrospective chart reviews of infants who were readmitted with severe hyperbilirubinemia provide an insight to the degree of risk. Newman et al[10] reviewed the outcome of all infants with TSBs of >30 mg/dL (513 μmol/L) who were readmitted to a large health care maintenance organization over a 4-year period. All 11 patients received aggressive bilirubin-reduction interventions. The TSB ranged from 30.7 mg/dL (525 μmol/L) to 45.5 mg/dL (778 μmol/L). None had documented acute symptoms of encephalopathy, and 9 were reported to have normal neurologic examinations when they were evaluated at ages ranging from 18 months to 5 years. One died of sudden infant death syndrome, and a second was receiving speech therapy but was otherwise normal.

Ahlfors and Herbsman[78] examined 8 term infants who were readmitted with TSBs ranging from 28.3 to 34.2 mg/dL. One infant with a TSB of 33.1 mg/dL (566 μmol/L) failed an "Algo" hearing screen, and a second, who was admitted with a TSB of 31.7 mg/dL (542 μmol/L), had acute symptoms of encephalopathy, seizures, and apnea and died with pathologic kernicterus that was found at autopsy. The infant had glucose-6-phosphate dehydrogenase (G6PD) deficiency but no evidence of hemolysis or infection. The remaining infants were asymptomatic, but follow-up was not reported. All surviving infants received exchange transfusion.

Harris et al[79] reported 6 readmissions of infants with TSBs of >25 mg/dL and acute signs of encephalopathy in their hospital but did not state the number of those who were admitted with TSBs of >25 mg/dL without symptoms during the same time interval. Admission TSBs ranged from 26.4 to 36.9 mg/dL (451–631 μmol/L). In contrast to the report by Newman et al,[10] 5 of the 6 infants were symptomatic on admission. MRI was abnormal in 3 of 4 infants tested (including an asymptomatic infant with a TSB of 26.4 mg/dL), and 2 of 5 infants had abnormal ABRs. Follow-up was normal for 4 infants, 1 had residual hearing loss, and 1 had severe cerebral palsy and mental retardation, which is atypical of kernicterus but consistent with global bilirubin encephalopathy observed in animals with a permeable BBB.

These 3 studies are summarized in Fig 1, which illustrates the wide spectrum of response to severe hyperbilirubinemia. Because the denominator of asymptomatic readmissions is uncertain in the latter 2 studies, Fig 1 should reflect the maximum risk for kernicterus in this TSB range. Most infants had no acute or residual effects from severe hyperbilirubinemia, and TSB did not discriminate which infants would or would not develop kernicterus (ie, low specificity). Most of these infants received aggressive phototherapy and/or exchange transfusion, which may relate to the good outcome.

If the goal of intervention in hyperbilirubinemia is to prevent all cases of kernicterus, one must choose a TSB (or B_f) with high sensitivity to include all infants at risk. Two recent studies examined the readmission or peak TSB level in infants with acute-phase bilirubin toxicity, residual neurologic injury, and/or autopsy-proven kernicterus in term and near-term infants. A literature search conducted by the Agency for Healthcare Research and Quality at the behest of the AAP reviewed 123 cases of kernicterus that were reported in 28 articles published in the English-language literature between 1955 and 2001.[5,7] Of 123 cases, 21 had idiopathic hyperbilirubinemia with chronic neurologic sequelae or kernicterus proven by autopsy (4 cases). The mean TSB was 37 mg/dL (range: 23–49.7 mg/dL) (Table 1). More than 90% of infants with kernicterus associated with idiopathic jaundice had TSBs >25 mg/dL. Twenty-five percent of the cases had a TSB <30 mg/dL, and 50% had a peak TSB 35 mg/dL (600 μmol/L). The review included 9 infants with kernicterus associated with sepsis (TSB range: 14.5–49.8 mg/dL) and 39 patients with ABO or Rh isoimmune hemolytic disease (mean TSB: ~32 mg/dL; range: 17.7–51 mg/dL). The mean TSB and lowest

