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The enigma of low bilirubin kernicterus in premature infants: Why does it still occur, and is it preventable?

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ABSTRACT

Low bilirubin kernicterus in preterm neonates, though rare, remains an unpredictable and refractory form of brain injury. Hypoalbuminemia, co-morbid CNS insult(s), infection, and inflammation are contributing causes that, in many cases, appear to interact in potentiating bilirubin neurotoxicity. Despite compulsive attention to serum bilirubin levels, and clinical and laboratory indices of neurotoxicity risk, low bilirubin kernicterus continues to be seen in contemporary NICUs. While efforts to refine and improve current treatment guidelines are certainly needed, such revision(s) will also have to take into account the risks and benefits of any intervention, including phototherapy.

Introduction

Chronic bilirubin encephalopathy, including kernicterus at postmortem, is currently a rare event in premature neonates.[1] However, in past decades, autopsy-proven kernicterus was often reported in sick, very low-birth-weight infants who had only modestly elevated total serum bilirubin (TSB) levels,[2,3] a condition termed low bilirubin kernicterus. Although not entirely clear, improvements in neonatal intensive care and the discontinuation of benzyl alcohol as a bacteriostatic agent in intravenous fluids and medications have been implicated in this marked reduction.[3–5]

However, recent case series of preterm neonates demonstrate that low bilirubin kernicterus has not completely disappeared,[6–12] its occurrence is enigmatic and unpredictable. In a study from the Netherlands, 5 sick, preterm infants (25–29 weeks' gestation) with peak TSB levels ranging from 8.7 to 11.9 mg/dL (148–204 μmol/L) developed the classical magnetic resonance imaging (MRI) findings of kernicterus.[6] Serum albumin levels in these infants were strikingly low, ranging from 1.4 to 2.1 g/dL.[6] Two other extremely low-birth-weight (ELBW) neonates with complicated neonatal courses, co-morbid CNS injury, and peak TSB levels of 7.5 mg/dL (128 μmol/L) and 9.9 mg/dL (168 μmol/L) developed neurologic sequelae and MRI findings consistent with chronic bilirubin encephalopathy.[7] Choreoathetosis and the classical MRI findings of kernicterus at follow-up were documented in 2 additional preterm infants of 31 and 34 weeks' gestation.[8] The mother of one showed clinical signs of chorioamnionitis but neither infant was acutely ill in the newborn period and their peak TSB levels were 13.1 mg/dL (224 μmol/L) and 14.7 mg/dL (251 μmol/L).[8]

What is it about these infants and their clinical courses that led to bilirubin-induced CNS injury at TSB levels

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conventionally felt to be non-neurotoxic? Clearly, some condition potentiated bilirubin neurotoxicity and/or they were innately vulnerable. Do low albumin levels, limited and/or impaired albumin–bilirubin binding, co-morbid CNS injuries, or other factors account for such enhanced bilirubin neurotoxicity? Can cases of low bilirubin kernicterus be anticipated and prevented? Our goal is to reflect critically on the clinical antecedents including co-morbid conditions that attend this rare but refractory form of bilirubin-induced brain injury in an attempt to better understand its pathogenesis. For the purposes of this review, low bilirubin kernicterus is defined as the occurrence of kernicterus at TSB levels below commonly recommended exchange transfusion thresholds.[6] Our comments focus on features of more recently reported cases.

Pathogenesis of low bilirubin kernicterus

Limited progress has been made in understanding the pathogenesis of low bilirubin kernicterus. A summary of current reports (Table 1) identifies several conditions that are frequently, albeit not invariably, observed. These include (i) hypoalbuminemia (<2.5 g/dL), (ii) the co-morbid CNS findings of intraventricular hemorrhage (IVH) and periventricular white matter injury (WMI), and (iii) infection/inflammation.[6–12] Each will be considered individually, although they often are seen together and may act synergistically to potentiate bilirubin neurotoxicity.

Hypoalbuminemia

Bilirubin is transported within the vascular space tightly but reversibly bound to serum albumin. Albumin-bound bilirubin is measured as TSB, whereas the small circulating fraction not bound to albumin or other serum proteins is the unbound or free circulating fraction. Unbound circulating bilirubin is in dynamic equilibrium with the extravascular tissues, including the CNS, and provides a measure of the relative amount of bilirubin that will exit the vascular space at a given level of TSB, albumin concentration, and albumin–bilirubin binding constant (s).[13,14] Two-thirds of total body albumin is in the extravascular space,[15,16] and considerable amounts of exchangeable bilirubin is present in the liver and the intestinal mucosa.[16] There is limited data on the flux of bilirubin across these pools in newborns, although unbound bilirubin is believed to be readily diffusible between them.[13,14,16] Notably, although unbound bilirubin has biologic effects in the brain, the unbound circulating bilirubin level alone does not dictate the risk of bilirubin encephalopathy. Bilirubin-induced neurotoxicity depends on the total amount of exchangeable bilirubin available (the miscible pool of bilirubin)[17] and a complex interaction between the level and duration of CNS bilirubin exposure and the innate cellular characteristics of the developing CNS that may predispose or protect against bilirubin-induced brain damage.[18,19]

