The Bilirubin Binding Panel: A Henderson-Hasselbalch Approach to Neonatal Hyperbilirubinemia

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abstract

Poor plasma bilirubin binding increases the risk of bilirubin neurotoxicity in newborns with hyperbilirubinemia. New laboratory tests may soon make it possible to obtain a complete bilirubin binding panel when evaluating these babies. The 3 measured components of the panel are the plasma total bilirubin concentration (B_{Total}), which is currently used to guide clinical care; the bilirubin binding capacity (BBC); and the concentration of non–albumin bound or free bilirubin (B_{Free}). The fourth component is the bilirubin-albumin equilibrium dissociation constant, K_D, which is calculated from B_{Total}, BBC, and B_{Free}. The bilirubin binding panel is comparable to the panel of components used in the Henderson-Hasselbalch approach to acid-base assessment. Bilirubin binding population parameters (not prospective studies to determine whether the new bilirubin binding panel components are better predictors of bilirubin neurotoxicity than B_{Total}) are needed to expedite the clinical use of bilirubin binding. At any B_{Total}, the B_{Free} and the relative risk of bilirubin neurotoxicity increase as the K_D/BBC ratio increases (ie, bilirubin binding worsens). Comparing the K_D/BBC ratio of newborns with B_{Total} of concern with that typical for the population helps determine whether the risk of bilirubin neurotoxicity varies significantly from the inherent risk at that B_{Total}. Furthermore, the bilirubin binding panel individualizes care because it helps to determine how aggressive intervention should be at any B_{Total}, irrespective of whether it is above or below established B_{Total} guidelines. The bilirubin binding panel may reduce anxiety, costs, unnecessary treatment, and the likelihood of undetected bilirubin neurotoxicity.

On the clinical horizon and the impetus for this review is the opportunity to measure plasma bilirubin binding routinely in the clinical management of neonatal hyperbilirubinemia. Although it was demonstrated more than half a century ago that poor bilirubin binding by plasma albumin significantly increases the risk of bilirubin neurotoxicity (kernicterus) in jaundiced newborns,[1–3] methods for the routine clinical laboratory or point-of-care measurement of bilirubin binding have been elusive until recently.[4–6] The purpose of the of this review is to introduce and review these tests after presenting the strong case for their expeditious incorporation into clinical care.[7] Bilirubin binding promises substantial medical, emotional, and financial benefits for jaundiced newborns, their families, and the health care system.[8–10]

The "new" issues in this review are (1) the emerging technologies and (2) laying to rest the long held, well-intentioned but unfortunately

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TABLE 1 A Plasma Bilirubin Panel Consists of B_{Total}, B_{Free}, BBC, and K_D

Component	Description	Relevant Equation	Comments
B_{Total} (mg/dL)	Albumin-bound + Free Bilirubin concentrations	$B_{Total} \pm B_{Free} \cong B_{Total}$	Clinical laboratory test
B_{Free} (μg/dL)	Non–albumin bound (free) bilirubin concentration	$\frac{K_D \cdot B_{Total}}{(BBC - B_{Total})}$	Peroxidase test[18] or fluorescent probe test[4]
BBC (mg/dL)	Concentration of bilirubin binding sites	$BBC = n \cdot 8.8 \cdot Alb_{Total}$	Peroxidase test[18] or front face fluorescence test[5,6]
K_D (μg/dL)	Bilirubin-albumin equilibrium dissociation constant	$\frac{B_{Free} \cdot (BBC - B_{Total})}{B_{Total}}$	Calculated
Alb_{Total}, g/dL	Plasma albumin concentration	$Alb_{Total} = \frac{BBC}{n \cdot 8.8}$	Clinical laboratory test
n	Bilirubin Binding Sites per Albumin molecule	$n = \frac{BBC}{8.8 \cdot Alb_{Total}}$	Calculated

The plasma albumin concentration (AlbTotal) is also included because it is related to the BBC as shown.

misguided notion that before bilirubin binding can be used clinically, prospective studies are required to prove that "the bilirubin binding test" predicts bilirubin neurotoxicity better than the conventional plasma total bilirubin concentration (B_{Total}) currently guiding clinical care.[11–15] The second issue stems from the misconception that the "bilirubin binding tests" and B_{Total} are somehow fundamentally different, which is not the case. The "bilirubin binding tests" are coming to aid, not compete with, their long-overworked and beleaguered colleague, B_{Total}. The "prospective" data needed to use bilirubin binding clinically are the bilirubin binding parameters (normal bilirubin binding values) for the various populations of jaundiced newborns (eg, term, premature, etc).