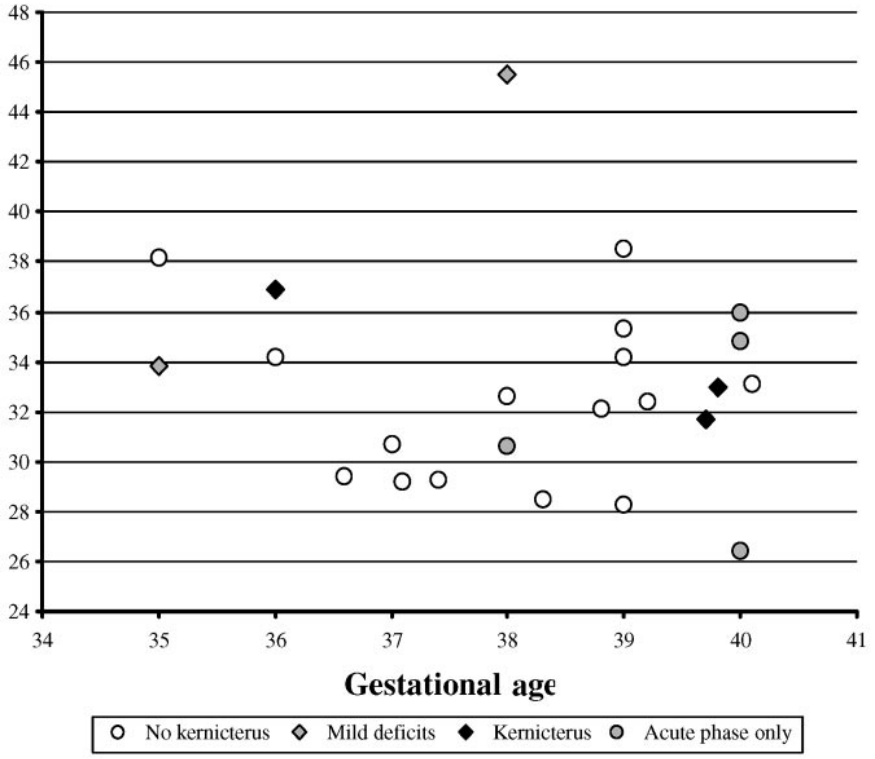

FIGURE 1
Outcome of newborns who were readmitted with severe hyperbilirubinemia, illustrating the low specificity of TSB in predicting bilirubin neurotoxicity.[10,45,46]

TABLE 1 **Peak TSB Associated With Acute, Chronic, or Autopsy-Confirmed Cases of Kernicterus Reported in the English-Language Literature: 1955–1999[7]**

Cause for Hyperbilirubinemia	*N*	Range, mg/dL (μmol/L)	Mean Peak TSB, mg/dL (μmol/L)
Idiopathic hyperbilirubinemia	21	23.0–49.7 (393–850)	37.0 (633)
Sepsis/infection	13[a]	14.5–47.8 (248–818)	31.8 (544)
Isoimmune hemolytic disease	52[b]	17.7–51.0 (303–872)	32.0 (547)

[a] Includes 3 patients with acute signs but no follow-up and 1 patient with normal follow-up.
[b] Includes 9 patients with acute signs but no follow-up and 2 patients with normal follow-up.

TABLE 2 **Readmission TSB Associated With Chronic or Autopsy-Confirmed Kernicterus in 111 Cases Reported to the Pilot Kernicterus Registry: 1991–2002**

Cause for Hyperbilirubinemia	*N*	Range, mg/dL (μmol/L)	Mean TSB, mg/dL (μmol/L)
Idiopathic hyperbilirubinemia	39	20.7–52.0 (354–889)	36.7 (628)
Sepsis/infection	5	21.5–48.0 (368–821)	30.1 (515)
G6PD deficiency	24	25.6–54.0 (438–924)	39.7 (679)
Hemolysis (other)	22	25.0–59.9 (428–1024)	38.9 (665)
Birth trauma	18	23.8–53.9 (407–922)	34.6 (592)
Other[a]	3	40.3–49.0 (689–838)	43.1 (737)
Total cases	111	20.7–59.9 (354–1024)	38.1 (652)
Acute-phase kernicterus with no sequelae	7	25.6–36.0 (438–615)	29.3 (501)

[a] Crigler-Najjar syndrome ($n = 2$) and galactosemia ($n = 1$).

TSB in these infants were ~5 mg/dL lower than in kernicteric infants with idiopathic hyperbilirubinemia.