Nevertheless, low serum albumin concentrations (1.4–2.1 g/dL) are frequently noted in cases of low bilirubin kernicterus and predict a reduced circulating bilirubin-binding capacity.[6] Serum albumin concentration decreases with illness and lower gestational age[17] and can vary greatly over time in a given neonate as illustrated by the ~20% coefficient of variation in serum albumin concentration when measured at birth and the onset of jaundice or of acute bilirubin encephalopathy observed in some cases of low bilirubin kernicterus.[6,9] But how uncommon are low serum albumin levels in preterm neonates?

Normative data on serum albumin levels in preterm infants are few in number, limited in scope, and therefore difficult to define. Nevertheless, mean serum albumin levels reported for the preterm infant below 30 weeks' gestation are approximately 1.9 g/dL (90% CI: 1.2–2.8 g/dL) and do not approach 2.5 g/dL until 36–37 weeks' gestation.[20,21] These data indicate a substantial overlap between that commonly seen in preterm neonates and those with low bilirubin kernicterus of identical gestational age (Fig. 1). This overlap demonstrates that other factors, in addition to hypoalbuminemia, must play a role in enhancing the neurotoxicity risk of bilirubin.

Limited innate albumin–bilirubin binding and clinical conditions that impair albumin–bilirubin-binding capacity are

Table 1 – Frequency of adverse conditions in recent (2001–2013) reported cases of low bilirubin kernicterus.[a]

References	Albumin <2.5 g/dL	Co-morbid CNS injury[b]	Infection/ inflammation	One risk factor[c]	Two or more risk factors	GA
Govaert et al.[6]	5/5	4/5	2/5	5/5	4/5	$25^{4/7}$–$29^{0/7}$
Odutolu and Emmerson[9]	1/1	0/1	1/1	1/1	1/1	$36^{6/7}$
Moll et al.[7]	N/A	2/2	1/2	2/2	1/2	$24^{0/7}$–$26^{0/7}$
Okumura et al.[10d]	N/A	1/5	N/A	1/5	0/5	$25^{0/7}$–$34^{0/7}$
Gkoltsiou et al.[11e]	N/A	3/3	3/3	3/3	3/3	$27^{0/7}$–$34^{0/7}$
Sugama et al.[8]	N/A	1/2	1/2	2/2	1/2	$31^{0/7}$–$34^{0/7}$
Kamel et al.[12]	1/2	0/2	N/A	1/2	0/2	$24^{6/7}$–$27^{0/7}$
Adverse condition per cases	7/8	11/20	8/13	16/20	10/20	

N/A—not available.
[a] Numbers are likely an underestimate, as conditions were not systematically screened across all the studies.
[b] PVL, IVH (grade II, III, and/or IV), and hydrocephalus *ex vacuo*.
[c] Presence of either hypoalbuminemia, one of the co-morbid CNS injuries, or infection/inflammation.
[d] Only 5 of 7 reported cases of kernicterus met the definition of low bilirubin kernicterus.
[e] Only 3 infants in reported cases of kernicterus met the definition of low bilirubin kernicterus.

2 such factors. The albumin–bilirubin binding constant varies significantly between newborns[17,22] and as a function of postnatal age.[23,24] Bilirubin-binding capacity is reduced in sick unstable infants[25–27] and is adversely affected by the presence of competing compounds[23,28–30] including unbound free fatty acids.[31,32] Notably, the seminal reports of low bilirubin kernicterus were in premature babies treated with sulfisoxazole,[33,34] a drug that displaces bilirubin from albumin, acutely lowering the TSB, while elevating levels of the unbound bilirubin that may then enter the CNS. Indeed, this bilirubin-displacing effect of sulfonamides is often used to induce acute bilirubin encephalopathy in a controlled fashion in the Gunn rat model of kernicterus.[35] Notably, a recent preliminary study suggests that unbound free fatty acid levels of oleate and linoleate may on occasions become markedly elevated in ELBW infants,[31] displace bilirubin from albumin as effectively as sulfisoxazole,[36] and lead to unbound bilirubin concentrations in the putative neurotoxic range (>75 nM) despite low TSB (<5.9 mg/dL).[31] These reports illustrate the importance of albumin–bilirubin binding characteristics in enhancing or limiting bilirubin neurotoxicity and suggest that measurement of unbound bilirubin and bilirubin-binding capacity may provide greater sensitivity and specificity than TSB for bilirubin-induced neurotoxicity.[32] They also suggest that in circumstances where bilirubin displacement is robust, even albumin concentrations of >2.5 g/dL in the preterm neonate may not protect against bilirubin encephalopathy.

