REVIEW ARTICLE

Review of bilirubin neurotoxicity II: preventing and treating acute bilirubin encephalopathy and kernicterus spectrum disorders

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Previously in Part I of this two-part review, we discussed the current and recent advances in the understanding of the molecular biology and neuropathology of bilirubin neurotoxicity (BNTx). Here in Part II, we summarize current treatment options available to treat the severely jaundiced infants to prevent significant brain damage and improve clinical outcomes. In addition, we review potential novel therapies that are in various stages of research and development. We will emphasize treatments for both prevention and treatment of both acute bilirubin encephalopathy (ABE) and kernicterus spectrum disorders (KSDs), highlighting the treatment of the most disabling neurological sequelae of children with mild-to-severe KSDs whose "rare disease" status often means they are overlooked by the clinical research community at large. As with other secondary dystonias, treatment of the dystonic motor symptoms in kernicterus is the greatest clinical challenge.

INTRODUCTION
Despite the availability of successful prevention strategies neurological disorders due to bilirubin neurotoxicity (BNTx) still occur throughout the world. Progress in eliminating the neurological sequelae of BNTx has been due to improved understanding of risk factors and causes of neonatal hyperbilirubinemia and effectively preventing excessive hyperbilirubinemia. We recently introduced the term kernicterus spectrum disorder (KSD) to refer to the broad spectrum of the permanent sequelae of BNTx to reduce some inaccuracies and ambiguity of terminology and to promote better estimates of disease prevalence. Better understanding and accurately predicting the risk and severity of KSDs occurring in a child with early-stage acute bilirubin encephalopathy (ABE) is important to determine the risks and benefits of potential new neuroprotective and neuro-rescue treatments. Basic and animal research, as discussed in Part I of this review, is an important pre-requisite to human studies because of the rarity of severe kernicterus and currently the length of time to assess the outcome, but with international multicenter collaborations, networks, and registries, progress can be accelerated.

Obviously, the most effective way to eliminate KSD is to prevent it. Much effort and success has gone into prevention of excessive hyperbilirubinemia and subsequent BNTx, yet KSDs still occur.[1] A recent publication by Rennie and co-workers[2] shows that the majority of KSD cases diagnosed in the UK had been discharged from the birth hospitals and experienced significant delays in readmission and management because hospital staff did not seem to take parental concerns seriously. In industrialized countries where early postnatal discharge is now prevalent, strategies to reduce the risk of KSD, which do not address follow-up and emergent management, are unlikely to succeed.

The goal of this review is to summarize recent advances in the understanding of BNTx in the context of preventing and treating KSDs. We feel that understanding BNTx and its neurological sequelae relates to three important clinical areas: (1) prevention, (2) treatment of ABE, and (3) treatment of KSDs. We contend that clear definitions of the outcomes of BNTx will have benefit for all three areas.

CURRENT STRATEGIES FOR TREATING HYPERBILIRUBINEMIA AND PREVENTING ABE
The current standards of treatment for treating hyperbilirubinemia in newborns are phototherapy and exchange transfusion. While these treatments are largely effective, some concerns exist regarding the safety of these treatments, especially in premature and extremely low birth weight infants.[3] Also, access to effective phototherapy and the ability to perform a double volume exchange transfusion is not universal. Phototherapy units can be costly or become ineffective if not properly maintained. These types of issue are especially common in low- and middle-income countries where instances of severe hyperbilirubinemia, ABE, and KSD are much higher than in more affluent nations.

Current hyperbilirubinemia treatment: crash-cart therapy
The so called "crash-cart approach" to ABE first described by Johnson et al. in 2009[4] and more recently by Hansen[5] implies emergent deployment of high-intensity phototherapy, possibly also with the addition of intravenous immunoglobulin in cases of blood group isoimmunization. Several case reports and series strongly suggest the possibility of reversing ABE with this strategy.[6,7] We continue to strongly advocate for this approach

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and, when possible, feel that every transport for neonatal jaundice should be equipped with the full range of therapies that can be initiated immediately upon arrival at the bedside including high-intensity phototherapy. We also advocate that, whenever unconjugated bilirubin is excessively high and a neonate has ABE, no matter how high and for how long, that the damage is ongoing and continuing and it is never too late to treat aggressively. The crash-cart approach can be implemented simply by establishing procedures and protocols.