The Pilot Kernicterus Registry, established by Brown and Johnson a decade ago,[4,6,80] contains 118 cases of acute and chronic bilirubin encephalopathy with documented readmission or peak TSB[6] occurring in term and near-term infants without Rh isoimmune hemolytic disease (7 additional infants with clinical evidence of kernicterus in the registry did not have a recorded TSB). Peak or admission TSB ranged from 20.7 to 59.9 mg/dL (Table 2). Seven infants (including cases reported by Harris et al[79] described above) had acute bilirubin toxicity, including seizures in 1 infant, but normal outcome. One hundred eleven infants developed residual neurologic injury or died with kernicterus. Nine of these infants (8.1%) had a TSB of ≤25 mg/dL. Fifteen percent of the patients with kernicterus had a TSB of <30.1 mg/dL (515 μmol/L), and 50% had a TSB of <38.5 mg/dL (658 μmol/L).

The readmission mean TSB (36.7 mg/dL) in kernicteric infants with idiopathic hyperbilirubinemia was similar to published levels in previous decades.[7] In the absence of Rh isoimmune hemolytic disease, there was no evidence that hemolysis, whether from G6PD deficiency or other causes (eg, bruising, nonisoimmune hemolytic anemia), lowered the threshold for kernicterus compared with idiopathic hyperbilirubinemia. Evidence of dehydration was prevalent in patients with kernicterus. Of 111 patients with kernicterus, 30 (27%) had a weight loss of >13% of birth weight on readmission, and 9 were

recorded to have serum sodium values ranging from 149 to 161 mEq/L. The registry data and the review by the Agency for Healthcare Research and Quality both suggest that some patients with sepsis may have a lower threshold for kernicterus. Although time of exposure and intervention with phototherapy or exchange transfusion are likely to be mitigating factors in the development of irreversible damage, there is no clear explanation for the wide variation of TSB that is found in infants both with and without residual injury. Gestational age and postnatal age may influence both serum binding and BBB function. Comorbid factors such as acidosis, asphyxia, dehydration, and hyperosmolarity, infection, or isoimmune hemolytic disease may also impair serum binding of bilirubin, but the degree to which altered binding contributes to the increased sensitivity to bilirubin is uncertain. The contribution of these variables as independent risk factors for kernicterus will never be known without measuring serum binding in these infants.

In summary, kernicterus is a rare complication of neonatal unconjugated hyperbilirubinemia, and most healthy term infants with a TSB of 25 to 40 mg/dL escape without significant permanent damage. Most of these infants received intensive therapy (phototherapy and/or exchange transfusion), so risks for kernicterus with prolonged exposure and without intervention is unknown. On the other hand, 8% to 9% of reported kernicterus cases occurred with a TSB of <25 mg/dL, with documented admission TSBs as low as 20.7 mg/dL (354 μmol/L). The recommendation for aggressive intervention in term infants with a TSB of ≥25 mg/dL balances the very low specificity of TSB for predicting kernicterus with an "acceptable" sensitivity that includes ~92% of patients with kernicterus.

ASSESSING RISK FOR NEUROTOXICITY AND KERNICTERUS BY USING B_f

After the discovery by Odell[81] that sulfisoxazole displaces bilirubin from albumin, which explains the high incidence of kernicterus in premature infants who are given the drug,[14] several methods for evaluating A binding were developed. These methods included the salicylate index,[82] HBABA dye binding,[83] erythrocyte bilirubin content,[84,85] Sephadex G25 gel filtration,[86,87] MADDS binding,[88] front-face fluorometry,[89] and the peroxidase method,[90] which measures B_f from the rate of enzymatic oxidation of TSB. A comparison of the various tests demonstrated good agreement between Sephadex gel filtration and the peroxidase method in predicting binding and B_f, although the value for B_f that was obtained with gel filtration was much higher.[90,91]

During the next 2 decades, a number of studies demonstrated positive correlations between B_f measured by Sephadex gel filtration and kernicterus,[92–94] and 2 small prospective studies suggested that binding tests might improve predictability of developmental outcome.[95,96] This flurry of interest subsided with the introduction of Rh immune globulin and phototherapy to prevent or treat hyperbilirubinemia, and the ensuing decreased prevalence of severe hyperbilirubinemia in term infants made studies to validate the predictive value of B_f logistically impossible. At the same time, several publications[15,17,23] (discussed in this review) questioned the value of binding tests, clinician's concerns about jaundice were transformed from fear to annoyance, and the measurement of B_f was relegated to a few research laboratories. The recent resurgence of kernicterus in both the United States and Europe and the recognition that TSB has limited predictive power for kernicterus have challenged us to reexamine whether measurement of B_f might improve our clinical management of hyperbilirubinemia.