Co-morbid CNS injury: A potential key to understanding low bilirubin kernicterus?

Co-morbid conditions, i.e., those that occur at the same time but independent of each other can also potentiate bilirubin-induced CNS injury. Evidence of major collateral CNS damage is common in low bilirubin kernicterus and most often includes periventricular WMI and IVH (Table 1).[6–8,10,11] The presence of such injuries in regions of the brain not typically affected by bilirubin (e.g., caudate, putamen, thalamus, germinal matrix, cerebral cortex, and periventricular white matter)[2,37–39] strongly suggests that additional pathogenic factors independent of bilirubin both precipitate the co-morbid CNS lesion and potentiate bilirubin neurotoxicity. Low bilirubin kernicterus in such cases represents a multi-hit phenomenon. Shapiro[40] alludes to such in his chronic bilirubin encephalopathy sub-categorization of "kernicterus plus syndrome."[41] These co-morbid CNS lesions merit comment in this regard, including an exploration of their pathogenesis in the hope of identifying a common pathway(s) to injury.

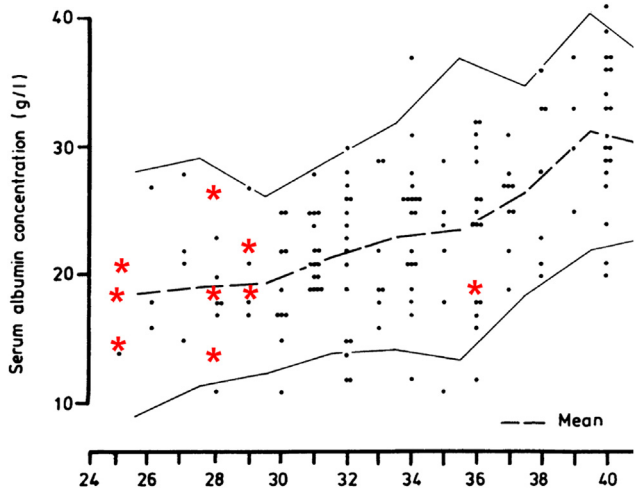

Fig. 1 – Serum albumin concentrations (g/L) from cases of low bilirubin kernicterus (large red asterisks)[6,9] plotted as a function of normative gestational age-specific albumin levels (small dark points) during the early neonatal period in infants of 25–40 weeks' gestation.[20] The normative data, mean, and 90% probability limits are shown with permission from BMJ Publishing Group Limited (Cartlidge and Rutter[20]).

Periventricular WMI

Periventricular WMI manifest by periventricular leukomalacia (PVL) is frequently reported in current cases of kernicterus in preterm neonates[7,11]; echoing reports from past decades.[2,42–44] When diffuse in nature, periventricular WMI leads to significant white matter volume loss demonstrated in neuroimaging studies by ventriculomegaly coupled with increased extra-axial volume and often thinning of the corpus callosum. Hydrocephalus *ex vacuo*, as such, may not always be highlighted as a notable feature in cases of low bilirubin kernicterus (Fig. 2) despite its overt appearance.[7]

Our understanding of the relationship between periventricular WMI and kernicterus continues to evolve. Previously, it was believed that bilirubin was primarily toxic to neurons and not the glial elements that predominate in the periventricular white matter. However, recent *in vitro* studies clearly demonstrate that glial elements including oligodendrocyte precursor cells are vulnerable to bilirubin cytotoxicity,[45,46] albeit less so than neurons. In addition, unconjugated bilirubin limits oligodendrocyte differentiation and impairs axonal myelination *in vitro*,[46] and demyelination and axonal loss are reported in the *cerebellum* of preterm infants with kernicterus.[47] As a result, some speculate that hazardous bilirubin levels are causally linked to *periventricular* WMI.[45,46]

However, an extensive literature on the comparative neuropathology of CNS injury in preterm neonates demonstrates distinctive bilirubin-induced cytopathology and topography that differ from other brain insults[2,38] and notably by an absence of *periventricular* white matter involvement.[2,38,39,48] The specific microscopic cellular alterations classically observed in kernicterus are not seen in *periventricular* tissue. When yellow staining of necrotic periventricular white matter is observed, it is considered secondary and the necrosis therein not bilirubin induced.[2,37,38,49] Moreover, periventricular white matter injury has not been reported in the Gunn rat[2,38] or more recently described mouse models of kernicterus,[50,51] consistent with the low bilirubin contents observed in Gunn rat pup cerebral hemispheres.[52] This absence is notable given the widespread use of mice and rats to study PVL.[53,54] Indeed, early postnatal murine CNS development (postnatal day 3–12), including oligodendroglia maturation and myelination, mirrors that of humans between 23 and 36