Rigorous quantification of bilirubin binding requires 3 (not 1) plasma measurements just as 2 measurements, pH and P_aCO_2, are needed to rigorously quantify acid-base balance.[16] B_{Total} is 1 of the measurements, and the other 2 are the bilirubin binding capacity (BBC) and the non–albumin bound or "free" bilirubin concentration (B_{Free}). The BBC is the concentration of plasma bilirubin binding sites and is related to the albumin concentration (Alb_{Total}) by the equation BBC (mg/dL) = $n \cdot 8.8 \cdot Alb_{Total}$, where Alb_{Total} is measured in grams/deciliter and *n* is the number of bilirubin binding sites per albumin molecule. It is often assumed that $n = 1$, but albumin molecules can bind more than one bilirubin molecule,[16] and endogenous ligands or drugs may occupy some of the bilirubin binding sites in plasma samples, effectively reducing the BBC.[1–3,5] When $B_{Total} \geq$ BBC, albumin is "saturated" with bilirubin and the risk of bilirubin neurotoxicity increases substantially.[1–3,7,16]

B_{Total}, BBC, and B_{Free} are used to calculate the fourth and final bilirubin binding component, the bilirubin-albumin equilibrium dissociation constant (K_D). K_D measures how long a bilirubin binding site that has captured a bilirubin molecule can restrain the struggling molecule before it frees itself and escapes back into solution. K_D is akin to the Henderson-Hasselbalch dissociation constant (K_a) that is used to quantify acid-base dissociation. However, K_D, unlike K_a, varies considerably in the newborn population.[17] Therefore, for each jaundiced newborn, both K_D and BBC must be determined to quantify the strength and number of plasma bilirubin binding sites, respectively. B_{Total}, B_{Free}, BBC, and K_D constitute a bilirubin binding panel with the properties and characteristics outlined in Table 1.

It is important to note that the failure to use all 4 bilirubin binding components to quantify bilirubin binding has seriously undermined every study evaluating the role of "bilirubin binding" in the pathogenesis of bilirubin neurotoxicity. A recent example is the failure of the B_{Total}/Alb_{Total} ratio (ie B_{Total}/BBC), billed as a "surrogate" bilirubin binding test, to predict bilirubin neurotoxicity better than B_{Total}.[19–21] The studies use just 2 of the bilirubin binding components to test the misguided hypothesis that the B_{Total}/Alb_{Total} ratio will predict bilirubin neurotoxicity better than B_{Total}.[19–21] Although the ratio is not better than B_{Total} as a general intervention guideline, some current guidelines do recommend lowering the intervention B_{Total} when Alb_{Total} is low in an effort to make the B_{Total} guidelines more consistent with respect to bilirubin binding.[14]

For most clinicians, the rub with bilirubin binding lies not in the concept that poor bilirubin binding increases the risk of bilirubin neurotoxicity but in the considerable confusion and angst as to whether measuring bilirubin binding can ever be a routine, practical, and helpful tool in the nursery.[11,12,22,23] The bad news is that a little algebra is required to show that the bilirubin binding panel can indeed become a routine, practical, and helpful tool in the clinical setting. The good news is that the calculations show beyond a doubt that the risk of bilirubin neurotoxicity at any B_{Total} increases as the K_D/BBC ratio increases, that is, as bilirubin binding worsens.