Several in vitro and animal studies, which most often use the peroxidase method, have compared the effects of TSB and B_f (or binding) on bilirubin uptake or toxicity. B_f, but not TSB, correlated with bilirubin uptake and toxicity in all studies regardless of whether they used cultured cells,[97–99] measured neurotoxicity (electroencephalogram) in animals with osmotic opening of the BBB,[18] or recorded ABR changes in Gunn rats.[100]

Clinical application of the peroxidase method was facilitated by the development of a US Food and Drug Administration–approved dedicated instrument (Arrows Co, Ltd, Osaka, Japan) that can measure both TSB and B_f using a 25-μL serum sample. A B_f of 20 nmol/L (1.2 μg/dL) was hypothesized to be a risk threshold for bilirubin toxicity in term infants on the basis of the highest B_f measured after titrating cord sera to a TSB of 20 mg/dL (R.P.W., unpublished data). Similar conclusions were made by a historically based analysis of kernicterus by Ahlfors.[101] These estimates were supported by 2 clinical studies of term infants[49,50] in which B_f correlated well with marked changes in the ABR (B_f range: 0.9–1.9 μg/dL), whereas TSB and TSB/A did not discriminate infants with and without toxicity.

Murki et al[102] prospectively studied 64 term infants without hemolytic disease who were readmitted for hyperbilirubinemia over a 1-year period. Fourteen infants had clinical evidence of acute toxicity with TSBs ranging from 17.5 to 46 mg/dL (299–787 μmol/L) and B_f levels ranging from 12.6 to 46 nmol/L (0.73–2.7 μg/dL). The mean B_f, TSB, and TSB/A were all statistically higher in the group of infants who were symptomatic. The authors did not report how many of the affected infants had residual damage.

In premature infants, B_f but not TSB correlated with ABR changes, impairment of ABR maturation,[58] and clinical signs of acute bilirubin toxicity,[103] although thresholds for toxicity seem to be somewhat lower than in term infants. In a study of 138 premature infants, Nakamura et al[104] identified 12 infants with clinical ev-

idence of bilirubin toxicity. TSBs ranged from 10.4 to 27.6 mg/dL (178–472 μmol/L), and B_f ranged from 0.8 to 1.76 μg/dL. A B_f of 1.0 μg/dL best discriminated toxic from asymptomatic low birth weight infants (1500–2500 g) with a sensitivity of 100% and specificity of 98%. In very low birth weight infants (<1500 g), a B_f of 0.8 μg/dL correctly identified 4 symptomatic infants with similar accuracy. One of these, a 644-g premature infants with a TSB of 11.5 mg/dL (197 μmol/L) and a B_f of 1.7 μg/dL at 14 days of age, died on day 15 with kernicterus proven at autopsy. This was the only 1 of 12 symptomatic infants who did not receive an exchange transfusion.

B_f in a group of 9 term infants who were readmitted for hyperbilirubinemia (TSB > 28 mg/dL) was evaluated recently with a modified peroxidase method using 2 peroxidase concentrations to correct for rate-limiting dissociation of bilirubin from albumin.[78] This modification yields a higher B_f than the standard method. One infant with classic kernicterus had a TSB of 31.7 mg/dL (542 μmol/L) and a B_f of 7.6 μg/dL, a value far exceeding the reported aqueous solubility of unconjugated bilirubin.[13] Eight additional infants with TSBs ranging from 28.3 to 34.2 mg/dL had B_f ranging from 1.8 to 4.4 μg/dL. One infant, with a B_f of 4.4 μg/dL, failed an Algo hearing screen, whereas the remaining infants were reported to be asymptomatic. Long-term follow-up was not reported.

The standard peroxidase method,[90] using a 1:41 serum dilution, has several limitations. First, serum dilution yields falsely low B_f results in infants with elevated serum levels of competing ligands (eg, sulfonamides, free fatty acids) that require high serum concentrations to effectively displace bilirubin from albumin. Second, serum dilution increases the affinity of albumin for bilirubin, possibly by decreasing albumin dimerization. The binding constant for very dilute human serum albumin is $>10^8$ L/mol, whereas K is closer to 5×10^6 L/mol in undiluted serum.[24,25,105,114] The B_f measured in minimally diluted serum is 5- to 10-fold greater than that measured by the standard method. Furthermore, the standard method uses a single peroxidase concentration, which underestimates B_f, an error that worsens as the B_f concentration increases. Accurate determination of B_f requires that the dissociation rate of bilirubin from albumin be much greater than the rate of oxidation, a condition that requires analysis using dilute peroxidase when the B_f is high. Finally, conjugated bilirubin, if present, will oxidize rapidly, which gives a falsely high value for unbound unconjugated bilirubin (because it is a direct spectrophotometric assay that measures the loss of yellow pigment). Ahlfors[105] recently developed an improved peroxidase assay that minimizes the problems noted above. The method uses minimally diluted serum (1:1 dilution), 2 enzyme concentrations, and a diazo reaction to exclude conjugated bilirubin.