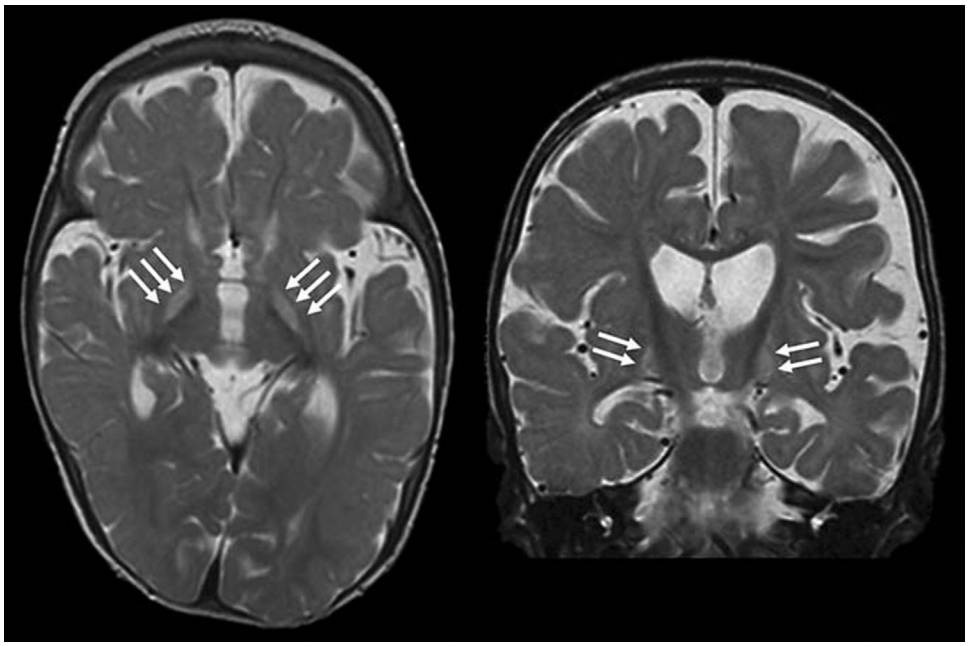

Fig. 2 – T2-weighted MRI of a 4-month-old corrected-age former $26^{0/7}$ week gestation preterm infant with low bilirubin kernicterus[7] demonstrating marked white matter volume loss as indexed by increased extra-axial fluid, ventriculomegaly (hydrocephalus *ex vacuo*), and thinning of the corpus callosum. The infant also shows increased signal intensity in the globus pallidus (arrows) consistent with chronic bilirubin encephalopathy.[7] The infant was 860 g at birth, had a peak TSB of 9.9 mg/dL on day of life 8, history of anemia, left heart failure, and grade II intraventricular hemorrhage and on developmental follow-up showed dyskinetic cerebral palsy, marked psychomotor retardation, and bilateral sensorineural hearing loss.[7] (Reprinted with permission from Moll et al.[7] Copyright© 2011 Karger Publishers, Basel, Switzerland.)

weeks' gestation,[53–55] making them suitable models to study white matter injury in preterm neonates. These observations do not preclude bilirubin-induced oligodendrocyte injury and impaired axonal myelination *in vivo* but suggest that such lesions will be restricted to brain regions classically affected in kernicterus. This appears to be the case.[38,47,56] Dysmyelination and degeneration in the globus pallidus, subthalamic nucleus, and cerebellum are neuropathologic findings of kernicterus in human neonates,[38,47] albeit characteristically late (>10 days) in onset.[38] Taken together, these data offer compelling evidence that *periventricular* white matter injury in kernicteric preterm neonates is related to co-morbid factor(s) and not bilirubin neurotoxicity.

IVH (grade II, III, and IV)

IVH is also reported in association with low bilirubin kernicterus albeit less frequently than periventricular WMI. Similar to perinventricular WMI, the neuropathology of IVH does not overlap with that of bilirubin-induced CNS injury and suggests instead that they share a common antecedent trigger.

Clinical antecedents to PWMI, IVH, and low bilirubin kernicterus

The major etiologies of periventricular WMI and IVH are hypoxia/ischemia and infection/inflammation. The former may manifest as asphyxia, a widely recognized neurotoxicity risk factor for kernicterus[57,58] and prerequisite co-morbid insult for bilirubin-induced brain injury in some animal models, including primates.[59] The neuropathology of hypoxic–ischemic encephalopathy (HIE) is different from that of kernicterus; these differences in topography and histopathology are well defined (Table 2).[2] The mechanism(s) by which HIE potentiates bilirubin-induced CNS injury is likely multifactorial and related at least in part to disturbed bioenergetics and acidosis.