Clinically, bilirubin binding can provide a far better "feel" about the risk of bilirubin neurotoxicity, for example, in a term infant with a "hazardous" B_{Total} of 35 mg/dL.[24–27] A better "feel" may seem too qualitative and vague for some, but keep in mind that the downside risk of a decision to treat a baby with a B_{Total} of 35 mg/dL with phototherapy alone[24–27] increases as K_D/BBC increases. It is unrealistic to expect the bilirubin binding panel to predict bilirubin neurotoxicity with 100% certainty, but the panel will provide important additional information at the time difficult treatment decisions are made and when those decisions are reviewed retrospectively once the outcome is known.[19,24–27] This may ultimately lead to more robust treatment recommendations based on B_{Total}, K_D/BBC ratios and clinical circumstances.[14,15,24–27]

K_D is the esoteric and often baffling member of the bilirubin binding panel, especially for those unfamiliar with the chemistry concept of mass action and its related equations.[16,17,28] Fortunately the more clinically familiar Henderson-Hasselbalch mass action approach to acid-base balance provides a convenient model for understanding K_D and its relationship to the measured bilirubin binding components. Mass action refers to the reversible chemical equilibria shown below for bicarbonate-carbonic acid and bilirubin-albumin, where $(BBC - B_{Total} + B_{Free})$ and $(B_{Total} - B_{Free})$ are the concentrations of unoccupied bilirubin binding sites and albumin-bound bilirubin, respectively.[7,28]

$$H^+ + HCO_3^- \rightleftarrows H_2CO_3$$

$$\overset{K_a}{\rightleftarrows} CO_2(gas) + H_2O$$

$$B_{Free} + (BBC - B_{Total} + B_{Free})$$

$$\overset{K_D}{\rightleftarrows} (B_{Total} - B_{Free})$$

Fortunately, B_{Free} is so small (μg/dL) compared with B_{Total} (mg/dL) that $B_{Total} \pm B_{Free} \cong B_{Total}$ and the bilirubin-albumin equilibrium can be simplified to:

$$B_{Free} + BBC - B_{Total} \overset{K_D}{\rightleftarrows} B_{Total}$$

The mass action equations derived from the equilibria follow, along with the familiar additional logarithmic derivation known as the Henderson-Hasselbalch equation. Equation 1 is used to calculate K_D from the measured bilirubin binding panel components.[16,17,28]

$$H^+ = \frac{K_a \cdot H_2CO_3}{HCO_3^-}$$

$$= \frac{K_a \cdot 0.03 \cdot P_aCO_2}{HCO_3^-};$$

$$pH = p\,K_a + \log\left(\frac{HCO_3^-}{0.03 \cdot P_aCO_2}\right)$$

$$B_{Free} \cong \frac{K_D \cdot B_{Total}}{BBC - B_{Total}}; K_D$$

$$\cong \frac{B_{Free} \cdot (BBC - B_{Total})}{B_{Total}}$$

At any B_{Total}, B_{Free} increases as K_D increases and/or BBC decreases, ie bilirubin binding worsens. Therefore, the K_D/BBC ratio is a continuous and robust indicator of the quality of plasma bilirubin binding, and, as noted earlier, all the measured variables (B_{Total}, B_{Free}, and BBC) are required to obtain the K_D/BBC ratio.

The Henderson-Hasselbalch approach to bilirubin binding exposes the incredible and unreasonable demands we have placed on B_{Total} for >60 years due to our inability to measure BBC and B_{Free}. Hsia et al[29] were the first to report that the incidence of kernicterus increased as B_{Total} increased in newborns with untreated hemolysis. Since then, countless studies have been undertaken with little success to correlate "maximum" B_{Total} with the risk of bilirubin neurotoxicity.[9,25–27,30,31] In retrospect, trying to distinguish "normal" B_{Total} (eg, "physiologic jaundice") with no risk of bilirubin neurotoxicity from "abnormal" B_{Total} (eg, "hyperbilirubinemia") with increased risk of bilirubin neurotoxicity is akin to having verified that the risk of acidosis is proportional to the P_aCO_2 and then trying to assess the risk of acidosis using only the P_aCO_2. As important and apropos to bilirubin-albumin binding, the Henderson-Hasselbalch equation shows us that the clinical use of both pH and P_aCO_2 to assess acid-base balance requires only the population parameters (normal values) for pH and P_aCO_2, not prospective proof that one predicts acidosis better than the other in a vain attempt to decide which of the 2 to use clinically.