Current hyperbilirubinemia treatment: filtered sunlight phototherapy
In studies of both mild-to-moderate and moderate-to-severely jaundiced patients in Nigeria, filtered sunlight phototherapy (FSPT) has been shown to be equally effective as intense electric phototherapy.[8–10] The main detraction for the use of FSPT is that it is dependent upon availability of the sun making continuous phototherapy impossible. However, studies have shown that intermittent phototherapy is as effective as continuous phototherapy at reducing total serum bilirubin levels and may, in fact, be preferred.[3,11] Therefore, the major detraction for FSPT is that, if a child is determined to need phototherapy at night or during a cloudy day, then the treatment cannot be started until the morning or until weather conditions improve.

NOVEL STRATEGIES FOR TREATING HYPERBILIRUBINEMIA AND PREVENTING ABE
Novel prevention strategy: improved access and methods to rapidly measure total and unbound (free) bilirubin and assess neurological function
The classical method for bilirubin determination is the measurement of total bilirubin (TB) in serum or plasma. TB is traditionally measured via a small sample of serum or plasma read via a spectrophotometer by reading the peak of bilirubin absorbance at 460 nm. Specialized spectrophotometers such as the bilirubin-ometer from Unistat (Reichert Technologies, Munich) are simple and easy to use for TB while others such as the BR2 bilirubin stat-analyzer from Advanced Instruments (Norwood, MA) are able to determine both direct (conjugated) and indirect (unconjugated) bilirubin values as well as the TB.

In addition to these serum-based methods, true Point-of-Care (POC) instruments have also been developed that do not require additional sample processing. A widely used POC device is a non-invasive transcutaneous bilirubin reader (BiliChek System, Philips, NV) that uses visible light multiwavelength reflectance spectro-photometric analysis to assess the bilirubin level in the skin and has been shown to be comparable to the TB measurement within its effective range (up to 15 mg/dL).[12–14] Additional POC devices include smartphone-based methods such as the Bilicam that has been shown effective up to 25 mg/dL,[15] and simple screening tools like the Bili-ruler may be useful in the initial determination of whether a child needs to be seen by a health-care professional.[16] Unfortunately, for higher levels of TB > 25 mg/dL, these tools are not sufficiently accurate. Fortunately, a new POC measure of plasma TB, the BiliStick, a simple, minimally invasive POC system requiring a 25-μL blood sample has been successfully field tested and validated in low-resource environments.[17–19]

As we have described previously, it is widely acknowledged that the TB measurement is a proxy measurement for the true toxic form of bilirubin, unconjugated, unbound "free" bilirubin (Bf). Therefore, no matter how accurate the various TB POC measurement devices are, they are still only estimating Bf exposure. Unfortunately, the current gold standard of measuring Bf, the "peroxidase method" is generally considered too difficult for most clinical laboratories to perform. However, recent technological advances in the form of a Bf-sensitive antibody may make routine Bf measurements a reality.[20] Recent reports have shown that this novel probe is

capable of reliably measuring Bf in neonatal blood samples.[21] We expect that in the future total serum bilirubin and Bf measurements will be used together to follow the production of bilirubin and determine the Bf exposure to the patient leading to a vastly improved estimation of risk of BNTx in the neonate. These values, combined with risk factors from history, physical exam, and signs of early neurophysiological abnormalities on auditory brainstem response (ABR; i.e., increased I–III and I–V interwave intervals and then loss of waves III and V) should help create new treatment guidelines. These new guidelines will help focus and optimize treatment decisions leading to reduce the need to err on the side of overtreatment while still preventing BNTx and KSD in all neonates.