Infection/inflammation

Perinatal and early postnatal infection/inflammation is increasingly recognized as an important contributor to brain structural and functional abnormalities later in life. Systemic inflammation may be sustained in nature[60] and its persistence associated with poorer neurodevelopmental outcomes.[61–63] Antecedents of perinatal and early postnatal inflammation include (i) microorganisms in the placenta, (ii) placental (chorioamnionitis) and umbilical (funisitis) inflammation, (iii) early bacteremia (sepsis), and (iv) intestinal injury including necrotizing enterocolitis.[64–67] Maternal infections remote from the genital tract, including periodontal disease, have also been implicated[68] as have severe fetal growth restriction and maternal obesity during pregnancy (BMI > 30 kg/m^2).[69–73] The association of these infectious/inflammatory conditions with low bilirubin kernicterus merits intensive investigation particularly in light of recent reports that hazardous bilirubin exposure itself can

Table 2 – Comparative neuropathology of kernicterus and hypoxic–ischemic encephalopathy in the preterm neonate.[2]

Topography of lesions	Kernicterus	HIE
Cerebral cortex	Absent	Present
Periventricular WM	Absent	Present
Corpus striatum	Globus pallidi	Putamen and caudate nuclei
Thalamus	Subthalamus	Anterior and lateral nuclei
Ammon's horn	Resistant sector (H_{2-3})	Sommer's sector (H_1)
Pons	Locus ceruleus	Basal pontine nuclei
Medulla	Vestibular and cochlear nuclei	Inferior olivary nuclei Superior olivary nuclei

Only topographic areas considered helpful for differential diagnosis are shown in this table. (Adapted with permission from Ahdab and Moosy.[2])

promulgate pro-inflammatory responses *in vitro*[74] and neuroinflammation *in vivo*.[51,75–77]

Chorioamnionitis

A notable contributor to the pathogenesis of both periventricular WMI and IVH, also reported in association with low bilirubin kernicterus, is histopathologic chorioamnionitis. Preterm birth is frequently associated with intrauterine inflammation of the chorioamniotic membranes[78] and antenatal inflammation linked to adverse CNS neonatal outcomes.[79] This is particularly the case when funisitis, the hallmark of fetal involvement and resultant fetal inflammatory response syndrome (FIRS), is evident.[79–81] FIRS involves systemic fetal inflammation that can be detected by perinatal elevations in plasma interleukin (IL) 6, IL-1 beta, and TNF alpha concentration.[65–67]

FIRS can lead to CNS injury via direct cytokine effects, via its potential adverse impact on hemodynamic status, and/or by potentiating injury from other perinatal insults including hypoxia–ischemia, sepsis, and IVH.[79,82] More specifically, infection/inflammation can predispose or sensitize the CNS to a second stress, i.e., make the brain more vulnerable to injury. For example, low-dose lipopolysaccharide that does not induce CNS damage itself has been shown to make the murine CNS exquisitely susceptible to a mild hypoxic–ischemic insult that would not itself induce brain damage.[61,83] We speculate that FIRS in an analogous fashion can lead to bilirubin-induced CNS damage at TSB levels not generally thought to pose a neurotoxic risk.

Surprisingly, the relationship between FIRS and bilirubin-induced CNS injury has not been extensively studied. More than 3 decades ago, Naeye[84] reported that histologic evidence of amniotic fluid infection at birth (infiltration of the placental subchorionic plate by neutrophils) potentiated the neurotoxicity of neonatal hyperbilirubinemia. This conclusion was based on the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke subjects who had placentas available for examination and neurodevelopmental follow-up, including motor and neurosensory data.[84] The combined effects of amniotic fluid infection and neonatal hyperbilirubinemia on motor and other neurologic outcomes were significantly greater than the arithmetic sum of their respective influences, indicating a potentiating impact. A significant increase in neurologic abnormalities was observed at peak TSB levels of 12–13 mg/dL (205–222 μmol/L) that was enhanced as placental inflammation became more severe and present only in children born before 34 weeks' gestation.[84] These findings are consistent with the known potentiating effects of neonatal infection on bilirubin neurotoxicity,[48,85–87] recently re-confirmed in clinical[88] and *in vitro* studies.[74,87,89–91] Such effects may be greater in less mature cells[74,90,91] and in preterm neonates.[49] One of the authors (J.F.W.) has reviewed recent cases of low bilirubin kernicterus in preterm neonates where maternal chorioamnionitis and funisitis featured prominently in the clinical presentation, suggesting that FIRS mirrors other conditions typified by infection and inflammation in potentiating bilirubin neurotoxicity.