Similarly, the clinical use of bilirubin binding to assess neonatal jaundice requires prospective determination of the bilirubin binding population parameters (normal values), not prospective studies of the relative abilities of the binding components to predict bilirubin neurotoxicity. If BBC and K_D did not vary in the newborn population and their values were known, only B_{Total} would be needed for a bilirubin binding panel (Equation 1) and to guide clinical care. Because this is not the case, bilirubin binding population parameters are required, as is the case for any new clinical laboratory test, to compare test results in an individual patient with the normal or typical values in the population at large.[32,33]

An example of bilirubin binding population parameters is provided in Table 2, which summarizes the statistical variation in the bilirubin binding panel components obtained from 117 jaundiced newborns ≥35 weeks' gestational age. B_{Total} and B_{Free} were measured as part of the routine evaluation for neonatal jaundice in a Clinical Laboratory Improvement Amendments–approved clinical laboratory using an older Food and Drug Administration–approved methodology that is currently used by clinical laboratories in Japan.[34] BBC

TABLE 2 Bilirubin Binding Panel Components in 127 Samples From 117 Newborns With Gestational Ages Ranging From 35 to 42 Weeks (mean 38 Weeks)

	B_{Total} mg/dL	B_{Free} µg/dL	BBC mg/dL	K_D µg/dL	K_D/BBC µg/mg
Median	19.4	1.01	33.6	0.644	0.019
25th percentile	16.5	0.60	30.9	0.486	0.014
75th percentile	22.8	1.55	36.2	0.913	0.027
Range	6.8–36.0	0.050–7.63	19.4–44.3	0.047–2.56	0.002–0.076

BBC was calculated from Alb_{Total} assuming $n = 1$ (see Table 1).

was calculated from Alb_{Total} assuming $n = 1$ for illustrative purposes as BBC measurements per se were not available at the time. The considerable variation in both K_D and BBC (ie, Alb_{Total}) shown in Table 2 indicates that a bilirubin binding panel at a B_{Total} of concern will add important additional information about the inherent risk of bilirubin neurotoxicity.

An overview of neonatal jaundice from the perspective of bilirubin binding focuses on how binding influences the distribution of the accumulating bilirubin between the blood (safe bilirubin) and the tissues, that is, brain (dangerous bilirubin).[7,35–37] If all the accumulating bilirubin were retained in the blood, bilirubin neurotoxicity could not occur, irrespective of the magnitude of B_{Total}. B_{Total} measures the "safe" bilirubin retained in the blood and B_{Free} determines how rapidly the "dangerous" bilirubin is accumulating extravascularly where it can potentially lead to bilirubin neurotoxicity.[7,37] B_{Free} is often misconstrued as the "toxic" bilirubin level, but it is more correctly viewed as the force driving bilirubin into the extravascular compartments (eg, brain, skin, etc). Therefore, the total accumulated bilirubin at any time will be the sum of the increase in B_{Total} (ΔB_{Total}) and the amount of bilirubin that has left the blood stream during that time. The latter can be quantified as $k_e \cdot \Delta B_{Free}$ as shown in Equation 2, where k_e is the rate constant governing how rapidly B_{Free} can leave the blood stream and ΔB_{Free} is the increase in B_{Free}.

$$\text{Total Accumulated Bilirubin} = \Delta B_{Total} + k_e \cdot \Delta B_{Free}$$

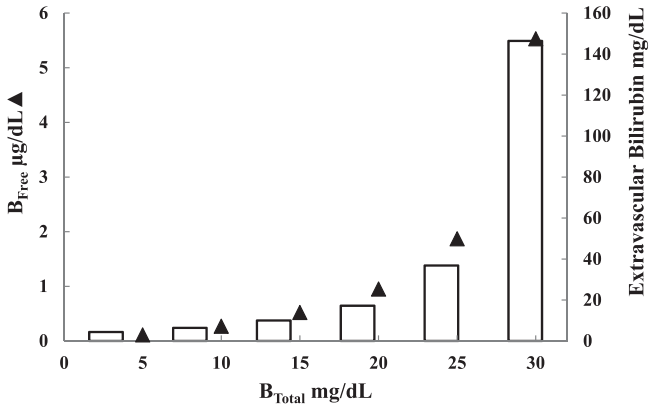

FIGURE 1
The change in B_{Free} (▲) and extravascular bilirubin (bars) as B_{Total} increases in 5-mg/dL increments. B_{Free} was calculated by using Equation 1 and the median K_D and BBC in Table 2. The extravascular bilirubin was calculated as 40 · ΔB_{Free} where ΔB_{Free} is the change in B_{Free} in each 5-mg/dL B_{Total} interval and assuming k_e = 40 mg/µg (see Equation 2). Both B_{Free} and the extravascular bilirubin increase exponentially as B_{Total} increases linearly.

