VALIDATION OF A TRANSCUTANEOUS BILIRUBIN (TcB) NOMOGRAM
IN IDENTIFYING HISPANIC NEONATES
AT RISK FOR HYPERBILIRUBINEMIA

by

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I dedicate this work to the memory of my late grandfather, Luther Gayle Pope. Although he only had a second-grade education, he worked tirelessly to provide a way for his five children to receive a college education. Four of his children received a bachelor degree. Two of those children, my aunt and mother graduated from college during World War II. Sadly, Luther left this world in 1961, but his inspiration continues to this day.
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Abstract

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All newborns are at potential risk of adverse outcomes if they are not monitored for hyperbilirubinemia or if treatment for this problem is inadequate. Strategies are needed to accurately identify those newborns with clinically significant hyperbilirubinemia. Transcutaneous bilirubin (TcB) nomograms offer a useful screening tool to identify and monitor newborns with jaundice, as well as a way to predict the subsequent development of hyperbilirubinemia; however, there have been few studies examining the accuracy of TcB nomograms. The primary purpose of this study was to validate a previously published but unvalidated TcB nomogram to independently determine its accuracy in identifying Hispanic newborns at risk for subsequent development of significant hyperbilirubinemia (TcB > 95th percentile).

A secondary analysis of an existing database was chosen to validate a TcB nomogram, and to examine dimensions relating to TcB and gestational age previously
unexamined in any known study. Hour-specific TcB values from an independent sample of Hispanic newborns ($n = 404$) were compared with the 50th and the 75th percentile values of the nomogram at three periods of postnatal age, and then each value was examined with regard to a subsequent TcB value that represented significant hyperbilirubinemia (i.e., $> 95$th percentile). In each analysis, the sensitivity and NPV of the 50th and the 75th percentiles was 100% sensitivity.

A secondary purpose of this study was to compare TcB values between early term and term newborns to determine if, because of their relative physiologic immaturity, early term neonates are a greater risk for hyperbilirubinemia compared to infants born after 38 weeks of gestation. Transcutaneous bilirubin values obtained at five epochs of postnatal ages in the two groups were compared. Although a significant difference in TcB values was found in one of the epochs, no significant difference was observed for the other four epochs.
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Chapter 1

Introduction

All neonates (infant ≤ 28 days of age) experience hyperbilirubinemia defined as total serum bilirubin (TSB) level > 2 mg/dL, which would be considered abnormal in an older infant or adult (Bhutani & Johnson, 2009a). All newborns are at potential risk of adverse outcomes if they are not monitored for hyperbilirubinemia or if treatment for this problem is inadequate. Current pediatric practice guidelines recommend a risk-based approach for the prevention and management of neonatal hyperbilirubinemia (American Academy of Pediatrics [AAP], 2004; Maisels et al., 2009a). Using an hour-specific bilirubin nomogram is considered the best documented method for assessing risk of neonatal hyperbilirubinemia; however, there have been limited studies examining the predictive ability of nomograms. A secondary data analysis study was undertaken to examine the predictive ability of a transcutaneous bilirubin (TcB) nomogram to identify the subsequent development of significant hyperbilirubinemia in Hispanic neonates. In addition, this study investigated whether, because of their relative physiologic immaturity, early term neonates had higher hour-specific TcB values than those infants born at term. This chapter includes a discussion on the following: (1) background and significance of the problem of neonatal hyperbilirubinemia and risk-based approaches to evaluate the risk of hyperbilirubinemia; (2) conceptual framework of the study; (3) purpose and assumptions of the study; and (4) research questions.
Background and Significance of the Problem

The clinical manifestation of hyperbilirubinemia is jaundice, a yellow discoloration of the skin that results from an increased concentration of bilirubin in the blood. Of the approximately four million infants born every year in the United States, between 60-80% will manifest jaundice during the first week of life (Bhutani, 2012; Martin et al., 2011). This transient icteric episode has been termed "physiologic jaundice" because it occurs in the majority of healthy term and late preterm neonates and there is no apparent pathologic process identified (Halamek & Stevenson, 2002). Although physiologic jaundice is generally a benign transitional phenomenon related to red blood cell, hepatic, and gastrointestinal immaturity, a select number of neonates, especially those who are less mature, infants from certain ethnic groups, and infants with a significant risk profile are at greater risk of developing potentially hazardous levels of bilirubin that may pose a direct threat of brain damage (Powers, Miller, & Shapiro, 2008; Watchko & Lin, 2012). In addition, timely detection of neonatal hyperbilirubinemia has become increasingly important due to shorter hospital stays following childbirth. Newborns with unrecognized or unmonitored hyperbilirubinemia represent a vulnerable population as they transition from the birth hospital to home, where families may not recognize the development of jaundice, and where postnatal follow-up care may be delayed or inconsistent (Bhutani & Johnson, 2009a; Profit, Cambric-Hargrove, Tittle, Pietz, & Stark, 2009).

Concerns regarding neonatal hyperbilirubinemia stem primarily from its potential to cause acute and chronic forms of bilirubin-induced neurological dysfunction (BIND). In neonates, prolonged exposure to significant levels of bilirubin can result in passage of bilirubin through the protective membrane that separates the blood from the brain and spinal cord, and cause reversible or irreversible cell damage or even cell death (McDonagh, 2010). Clinical features of acute BIND include poor feeding, lethargy,
irritability, abnormal muscle tone and posture, apnea (temporary cessation of breathing), and seizures (Shapiro, 2010). The chronic and permanent form of BIND, also known as kernicterus, is associated with athetoid cerebral palsy, deafness, vision loss, dental dysplasia, and mortality in severe cases (Ip et al., 2004). The diagnosis of both the acute and chronic forms of BIND is made based on clinical signs and symptoms and TSB. No singular TSB value has been shown to be associated with an increased risk of BIND in otherwise healthy neonates (Ip et al., 2004; Johnson, Bhutani, Karp, Sivieri, & Shapiro, 2009; Shapiro, 2010). The exact level of TSB that causes neurotoxicity in neonates is likely to depend on confounding factors such as gestational age (i.e., maturity), postnatal age (in hours), and the incremental rate of bilirubin elevation (i.e., rate of rise) (Bhutani, Johnson, & Keren, 2005). Neonates diagnosed with significant hyperbilirubinemia, if promptly identified, can be effectively and safely treated with phototherapy (AAP, 2004).

According to patient safety and public health organizations, all newborns are at potential risk of adverse outcomes if they are not monitored for hyperbilirubinemia or if treatment for this problem is inadequate (Centers for Disease Control and Prevention [CDC], 2001; Joint Commission on Accreditation of Healthcare Organizations [JCAHO], 2001). Concerns about the continued occurrence of kernicterus in otherwise healthy newborns prompted JCAHO (2001) to issue a Sentinel Event Alert that all neonates are at potential risk of brain damage if hyperbilirubinemia is unmonitored or progresses untreated.

While the incidence of kernicterus is relatively rare, it continues to occur in developed countries. Population-based estimates of the incidence of kernicterus in North America and Europe range from 0.5 to 2.7 cases per 100,000 live births; however, the true incidence of kernicterus in the U.S. is unknown because it is not a condition that must be reported to public health agencies (Bjerre, Petersen, & Ebbesen, 2008; Burke et
al., 2009; Manning, Todd, Maxwell, & Platt, 2007; Sgro, Campbell, & Shah, 2006). In addition, more than 20 years of clinical case reports reveal that many of the victims are infants who have been discharged from the hospital newborn nursery as healthy, yet have returned to a pediatrician’s office or an emergency department with TSB values in hazardous ranges (e.g., ≥ 30 mg/dL) and have subsequently been diagnosed with kernicterus (Ip, Chung, Trikalinos, DeVine, & Lau, 2009).

Risk-based Strategies to Assess the Risk of Neonatal Hyperbilirubinemia

Transcutaneous bilirubin (TcB)

Bilirubin levels can be determined by either laboratory analysis of the blood (TSB) or by a noninvasive method (TcB). Transcutaneous bilirubin measurement was conceived after it was discovered that a close linear relationship between TcB and TSB could be quantified between TSB concentration and the intensity of the yellowness of the skin (Yamanouchi, Yamauchi, & Igarashi, 1980). Worldwide, studies have demonstrated a strong linear correlation between TcB and TSB measurements; however, transcutaneous bilirubinometry should be regarded as a screening test since it measures the amount of bilirubin present in the skin and subcutaneous tissues (Bhutani et al., 2000; Ho, Ng, Tsui, & Lo, 2006; Kaplan & Hammerman, 2013; Maisels et al., 2004; Rubaltelli et al., 2001; Stoniene, Buinauskiene, Markuniene, 2009; Wainer, Rabi, Parmar, Allegro, Lyon, 2009). Obtaining a TcB can facilitate the decision whether or not to perform a more accurate, albeit painful and time-consuming, TSB determination (Kaplan & Hammerman, 2013).

Currently, the two U.S. Food and Drug Administration approved TcB bilirubinometers are the BiliChek® (or BiliCheck®; Respironics, Murrysville, PA) and the third generation, Dräger Jaundice Meter, JM-103™ (Drägerwerk, Lubeck, Germany).
The BiliChek measures TcB by directing the entire spectrum of visible light (i.e., white light: 380 to 750 nanometers [nm]) into the skin. The reflected light is returned to the device where an internal microprocessor analyzes information about the spectral properties (wavelengths) of substances (e.g., melanin pigment, hemoglobin, and bilirubin) within the capillary beds and the subcutaneous tissues. Combined, these substances create a complex profile from which the bilirubinometer mathematically isolates and deducts the contribution of each element until the only variable remaining is bilirubin. Once the wavelengths have been quantified, a microprocessor analyzes the pattern of the optical densities and generates a TcB measurement. The bilirubin result is then displayed on a liquid crystal display (LCD) screen in either mg/dL or μmol/dL. The accuracy of the BiliChek was examined in two large studies, which included neonates from diverse races/ethnicities (Bhutani et al., 2000; Rubaltelli et al., 2001). Investigators compared TcB measured with the BiliChek and TSB measured with high-performance liquid chromatography (HPLC; the gold-standard in bilirubin measurement in research laboratories), as well as the more conventional methods used in hospital laboratories. The correlation between TcB and TSB ranged from $r = 0.87$ to $r = 0.91$.

The measurement technique of the JM-103 is different from the BiliChek. The JM-103 uses a dual optical path system and analyzes optical differences in the blue (450 nm) and the green (550 nm) wavelength regions. The two optical paths allow light scattered from shallow areas of dermal and epidermal tissue and light from deeper subcutaneous tissue to return to the device through different paths. By measuring the difference in optical densities from the two pathways, the parts that are common to the more shallow areas of tissue are deducted from the deeper areas. Thus, the measurement of bilirubin, accumulated primarily in the deeper subcutaneous tissues, should decrease the effect of melanin and hemoglobin. The JM-103 was examined in a
large clinical study of 849 late preterm and term neonates with a diverse ethnic background (Maisels et al., 2004). Correlation between TcB and TSB values was $r = 0.95$. These investigators also performed a comparison of the BiliChek versus the JM-103 in a subset of newborns. In this sample, all of SB measurements were determined by the same clinical laboratory using one method of analysis. The correlation for each of the two devices was very strong ($r = 0.97$) and identical.

The use of transcutaneous bilirubinometry in hospital newborn nurseries has become commonplace (Bhutani et al., 2000; Maisels & Kring, 2006; Wainer, Parmar, Allegro, Rabi, & Lyon, 2012). Several advantages to using TcB have been described: (a) it is a noninvasive method that significantly reduces the need for painful heel stick or venipuncture blood specimens (Ebbesen, Rasmussen & Wimberley, 2002; Maisels & Kring, 1998); (b) it offers an immediate and valid estimate of TSB (Bhutani et al., 2000; Ip et al., 2004; Ebbesen et al., 2002; Maisels et al., 2004; Rubaltelli et al., 2001); (c) it provides an efficient screening tool to identify infants who need to be evaluated with a TSB, thus reducing the likelihood that a clinically significant TSB value will be missed (Maisels 2006; Maisels et al., 2009a); (d) it is a portable, point-of-care device potentially useful in a variety of settings, such as home (Wainer et al., 2012); and (e) its use may reduce healthcare costs and resource utilization (McKenzie & Palmer, 2010).