Novel prevention strategy: improved screening of patients with genetic predisposition toward BNTx sensitivity
The final piece of this risk assessment puzzle will likely be an analysis of the genetic background of the child. We have previously hypothesized that there exists a genetic predisposition to BNTx sensitivity that would explain in part why KSD severity does not correlate well with peak TB levels.[22] Testing of this hypothesis is still ongoing with the ultimate goal to identify genetic signatures that could be used to create a genetic screening "chip" capable of characterizing jaundiced newborns as hypersensitive or hyposensitive to BNTx. BNTx hypersensitive newborns could then be placed on closer monitoring protocols and more aggressive anti-bilirubin therapies. Conversely, treatment plans for BNTx hyposensitive newborns could be adjusted to avoid unnecessary risk, such as unnecessary double volume exchange transfusions.

Novel prevention strategy: improved rapid assessment of neurological function
In the future, new methods to anticipate BNTx in babies with hyperbilirubinemia will be helpful to focus treatment to those who need it the most. First, there is the availability of ABR and the use of ABR and Bf to determine how aggressive to treat neonatal with extreme hyperbilirubinemia[23] using the automated ABR (AABR) to assess central nervous system (CNS) function. The AABR is a screening version of the full (diagnostic) ABR, sensitive only to the presence or absence of an ABR response. It is designed to assess hearing loss but not auditory neuropathy spectrum disorder (ANSD), nor the earliest signs of ABE, increased I–III and I–V interwave intervals, and decreased amplitudes of waves III and V. Modern "Next Gen" ABR technology and signal processing can be designed specifically to detect these early CNS dysfunctions due to BNTx. These technologies could be used in neonatal intensive care units, nurseries, emergency departments, and neonatal transfer vehicles to assess those with worrisome hyperbilirubinemia to help determine the urgency of treatment. We suggest this test be called the "Bili-ABR" to distinguish it from the current AABR. There is no reason that this test could not be offered as a software add-on to existing newborn AABR machines for the purpose of evaluating BNTx and ABE in neonates with hyperbilirubinemia. Further, new developments in more effective ABR stimuli, such as the CE-Chirp stimulus, and "next generation" signal processing could make collecting of electrophysiological data faster and more efficient.[24] These improvements could be incorporated into an instrument no more difficult to use than the amplitude electroencephalogram (aEEG) or AABR. The improved functionality of these instruments would make them useful in the initial and serial evaluation of neonates with extreme hyperbilirubinemia and premature infants with worrisome hyperbilirubinemia.

NEW STRATEGIES FOR TREATING ABE AND REDUCING THE SEVERITY OF KSD
Novel ABE treatment: minocycline
Whereas double volume exchange transfusion is the gold-standard treatment for hazardous hyperbilirubinemia with ABE,

334

there is often a 3–6-h delay from the identification of the critical situation until the start of the exchange. If an effective neuroprotective drug that could be given immediately while preparations are made for the exchange, it could potentially decrease the kernicteric damage significantly and have major lifetime benefit to the individual.

The anti-inflammatory and anti-microbial drug minocycline is protective against BNTx in the jj–sulfa Gunn rat model of kernicterus.[25–27] Recent reports showed the effectiveness of minocycline in preventing kernicterus death in the Gunn mouse model of hyperbilirubinemia,[28,29] whereas intraperitoneal injection of the antioxidant drug N-acetyl cysteine was ineffective, suggesting that inflammation and not oxidative stress is essential for BNTx. Despite its effectiveness, it is unlikely that minocycline will be used prophylactically for the treatment of hyperbilirubinemia to prevent ABE because of its known side effects, including tooth discoloration, hyperpigmentation of skin and nails, skin rash, and increased skin sensitivity to light.[30] Without a better understanding of the risk of developing severe KSD vs. the risk of minocycline side effects, the physician managing a child with ABE is unlikely to recommend a treatment like minocycline resulting in potentially unnecessary complications for the child.