For example, if B_{Total} increases from 1 mg/dL to 5 mg/dL in 24 hours (ΔB_{Total} = 4 mg/dL) and 4 mg/dL has accumulated in the extravascular space during that time ($k_e \cdot \Delta B_{Free}$ = 4 mg/dL), then the total accumulated bilirubin 8 mg/dL.[35,36] An important but underappreciated consequence of Equations 1 and 2 is that as B_{Total} increases linearly, both B_{Free} and the extravascular bilirubin increase exponentially as illustrated in Fig 1.[7,16]

In the current clinical setting where only B_{Total} is available, tools such as the Bhutani-Johnson hour-specific B_{Total} nomogram (Fig 2) are used to determine the likelihood that bilirubin accumulation and the risks of "hyperbilirubinemia" and bilirubin neurotoxicity are increased.[38,39] However, Equation 2 shows that the total bilirubin accumulation rate is also proportional to B_{Free}, which means that the bilirubin accumulation rate needed to achieve any hour-specific B_{Total} increases as B_{Free} increases. Because B_{Free} increases as the K_D/BBC ratio increases (Equation 1), the likelihood of an elevated bilirubin accumulation rate at any hour-specific B_{Total} will increase as the K_DBBC ratio increases, as illustrated by the vertical dashed lines in Fig 2 (see Appendix for more details).

A similar argument applies when B_{Total} reaches or exceeds current B_{Total} intervention guidelines.[14,15] However, in this case, the primary clinical concern is the inherent risk of bilirubin neurotoxicity rather than excessive bilirubin accumulation. In term newborns, for example, the risk of bilirubin neurotoxicity at "hazardous" B_{Total} ≥30 mg/dL is probably somewhere between 10% and 50% depending on the clinical circumstances.[19,25–27,29] Equation 2 shows that the extravascular accumulation of bilirubin and therefore the inherent risk of bilirubin neurotoxicity at B_{Total} ≥30 mg/dL will increase as

K_D/BBC (B_{Free}) increases. If the "average" or inherent risk of bilirubin neurotoxicity occurs at the median K_D/BBC ratio for the population (Table 2), the K_D/BBC ratio of a newborn with a B_{Total} ≥30 mg/dL can be compared with the median K_D/BBC to determine whether the risk of bilirubin neurotoxicity (ie, the accumulated bilirubin needed to achieve that B_{Total}) is significantly different from the "average" risk in the population at large. In addition, the actual B_{Total} at which the "average" risk of bilirubin neurotoxicity would occur in a newborn can be calculated by using Equation 1, individualizing B_{Total} intervention guidelines and helping to identify those few newborns that may be at increased risk for bilirubin neurotoxicity at B_{Total} below current intervention guidelines[25–27] (see Appendix for more details).