**Transcutaneous bilirubin (TcB) nomograms**

The AAP (2004) recommends predischarge bilirubin measurement (TSB or TcB) with the results plotted on a nomogram, similar to the Bhutani nomogram, as the best documented method for evaluating a neonate’s risk for the subsequent development of hyperbilirubinemia. This recommendation is the result of decades of research that has shown that the ability of nurses and physicians to recognize clinically significant jaundice or to predict bilirubin levels based on the visual assessment of jaundice is limited (Ip et
al., 2004; Keren, Tremont, Luan, & Cnaan, 2009; Moyer, Ahn, & Sneed, 2000). The use of a clinical tool, such as a bilirubin nomogram potentially offers an accurate and discriminatory tool for predicting the risk of the development of hyperbilirubinemia in neonates (Romagnoli et al., 2012).

The availability of modern bilirubinometers that measure bilirubin concentration in dermal and subcutaneous tissues has made it possible to obtain serial, non-invasive (i.e., painless) TcB measurements. With the increased use of TcB measurement; however, concerns have been raised regarding the appropriateness of plotting TcB values on a nomogram developed using TSB values. The predictive accuracy of TcB values, performed using the BiliChek or the JM-103, was evaluated on the Bhutani nomogram (Rodriguez-Capote, Kim, Paes, Turner, & Grey, 2009). Using the 40th, 75th, and 95th percentiles, investigators obtained a 6%, 0%, and 1% false-negative rate for BiliChek and 62%, 74%, and 81% for the JM-103 device, respectively. The authors concluded that TcB measurements cannot be directly applied (i.e., plotted) to a TSB nomogram. In part, this limitation led to the creation of TcB nomograms. These nomograms have been developed using serial TcB values measured at various postnatal ages in hours (i.e., hour-specific), and then calculating percentile values for each designated period. Transcutaneous bilirubin values obtained for clinical purposes during postnatal hospitalization can then be plotted on a TcB nomogram, analogous to the manner in which a clinician plots height and weight values on a growth chart, with the expectation that infants starting out on a certain percentile will stay on or near that percentile as time passes (Keren & Bhutani, 2007).

The evaluation of child growth trajectories is highly dependent on the growth chart used (de Onis, Garza, Onyango, & Borghi, 2007). For example, the birth weight of an extremely premature infant should not be evaluated using a chart developed in term
neonates. Similarly, the evaluation of TcB values using a nomogram developed in a newborn population with a different race/ethnic origin or a significantly different risk profile may bias the proper assessment and management of jaundice (DeLuca, Romagnoli, Tiberi, Zuppa, & Zecca, 2008). Trends in TcB values can vary significantly among different populations. DeLuca, Jackson and colleagues (2009) described the trends in TcB levels used in the development of TcB nomograms across four diverse populations of newborns, including Thai, Italian, North American white, and North American Hispanic. Findings revealed that maximum (peak) TcB levels occurred earliest in Thai neonates, latest in Hispanic neonates, and at intermediate epochs in European and North American populations. Two other investigations have found significant differences in the trends in TcB levels between late preterm and term neonates, as well as between infants who did and did not require treatment for hyperbilirubinemia using phototherapy (Fouzas, Skylogianni, Mantagou, & Varvarigou, 2010; Fouzas, Mantagou, Skylogianni, Mantagos, & Varvarigou, 2010).

Transcutaneous bilirubin nomograms have been developed for newborn populations from different parts of the world, including China, Europe, Greece, India, Israel, and Thailand (Bental et al., 2009; DeLuca et al., 2008; Fouzas, Karatza et al., 2010; Mishra, Chawla, Agarwal, Deorari & Paul, 2010; Sanpavat, Nuchprayoon, Smathakanee, & Hansuebsai, 2005; Yu et al., 2011). Two TcB nomograms have been developed for neonatal populations in the United States: one for a primarily white (73%) population in the Midwest and one for a Hispanic population in the Southwest (Engle, Lai, Ahmad, Manning, & Jackson, 2009; Maisels & Kring, 2006). Although approximately 10 TcB nomograms have been published, only one has been validated in an independent population of neonates (Romagnoli et al., 2012). The result of this study showed that infants with TcB values at or above the 75th percentile were at increased risk of
subsequent significant hyperbilirubinemia. The predictive ability of the other nomograms, however, remains uncertain.

**Gestational Age**

A risk-based approach to the management of hyperbilirubinemia requires an understanding of non-pathologic factors that affect bilirubin levels in the otherwise healthy neonate (Maisels & Newman, 2012). Bilirubin levels vary considerably between neonatal populations depending on certain environmental and epidemiologic factors such as certain ethnicities, decreased gestational age, and exclusive breastfeeding (Maisels, De Ridder, Kring, & Balasubramaniam, 2009b; Watchko & Lin, 2012). Investigators studying neonatal hyperbilirubinemia have reported significantly higher maximum TSB levels in infants of East Asian, Native American, and Hispanic parentage compared to white infants (Engle, W.D., Jackson, Sendelbach, Manning, & Frawley, 2002; Engle, W.D. et al., 2009; Ho, 1991; Johnson, Angelus, Aldrich, & Skipper, 1986; Saland, McNamara, & Cohen, 1974).

Of all the risk factors for hyperbilirubinemia that have been reported, decreased gestational age is the factor that has been identified most consistently (Keren et al., 2008; Maisels et al., 2009b; Newman, Xiong, Gonzales, & Escobar, 2000). Premature infants born before 33 weeks' gestation are at increased risk for neurologic dysfunction at lower TSB levels (Mazeiras et al., 2012). These observations reflect the elevated bilirubin production, decreased ability to metabolize and eliminate bilirubin, and increased central nervous system sensitivity to bilirubin in the immature, low birth-weight neonate (Cashore, 2000). However, it is important to note that the degree of prematurity does not need to be that pronounced. Infants with gestational ages between 34 and 36 weeks (i.e., late preterm) have been shown to have higher TSB levels compared to term neonates, and have an approximately eightfold increased risk for the development of
hyperbilirubinemia, defined as a TSB level of 20 mg/dL or higher (Newman et al., 1999; Sarici et al., 2004). In addition, maximum TcB levels are higher and sustained longer in late preterm infants compared with term infants (DeLuca et al., 2008; Fouzas, Mantagou, et al., 2010; Maisels & Kring, 2006).

More recently, attention has been focused on a subgroup of term infants known as “early term,” defined as infants born from 370/7 through 386/7 weeks of gestation (Fleischman, Oinuma, & Clark, 2010). A growing body of evidence suggests that this group of newborns may be at greater risk of neonatal morbidity compared with infants born at ≥ 39 weeks of gestation. Population-based studies have shown that early term infants are at increased risk for a composite outcome measure of various conditions or events that warranted admission to the neonatal intensive care unit, including neonatal jaundice (Bastek et al., 2008; Bates et al., 2010; Kamath, Marcotte, & DeFranco, 2011; Tita et al., 2009; Wilmink et al., 2010). Yet, it is difficult to make comparisons between investigations or to interpret the severity of neonatal jaundice in early term neonates.

The first challenge is the lack of consistency in defining significant hyperbilirubinemia in previous research. Not only did multiple studies use different terms to describe the outcome measure, including “hyperbilirubinemia,” “hyperbilirubinemia requiring phototherapy,” “treatment for hyperbilirubinemia,” and “phototherapy,” none of the studies provided additional information, such as how bilirubin levels were described (e.g., mg/dL or µmol/L) or the thresholds of bilirubin levels that were used for the initiation of phototherapy. A second challenge is the heterogeneity of the study populations. Although infants were defined as term and late preterm, which are mutually exclusive categories that have been defined precisely according to week and day (e.g., 370/7) of gestation by the AAP and the American College of Obstetricians & Gynecologists (ACOG), investigators grouped together infants from different gestational age
classifications (W.A. Engle, Tomaskek, Wallman, & the Committee on Fetus and Newborn, 2007). For example, infants born at 36 and 37 weeks of gestation were grouped together and infants born at 38 weeks were grouped with those born at 39 and 40 weeks’ gestation (Bastek et al., 2008; Bates et al., 2010). Recently, the March of Dimes, Society for Maternal-Fetal Medicine, and the American College of Obstetricians and Gynecologists have recommended a standardization of the nomenclature used for gestational age classification as presented in Figure 1.1 (Fleischman et al., 2010).

![Figure 1.1 Concepts of Completed Weeks for Late Preterm, Early Term, and Term Gestation. Reprinted with permission from “Concept of gestational age in ‘completed weeks’: Lost in translation,” by Chabra, S., 2012, Obstetricians and Gynecologists, 119(1), p. 184](image)

**Framework**

*The Engle Model of Transitional Neonatal Hyperbilirubinemia*

The model that guided this study (Figure 1.2) was created using the physiologic principles of neonatal hyperbilirubinemia as described by Stevenson, Dennery, and Hintz (2001) and Hansen (2010). Following birth, the newborn must make the transition from the intrauterine to the extrauterine pattern of heme catabolism and bilirubin physiology. Neonatal hyperbilirubinemia results from a predisposition to the production of bilirubin in newborn infants and their limited ability to eliminate bilirubin from the body, which is temporarily intensified during the transition after birth. This can result in an imbalance between bilirubin production and bilirubin elimination. Thus, the value of the bilirubin
concentration measured at any point in time is due to the net balance between bilirubin production and bilirubin elimination (Bhutani & Johnson, 2003).

Bilirubin production

Overall, three processes can lead to an increased production of bilirubin in the neonate: increased hemoglobin destruction, altered albumin-bilirubin binding, and increased entero-hepatic circulation. Together, these processes contribute to a two-to three-fold increase in bilirubin production in the neonate versus that observed in adults (Stevenson et al., 1994). Bilirubin is the end product of heme catabolism, with the primary source being hemoglobin derived from senescent red blood cells (RBC). The healthy newborn produces 8 to 10 mg/kg of bilirubin per day, and this increased production can be explained primarily by a shortened lifespan of the RBC (Wong, Stevenson, Ahlfors, & Vreman, 2007). Compared to the average life span of the RBC in the adult (120 days), RBCs have an average life span of about 60-70 days in the term infant and about 35-50 days in the premature infant (Brugnara & Platt, 2009). In addition, the newborn has a greater circulating RBC volume resulting from residual fetal hematopoietic tissue.

In blood and extravascular fluid, bilirubin is reversibly bound to albumin. Because albumin has a strong binding affinity for bilirubin, concentrations of unbound or “free” bilirubin are minute; however, when the concentration of bilirubin exceeds the amount that can be bound to albumin, free bilirubin concentrations may increase significantly (Brumbaugh & Gourley, 2012; Wong et al., 2007). It is this form of bilirubin that can enter the brain, interstitial fluid, and cerebrospinal fluid, and it is free bilirubin that is believed to be responsible for bilirubin neurotoxicity (Shapiro, 2003). In the healthy, term newborn albumin levels are lower and there is decreased albumin-binding capacity for bilirubin and other substances (e.g., medications), than in the older child or adult. Albumin levels increase by about 30% in the first week of life, but do not reach adult levels until about 5
months of age. In addition, the affinity of albumin to bind with bilirubin is reduced in sick, low birth weight, and/or premature infants. These deficiencies can result in higher levels of free bilirubin in the circulation that can potentially diffuse into the central nervous system (Kanakoudi et al., 1995).