Novel ABE treatments: hypothermia

Therapeutic hypothermia as a method to treat neonatal hypoxic–ischemic encephalopathy is a well-established standard of treatment.[31] Kuter et al. treated primary mouse neuron cultures with bilirubin (1:1 bilirubin-to-albumin ratio) and then altered the temperature between 29 °C and 37 °C.[32] Under the same bilirubin exposure conditions, lowering temperature from 37 °C to 34 °C lowered the neuronal death rate significantly from $44 \pm 2\%$ (mean $\pm$ SD) to $30 \pm 4\%$ at 34 °C and to $18 \pm 4\%$ at 32 °C, but the trend reversed at 29 °C with death rate rising to $28 \pm 4\%$.[32] One possible mechanism for the effectiveness of hypothermia is that reduced temperature may make mitochondria more efficient, which would support the hypothesis that mitochondrial function is important to the mechanism of BNTx.[33]

Novel ABE treatments: caffeine

Currently, the only known sign of ABE in premature infants is either apnea or no clinical signs et al.[34] Apnea in these patients is often treated with caffeine. Deliktas et al. used primary astrocyte cell cultures from 2-day-old Wistar albino rats to test the prophylactic properties of caffeine in preventing bilirubin-induced cell death.[35] The authors showed that apoptosis due to bilirubin (measured by deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL staining)) was decreased from 34.2 ± 2.6 to $5.8 \pm 1.4\%$ with prophylactic or $9.2 \pm 2.6\%$ with therapeutic caffeine. However, without more information on how bilirubin was administered and whether of Bf or TB was measured, it is difficult to assess the exposure of these cell cultures to BNTx.

CURRENT TREATMENT OPTIONS FOR KSD PATIENTS

While we agree with and wholeheartedly support the importance of prevention, we are aware that there is very little research and literature on treatment for the very unfortunate children and adults who suffer from KSDs. Therefore, we would like to move beyond prevention to discuss current and future treatment options for those with KSD.

As we have previously described, the term kernicterus covers a wide spectrum of disorders characterized by various levels of severity in auditory and motor dysfunction.[36] Importantly, there does not appear to be any obvious damage to the higher functioning regions of the brain, such as the neocortex. This lack of damage would explain the clinical observations that, while severe kernicterus patients are often severely physically disabled including being unable to speak or effectively communicate without assistance, they appear to be cognitively intact and even extremely intelligent. It is therefore especially disheartening that there have not been more advancements made in the treatment of the movement disorders that severe KSD children suffer from and that a reliable communication system has not been developed that does not rely on voluntary control of the voice, or eye, head, or hand movements.

Current KSD treatments: auditory

The auditory dysfunction associated with KSD has been well characterized as a form of ANSD.[37–39] Experiments in the jj–sulfa Gunn rat show that kernicteric ANSD results from the BNTx causing errors in auditory signal processing at the level of the auditory brainstem,[40–42] with more severe BNTx also affecting the large, myelinated fiber of the primary auditory neurons.[43] To promote language development and reading in children with ANSD, we recommend using "cued" speech in infants and toddlers and at school age using educational programs that teach the phonemic content of language based on recommendations of experts in auditory neuropathy and reading. However, to date we are unaware of any evidence-based studies showing the benefits of these programs.

There is evidence for the benefits of cochlear implantation (CI) in children with ANSD.[38] While it is not completely understood, it is postulated that the cochlear implants restore synchrony in ANSD patients,[44] and numerous KSD patients with ANSD report a positive response to this treatment. For individuals with severe ANSD and hearing loss who are not developing language, we recommend evaluation for CI. In one of the author's experience (S. M.S.), over 90% of KSD patients respond to CI. Caveats are that there are some children with "Kernicterus Plus", i.e., kernicterus plus other conditions that cause brain injury, e.g., kernicterus plus hypoxic–ischemic encephalopathy or genetic conditions that could interfere with the effectiveness of CI. When neocortical auditory or cognitive function is impaired by these "plus" syndromes, the response to CI may be suboptimal. Further, there are cases of spontaneous recovery of auditory function in patients with ANSD, so the natural course of ANSD in children with KSDs is not well characterized and may be influenced by factors such as the severity, etiology, treatment, and gestational age at the time of BNTx.