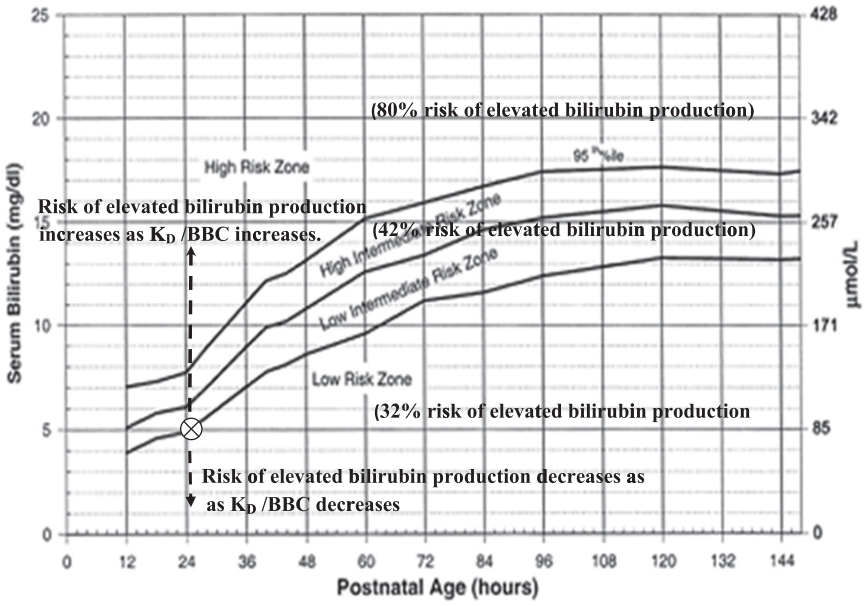

FIGURE 2

A 24-hour B_{Total} of 5 mg/dL plotted on the Bhutani-Johnson nomogram.[38] The risks of an elevated bilirubin production rate at B_{Total} in the low, intermediate, or high zone are 32%, 42%, and 80%, respectively.[39] The B_{Total} of 5 mg/dL falls between the low and intermediate risk zones, but bilirubin binding (K_D/BBC) further alters the risk as indicated by the dashed arrows (see Appendix for more details).

Bilirubin binding population parameters will vary depending on the newborn population under consideration. For example, the population of jaundiced premature newborns has an inherent increased risk of bilirubin neurotoxicity, increased bilirubin production when ventilated, and (perhaps) more complications from phototherapy than term newborns.[40–42] Table 3 provides an example of bilirubin binding population data for 48 premature newborns weighing ≤1 kg at birth. This population clearly does not bind bilirubin as well as term newborns,[43] and exchange transfusion in this group has been recommended at B_{Total} anywhere from 10 to 14 mg/dL.[15,44] As with term newborns, bilirubin binding population parameters can be used to establish "standard" risks of bilirubin neurotoxicity and to individualize intervention B_{Total}[45,46] (see Appendix for more details).

The bilirubin binding panel creates an analog expansion of the current "one size fits all" B_{Total} guidelines that are arbitrarily digitalized according to clinical status, birth weight, or gestational age.[14,15] It also increases the vascular bilirubin variables available for studies attempting to link adverse outcomes with "brain bilirubin exposure."[25–27,47] As with any clinical laboratory test, the bilirubin binding panel must be interpreted in the context of the clinical circumstances. Poor bilirubin binding will have little clinical impact if bilirubin accumulation is minimal,[2] and excellent bilirubin binding may not prevent kernicterus when excessive bilirubin accumulation is present.[29]

Although several methods for measuring the various bilirubin binding components have been published over the years,[18,48–56] bilirubin binding has never been packaged as a panel, as outlined in this review. The most widely used "bilirubin binding test" is the peroxidase test for measuring B_{Total} and B_{Free} as first described by Jacobsen and Wennberg in 1974.[53] A BBC can also be obtained using this test if B_{Free} is measured at 2 B_{Total}, which provides 2 equations that can be solved for the 2 unknowns K_D and BBC (Equation 1).[43,53]

TABLE 3 Bilirubin Binding Panel Components in 64 Samples From 48 Premature Newborns Weighing ≤1 kg at Birth and With Gestational Ages Ranging From 23 to 31 Weeks (Mean 26 Weeks)

	B_{Total} mg/dL	B_{Free} µg/dL	BBC mg/dL	K_D µg/dL	K_D/BBC µg/mg
Median	6.0	0.36	24.7	1.09	0.050
25th percentile	4.4	0.22	22.7	0.695	0.030
75th percentile	7.5	0.62	28.5	1.70	0.069
Range	2.8–15.2	0.05–2.46	12.4–34.5	0.338–4.92	0.012–0.169

BBC was calculated from Alb_{Total} assuming n = 1 (see Table 1).