Notwithstanding these limitations, B_f measured by the dilute-peroxidase method has correlated remarkably well with various indicators of bilirubin toxicity,* probably because dilution yields a rather consistent systematic change in bilirubin–albumin binding and apparent B_f in normal infants. This is not true when binding competitors are present. Symptomatic infants reported by Murki et al[102] had elevated free fatty acid levels compared with well infants (1.58 vs 0.88 mmol/L), which suggests that the B_f concentration may have been even higher than measured, because free fatty acids are known to alter bilirubin-albumin binding.[44]

Ritter et al[15] measured B_f in 30 premature infants who were autopsied during the benzyl-alcohol era in which elevated plasma levels of benzoate displaced bilirubin from albumin. Seven of these infants had kernicterus with a mean B_f of 18.2 nmol/L (1.1 μg/dL) and mean TSB of 7.3 mg/dL compared with mean values of B_f and TSB in nonkernicteric infants of 10.9 nmol/L (0.64 μg/dL) and 6.1 mg/dL, respectively. Although most B_fs in the kernicterus group were in a range predicted to place the infant at risk, the differences in B_f were not significant when using the Mann-Whitney U test ($P = .07$); they were significant when using the Student's t test. However, yellow staining was more intense in brains exposed to higher B_f. It was not documented whether these infants received benzyl alcohol, but the high mean B_f relative to mean TSB (expected B_f would be <8 nmol/L) in kernicteric patients is consistent with the presence of strong competitors and/or marked alterations of binding constants. Cashore and Oh[103] reported similar findings in 13 autopsied premature infants. Mean TSB and B_f values in 5 infants with kernicterus were 8.6 mg/dL and 27 nmol/L (1.6 μg/dL), respectively, compared with 8.0 mg/dL and 13 nmol/L (0.76 μg/dL), respectively, in 8 infants without kernicterus ($P < .05$).

In summary, pharmacokinetic principles, in vitro and animal experiments, and limited clinical data in both term and premature infants with clinical evidence of early encephalopathy or frank kernicterus consistently support the propositions that B_f is superior to TSB in predicting risk for bilirubin toxicity and that clinical management might be improved significantly by measuring both variables. Biologically, this is not surprising, because TSB is not the principal determinant of bilirubin entry into brain but rather is a dependent variable, the effect of which is greatly modified by variations in serum binding.

THE CHALLENGE: DEVELOPING A MORE RATIONAL APPROACH TO HYPERBILIRUBINEMIA

The 1994 AAP practice parameter for neonatal jaundice[75] was predicated on available evidence that kernicterus was rare in healthy term infants with elevated

*Refs 47, 49, 50, 58, 60, 102–104, and 106.

TSBs even beyond 30 mg/dL and the assumption that the risk of intervention at TSB levels of <24 to 30 mg/dL may exceed the risk of encephalopathy. Subsequent information supports the contention that kernicterus is a rare event but also reveals that TSB is a poor discriminator of patients at risk.

A resurgence of kernicterus in the past decade[107] has forced a reevaluation of the 1994 practice parameters. The current AAP guidelines,[3] which promote greater parent education and closer predischarge and postdischarge surveillance for jaundice, address the need to prevent infants with hyperbilirubinemia from "falling through the cracks."[4] However, the 2004 guidelines continue to base intervention strategies on TSB even while acknowledging that clinical evidence providing a specific risk threshold is weak.