Current KSD treatments: motor

For those KSD patients with moderate-to-severe motor dysfunction, there are unfortunately very few established options for relief of the severe dystonia and abnormal movements. The current treatments for these patients include oral medications, especially benzodiazepines, e.g., diazepam or clonazepam, and baclofen, which stimulates GABA-B. Other medications, e.g., trihexyphenidyl (anticholinergic) and tetrabenazine (decreases dopamine), have had limited benefit. Selective botulinum toxin is used to improve function or relieve painful muscle hypertonia. Since baclofen does not cross the blood–brain barrier, intrathecal baclofen delivered by a pump has been successful in reducing tone and muscle spasm when oral medications have failed.

NOVEL TREATMENTS FOR KSDS

Novel KSD treatment: motor—new medications

Without question, the most debilitating aspect of KSD is severe motor dysfunction characterized as abnormal muscle tone with or without writhing movements (athetosis). The abnormal tone seen in KSD patients makes this dystonic motor kernicterus a subset of cerebral palsy. Like other forms of cerebral palsy, there is no effective cure for these symptoms. Patients with severe motor and classical KSDs are unable to ambulate, feed themselves, speak, or use sign language. Further, owing to issues with head control and

controlling eye movements, they often cannot use communication devices, such as communication boards or eye movement-based devices. The lack of effective treatment for the motor dysfunction associated with severe KSDs has led many families to experiment with novel drug therapies. Patients visiting our clinic have reported some relief of symptoms through the use of various medications approved in a similar patient population such as valbenzine, a long-acting dopamine release inhibitor used to treat adult tardive dyskinesia. Our patients and families also report of cannabinoid oil bringing relief to various symptoms. However, without controlled clinical trials, these reports should be viewed with caution.

We hope that trials of potentially beneficial medications such as these are soon tested in the KSD patient population. The availability of a national or international KSD registry, with a mechanism to validate the diagnosis, e.g., the KSD diagnostic criteria and toolkit we have proposed,[36,38] will allow evidence-based studies of these new treatments.

Novel KSD treatment: deep brain stimulation (DBS)
DBS is a popular area of investigation for the treatment of dystonia due to KSD. DBS has been successfully used to obtain improvements in tone and voluntary motor control in pediatric primary dystonia patients,[45,46] although less evidence exists of its benefits in pediatric secondary dystonia.[47] DBS implantations have been trialed in KSD patients for at least the past ten years.[48] Anecdotal evidence from patients we follow shows small improvements in tone, motor control, and sleep that are nonetheless significant and impactful to patients and their caregivers. Improvements may take months to manifest after surgery and are to some extent dependent on the time and skill with which the DBS is programmed. Like primary dystonia, bilateral DBS leads are usually placed in the globus pallidus interna (GPi), with some receiving additional leads in output projections of the GPi.[49] The reasoning for this placement is that this is the region of the brain that is usually associated with bilirubin damage in these patients and recordings from kernicterus jj–sulfa Gunn rats have shown both a loss of GPi neurons and abnormal excessive firing of remaining neurons.[50]

Novel KSD treatment: brain–computer interfaces (BCIs)
BCIs can potentially provide communication for individuals with severe neuromotor impairments that eliminate speech and prevent use of augmentative and alternative communication devices. Children with the most severe cases of classical or motor-predominant kernicterus have severe dystonia with no voluntary movement and are functionally locked-in, but they are cognitively intact. We are currently piloting BCI feature-matching protocols to determine appropriate BCIs for these individuals.

Novel KSD treatment: stem cell treatment
With the advent of autologous stem cells allowing for one's own cells to be used to derive stem cells for treatment, the rejection of a stem cell treatment is essentially eliminated. However, concerns still exist as to the safety and efficacy of this treatment for KSD patients. Despite these concerns, reports of KSD patients receiving intrathecal autologous bone marrow-derived stem cell treatments were recently published.[51] Two patients of unknown severity were treated using intravenous injections of these autologous stem cells. One of the patients was described as having improvements in "talking and power," though specific metrics of how this was measured were not described. The other patient was said to have improved "urine and stool sensation," "seven months after stem cell therapy".[51] While these reports are encouraging, the lack of detail regarding the treatment, patients, and how the outcomes were measured makes it difficult to interpret these results.