The peroxidase test, with a few modifications, has been used for many clinical and laboratory research studies.[13,16.17,45,46,57–68] It has also been used for many years in the routine clinical management of newborn jaundice in Japan, with good results.[45,59,66,67] Several studies using this test show that the magnitude of B_{Free} (ie, poor bilirubin binding) is indeed more closely associated with or predictive of bilirubin neurotoxicity than the magnitude of B_{Total}.[13,46,58,60–63,69] These studies support the principle that the accumulated bilirubin and risk of bilirubin neurotoxicity at a given B_{Total} increase as bilirubin binding worsens (Equation 2) but as discussed earlier, are not evidence that B_{Free} rather than B_{Total} should guide jaundice management.[22] The test has not been automated for routine clinical laboratory use in the United States and is not well suited for point-of-care testing.

Two emerging tests that measure BBC[5,6] and B_{Free}4 overcome the practical limitations of the peroxidase test. Although these newer tests are well suited for point-of-care use, both will require additional clinical tests to obtain a bilirubin binding panel. The BBC test is based on the ability of bilirubin to quench (reduce) albumin fluorescence as it binds.[5,6] The fluorescence before and after adding an excess of bilirubin is used to obtain B_{Total} and the BBC, respectively. B_{Free} must be measured by another method, but the BBC alone can be helpful. For example, the concern about a 24 hour-specific B_{Total} of 5 mg/dL (Fig 2) may be greater if the if BBC is 25 versus 34 mg/dL. The test measures BBC in seconds using a drop of whole blood for which the HCT has been determined. This makes it ideal for point-of-care use in either intensive care or well-infant nurseries. Although the BBC is less likely to be affected by pH and temperature as compared with B_{Free},[34,64] the effects of these factors have not been assessed. There has been relatively little clinical experience or research with the original version of this test,[5] and the variation between the BBC and Alb_{Total} has not been established. Studies are also needed to determine how test performs when ligands such as sulfisoxazole or benzoate that also bind at bilirubin binding sites are present.[46,58]

The newly described B_{Free} test uses a probe whose fluorescence is quenched when it binds bilirubin.[4] In plasma, only B_{Free} binds with the probe and can therefore be quantified from the change in probe fluorescence. B_{Free} measured by this method compares favorably with B_{Free} determined by the peroxidase test,[4] and the test has been used in 1 clinical study.[69] Both B_{Total} and the BBC are still needed to complete the bilirubin binding panel, and studies are also needed to determine how B_{Free} is affected by the presence of other ligands.[46,58] Ideally, both of the new tests and a standard clinical laboratory or transcutaneous B_{Total} would be readily available for the assessment of a jaundiced newborn.

The bilirubin binding panel provides the long missing companions of B_{Total} that are needed to maximize the information available from vascular bilirubin metrics when evaluating neonatal hyperbilirubinemia. Integrating the panel with other tests for assessing the likelihood of bilirubin neurotoxicity, such as end-tidal carbon monoxide,[39,41] automated auditory brainstem response MRI,[70] and G6PD screening,[71,72] will provide even more clinical guidance, promising to reduce the cost of jaundice management and the incidence of bilirubin neurotoxicity by greatly improving our ability to identify those jaundiced newborns truly needing treatment.[47,73]

In summary, neonatal hyperbilirubinemia is a normal occurrence that on rare occasions causes bilirubin neurotoxicity. The emerging tests that can make the bilirubin binding panel a clinical reality shift the clinical focus from the magnitude of B_{Total} (hyperbilirubinemia) to the magnitude of the extravascular bilirubin accumulation and the risk of bilirubin neurotoxicity at any B_{Total}. The underlying principle governing clinical use of bilirubin binding is that the extravascular (brain) bilirubin level and the corresponding risk of bilirubin neurotoxicity at any B_{Total} increase as the K_D/BBC ratio increases. Prospective determination of population bilirubin binding parameters, not prospective studies comparing the relative abilities of bilirubin binding components to predict bilirubin neurotoxicity, are required to use the bilirubin binding panel clinically. The panel improves and individualizes the current laboratory tools used to guide the clinical management of neonatal hyperbilirubinemia by further delineating the risk of bilirubin neurotoxicity at any B_{Total}.