An additional source of bilirubin production in the neonate is the re-absorption of bilirubin from the intestinal mucosa (i.e., enterohepatic circulation) (Maisels, 2005). The enterohepatic circulation of bilirubin is promoted by reduced intestinal flora, high levels of \( \beta \)-glucuronidase, and decreased intestinal motility (Ives, 1997). The neonate is born with a sterile gut, and the intestinal flora that normally contributes to the breakdown of bilirubin is not established for several days to weeks. In addition, the concentration of \( \beta \)-glucuronidase, an intestinal enzyme that hydrolyzes or “deconjugates” conjugated bilirubin, is approximately 10 times higher in newborns than in adults (Gourley & Odell, 1989). The longer conjugated bilirubin remains in the small intestine, the more likely it will be deconjugated and reabsorbed. This process frequently occurs in exclusively breast fed infants until lactation is well established or in newborns who have delayed feedings. The cycle of deconjugation and reabsorption of bilirubin in the enterohepatic circulation increases the bilirubin load to the liver, thus increasing serum bilirubin levels (Watchko, 2000).
This model illustrates the imbalance between bilirubin production and bilirubin elimination in the transition after birth. On the left side of the diagram are the various processes that can result in an increased rate of bilirubin production. On the right side of the diagram are the processes that can result in decreased bilirubin elimination from the body. RBC = red blood cell.
Bilirubin elimination

The ability of the neonate to efficiently eliminate bilirubin is limited due to a diminished capacity of the liver to metabolize bilirubin. This metabolic process involves hepatic uptake of bilirubin and hepatic bilirubin conjugation (Gourley & Odell, 1989). Hepatic uptake occurs when the bilirubin is released from albumin into the liver and binds to an intracellular carrier protein, i.e., ligandin. The binding of bilirubin to ligandin helps prevent reflux of bilirubin from the liver back into the extracellular circulation, and it also facilitates conjugation. Ligandin levels in the neonate do not reach adult levels until several weeks after birth (Muslu et al., 2008). This transitional deficiency results in a slower rate of hepatic uptake of bilirubin, which can decrease bilirubin elimination. The importance of the conjugation process is that it makes bilirubin water-soluble so that it can be eliminated from the body (Berk, 2004). This biotransformation involves the action of the hepatic conjugation enzyme (UDPGT). Between 17 to 30 weeks of gestation, UDPGT activity is only 0.1% of adult values, increasing to 1% of adult values between 17-30 weeks’ gestation, and reaching adult values by 14 weeks after birth (Kawade & Onishi, 1981).

Purpose

Timely detection of jaundice and the identification of those infants at risk developing hyperbilirubinemia are essential to avoid bilirubin-induced morbidities. Transcutaneous bilirubin nomograms offer a clinically useful tool to identity infants at risk for the subsequent development of hyperbilirubinemia; however, there have been scant studies examining the predictive ability of nomograms. The primary purpose of this study was to validate a previously published but unvalidated TcB nomogram (hereafter referred to as the Parkland Hispanic TcB nomogram) to independently determine its accuracy in identifying Hispanic neonates at risk for the subsequent development of significant
hyperbilirubinemia (TcB > 95th percentile). A secondary purpose of this study was to compare TcB values between early term and term newborns to determine if, because of their relative physiologic immaturity, early term neonates have higher TcB values compared to infants born after 38 weeks of gestation.

**Research Questions**

In this study the following research questions were addressed:

1. Is the 50th or the 75th percentile of the Parkland Hispanic TcB nomogram able to identify those Hispanic neonates who develop subsequent significant hyperbilirubinemia (TcB > 95th percentile) during their birth hospitalization?

2. Are there significant differences in hour-specific TcB values between early term and term neonates?

**Assumptions**

1. The Parkland Hispanic TcB nomogram correctly identifies percentile values for TcB in Hispanic neonates up to the third day of life.

2. The 95th percentile of a TcB nomogram has clinical relevance, and as a group, infants with TcB values greater than the 95th percentile are at greater risk than infants with TcB values at lower percentiles; therefore, this assumption allows the use of the 95th percentile as a relevant outcome in the assessment of predictive statistical measures, such as sensitivity and specificity.

**Summary**

Transitional neonatal hyperbilirubinemia is an almost universal finding during the first postnatal week. Following birth, the metabolism of bilirubin is in transition from the fetal stage of development to the adult stage. Elevated bilirubin levels are the result of an imbalance between increased production and excretion of bilirubin, which reflects the transitional immaturity of hematologic, hepatic, and gastrointestinal systems following
birth. Transcutaneous bilirubin measurement is widely used as a painless, instantaneous, and accurate screening method for neonatal hyperbilirubinemia. Screening of neonates prior to hospital discharge can detect bilirubin levels that require treatment and predict those infants who are at increased risk of the subsequent development of significant hyperbilirubinemia. The validity of a TcB nomogram should be established in a second, independent population of neonates with similar known risk factors. The use of a validated TcB nomogram can provide a useful clinical tool to help in the identification and management of neonatal hyperbilirubinemia. Research is needed to fill this gap, as well as to describe differences in TcB values between neonates of different gestational ages during the early postnatal period.
Chapter 2
Critical Review of Relevant Literature

Introduction

The purpose of this chapter is to review the current level of knowledge about risk-based strategies used to assess a neonate's risk for the development of hyperbilirubinemia. This review begins by addressing the current American Academy of Pediatrics’ (AAP, 2004; Maisels et al., 2009a) clinical practice guidelines for the management of neonatal hyperbilirubinemia. The remaining sections of the review are organized according to research related to the various risk-based strategies recommended by the AAP. This chapter concludes with a comparison of studies of transcutaneous bilirubin (TcB) value plotted on a TcB nomogram, and will identify some of the gaps in the current knowledge relating to transitional neonatal hyperbilirubinemia, including implications for nursing research.

Review of Relevant Literature

Risk-based Strategies to Assess the Risk of Neonatal Hyperbilirubinemia

Early diagnosis of neonatal hyperbilirubinemia has become increasingly important due to shorter hospital stays following childbirth. By the mid-1990s, the average length of hospital stays for women and their newborns following vaginal birth had dropped to two days compared with four days in 1970 (Martell, 2000). In transitional neonatal hyperbilirubinemia, rising bilirubin levels usually reach maximum concentration (i.e., peak) between age 72 and 120 hours (Bhutani & Johnson, 2009b).
Discharge of newborns within 72 hours of birth has been associated with increased hospital readmission rates of infants for neonatal conditions, such as hyperbilirubinemia, that may not give rise to signs or symptoms shortly after birth (Bravo, Uribe, & Contreras, 2011; Burgos, Schmitt, Stevenson, & Phibbs, 2008; Maisels & Kring, 1998). Consequently, shortened postnatal hospital stays potentially increase the risk of not detecting significant hyperbilirubinemia as discharge occurs when serum bilirubin is still increasing (Bhat & Rao, 2008).

In the current environment of early postnatal discharge, efforts have been made to reduce the frequency of severe neonatal hyperbilirubinemia and bilirubin-induced encephalopathy by instituting systematic risk-based strategies that promote the timely identification of jaundice in the hospital nursery and evaluation of an infant’s risk for developing severe hyperbilirubinemia (AAP, 2004). In 2004, the AAP published clinical practice guidelines on the prevention and management of neonatal hyperbilirubinemia in late preterm (defined as birth from 34\textsuperscript{0/7} through 36\textsuperscript{6/7} weeks of gestation), and term (defined as birth from 37\textsuperscript{0/7} through 41\textsuperscript{6/7} weeks of gestation) neonates. The guidelines recommend two risk-based strategies, used individually or in combination prior to hospital discharge, to assess a neonate’s risk for the subsequent development of hyperbilirubinemia: (1) an assessment of an neonate’s clinical risk factors for the development of hyperbilirubinemia, and (2) measurement of either total serum bilirubin (TSB) or TcB with the resulting values plotted on an hour-specific bilirubin nomogram.

**Clinical Risk Factors**

Despite decades of research, defining what represents a normal or safe bilirubin level in the term and late preterm neonate has proven elusive. Large epidemiologic studies have identified multiple factors associated with some effect on neonatal bilirubin levels. However, because these risk factors are common and the risk of extreme
hyperbilirubinemia is small, these factors may be individually of limited use as predictors of significant hyperbilirubinemia (Maisels & Newman, 2012; Newman, Easterling, Goldman, & Stevenson, 1990). Nevertheless, maximum bilirubin values vary considerably by race or ethnicity, whether or not the infant was exclusively breast-fed, and decreased gestational age (Maisels & Kring, 2006).

Race or ethnicity

While most neonates experience transitional hyperbilirubinemia, the incidence of significant levels of bilirubin is not equal across all populations of neonates and varies significantly with race or ethnicity (Wasser & Hershakovitz, 2010). Neonates of East Asian, American Indian, and Native Canadian ancestry have a higher incidence of hyperbilirubinemia than Caucasian or African American neonates (Huang, Tai, Wong, Lee, & Yong, 2009; Lin et al., 1985; Maisels & Newman, 1999; Munroe, Shah, Badgley, & Bain, 1984; Setia, Villavaces, Dhillon, & Meueller, 2002). Similarly, a recent study showed that maximum TSB concentrations in Hispanic neonates, primarily of Mexican descent, are significantly higher than those of white infants (W.D. Engle et al., 2002).

In a nested case-control study of a cohort of 51,387 newborns, (Newman et al., 2000) biological predictors of extreme hyperbilirubinemia were examined in a large Californian health maintenance organization. Cases (n = 73) were newborns with peak TSB levels ≥ 25 mg/dL, and controls (n = 428) were a random sample of newborns from the cohort with a peak TSB level < 25 mg/dL. The investigators found that an infant born to a woman of Asian ethnicity was greater than three times more likely to have a TSB level ≥ 25 mg/dL (Odds Ratio [OR] = 3.1, 95% CI [1.5-6.3]).

Ethnic variation in the incidence of neonatal jaundice diagnosis was examined in a population-based cohort in Washington State (Setia et al., 2002). Diagnoses of jaundice and severe jaundice were identified using International Classification of
Diseases, Ninth Revision (ICD-9) diagnosis and procedure codes. Study participants were composed of infants of full East Asian parentage (n = 3000), maternal Asian parentage (n = 2997), paternal Asian parentage (n = 2048), and full white parentage (n = 3000). The investigators found that infants of full East Asian parentage were more likely to receive a diagnosis of jaundice than were white infants (relative risk [RR] = 1.37, 95% CI [1.16-1.62]). For infants with Asian mothers and white fathers, the RR was 1.09 (95% CI [0.91-1.30]), and infants with Asian fathers and white mothers had an RR of 1.26 (95% CI [1.05-1.52]). The risk of severe jaundice, defined as jaundice requiring phototherapy, exchange blood transfusion, or rehospitalization, was significantly increased only for infants of full East Asian parentage (RR = 1.7, 95% CI [1.12-2.58]).

Studies conducted in the 1970s and 1980s revealed that American Indian, Alaskan and Canadian Native infants had higher TSB levels compared to those infants in other races or ethnicities. Saland and colleagues (1974) compared TSB levels in Navajo infants (n = 47) with a control group of infants (n = 36), composed of infants born to black, Puerto Rican, and white mothers. Maximum TSB levels in Navajo neonates were significantly higher than those of the control group throughout the first four days of life.

Total serum bilirubin levels and rates of bilirubin production were compared in Navajo and Caucasian neonates (Johnson et al., 1986). Rates of bilirubin production were determined by measuring the end tidal endogenous excretion of carbon monoxide (ETCOc), which closely approximates the rate of bilirubin production (Stevenson, Bartoletti, Ostrander, & Johnson, 1980). Navajo newborns had significantly higher TSB levels (8.6 ± 2.5 mg/dL, p < .05) and ETCOc excretion (27.6 ± 6.6 μL/kg/h, p < .001) compared with Caucasian infants (TSB 6.9 ± 2.3 mg/dL; ETCOc 21.0 ± 4.5 μL/kg/h).

Total serum bilirubin levels obtained during first week of life in Alaskan Inuit (n = 119) and Canadian Caucasian (n = 25) newborns were compared to explore differences
in patterns of physiologic jaundice (Postl, Nelson, & Carson, 1982). Maximum TSB levels in the Inuit neonates were significantly higher than in the Caucasian infants (8.76 versus 6.04 mg/dL, \( p < .05 \), respectively). In addition, peak TSB levels occurred later in the week for the Inuit infants compared to the Caucasian infants (day 3 versus day 2, respectively).

A limitation of previous studies of neonatal hyperbilirubinemia has been the inclusion of relatively few Hispanic infants; despite the fact the Hispanics comprise 16% of the total U.S. population (U.S. Census Bureau, 2011). One contemporary study of hyperbilirubinemia included a large percentage of newborn patients born to mothers of Hispanic origin (W.D. Engle et al., 2002). Total serum bilirubin levels obtained from 248 Hispanic (82%) and 56 non-Hispanic (18%) neonates were compared during the first week of life either during postnatal hospitalization or at a hospital-based follow-up clinic. Hispanic infants had significantly \( (p < .001) \) higher maximum TSB levels compared with non-Hispanic infants. For example, 31% of the Hispanic infants \((n = 248)\) had TSB levels \( \geq 15 \) mg/dL compared with 16% of the non-Hispanic neonates \((n = 56)\).