The challenge in defining national guidelines for the management of patients with hyperbilirubinemia is to minimize both the risk for bilirubin encephalopathy and the need for intervention.[74] It has been estimated that between 1 and 3 per 100 000 otherwise well newborns would develop kernicterus if untreated[108] (35–105 cases per year in the United States with ~3.5 million term deliveries). Approximately 70 000 term newborns (2% of births) will develop a TSB of >20 mg/dL.[109] It is clear that a health care policy that demands intervention at a TSB yielding 100% sensitivity (ie, 20–21 mg/dL) would result in an enormous number of unnecessary interventions. Approximately 5000 infants (0.15% of term infants) will exceed AAP guidelines (TSB: 25 mg/dL) each year and require intervention with aggressive phototherapy or exchange transfusion to prevent 92% of kernicterus cases (32–96 cases; 8% will have a TSB <25 mg/dL).

Available clinical data indicate that B_f or B_f combined with TSB should improve risk assessment for neurotoxicity in both term and premature infants. If B_f rather than TSB were used as an indicator for aggressive interventions,[109] Ahlfors and Wennberg[105] have estimated that the number of interventions, including exchange transfusion with its associated mortality and morbidity,[7,110–112] would be greatly reduced. If verified by clinical trials, guidelines based on B_f could yield significant savings in health care dollars, neonatal morbidity, possibly mortality, and family stress.

Although both the AAP[3] and the National Institute of Child Health and Human Development[113] have identified the need for studies of B_f to improve intervention criteria, the current guidelines based on TSB effectively preclude conducting such studies in patients who are at risk for kernicterus. How then do we proceed? Ahlfors and Wennberg[109] suggested a stepwise approach for clinical evaluation of B_f in infants. First, it is important to obtain population data regarding interindividual variations in binding in different clinical settings. This does not require obtaining serum from jaundiced infants but can be done by titrating cord serum with bilirubin in vitro and measuring B_f at TSB levels thought to be "at risk." A second step would be to evaluate day-to-day intraindividual variation in binding. Unpublished clinical experience using the standard peroxidase method suggests that, in the absence of intervening severe illness, binding parameters change little in a given patient. A systematic assessment of day-to-day variation in the B_f level linked to age-specific changes in the TSB[6] might improve predictability of severe hyperbilirubinemia, because the B_f should be a better indicator of bilirubin load.

Although controlled, randomized trials that test the efficacy of TSB versus B_f in predicting outcome cannot be performed ethically or logistically, it is possible to compare TSB and B_f as predictors of "reversible" toxicity using bilirubin-induced neurologic dysfunction (BIND) scores,[6,59] MRI, or the ABR as dependent variables. The ABR may be the best clinical indicator that we have for evolving bilirubin neurotoxicity[53,58] and can identify patients in need of close follow-up for hearing loss or auditory processing difficulties. Because deafness is a common manifestation of kernicterus, it would seem incumbent on those who dismiss the ABR as a transient bilirubin "effect" to show that an abnormal ABR is independent of progressive neurotoxicity leading to hearing loss or auditory processing difficulties.

The greater challenge continues to be in relating B_f and/or TSB to long-term outcome. Because kernicterus is a rare complication of idiopathic hyperbilirubinemia, outcome studies measuring TSB and using kernicterus as the primary outcome variable have produced inconclusive results even in large collaborative projects. Likewise, long-term outcome studies conducted within large health care maintenance organizations have thus far only determined that the risk for permanent sequelae in healthy term patients with high TSBs is very small.[10,114] It is unlikely that data allowing meaningful sensitivity, specificity, and receiver operating characteristic curve calculations in this population will be forthcoming without a national reporting system for infants with very high TSB (eg, >25 mg/dL) and available laboratories that can measure B_f and relate it to outcome. It may be more feasible to collect such data for premature infants in newborn intensive care settings in which the sporadic occurrence of kernicterus in sick low birth weight infants with low TSB continues to be reported,[115] the effect size of TSB on neurodevelopmental outcome is small,[116] and a national consensus for intervention criteria is (correctly and fortunately) not established.

CONCLUSIONS

TSB is, at best, a poor risk indicator for kernicterus and, at worst, an excuse for not intervening when intervention is necessary. Laboratory and available clinical data are consistent with basic pharmacological principles in demonstrating that B_f rather than TSB (analogous to free

thyroxine and thyroxine) is a critical serum factor involved in brain uptake of bilirubin and subsequent neurotoxicity. To improve guidelines for managing hyperbilirubinemia and minimize the number of unnecessary and at times dangerous therapeutic interventions, there is need for a national strategy to obtain prevalence and incidence kernicterus data and to identify B_f and TSB levels and independent comorbid factors associated with reversible and irreversible bilirubin encephalopathy.

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