Animal models have also been used to study the effectiveness of stem cell treatments to reverse the auditory and motor dysfunction. Our group has shown that neuron-like progenitor cells derived from WA09 human embryonic stem cells can be successfully implanted stereotactically into the brains of jaundiced Gunn rats[52,53] and survive. Another group has reported a new model of injecting phenylhydrazine into 7-day-old normal Wistar rats to create hemolysis and then giving sulfisoxazole to create kernicterus and then correcting the kernicterus using human adipose tissue-derived stem cells injected intrathecally.[54,55] We have used phenylhydrazine to create ABR abnormalities and dystonia in our jj Gunn rat pups but were unable to create any neurological abnormalities in non-jaundiced heterozygote control Gunn rats given high doses of phenylhydrazine. Further, these studies did not provide convincing evidence that reproducible ABRs were recorded in either experimental or control animals, and there was no evidence presented of dystonia in the treated animals. We have hypothesized that BNTx selectively targets parvalbumin expressing GABA-ergic neurons in the basal ganglia, especially the globus pallidus (see Part 1, Section 3.2, Selectivity of BNTx). We believe that the method of targeted stereotactic delivery of specific neural progenitor cells into the globus pallidus by directed implantation is most likely to be effective and that if the return of function in a dystonic animal could be demonstrated, then the efficacy of other less invasive methods of delivery, e.g., intrathecal or intravenous, could be compared.

CONCLUSIONS
The future holds new revelations from new cell culture and animal models as well as clinical research. Bilirubin exposure in cell culture was revolutionized in the early 2000s that highlighted the importance of measuring Bf in culture and using clinically relevant doses.[56,57] Animal models are being revolutionized by new genetic strains of mice and rats that can expand the kinds of questions that can be asked. Translational and clinical studies can benefit from new magnetic resonance imaging and analysis techniques and tractography, new neurophysiological testing methods, and new genomic and expression RNA studies to answer fundamental questions. Refining and objectifying definitions of KSDs will allow multicenter sharing of data. For example, DBS has proved extremely beneficial for primary (genetic) dystonia but less so for the secondary dystonia, e.g., hypoxic–ischemic, traumatic, metabolic, and kernicteric encephalopathies. The remarkable homogeneity of the CNS lesions in kernicterus provides the hope that finding an optimal location and programming parameters for DBS for one or a few dystonic KSD patients will be applicable to all.

Collaboration and cooperation on a KSD database and registry will support evidence-based research and move this and other treatments of kernicterus forward at an accelerated pace. BCIs to enable communication and functional voluntary movement in individuals with severe KSD will be a major advance for these cognitively intact individuals who are functionally "locked in." The ultimate treatment, neural repair with neural progenitor or stem cells, will be possible only with the support of the type of preclinical animal model research that we have described and advocate.

Understanding and accurately predicting the risk and severity of KSD occurring in a child with early-stage ABE is critically important to determine the risks and benefits of potential new neuroprotective and neuro-rescue treatments. For example, knowledge of the risk of a KSD in a child and accurate prediction of its severity plus the risk of a neuroprotective treatment could provide the clinician and family the basis to make an informed decision of whether the benefit outweighs side effects. A risk algorithm including (a) history with risk factors; (b) focused exam; (c) a Bilirubin Risk Chip with measurement of TB, Bf, albumin, and genetic risk factors regarding production and elimination of and susceptibility to bilirubin; and (d) measures of neurophysiological

function highly sensitive to BNTx with a "Next Gen" bilirubin-focused AABR would represent a rapid, sensitive, and more complete method for assessing BNTx and determining the most appropriate treatment. Basic and animal research will be a pre-requisite to human studies because of the rarity of severe kernicterus and currently the length of time to assess the outcome, but with international multicenter collaborations, networks, and registries, progress can be accelerated.

Finally, we note that research in neonatal hyperbilirubinemia, BNTx, and KSDs, far from being a disease of the past, has recently become a condition of new research using modern molecular biology, Next Gen genetics, and outcomes research, and we are excited and heartened by new young researchers being attracted to the field.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS
Both the authors contributed to the conception, design, and drafting of this article, revising it critically for important intellectual content, and gave final approval of the version to be published.

ADDITIONAL INFORMATION
Competing interests: The authors declare no competing interests.

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