Breastfeeding

Despite the advantages of breastfeeding, there is evidence of a strong association between exclusive breastfeeding (i.e., no formula supplementation) and an increase in risk for neonatal hyperbilirubinemia (Gourley, 2002). The association between breastfeeding and maximal TSB levels \( \geq 12.9 \) mg/dL was examined in 2,416 primarily white (> 95%) infants consecutively admitted to a hospital well-baby nursery (Maisels, Gifford, Antle, & Leib, 1986). Significant hyperbilirubinemia was defined as TSB \( > 12.9 \) mg/dL, and was identified in 147 infants (6.1%). Infants identified with significant hyperbilirubinemia were compared with 147 randomly selected control infants with maximum TSB levels \( \leq 12.9 \) mg/dL. Breastfeeding was significantly associated with hyperbilirubinemia during the first three days of life. Of infants in the study group, in
whom no other cause for hyperbilirubinemia was found, 82.7% were exclusively breast-fed compared with 46.9% in the control group ($p < .00001$). In addition, maximum TSB levels were higher in the breast-fed group compared to the formula-fed group. The 95th percentile for formula-fed infants was a TSB value of 11.4 mg/dL versus 14.5 mg/dL in the breast-fed group, and the 97th percentiles were 12.4 and 14.8 mg/dL, respectively.

One review of 12 studies involving more than 8,000 neonates in the first week of life revealed that compared to formula-fed infants, breast-fed infants had significantly higher maximum TSB levels (Schneider, 1986). "Moderate" hyperbilirubinemia, defined as TSB ≥ 12 mg/dL, was present in 12.9% of the breast-fed infants compared to 4% of the formula-fed infants ($p < .00001$).

Gestational age

According to the AAP (2004) regarding the risk for the development of hyperbilirubinemia, birth from 35\(0/7\) to 36\(6/7\) weeks of gestation (i.e., late preterm) is delineated a "major" risk factor, birth from 37\(0/7\) through 38\(6/7\) weeks of gestation (i.e., early term) is delineated a "minor" risk factor, while birth at ≥ 41 weeks of gestation (i.e., term) is considered to decrease risk. Previous studies of neonatal hyperbilirubinemia in term and late preterm infants have confirmed that decreased gestational age is the most important factor associated with the development of significant hyperbilirubinemia (Gale, Seidman, Dollberg, & Stevenson, 1990; Linn et al., 1985; Maisels & Kring, 1998; Newman et al., 1999).

In a retrospective study, the incidence of “identified hyperbilirubinemia” was investigated in a multicenter cohort of 51,387 late preterm and term infants (Newman et al., 1999). Bilirubin levels (TSB) were obtained at the discretion of the medical provider when an infant was visually observed to have jaundice (i.e., identified hyperbilirubinemia), rather than routine screening with a TSB. Maximum TSB levels of ≥ 20 mg/dL in the first
30 days of life were identified in 2.0% of all births, and maximum TSB levels ≥ 25 mg/dL were identified in 0.15%. A strong association was found between hyperbilirubinemia and gestational age. Compared with infants born at 41 weeks (0.7%), infants born at 37 weeks (5.7%) were at an eight-fold increased risk, and infants born at 38 weeks (3.2%) were at an almost 5-fold increased risk for developing TSB levels ≥ 20 mg/dL (p < .001).

The effect of early discharge following birth on the risk of readmission to the same hospital was examined in a cohort of over 29,000 infants, with specific reference to readmission for hyperbilirubinemia (Maisels & Kring, 1998). During the study period, they found that the incidence rate for readmission for hyperbilirubinemia was 4.2 per 1000 discharges. Factors associated with an increased risk for readmission included gestational age and a birth hospital length of stay less than 72 hours. Early term infants were more than seven times more likely (OR = 7.2, 95% CI [3.05-16.97]) to be readmitted for jaundice compared with infants born at 40 weeks’ gestation or greater. This association, however, was partly due to lower treatment thresholds for phototherapy in less mature infants.

Recently, the magnitude of gestational age as a risk factor for the development of hyperbilirubinemia has been quantified; however, the results are conflicting. In a nested case-control study from a cohort of 51,387 late preterm and term infants, the accuracy of a risk index model in predicting “extreme” hyperbilirubinemia (TSB levels ≥25 mg/dL) was examined (Newman et al., 2000). Cases (n = 73) were newborns with peak TSB levels ≥25 mg/dL. Controls (n = 423) were a random sample of newborns from the cohort with peak TSB levels <25 mg/dL. Decreasing gestational age was found to be a strong predictor of extreme hyperbilirubinemia. For each decreasing week of gestation, extreme hyperbilirubinemia was associated with case status 0.6 per week (95% CI [0.4-0.7]). The OR was converted to a “risk index” for predicting the outcome, and findings revealed that
for each decreasing week of gestation below 40 weeks the risk of an infant developing extreme hyperbilirubinemia increased by a factor of approximately 1.6. Thus, an infant born early-term is about three to four times more likely to develop the outcome than an infant born after 40 weeks. The authors concluded that because there was a high prevalence of the strongest risk factors (e.g., exclusive breastfeeding and decreased gestational age) among cases, and the incidence of extreme hyperbilirubinemia in this population was relatively rare, clinical variables were better at predicting infants at low risk than at high risk for extreme hyperbilirubinemia.

In a retrospective study of late preterm and term infants (n = 2,840), infants born at less than 38 weeks' gestation were almost three times more likely (OR = 2.6, 95% CI [0.5-4.5]) to develop significant hyperbilirubinemia, defined as a post-discharge TSB > 95th percentile on a TSB nomogram, compared with the reference group of infants born at 38 and 39 weeks (Keren et al., 2005). A limitation of this study is that gestational age was rounded to the nearest week.

In a nested case-control study, the effect of different clinical risk factors was examined in late preterm and term neonates with a qualifying predischarge TSB levels between 17 and 22.9 mg/dL at postnatal age ≥ 48 hours on the subsequent development of significant hyperbilirubinemia, defined as TSB ≥ 25 mg/dL (Kuzniewicz et al., 2008). Cases were defined as newborns (n = 62) who had TSB ≥ 25 mg/dL after the qualifying predischarge TSB. Four randomly selected controls (n = 248) were matched to each case based on their risk group for phototherapy as defined by the AAP (2004). Among the important predictors of case status was lower gestational age and exclusive breastfeeding. When adjusted for the presence of additional risk factors, birth from 38 to 39 weeks’ gestation was a significant predictor for developing significant hyperbilirubinemia (adjusted odds ratio [aOR] = 3.12, 95% CI [1.21-8.03]). A similar trend
was seen in infants born at 34 to 37 weeks’ gestation, although it was not statistically significant ($aOR = 3.74$, 95% CI [0.62-22.7]). It should be noted that gestational age categories were not stratified based on the mutually exclusive, standardized classifications such as late preterm, early term, and term (W.A. Engle et al., 2007; W.A. Engle & Kominiarek, 2008; World Health Organization [WHO], 2010).

In a nested case-control study, infants born at 37 weeks' gestation were almost 15 times more likely to be readmitted to the hospital for significant hyperbilirubinemia ($aOR = 14.86$, 95% CI [1.91-115.38]) compared with infants born at ≥ 40 weeks (Maisels et al., 2009b). This observation was similar to the risk for late preterm infants ($aOR = 20.79$; 95% CI [2.34, 184.74]); however, infants born at 38 weeks were not at significantly increased risk ($aOR = 1.78$; 95% CI [0.29, 7.22]).

**Predischarge Total Serum Bilirubin (TSB) Measurement**

Jaundice appearing in the first 24 hours after birth is generally considered pathologic, and it always requires further evaluation (AAP, 2004). Newborns observed to have jaundice within the first 24 postnatal hours are more likely to subsequently develop significant hyperbilirubinemia (Newman, Liljestrand, & Escobar, 2002). In addition, infants experiencing jaundice in the first or second day of life are more likely to be readmitted for hyperbilirubinemia (Maisels & Kring, 1998). According to the AAP (2004), the best documented method for predicting a newborn’s risk for the subsequent development of hyperbilirubinemia is to measure the TSB or TcB level and plot the results on an hour-specific bilirubin nomogram. The first bilirubin nomogram, published in 1999 by Bhutani and associates, is included in the 2004 AAP clinical practice guidelines.

**Total serum bilirubin nomogram**

Bhutani and associates (1999) introduced universal TSB determination in their landmark study to determine if bilirubin values obtained on all newborn patients before
hospital discharge would predict significant hyperbilirubinemia (defined as TSB levels ≥ 95th nomogram percentile value) during the first week of life. Prior to discharge from the birth hospital, TSB determinations were obtained at the time of the routine metabolic screen in 13,003 late preterm and term neonates. Study participants (n = 2,840) included infants who were eligible for early discharge, defined as prior to 72 hours of age, did not require phototherapy prior to discharge, and were enrolled in an optional outpatient follow-up program. In the study group, additional TSB levels were measured at least once in the six days following discharge from the birth hospital. All TSB values obtained from the study participants were plotted against the infant’s postnatal age (in hours) and a TSB nomogram was constructed, hereafter referred to as the Bhutani nomogram. Percentiles were identified that define four risk zones used to predict subsequent hyperbilirubinemia including the high risk zone: above the 95th percentile; low-risk zone: values below the 40th percentile; and two intermediate risk zones: values between the 40th to 95th percentiles, which were subdivided by the 75th percentile into upper- and lower-intermediate risk zones. The investigators reported that an hour-specific TSB measured prior to hospital discharge can predict which newborn is at high, intermediate or low-risk for subsequently developing significant hyperbilirubinemia.

The findings from the study by Bhutani et al. (1999) revolutionized the clinical management and research of neonatal jaundice in several ways. First, the Bhutani nomogram provides an easily understood and graphic clinical tool for identifying infants who need additional evaluation during postnatal hospitalization and surveillance following discharge (Maisels, 2006). In addition, the nomogram visually highlights the physiologic principle that in transitional neonatal hyperbilirubinemia, TSB is changing constantly and should be interpreted only in relationship to the infant’s postnatal age in hours not days, i.e., “hour-specific” (Maisels & Newman, 1999).
Although the Bhutani nomogram has impacted the care of the jaundiced neonate, its use as a clinical tool has been questioned due to concerns regarding methodological flaws of the original study (Maisels & Newman, 1999). The presence or absence of neonatal hyperbilirubinemia following hospital discharge was not determined in 10,163 (78.2%) of the 13,003 infants undergoing pre-discharge TSB testing. Only 2,976 (22.8%) of the cohort had TSB levels measured as an outpatient, which occurred at the physician’s discretion and for noncompulsory enrollment in the outpatient follow-up program. The fact that TSB testing after discharge was optional rather than universal appears to have had a significant effect on the nomogram, especially if only the most jaundiced patients returned for follow-up. For example, TSB levels at the low-risk zone (i.e., 40th percentile) obtained following discharge reported are much higher than maximum TSB levels reported in any previous study (Maisels & Gifford, 1986; Saigal, Lunyk, Bennett, & Patterson, 1982; Stevenson, Dennery et al., 2001), including values for exclusively breast-fed Japanese infants (Yamauchi & Yamanouchi, 1989a). Likewise, the generalizability of the Bhutani nomogram to the overall U.S. or international population is questionable, since it was developed in a select population of primarily white (43.4%) and black (41.2%) infants born at a single hospital in Pennsylvania. Therefore, it would not be appropriate to use this nomogram to determine risk in other populations of infants where different risk factors may be present (Maisels & Kring, 2006).