Sepsis and necrotizing enterocolitis

Sepsis is a well-characterized risk factor for kernicterus,[86,88,92] including low bilirubin kernicterus.[9,85,93] This risk is quite robust as recently confirmed in a large cohort of neonates in Cairo, Egypt [OR = 20.6 (95% CI: 4.9–87.5)].[88] Umbilical infections[87] and necrotizing enterocolitis[85,93] are also reported in association with kernicterus. These conditions are associated with severe systemic inflammation that may potentiate bilirubin neurotoxicity via several mechanisms detailed above and hypoalbuminemia and disturbed bilirubin–albumin binding linked with these conditions.[26] Together, the effects of sepsis and hypoalbuminemia may pose more than an additive risk for bilirubin neurotoxicity as highlighted in a recent case report of kernicterus.[9]

Bilirubin and neuroinflammation

Immunostimulatory and immunotoxic effects of unconjugated bilirubin are attracting increasing interest in the efforts to better understand bilirubin neurotoxicity.[74] Hazardous levels of unconjugated bilirubin *alone* induce acute and chronic microglial activation *in vivo*[51,75–77] (Fig. 3),[75] directly upregulate pro-inflammatory gene expression,[51] and trigger the cellular (microglia and astrocyte) release of TNFα, IL-1β, and IL-6 cytokines,[51,74,94] producing a pro-inflammatory milieu. Notably, unconjugated bilirubin is as potent as lipopolysaccharide endotoxins in inducing pro-inflammatory cytokine release *in vitro*.[74,89–91] One would predict that these bilirubin-induced responses might contribute to neurologic damage[95] and/or exacerbate or prolong the neuroinflammation that accompanies FIRS, sepsis, and NEC. Pro-inflammatory cytokines do enhance bilirubin-induced neuronal apoptosis and necrosis in monoculture.[74,91,96] Activated

microglia release (i) reactive oxygen species, (ii) reactive nitrogen species, (ii) glutamate, and (iv) cytokines that could individually or together enhance bilirubin neurotoxicity.

It is intriguing that minocycline (MNC), which demonstrates robust neuroprotection against bilirubin-induced CNS injury in Gunn rat pups,[75,77,97–99] has potent immunomodulatory effects and prevents microglial recruitment and activation in hyperbilirubinemic Gunn rat pups treated with sulfadimethoxine (Fig. 3).[75,99] Whether this reflects a direct anti-inflammatory effect of MNC or simply the lack of neuroinflammation in the absence of injury prevented by some other MNC-mediated neuroprotective mechanism is unclear but lends credence to the importance of bilirubin-induced neuroinflammation in the pathogenesis of kernicterus.

However, recent study suggests a more complex dynamic including evidence that early bilirubin-induced pro-inflammatory responses can paradoxically be neuroprotective.[51,100] The signaling pathway involving toll-like receptor 2 (TLR2) in the CNS appears to be linked to hyperbilirubinemia-induced activation of microglia and astrocytes, resultant reactive gliosis; upregulation of *TNF-α, IL-1β, and IL-6* gene expression; and pronounced neuroinflammation *in vivo*.[51] However, deletion of TLR2 in mice blocks the induction of these inflammatory cytokine genes and is associated with enhanced cerebellar apoptosis and higher neonatal death rates.[51] These data suggest that TLR2 signaling and early neuroinflammation are neuroprotective *in vivo*.[51] Consistent with this complex dynamic, astrocytes in a co-culture model with neurons can protect or aggravate bilirubin-induced neurotoxicity depending on the duration of the cell–cell communication (preconditioning) and bilirubin exposure.[100,101]

Chronic bilirubin-induced neuroinflammation?

Persistent inflammation is now recognized as an important risk factor for CNS injury in preterm neonates.[60,63] Notably, prolonged or chronic bilirubin-induced neuroinflammation (microglial activation) is reported in the Gunn rat model of kernicterus and appears to have an adverse effect on brain development.[76,77] The possibility that bilirubin-induced CNS injury may extend beyond the initial insult is not widely appreciated and suggests that there may be a therapeutic window following the acute period of bilirubin encephalopathy. This possibility merits clarification as does the study of