APPENDIX

Bilirubin Binding and the Hour-Specific B_{Total}

Consider an accumulated bilirubin of 8 mg/dL at 24 hours of age in a newborn with a B_{Total} of 1 mg/dL at birth. If the 24-hour specific B_{Total} is 5 mg/dL (Fig 2), 4 mg/dL of bilirubin has left the vascular space and $k_e \cdot \Delta B_{Free}$ = 4 mg/dL (Equation 2). At the median K_D (0.644 μg/dL) and BBC (33.6 mg/dL) from Table 2, B_{Free} calculated from Equation 1 increases from 0.02 μg/dL at birth to 0.12 μg/dL at 24 hr, and ΔB_{Free} = 0.10 μg/dL and k_e = 40 mg/μg (Equation 2). However at the 75th and 25th percentiles of K_D (0.913 μg/dL) and BBC (30.9 mg/dL), respectively, ΔB_{Free} = 0.14 μg/dL and $k_e \cdot \Delta B_{Free} = 40 \times 0.15 = 6$ mg/dL, requiring a 25% higher accumulated bilirubin of 10 mg/dL to achieve the 24-hour B_{Total} of 5 mg/dL. Similarly, at the 25th and 75th

percentiles of K_D (0.486 μg/dL) and BBC (36.2 mg/dL), respectively, ΔB_{Free} = 0.064μg/dL and $k_e \cdot \Delta B_{Free}$ = 40 × 0.065 = 2.6 mg/dL, requiring a 17.5% lower accumulated bilirubin of 6.6 mg/dL to achieve the 24-hr B_{Total} of 5 mg/dL. In general the accumulated bilirubin needed to achieve any hr-specific B_{Total} increases as the K_D/BBC ratio increases as indicated by the dashed lines in Figure 2.

Bilirubin Binding and Guideline Intervention B_{Total}, Term Newborns

A B_{Total} ≥30 mg/dL is considered "hazardous" in the term newborn population,[25–27] and B_{Free} calculated using Equation 1 at the B_{Total} of 30 mg/dL and the median population K_D and BBC from Table 2 is 5.37 μg/dL. Because the volume of distribution for bilirubin varies with birth weight,[74] B_{Free} is about 1.5 μg/dL per kg at the average term newborn birth weight of 3.5 kg (5.37 μg/dL ÷ 3.5 kg). Measuring the bilirubin binding panel makes it possible to calculate the B_{Total} at which the "standard" risk B_{Free} of 1.5 μg/dL per kg will occur using the following rearrangement of Equation 1:

$$\text{"Standard" Risk } B_{Total} = \frac{BBC(1.5 \cdot weight,\text{kg})}{K_D + (1.5 \cdot weight,\text{kg})}$$

For example, an otherwise well 3-kg term newborn with a B_{Total} of 20 mg/dL, a K_D of 0.91 μg/dL, and a BBC of 24 mg/dL would have a B_{Free} of 1.5 μg/dL per kg and all else being equal would be at about the same inherent risk of bilirubin neurotoxicity as a 3.5 kg newborn with a B_{Total} of 30 mg/dL and the median K_D and BBC.[14,17]

Bilirubin Binding and Guideline Intervention B_{Total}, Premature Newborns:

In premature newborns, exchange transfusion has been recommended at B_{Total} anywhere from 10 to 14 mg/dL.[15,45] B_{Free} calculated using Equation 1 at these B_{Total} and the median K_D and BBC from Table 3 ranges from 0.74 to 1.43 μg/dL, respectively (Equation 1). An individualized "standard" risk B_{Total} for this group could be established in the manner described for term newborns, for example, the B_{Total} at which the B_{Free} reaches 1.0 μg/dL per kg.[45,46]

ABBREVIATIONS

Alb_{Total}: plasma concentration of albumin
BBC: plasma bilirubin binding capacity
B_{Free}: non–albumin bound (free) bilirubin concentration
B_{Total}: plasma total bilirubin concentration
K_a: Henderson-Hasselbalch acid-base equilibrium dissociation constant
k_e: rate constant for free bilirubin leaving the blood
K_D: bilirubin-albumin equilibrium dissociation constant
n: number of bilirubin binding sites per albumin molecule

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