The outcome targeted for prediction in the Bhutani nomogram study was a post-discharge bilirubin level ≥ the 95th percentile, termed “significant hyperbilirubinemia” (Bhutani et al., 1999). Two studies examined the incidence of false-negative results of predischarge screening based on the Bhutani nomogram risk zones. A retrospective review was performed to identify infants who were readmitted for TSB > 17 mg/dL in a Southeastern pediatric tertiary care center (Slaughter, Annibale, & Suresh, 2009).
Readmitted infants whose predischarge bilirubin levels (TSB or TcB) were in the low-risk (< 40th percentile) and low-intermediate (40-75th percentile) risk zones of the Bhutani nomogram were considered false-negatives. During the 51-month study period, 28 of 6,220 (0.45%) were readmitted for treatment of hyperbilirubinemia, and predischarge bilirubin values were in the low-intermediate risk zone in 12 of these infants (43%). The authors concluded that nearly half of readmitted infants had predischarge bilirubin values in the low or low-intermediate risk zones of the AAP-recommended nomogram; therefore, infants classified as “low-risk” really may be at significant risk.

In Israel, the incidence of false-negative results, defined as predischarge TSB or TcB ≥ 75th percentile, among infants who were readmitted for primary phototherapy (i.e., had not been treated with phototherapy during birth hospitalization) was examined (Bromiker, Binn-Nunn, Schimmel, Hammerman, & Phibbs, 2012). Of 25,439 neonates born between 2008 and 2009, 145 (0.56%) were readmitted with a mean TSB 18.7 mg/dL. False-negative predischarge bilirubin values were identified in 46 (32.2%) infants who were readmitted. Of these, six (4.2%) had a predischarge bilirubin value in the low-risk zone (RR = 1) and 40 (28%) had a predischarge bilirubin value in the low-intermediate risk zone (RR = 7.82, 95% CI [3.23-17.96]). The authors concluded that predischarge bilirubin levels classified as low risk did not eliminate the risk of readmission for significant neonatal hyperbilirubinemia.

**Combining Clinical Risk Factors with Predischarge Bilirubin Measurement**

One of the AAP’s recommended strategies for evaluating the risk of neonatal hyperbilirubinemia is using a combined approach. This approach involves the combined assessment of a TSB or TcB expressed as a percentile risk zone on the Bhutani nomogram and an evaluation of an infant’s clinical risk factors for hyperbilirubinemia (AAP, 2004).
Clinical risk factors and TSB

Two studies examined the effectiveness of using a combined approach to evaluate infants for the risk of hyperbilirubinemia; however, the results were mixed. Except for exclusive breastfeeding and gestational age, there were no common clinical risk factors between the two studies. Both of the studies used a retrospective approach for data collection; therefore, the accuracy of the evaluations of clinical risk factors may have been attenuated by any missing data (Newman, Liljestrand, & Escobar, 2005). In addition, TSB determinations were made based on visual assessment of jaundice, rather than universal screening.

In a retrospective cohort of neonates ≥ 36 weeks of gestation (n = 5,706), the accuracy of combining clinical risk factors and TSB levels measured prior to hospital discharge to predict a subsequent (first 30 days after birth) TSB level of ≥ 20 mg/dL was examined (Newman et al., 2005). They reported a c-statistic [c] of 0.69 (no data reported for the 95% CI) for the risk index in predicting a TSB level ≥ 20 mg/dL at 48 hours. Combining the predischarge TSB level with clinical risk factors improved prediction over the TSB alone (c = 0.86 versus 0.79, respectively), largely due to the effect of gestational age. For example, when the predischarge TSB level was ≥ 95th percentile (i.e., high risk zone), the risk of subsequently developing hyperbilirubinemia increased from approximately 10% for neonates born at ≥ 40 weeks to over 40% for those born at 36 weeks. For infants whose predischarge TSB was in the high risk zone, birth at 37, 38, and 39 weeks of gestation was associated with a decreased chance of subsequently developing hyperbilirubinemia (35%, 20%, and 15%, respectively).

In a retrospective cohort of late preterm and term neonates (n = 996), the accuracy of combining a clinical risk factor index and predischarge TSB levels to predict significant hyperbilirubinemia, defined as TSB > 95th percentile was examined (Keren et
The predictive accuracy of predischarge TSB risk zone was evaluated separately from the risk factor model. Findings revealed that the predischarge TSB level expressed as a risk zone had better discrimination ($c = 0.83$, 95% CI [0.80-0.86]) than the clinical risk factor model alone ($c = 0.71$, 95% CI [0.66-0.76]) for predicting significant hyperbilirubinemia. Neither the risk index nor the predischarge TSB risk zone predicted the outcome with $\geq 98\%$ sensitivity without severely compromising specificity. For example, using the 40th percentile reached 99% sensitivity, but incorrectly labeled 79% of infants without hyperbilirubinemia as being at risk for developing the outcome (i.e., specificity = 0.21).

Clinical risk factors and TcB measurement

Three groups of investigators examined the efficacy of using clinical risk factors combined with universal TcB measurements, expressed as a risk zone on the Bhutani nomogram, in predicting the need for phototherapy in three diverse populations of neonates. It should be noted that bias may be present in using this outcome variable because the level of bilirubin at which phototherapy treatment is recommended for infants born from 35 through 37 weeks’ gestation is about 2.5 mg/dL lower than for infants born at 40 weeks’ gestation (AAP, 2004).

A cohort of 812 late preterm and term infants were recruited to prospectively compare the accuracy of an alternate risk assessment strategy to predict significant hyperbilirubinemia (Keren et al., 2008). The study population was composed of primarily black (53%), white (34%), and East Asian (8%) infants born at a single center in Pennsylvania. Significant hyperbilirubinemia was defined as a TcB level that exceeded or was within 1 mg/dL of the hour-specific TSB threshold for phototherapy recommended by the AAP (2004). Three risk assessment strategies were evaluated: (a) a predischarge TcB value expressed as risk zone on the Bhutani nomogram; (b) clinical risk factors, and
(c) a combination of the predischarge risk zone, gestational age and percentage of postnatal weight loss. For purposes of analysis, the investigators rounded gestational age to the nearest whole week and used infants born from 38 to 39 weeks as the referent group. They found that combining the predischarge TcB risk zone with clinical risk factors (c = 0.96, 95% CI [0.93-0.98]) had better overall predictive accuracy than the strategy that used the TcB risk percentile zone alone (c = 0.88, 95% CI [0.85-0.91]) or the clinical risk factor strategy alone (c = 0.91, 95% CI [0.86-0.97]). Further analysis revealed that gestational age was the only clinical risk factor that, when added to the risk zone, significantly improved predictive accuracy (c = 0.952 versus 0.954 for full model). For neonates with a predischarge bilirubin in the high-risk zones, infants born at less than 38 weeks were 9 times more likely to develop significant hyperbilirubinemia (OR = 9.2, 95% CI [4.4-19.0]) compared with infants born at 38 to 39 weeks. The authors noted that because black race is associated with a lower risk of significant hyperbilirubinemia, and because approximately half of the study infants were born to black mothers, the generalizability of this study to populations with fewer black infants may be limited.

In Portugal, a cohort of 463 term and late preterm newborns were recruited to examine the risk-based strategy proposed by Keren et al. (2008) (Goncalves et al., 2011). The predischarge TcB measurement and gestational age was combined to predict significant neonatal hyperbilirubinemia, defined as a TcB/TSB level that was ≥ 1 mg/dL of the AAP (2004) recommended TSB threshold for phototherapy. Similar to the method of analysis used by Keren et al. (2008), investigators rounded gestational age to the nearest whole week and used infants born from 38 to 39 weeks as the referent group. Overall, the investigators found that infants born at < 38 weeks’ gestation had significantly greater risk (RR = 3.41, 95% CI [1.68-6.98]) for significant hyperbilirubinemia compared with infants born at 40 weeks’ gestation or greater. They concluded that the risk assessment
strategy of combining gestational age and predischarge bilirubin risk zone significantly increased the predictive ability compared with predischarge bilirubin zone alone (c = 0.90 versus 0.86, respectively).

In a prospective cohort of late preterm and term neonates (n = 931) born at a hospital in India, the accuracy of combining predischarge TcB level and gestational age was examined for predicting hyperbilirubinemia requiring phototherapy within the first seven postnatal days (Kaur, Chawla, Pathak, & Jain, 2011). Overall, a total of 199 (20%) neonates developed hyperbilirubinemia that required phototherapy. Plotting TcB values on the 75th and 95th percentiles of the Bhutani nomogram to predict hyperbilirubinemia resulted in a sensitivity of only 50.2% and 14.1%, respectively. The 40th percentile had the best accuracy of predicting neonates who did not need subsequent treatment for hyperbilirubinemia (negative predictive value = 92.1% and specificity = 84.9%). Logistic regression analysis revealed that the discriminating abilities of the combined strategy performed better (c = 0.75) than the percentile (c = 0.72) or clinical risk factors (c = 0.58) alone. The investigators concluded that the lower discriminating ability of their various strategies may have been due to the higher proportion of late preterm (mean gestational age 37.8 ± 1.5 weeks) and infants with low birth weight (mean birth weight 2,627 ± 536 g), as well as a higher rate of exclusive breastfeeding (> 80%) in their study population.

The only study to date that has evaluated a combined risk-based strategy using a TcB-based nomogram was conducted by Maisels and associates (2009b). In a nested case-control study of late preterm and term infants, the accuracy of universal predischarge TcB measurements, expressed as risk zones on a previously developed TcB nomogram, combined with clinical risk factors was examined to predict the risk of subsequent hyperbilirubinemia (defined as hospital readmission with a TSB ≥ 17 mg/dL). The three variables that provided the best prediction of case status (c = 0.885) were the
maximum predischarge TcB percentile zone, exclusive breastfeeding, and gestational age. This prediction was significantly better than the use of gestation and exclusive breastfeeding alone ($c = 0.770$, $p < 0.001$) or the TcB percentile zone alone ($c = 0.766$, $p = 0.001$). In addition, compared with formula-fed infants, infants who were exclusively breastfeed were more than 10 times more likely ($aOR = 10.75$, 95% CI [2.37-48.82]) to be readmitted for significant hyperbilirubinemia.

The results of previously described studies prompted an expert consensus group to recommend an even more structured approach to the management of neonatal jaundice including predischarge bilirubin screening for all newborns (i.e., universal) using either TSB or TcB determination and an assessment of risk factors for hyperbilirubinemia (Maisels et al., 2009b). However, the United States Preventive Services Task Force (USPSTF, 2009), supported by a systematic review, concluded that evidence is insufficient to make that recommendation an official clinical practice guideline.

Transcutaneous Bilirubin (TcB) Nomogram

A systematic risk-based approach to neonatal jaundice requires an understanding of transitional physiologic factors that can affect bilirubin levels in the normal, healthy infant as well as an understanding of the natural history of neonatal jaundice (Maisels & Newman, 2012). The natural history or course of neonatal jaundice involves a distinct pattern of changes in bilirubin levels following birth, which includes an incremental rate of increase (i.e., rate of rise, mg/dL/hour), a peak (maximum level), and a plateau followed by gradual resolution (Maisels, 2005). The availability of modern bilirubinometry has made it possible to obtain serial (as frequently as every 6-12 hours), hour-specific TcB values, and to consolidate the values by age in hours into percentiles on a TcB nomogram. These nomograms provide a visual perspective of the magnitude
and trends in TcB in the context of postnatal age in the population from which they are developed (Maisels, 2012).

Transcutaneous bilirubin nomograms can graphically describe the natural history of bilirubin levels, thus providing a better understanding of hyperbilirubinemia in a given population of infants (DeLuca, Jackson et al., 2009). Knowing the trend (patterns) of bilirubin levels in a population of neonates may offer a more accurate predictor for the risk of the subsequent development of significant hyperbilirubinemia. Hour-specific TcB nomograms have been developed in various neonatal populations worldwide.

In 2006, the first TcB-based nomogram was reported (Maisels & Kring, 2006). Using the JM-103, TcB determinations were obtained from a sample \( n = 3,984 \) of primary white (73.1%) late preterm and term infants, who were admitted to a hospital newborn nursery in the Midwest and who did not require phototherapy prior to discharge. Over 60% of the infants were exclusively breast-fed. A nomogram was developed using TcB values from the entire study sample, as well as a nomogram for each of the following groups of gestational age (in completed weeks): 35-37 weeks, 38-39 weeks, and 40 weeks or greater. The 95th percentile TcB values at 24, 48, and 72 postnatal hours were 6.5, 10.0, and 12.0 mg/dL, respectively. Decreased gestational age and breastfeeding were associated with significantly higher mean TcB levels. Compared with infants born at 40 weeks of gestation or greater, infants born from 35 through 37 weeks had consistently higher TcB levels in the first 24 hours following birth \( (p < .01) \), as well as at the other periods \( (p < .00001) \). Although TcB levels in the first 24 hours were not significantly different compared with infants born at \( \geq 40 \) weeks, infants born between 38 and 39 weeks did have significantly higher TcB levels from 48 to 96 hours of life \( (p < .00001) \).