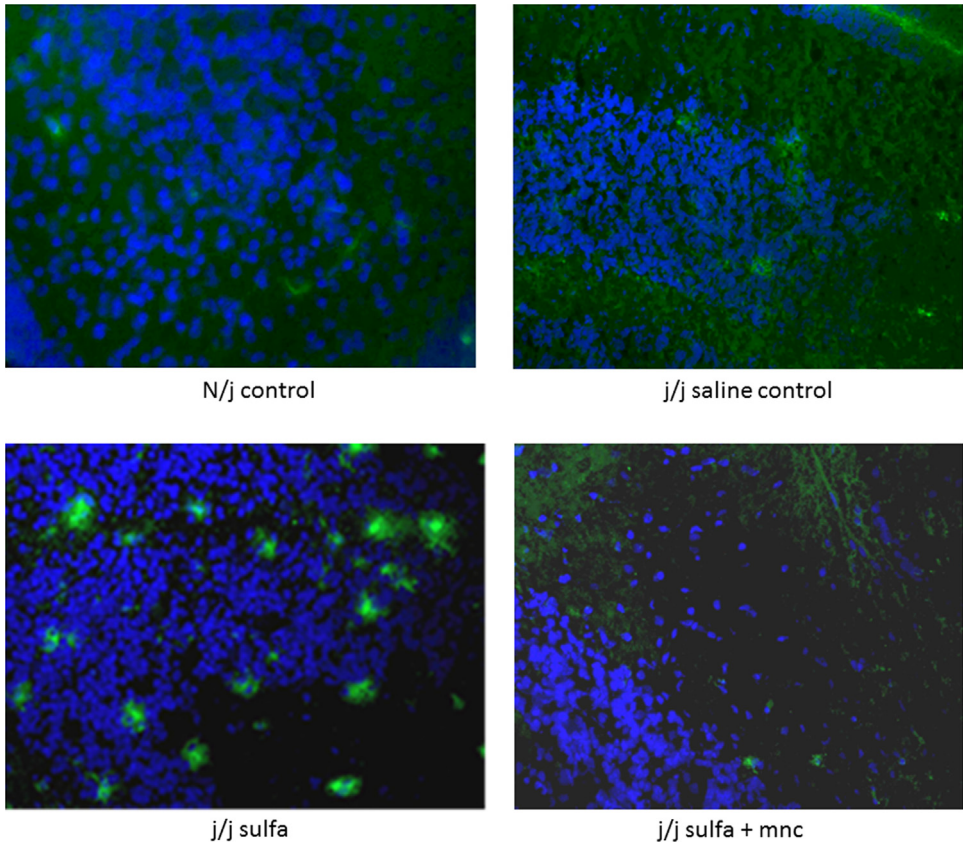

Fig. 3 – Microglial reactivity (OX-42 staining in green; counterstained with DAPI; 200× magnification) as indexed by the presence, number, and ameboid phenotype of activated microglia in cerebellar section from a hyperbilirubinemic j/j Gunn rat pup following 24 h of acute bilirubin encephalopathy (ABE) induced by sulfadimethoxine administration (200 mg/kg ip) (j/j sulfa). Littermate-matched non-jaundiced N/j (N/j control) and jaundiced j/j saline-treated pups (j/j saline control) are shown as controls as is a littermate-matched j/j sulfadimethoxine-dosed pup pretreated with minocycline (j/j sulfa + mnc); none of these littermate pups showed microglial reactivity or ABE. In preliminary studies, activated microglia were seen as early as 3 h after induction of ABE using sulfadimethoxine and became increasingly evident 6 h postinduction and uniform following 18 h of ABE.[75] (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

putative neuroprotective intervention(s) targeted to modulate inflammatory responses that may be effective within the complex "yin and yang" of neuroinflammation.[102,103]

Can low bilirubin kernicterus be prevented?

Low bilirubin kernicterus appears to result only when a rare constellation of co-morbid conditions and/or impaired albumin–bilirubin binding befall a vulnerable premature neonate (analogous to a "perfect storm"). By definition, low bilirubin kernicterus occurs at TSB levels not considered neurotoxic (i.e., below those commonly recommended for exchange transfusion).[6] Current exchange transfusion treatment thresholds reflect both the TSB and the presence of neurotoxicity risk factors. The latter increases the risk of bilirubin neurotoxicity in a hyperbilirubinemic infant and commonly include (1) lower gestational age, (2) serum albumin levels <2.5 g/dL, (3) rapidly rising TSB levels, suggesting hemolytic disease, (4) sepsis, and (5) clinical instability.[104–106] As set forth in one approach, infants are considered to be clinically unstable if they have one or more of the following conditions: (a) blood pH $<$ 7.15, (b) blood culture-positive sepsis in the prior 24 h, (c) apnea and bradycardia requiring cardio-respiratory resuscitation (bag mask ventilation and or intubation) during the previous 24 h, (d) hypotension requiring pressor support during the previous 24 h, and (e) mechanical ventilation at the time of blood sampling.[105,106] What modification(s) to existing approaches could be entertained that would be implementable, not pose added risk, and potentially prevent at least some cases of low bilirubin kernicterus? Would the timely identification of additional neurotoxicity risk factors and/or measurement of unbound bilirubin and bilirubin binding characteristics enhance outcomes?