To date, the only TcB nomogram developed in a cohort of 2,005 Hispanic newborns was reported in 2009 (W.D. Engle et al., 2009). The sample was comprised of
term and late preterm infants (10%) who were admitted to the newborn nursery of a large public hospital in the Southwest and who did not require phototherapy prior to discharge. The 95th percentile values at 24, 48, and 72 postnatal hours were 7.6, 11.0, and 12.4 mg/dL, respectively. Investigators compared hour-specific TcB values at 11 epochs or periods of postnatal age (in hours) from their Hispanic population with TcB data from a predominately white, North American population (Maisels & Kring, 2006). Hour-specific TcB values in the Hispanic population were significantly higher at the majority (82%) of epochs analyzed than the corresponding TcB values in the white, non-Hispanic population. The authors concluded that these findings were strengthened by the increased gestational age and lower incidence of exclusive breastfeeding in their population, factors that should have resulted in lower, not higher TcB values.

In Rome, a TcB nomogram was developed in a predominately breast-fed, Italian population (n = 2,198) of preterm and term newborns during postnatal hospitalization (DeLuca et al., 2008). For infants who subsequently required phototherapy, only pre-treatment TcB values were included in the development of the nomogram. Two TcB nomograms were developed: one for late preterm infants and one for term infants; however, no further analysis regarding the effect of gestational age was conducted. For term infants, the 95th percentile TcB values at 24, 48, and 72 hours of age were 12.0, 13.0, and 14.9 mg/dL, respectively. For late preterm infants, the 95th percentile TcB values at 24, 48, and 72 hours of age were 9.0, 13.7, and 14 mg/dL, respectively. The authors concluded that the relatively higher TcB values at the 95th percentile could be explained by the inclusion of pre-treatment TcB values in infants who subsequently required phototherapy.

A TcB nomogram was developed in a group of healthy, late preterm Greek infants (n = 793) to provide data on the natural history of TcB levels for the first 120
postnatal hours in this population (Fouzas, Skylogianni, Mantagou, & Varvarigou, 2010). The 95th percentile at 24, 48, and 72 hours of age were 8.3, 11.6, and 14.0 mg/dL, respectively. In addition, mean TcB rates of rise between the neonates who did and did not require phototherapy was compared. Between 24 and 48 hours of age, the rates of increase were similar; however, in neonates who did not require phototherapy, the incremental rates decreased, close to zero by the fourth day of life. This is contrasted with infants who subsequently required phototherapy, in whom the rate of rise remained high throughout the fourth day of life. In contrast to other studies, breastfeeding was not related to an increased risk for developing significant hyperbilirubinemia ($OR = 1.09$, 95% CI [0.51-2.38]).

*Predicting Neonatal Hyperbilirubinemia Using a TcB Nomogram*

Over the past 20 years, several hour-specific bilirubin nomograms have been developed in diverse neonatal populations and are available for clinical use. This most likely is in response to the AAP (2004) request for nomograms specifically designed for populations that differ with regard to risk factors for significant hyperbilirubinemia. Investigators have reported to have validated the ability of TcB nomograms to predict the development of significant neonatal hyperbilirubinemia. Yet, after their development, none of these nomograms have been formally validated in a separate, independent population of neonates (Sackett, Straus, Richardson, Rosenberg, & Haynes, 2000).

**Thailand**

A cohort of 392 healthy, term neonates who were delivered by cesarean section in a Thai hospital were recruited to develop an hour-specific TcB nomogram and to identify the percentile that would predict significant hyperbilirubinemia, defined as a the need for phototherapy (Sanpavat et al.). Hour-specific TcB measurements were obtained daily until four days (96 hours) after birth or until phototherapy was required. Although
infants who were ill or had birth asphyxia were excluded from the nomogram development, a few infants \((n = 4)\) with fetal distress were enrolled. The 90\(^{th}\) percentile had the greatest ability in predicting infants at high risk for having hyperbilirubinemia severe enough to require treatment, with the sensitivity of 96.9\% and specificity of 78.8\%.

Criteria used to determine the need for phototherapy was based on a staff-developed guideline, which may overestimate sensitivity. When the Thai thresholds for phototherapy are compared to the AAP thresholds, the level of bilirubin at which treatment is recommended in Thai infants is 2 to 3 mg/dL lower than those of the 2004 AAP clinical practice guidelines. A limitation of the study is that investigators evaluated the accuracy of nomogram to predict hyperbilirubinemia in the same population used to construct the nomogram (Sackett et al., 2000).

Israel

A TcB nomogram was developed in a sample of 628 late preterm and term neonates admitted to an Israeli hospital nursery (Bental et al., 2009). Neonates who were determined to be jaundiced were consecutively added to the study group whenever an investigator was on duty, at which time, a laboratory technician would obtain paired TcB and TSB values. Infants needing phototherapy (before treatment), infants with known ABO and/or Rh incompatibility, or infants with positive direct antibody test (DAT) were included. The 75\(^{th}\) percentile was able to predict significant hyperbilirubinemia after 48 hours of life, defined as TSB > 95\(^{th}\) percentile, with 100\% sensitivity and 75.91\% specificity; however, the sensitivity was reduced to 83.3\% (~16.7\% false negative rate) at 24 to 48 hours of life.

China

As noted previously, maximum TSB levels in East Asian infants are known to be significantly higher than those in white infants. A group of investigators developed the first
hour-specific TcB nomogram in a sample of 6,035 healthy, Chinese late preterm and term newborns (Yu et al., 2011). Infants who were admitted to the neonatal intensive care unit (NICU), those with positive DAT, and those requiring phototherapy before hospital discharge were excluded. In the same group of neonates, the investigators analyzed the ability of their TcB nomogram to identify infants who subsequently developed significant hyperbilirubinemia following hospital discharge (defined as TSB above the 95th percentile on the Bhutani nomogram). The authors concluded that the 75th percentile of the TcB nomogram was an ideal cutoff point for identifying infants at risk for the outcome who would need intensive follow-up because the negative predictive value was 98.5%; however, the sensitivity only reached 78.7% with 119 false negatives. A sensitivity of 100% was reached only using the 40th percentile. That is, there were no infants in this percentile who subsequently developed hyperbilirubinemia which required phototherapy.

India

A nomogram was developed in a sample of 625 healthy late preterm and term Indian neonates (Mishra et al., 2010). The predictive accuracy of the nomogram performed lower than previous studies. The sensitivity for the 75th percentile only reached 86.1% with 11 false negative results, and the 50th percentile had 1 false negative (sensitivity 98.7%). Only when the 25th percentile was chosen as the risk predictor for significant hyperbilirubinemia, defined as the 2004 AAP TSB thresholds for phototherapy, were all false negatives avoided. The authors concluded that the need for phototherapy in the late preterm neonates (19/65, 29.2%) was significantly higher as compared to term neonates (60/150, 10.7%, p < 0.001), which could explain this performance. Future research to prospectively validate their nomogram was recommended.
Greece

In Greece, a TcB-based nomogram was developed in a sample of 2,818 late preterm and term neonates (Fouzas, Mantagou, Skylogianni, Mantagos, & Varvarigou, 2010). Less than half of the study population was exclusively breast-fed. Three different nomograms that extended up to 120 hours of age were constructed including one for the whole population, as well as a nomogram for each of two categories of gestational age, < 37 weeks and ≥ 37 weeks. In addition, the lowest TcB percentile for the subset of infants who required phototherapy (not included in the development of the nomogram) was compared with the general nomogram population. A different pattern for rates of increase in TcB was found among infants who did and did not require phototherapy. For example, during the first 24 hours of age, the 5th percentile TcB curve from infants who required treatment rested constantly below the 75th percentile curve from those who did not require phototherapy, resulting in an overlap of TcB values between the two groups, which persisted up to the age of 60 hours. After 60 hours of postnatal age, the 5th percentile TcB curve from neonates who required phototherapy exceeded the 95th percentile TcB curve of the nomogram. The authors concluded that using the 95th percentile track of previously published TcB nomograms as the risk demarcator for significant hyperbilirubinemia failed to differentiate reliably between infants who would and would not require phototherapy.

Comparison of TcB Nomograms

A systematic review of four TcB nomograms was examined in which trends in the natural course of transcutaneous bilirubin among various populations of neonates were compared (DeLuca et al., 2009). The four populations of neonates included European, North American Hispanic, North American white, and Thai (DeLuca et al., 2008; W.D. Engle et al., 2009; Maisels & Kring, 2006; Sanpavat et al., 2005). Significant differences
in mean TcB values across the populations were found, as well as substantive variability in the TcB rate of rise. In all four populations of neonates, the TcB rate of rise reached a plateau followed by a decrease at an average of 96 hours of age; however, peak TcB levels occurred earliest in Thai neonates, later in Hispanic newborns, and intermediately in the European and the white North American populations. The plateau of bilirubin level conceptually relates to the postnatal age when there is equilibrium between bilirubin production and elimination. Additionally, across the four studies the gestational age of study participants differed slightly but significantly ($p < .001$). The mean ± SD gestational age among study subjects in Europe (38.3 ± 1.9) and Thailand (38.6 ± 1.2) was younger compared with infants in the North American studies (39.2 ± 1.4 and 39 ± 1, respectively).

**Validated TcB Nomogram**

The validity of a nomogram is increased when its predictive accuracy has been evaluated in a second, independent population of neonates (Sackett et al., 2000). Of the TcB nomograms that have been published, only one has been validated in an independent study population of neonates (Romagnoli et al., 2012). The validity of the other TcB nomograms is uncertain.

The European TcB nomogram developed by DeLuca and colleagues (2008) was validated prospectively in a multi-center study by Romagnoli et al. (2012). They verified the ability of the nomogram to identify infants who are not at risk for severe hyperbilirubinemia (defined as TSB > 17 mg/dL and/or the need for phototherapy according to AAP guidelines) in neonates admitted to five different newborn nurseries in Italy. The sample consisted of 2,167 newborns, of which 184 (8.5%) were late preterm. The majority of infants were Caucasian (90.1%) and exclusively breastfed. The simultaneous measurement of TcB and TSB was made when jaundice was observed and/or prior to hospital discharge. In the first 48 hours of age 100% sensitivity was
reached only with the 50th percentile of the TcB nomogram. After the 48th hour time period, the 75th percentile of the nomogram was able to identify all newborns with subsequent significant hyperbilirubinemia.

Summary

Newborn jaundice is an extremely common problem which occurs in an environment of healthcare that emphasizes cost containment. Early discharge of the healthy mother and her newborn is viewed as one way to achieve this goal; however, for most infants early discharge occurs before bilirubin has reached maximum concentration. Potentially, all neonates must be regarded as at risk for the subsequent development of significant hyperbilirubinemia. These concerns have led to the recommendation of predischarge risk assessment for the subsequent development of significant hyperbilirubinemia as a potential strategy to reduce the incidence of acute BIND or kernicterus (AAP, 2004). Current practice guidelines give clinicians a choice of three predischarge risk-based strategies: a) bilirubin measurement interpreted using an hour-specific nomogram, b) assessment of clinical risk factors, or c) a combination of the two. Research reviewed in this chapter relating to the use of the recommended predischarge risk-based strategies demonstrates conflicting results. In addition, it is difficult to draw conclusions regarding the accuracy of various strategies for prediction of neonatal hyperbilirubinemia (Ip et al., 2004).

The first challenge is the lack of consistency in defining significant neonatal hyperbilirubinemia. For example, in various studies significant levels of TSB were defined as more than or equal to 12.9, 15, 17, 20, and 25 mg/dL (Ho, 1991; Kuzniewicz et al., 2008; Maisels & Gifford, 1986; Maisels et al., 2009; Newman et al., 1999; Newman et al., 2000; Newman et al., 2005). Other studies defined significant hyperbilirubinemia according to percentiles on the Bhutani nomogram, such as ≥ 95th percentile (Bhutani et
al., 1999; Keren et al., 2005). Still other studies defined significant hyperbilirubinemia according to threshold bilirubin levels used to initiate treatment with phototherapy (Goncalves et al., 2011; Kaur et al., 2011; Keren et al., 2008).

A second challenge that makes it difficult to compare between investigations or interpret the risk for hyperbilirubinemia according to gestational age is the heterogeneity of the gestational age of study populations. In some studies, neonatal populations were stratified according to the mutually exclusive categories (e.g., preterm, late preterm, and term) that have been defined precisely according to week and day of gestation by the AAP and the American College of Obstetricians & Gynecologists (ACOG) (W.A. Engle, et al., 2007). In other studies, although infants were described as term and “near-term,” the study population included late preterm and early term neonates (e.g., 36 and 37 weeks’ gestation).

Research findings suggest that an hour-specific TcB nomogram has the potential for being a useful clinical tool to identify infants at risk for the subsequent development of hyperbilirubinemia; however, it requires the most appropriate nomogram for a particular population (DeLuca, Carnielli, & Paolillo, 2009; DeLuca, Jackson, et al., 2009). This means choosing a nomogram developed in a population as similar as possible to the infant that will be evaluated, especially regarding important clinical risk factors such as decreased gestational age, ethnicity, and the source of the bilirubin value (TSB versus TcB). Research is needed to validate previously developed TcB nomograms in separate, independent populations of newborns.

Research investigating TcB measurement has demonstrated that bilirubin levels in the late preterm infant are more pronounced and sustained over a longer period compared with term newborn (Fouzas, Mantagou, et al., 2010). Although these investigations are important, these findings do not describe differences in neonatal
jaundice between early term and term neonates. Furthermore, to date no study has compared TcB levels between early term and term neonates. Research is needed to help fill the gaps in the literature related to the differences in bilirubin levels between early term and late term neonates during the early postnatal period
Chapter 3
Methods and Procedures

Introduction

This secondary data analysis study examined the predictive ability of the previously reported but unvalidated Parkland Hispanic TcB nomogram to identify Hispanic neonates at risk for the subsequent development of significant hyperbilirubinemia. In addition, this study compared differences in hour-specific TcB values between early term and term newborn patients, which had not been previously investigated. This chapter begins with a description of the research design, sample, and setting. In addition, measurement methods, procedures, and ethical considerations are discussed. This chapter concludes with statistical analyses and delimitations.

Research Design

The design chosen for this study was a secondary analysis of an existing clinical database. Secondary data analysis (SDA) refers to the use of existing data, which were collected for another study, and then re-examined to answer a research question other than the question(s) for which the data were originally collected (Portney & Watkins, 2009). The reasons this design was chosen are twofold: (a) to validate reported findings in the original study of the Parkland Hispanic TcB nomogram, and (b) to examine dimensions of TcB and gestational age previously unexamined (W.D. Engle et al., 2009).
There are advantages and disadvantages to a SDA design. A major advantage of using a SDA research design is its potential for resource savings and cost-effectiveness (Kiecolt & Nathan, 1985). Secondary data analyses have the potential for economy of time and expense related to data collection and analyses (Newman, Browner, Cummings, & Hulley, 2007; Polit & Beck, 2010). Additionally, SDA provides an opportunity to generate knowledge for clinical practice and to evaluate patient and nursing outcomes (Castle, 2003; Magee, Lee, Giuliano, & Munro, 2006). The primary disadvantage of a SDA design is inherent in its nature: (a) data were not collected to answer the current investigator’s specific questions or hypotheses, (b) the investigator has less control over the population or variables selected to study, and (c) the investigator is dependent on the accuracy of the original data collection and data entry (Kiecolt & Nathan, 1985).

Appraising Diagnostic and Screening Tests

Healthcare clinicians want to use the most accurate tools or tests in order to help screen for and accurately determine the presence or absence of a disease or target condition (Sackett et al., 2000). The accuracy of a clinical test or tool used to screen for or to confirm a diagnosis is evaluated in terms of its ability to classify patients in two groups according to the presence or absence of a symptom or a disease as compared with a gold standard (Altman & Bland, 1994a). Two statistical measures used to evaluate the accuracy of a diagnostic test are sensitivity and specificity (Haynes, Sackett, Guyatt, & Tugwell, 2006; Newman et al., 2007; Sackett et al., 2000). Such a test can have four possible outcomes: (a) true positive, which accurately identifies the presence of the disease being tested for; (b) false positive, which incorrectly indicates the presence of the disease being tested for; (c) false negative, which incorrectly indicates the absence of the disease being tested for; and (d) true negative, which accurately identifies the absence of
the disease being tested for (Sackett et al., 2000). The outcomes can be summarized in a 2x2 classification table (Table 3.1).

The sensitivity of a diagnostic test (also called the true positive rate) is defined as the probability an individual with the target disease will have a positive test result. It measures the proportion of “positives” that are correctly identified (i.e., the percentage of “diseased” individuals who are correctly identified as having the disease. In clinical situations, such as neonatal hyperbilirubinemia, when serious consequences of missing a true positive are present, having a test with high sensitivity is extremely important (Bhutani, 2012). Conversely, the specificity of a test (also called the true negative rate) is defined as the probability that an individual who does not have the target disease will have a negative test result (i.e., true-negatives). Specificity measures the percentage of “negatives” which are correctly identified as such (e.g., the percentage of “non-diseased” individuals who are correctly identified as such) (Haynes et al., 2006).

Additional statistical measures that can help appraise the accuracy of a diagnostic test are the likelihood ratios (LRs). Likelihood ratios are calculated from sensitivity and specificity and are unaffected by disease prevalence (Haynes et al., 2006). These measures are calculated to determine the “likelihood that a positive test result is a true positive and a negative test result is a true negative” (Grove et al., 2013, p. 408). The positive LR (LR+) can be calculated by dividing the sensitivity by 1 – specificity. The LR+ answers the question, “How much more likely is a positive test to be found in a person with the disease than a person without it?”
Table 3.1 Summary of Analysis for Diagnostic or Screening Test

<table>
<thead>
<tr>
<th>Target Disease</th>
<th>Present</th>
<th>Absent</th>
<th>Totals</th>
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</thead>
<tbody>
<tr>
<td><strong>Test Result</strong></td>
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</tr>
<tr>
<td>Positive</td>
<td>a</td>
<td>b</td>
<td>a + b</td>
</tr>
<tr>
<td>True positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False positive</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Negative</td>
<td>c</td>
<td>d</td>
<td>c + d</td>
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<td>False negative</td>
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<tr>
<td>True negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>a + c</td>
<td>b + d</td>
<td>a + b + c + d</td>
</tr>
</tbody>
</table>

Sensitivity (%) = a/(a + c) x 100  
Specificity (%) = d/(b + d) x 100  
Positive Likelihood Ratio (LR+) = sensitivity/100% - specificity  
Negative Likelihood Ratio (LR-) = 100% - sensitivity/specificity  
Positive Predictive Value (PPV) = a/(a + b)  
Negative Predictive Value (NPV) = d/(c + d)

The negative LR (LR-) is calculated by 1 – sensitivity divided by the specificity. The LR of a positive test is always greater than 1.0, and the larger the LR+ the more likely the patient to have the disease. Conversely, LR- is always less than 1.0, with smaller numbers indicating a lower risk for the patient to have the disease (Haynes et al., 2006).

Two additional measures used to determine the accuracy of diagnostic or screening tests are the positive and negative predictive values (PPV and NPV, respectively) (Altman & Bland, 1994b). The PPV and NPV represent the proportions of patients who are correctly diagnosed. The positive predictive value (PPV) of a diagnostic test answers the question, “If the patient’s test is positive, what is the probability that they have the target disease.” Similarly, the negative predictive value (NPV) of a test is the probability that a patient who has a negative test result is actually free of the target disease (Newman et al., 2007).
The Early TcB Database

Data analyzed in this study were abstracted from an available clinical database, hereafter referred to as the Early TcB database. The subjects (n = 560) selected for inclusion in the database were late preterm (birth from 34\(^{0/7}\) through 36\(^{6/7}\) weeks of gestation), early term (birth from 37\(^{0/7}\) through 38\(^{6/7}\) weeks), term (birth from 39\(^{0/7}\) through 41\(^{6/7}\) weeks), and post-term (birth from \(\geq\) 42 weeks of gestation) infants who were admitted to a hospital newborn nursery (NBN) from June 1-30, 2011 or May 1-31, 2012. The Early TcB database was chosen based on the fit of variable selection with this study’s conceptual framework and data availability. The Early TcB database was developed for two reasons: a) to pilot a change in the standard procedure of the newborn nursery to include early (defined as TcB determinations performed prior to 6 hours of age) TcB determinations (utilizing the JM-103™ bilirubinometer), and b) to examine the relationship of early TcB determinations and the need for a higher level of care (Jackson, Saumur, & Engle, 2013). The change in hospital policy was made to augment the existing standard procedure, which includes at least once daily TcB determinations performed by nursing personnel to screen infants for hyperbilirubinemia.

Description of Database Setting

The setting for the Early TcB database is a NBN in Dallas, Texas. The 181-bed NBN is a part of a large, university-affiliated public hospital that serves a county of almost 3 million people, has approximately 968 licensed beds, and serves as an academic setting for medical students, interns, residents, fellows, and nursing students (U.S. News & World Report, 2012). In 2011, over 11,000 newborns were discharged from the NBN (Parkland Health & Hospital System, 2012). The racial/ethnic composition of the general population of women who deliver at the hospital consists of 84% Hispanic, 10% African-American, 4% white, and 3% other (Chao, Bloom, Mitchell, McIntire, & Leveno, 2011).
addition, over 90% of the neonates admitted to the NBN are enrolled in the state’s Medicaid program.

Sample

For the primary and secondary purposes of this study, a convenience sample who were included in the Early TcB database and met the criteria for this study were selected. Inclusion and exclusion criteria for both research purposes are presented in Table 3.2.

Table 3.2 Inclusion and Exclusion Criteria for Sample

<table>
<thead>
<tr>
<th>Primary Research Purpose</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infant admitted to NBN nursery from June 1-30, 2011 or May 1-31, 2012 and included in Early TcB database</td>
<td>Infant with significant congenital abnormalities noted either antenatally or at the time of delivery</td>
</tr>
<tr>
<td></td>
<td>Successful transition in delivery room</td>
<td>Infant who were admitted to the Neonatal Intensive Care Unit (NICU) following delivery or transferred to NICU from newborn nursery</td>
</tr>
<tr>
<td></td>
<td>$\geq$ 35 weeks’ gestation</td>
<td>Infant received phototherapy treatment prior to or at time of TcB measurement</td>
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<td></td>
<td>$\geq$ 2100 grams in birth weight</td>
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<td></td>
<td>2 or more TcB determinations performed during postnatal hospitalization between 6 and 66 hours of age</td>
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<tr>
<td></td>
<td>Mother of infant self-identified as Hispanic upon admission to labor &amp; delivery unit</td>
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</table>

<table>
<thead>
<tr>
<th>Secondary Research Purpose</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infant admitted NBN from June 1-30, 2011 or May 1-31, 2012 and included in Early TcB database</td>
<td>Infant with significant congenital abnormalities noted either antenatally or at the time of delivery</td>
</tr>
<tr>
<td></td>
<td>Apparent successful transition in delivery room</td>
<td>Infant who were admitted to the Neonatal Intensive Care Unit (NICU) following delivery or transferred to NICU from newborn nursery</td>
</tr>
<tr>
<td></td>
<td>$\geq$ 2100 grams in birth weight</td>
<td>Infant received phototherapy treatment prior to or at time of TcB measurement</td>
</tr>
<tr>
<td></td>
<td>Infant with 1 or more TcB determinations performed during postnatal hospitalization between 6 and 66 hours of age</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infant born from 37(^{0/7}) through 38(^{6/7}) weeks of gestation or from 39(^{0/7}) through 41(^{6/7}) weeks of gestation</td>
<td></td>
</tr>
</tbody>
</table>
Power Analysis

A power analysis was conducted for each of the two research questions.