Risk factor assessment

Risk stratification based on clinical factors has been an integral part of neonatal hyperbilirubinemia management for decades.[107,108] In addition to the neurotoxicity risk factors detailed above, the current review identified co-morbid CNS insults and chorioamnionitis accompanied by FIRS as potential markers for low bilirubin kernicterus risk. The challenge of incorporating them as additional neurotoxicity risk factors are many, including the fact that they have not been validated and are relatively common, whereas low bilirubin kernicterus is rare. The latter implies these risk factors may be sensitive but not specific. In addition and of practical concern is the timing of their identification relative to the period of bilirubin neurotoxicity risk. More often than not, cranial ultrasound screening to detect IVH and early sonographic evidence of WMI is not performed until the end of the first week of postnatal life, even in extremely low-birth-weight neonates; a timeframe typically beyond that of acute bilirubin-induced CNS injury risk. Similarly, placental pathology to detect FIRS takes several days to complete, and final reports are often not available until a week or so of postnatal life. Clinical evidence of maternal chorioamnionitis identifiable intrapartum could potentially serve as a proxy for FIRS but is not robust in predicting histopathologic chorioamnionitis, let alone FIRS.[81]

Measurement of unbound bilirubin and/or bilirubin-binding capacity

Routine measurement of unbound bilirubin might help if we had firm guidelines for intervention and if we knew that the intervention was safe and effective in lowering the unbound bilirubin concentration. However, the measurement of unbound bilirubin as part of clinical management remains hampered by several challenges. First and foremost, there is currently no commercially available device to perform the measurement, albeit some are being tested. Should unbound bilirubin measurements become clinically available, it will be important to remember as highlighted by Ahlfors,[13,17] that unbound bilirubin and TSB are not competing, independent determinants of bilirubin toxicity but are critically interrelated and interdependent in estimating risk. TSB is needed to gauge the size of the neonate's bilirubin load and unbound bilirubin in its distribution.[13] Ahlfors[13] suggests that the unbound bilirubin to TSB ratio may be the best index of risk.[109] Ongoing efforts are necessary to bring the measurement of unbound bilirubin to the clinical arena and to validate its utility, either alone or in conjunction with TSB.

The bilirubin/albumin ratio can serve as a proxy for unbound bilirubin but has a limited and conflicting track record in predicting adverse neurodevelopmental outcome.[23,110] The recent Bilirubin–Albumin Ratio Trial (BARTrial) from the Netherlands is a case in point.[111] This prospective, randomized, controlled trial compared treatment based on the bilirubin–albumin ratio or TSB (whichever was exceeded first) versus TSB only (control group) and failed to demonstrate an improved neurodevelopmental outcome in preterm neonates managed using the bilirubin–albumin ratio,[111] but this study might have been under-powered. Only 30% of infants in the experimental group were treated on the basis of bilirubin–albumin ratio as opposed to TSB.[111] It is also possible that bilirubin–albumin ratios that are lower than those used as criteria for intervention in the BARTrial might, nevertheless, place an infant at risk for neurotoxicity. In preliminary data, Japanese investigators found that a bilirubin–albumin ratio of $\geq$0.50 μmol/L/μmol/L ($\geq$4.25 mg/dL/g/dL) for infants of 30–34 weeks' gestation and $\geq$0.40 μmol/L/μmol/L ($\geq$3.4 mg/dL/g/dL) for infants of $<$30 weeks' gestation predicted putative neurotoxic unbound bilirubin levels of $\geq$1.0 μg/dL.[112] Having said that, 7 of the 8 low bilirubin kernicterus infants in Table 1 who had TSB and albumin levels reported had bilirubin–albumin ratios in excess of exchange transfusion thresholds previously outlined by Ahlfors[17] and used in the BARTrial.[111] Effects on central auditory pathways may be seen at even lower bilirubin–albumin ratios of 0.30–0.40 μmol/L/μmol/L (2.6–3.4 mg/dL/g/dL).[6,113] Detection of deficient bilirubin–albumin binding and reserve binding capacity may also prove useful in identifying neonates at risk.[32,114,115]

Given the complex, multifactorial nature of low bilirubin kernicterus, there is no guarantee that even these measures will be effective in preventing this CNS insult, to say nothing of the problems involved in identifying an intrinsically vulnerable host. Indeed, it is impossible to index the innate susceptibility of the cells of the CNS to bilirubin-induced injury and to factor this into the risk calculus, although, almost certainly, differences in CNS vulnerability exist. While efforts to refine and improve current treatment guidelines are certainly needed, such revision(s) will also have to take into account the risks and benefits of any intervention, including phototherapy.[116,117]

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