Browner et al. (2007) published a formula for determining sample size for a descriptive study that investigates the sensitivity and specificity of a diagnostic or screening test:

\[ N = 4z_{\alpha/2}^2 P (1 - P) / W^2, \]

Using this formula, a minimum of 73 subjects were required to determine the accuracy of the Parkland Hispanic TcB nomogram for identifying infants with subsequent significant hyperbilirubinemia (TcB > 95th percentile). The expected proportion of infants with significant hyperbilirubinemia was based on the findings of Bhutani and colleagues (2000) who examined the predictive ability of TcB measurements obtained between 24 and 48 hours of age to detect the predischarge high-risk group, defined as TcB > 95th percentile on the Bhutani nomogram. Findings revealed that the proportion of infants in the high-risk group was 23 out of 419, that is, a proportion of .05 (5%). Therefore, the total width for confidence intervals chosen was 0.10 (0.05 below and 0.05 above), and the confidence level selected was 95%.

The secondary purpose of this study was to compare differences in hour-specific TcB values between two groups of newborn patients: early term and term. Statistical power was calculated using G*Power 3.1.5 for Windows for an a priori sample size estimation for a two-tailed Mann-Whitney U-test between two groups (Faul, Erdfelder, Buchner, & Lang, 2009). A minimum of 208 subjects (104 in each group) were required to achieve a statistical power of .80 and alpha level of .05, based on an anticipated effect size of \( d = 0.4 \).

---

1. \( N = \) sample size; \( z_{\alpha} \) = the standard normal deviate for a two-sided \( \alpha \), where \((1 - \alpha)\) is the confidence level (e.g., since \( \alpha = 0.05 \) for a 95% confidence level, \( z_{0.05} = 1.96 \)); \( P \) = expected proportion of population who have the variable of interest; \( W \) = desired total width of confidence interval.
The effect size was based on findings reported by Maisels and Kring (2006), which compared TcB levels among three categories of gestational age at delivery: 35\( \frac{0}{7} \) through 37\( \frac{6}{7} \), 38\( \frac{0}{7} \) through 39\( \frac{6}{7} \), and ≥ 40 weeks of gestation.

For both research questions, a convenience sample of newborn patients was selected from the Early TcB database. A sample of 404 Hispanic neonates (≥ 35 weeks of gestation) was selected for the primary research question, and a sample of 513 neonates (131 early term group and 382 term group) was selected for the second research question. Thus, the minimum sample size required for both power analyses was satisfied.

Measurement Methods

Data from the Early TcB Database

The method of data collection was an abstraction of existing data from the Early TcB database, which were originally collected from the electronic medical record (EMR) of the hospital healthcare system (Jackson et al., 2013). The demographic variables abstracted from the Early TcB database included gestational age, birth weight, mother’s ethnicity, and method of infant feeding. These variables may reflect non-pathologic factors, such as less mature gestations and exclusive breastfeeding, which can affect bilirubin levels in neonates. In the original Parkland Hispanic TcB nomogram study, in addition to the demographic variables described previously, mode of delivery and infant gender were analyzed; therefore, measurement of these two variables is warranted (W.D. Engle et al., 2009). The dependent variable of hour-specific TcB values (i.e., postnatal age in hours at time of TcB determination) was abstracted from the Early TcB database. Compared to the original nomogram study, fewer TcB values for any given period in the Early TcB database were available; therefore, TcB values obtained ± 6 hours of the following postnatal ages were analyzed: 12, 24, 36, 48, and 60 hours. Hour-specific TcB
values reflect the physiologic principle that in transitional neonatal hyperbilirubinemia, bilirubin levels are changing constantly and should be interpreted only in relationship to the infant’s chronological age in hours not days. The conceptual and operational definitions and codes for the study variables are presented in Table 3.3.

Table 3.3 Study Variables

<table>
<thead>
<tr>
<th>Study Variables &amp; Level of Measurement</th>
<th>Conceptual Definition</th>
<th>Operational Definition &amp; Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (GA) Interval</td>
<td>Estimated age of the fetus or neonate. Defined as the time in days or weeks from fertilization to birth. Since the exact date of fertilization is seldom known, GA is quantified by the time elapsed since the first day of the last mother’s last menstrual period (LMP). GA is assigned in completed weeks of gestation, indicating the number of 7-day intervals that have passed after onset of the LMP. GA should never be rounded up.</td>
<td>Estimated age of neonate in completed weeks as recorded in the electronic medical record (EMR).</td>
</tr>
<tr>
<td>Gestational age classification Nominal</td>
<td>Classification of newborn infants based on gestational age at birth; designated by WHO and expert groups; consists of late preterm (34(^0/7)-36(^6/7) wks), early term (37(^0/7)-38(^6/7) wks), term (39(^0/7)-41(^6/7) wks), or post-term (≥ 42 wks)</td>
<td>Classification groups assigned according to infant’s estimated GA as recorded in the EMR: Late Preterm = 1; Early Term = 2; Term = 3; Post-term = 4</td>
</tr>
<tr>
<td>Postnatal age Interval</td>
<td>Chronological age of infant. Represents time elapsed since birth</td>
<td>Age of infant (in hours) calculated using date and time of birth and time of interest (e.g., TcB determination) as recorded in the EMR.</td>
</tr>
<tr>
<td>Birth weight Interval</td>
<td>The weight of a neonate determined immediately after delivery</td>
<td>Birth weight as recorded in the EMR, expressed to the nearest gram.</td>
</tr>
<tr>
<td>Mode of delivery Nominal</td>
<td>Vaginal or cesarean delivery (C/S)</td>
<td>Mode of delivery as recorded in the EMR: Vaginal = 1 C/S = 2</td>
</tr>
</tbody>
</table>
Table 3.3 – Continued

<table>
<thead>
<tr>
<th>Study Variables &amp; Level of Measurement</th>
<th>Conceptual Definition</th>
<th>Operational Definition &amp; Code</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>Assigned gender of neonate at delivery</td>
<td>As recorded in the EMR: Male = 1; Female = 2</td>
</tr>
<tr>
<td><strong>Nominal</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Method of Infant feeding**           | Type of enteral nutrition provided to the newborn, either breast and/or formula | Based on nursing notes as recorded in the EMR: Exclusively breast-fed or breast-fed & < 30 ml formula = 1; Both: breast-fed & > 30 ml formula = 2; Exclusively formula-fed = 3 |
| **Nominal**                            |                       |                              |

| **TcB values**                          | TcB values obtained at various postnatal ages (i.e., hour-specific) | Hour-specific TcB values obtained ± 6 hours of the following postnatal ages as recorded in the EMR: 12, 24, 36, 48, and 60 |
| **Interval**                            |                       |                              |

| **Significant hyperbilirubinemia**      | An abnormally high concentration of the endogenous pigment, bilirubin in the blood, dermal, and subcutaneous tissues | TcB value > 95th percentile on Parkland Hispanic TcB nomogram |
| **Interval**                            |                       |                              |

**Data from the Parkland Hispanic TcB Nomogram Study**

Data from the original Parkland Hispanic TcB nomogram study were made available for use in this study by Dr. G.L. Jackson. In the original study, the nomogram was developed using hour-specific TcB values obtained from a convenience sample ($n = 2,005$) of Hispanic neonates: (a) who were admitted to the NBN from May 2005 through April 2007; (b) were ≥ 35 weeks of gestation and ≥ 2,100 grams in birth weight; (c) had successful transition in the delivery room; (d) who had not received treatment with phototherapy prior to, or at the time of TcB measurement; and (e) had no significant congenital anomalies (W.D. Engle et al., 2009). For development of the nomogram, only TcB values ($n = 3,284$) obtained ± 2 hours of the following postnatal ages were used: 12,
18, 24, 30, 36, 42, 48, 52, 60, 66, and 72 hours. Actual nomogram percentile values from 12 to 66 postnatal hours are shown in Table 3.4.

Table 3.4 Percentile Values for TcB between 12 and 66 Postnatal Age

<table>
<thead>
<tr>
<th>Postnatal Age (Hours)</th>
<th>50th</th>
<th>75th</th>
<th>95th</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>2.0</td>
<td>2.8</td>
<td>4.4</td>
</tr>
<tr>
<td>24</td>
<td>4.2</td>
<td>5.4</td>
<td>7.6</td>
</tr>
<tr>
<td>36</td>
<td>5.7</td>
<td>7.0</td>
<td>9.1</td>
</tr>
<tr>
<td>48</td>
<td>7.0</td>
<td>9.0</td>
<td>11.0</td>
</tr>
<tr>
<td>54</td>
<td>7.2</td>
<td>8.8</td>
<td>10.6</td>
</tr>
<tr>
<td>60</td>
<td>7.3</td>
<td>8.8</td>
<td>10.9</td>
</tr>
<tr>
<td>66</td>
<td>7.5</td>
<td>9.1</td>
<td>12.0</td>
</tr>
</tbody>
</table>


Data Collection Procedures

Data contained in the database were originally abstracted from the EMR of the hospital healthcare system. The medical director of the NBN, Dr. Jackson is the developer and administrator of the database. Data collectors for the database consisted of the medical director and two medical students from the hospital-affiliated medical school. Data include antenatal information (e.g., mother’s ethnicity, age, pregnancy and birth history, prenatal complications), intrapartum information (e.g., maternal medications during labor, mode of delivery), neonatal demographic information (e.g., date/time of birth, gender, birth weight, gestational age, Apgar scores), and other patient information that are generated as a result of routine newborn care (e.g., TcB values, method of infant feeding). The following techniques were used to assure that database errors were eliminated: (a) each field was sorted and outliers for fields were found after sorting, (b) records in which dates and values appeared incorrect were re-assessed by reviewing the individual EMR, (c) duplicate records in the database were identified and deleted, and (d)
queries were run to double-check values and groupings (G. Jackson, personal communication, August 28, 2013). A sample of neonates for each of the study questions was constructed from the database. A copy of the data collection tool is provided in Appendix A.

Ethical Considerations

Written permission to conduct this study was obtained from the University of Texas Southwestern Medical Center (UTSWMC) and the University of Texas at Arlington (UTA) Institutional Review Boards (IRBs) (Appendices B & C). A waiver of consent was approved from both IRBs. In addition, written permission for research site approval (Early TcB database) was obtained from Parkland Health and Hospital System (Appendix D). This study performed secondary analysis of existing data; therefore, the only risk to study participants was loss of confidentiality. The only protected health information in the data was the birthdate and the medical record number. To protect confidentiality of study participants the medical record number (MRN) was de-identified and participants were assigned a unique study identifying number. A list of the MRNs and associated with study identifying numbers were kept in a separate file from the study data. Study participants’ birthdates were not used. The original Microsoft Excel files were stored, unaltered, in a locked file cabinet with access only to this researcher.

Statistical Analysis

Following approval from the University of Texas Southwestern Medical Center (UTSWMC) and the University of Texas at Arlington (UTA) Institutional Review Boards (IRBs), data from the Early TcB database and data from the previously reported study of the Parkland Hispanic TcB nomogram were received as Microsoft Excel (2010) files from the identified sources and then imported into SPSS, version 21 (IBM Corp., 2012).
Statistical analysis was computed using SPSS Statistics for Windows, version 21.0 and VassarStats (Lowry, 2013).

Descriptive statistics were computed for the study sample on the categories of gender, mode of delivery, method of infant feeding, gestation, birth weight, and hour-specific TcB values. Prior to statistical analyses, interval data from the Early TcB database and the previously published study of the Parkland Hispanic TcB nomogram were tested for normality with the Shapiro-Wilk W-test. Results of this analysis showed that the distributions for these variables significantly deviated from normal. To assess comparability between this population and that of the original nomogram study, univariate analyses of quantitative variables were performed using the Mann Whitney U-test and chi-square test, whichever was appropriate based on the variable’s level of measurement. The significance was set at a level of 0.05.

The primary purpose of this study was to validate the Parkland Hispanic TcB nomogram by describing its accuracy as a screening tool to identify Hispanic newborn patients at risk for the subsequent development of significant hyperbilirubinemia, defined as TcB > 95th percentile. The predictive accuracy of the 50th and the 75th percentile values of the nomogram for hour-specific TcB values at 12 ± 6, 24 ± 6, and 36 ± 6 postnatal hours was determined by whether subsequent TcB values at postnatal ages (36 ± 6 and 60 ± 6 hours) were > or ≤ 95th percentile of the nomogram. Estimates of sensitivity (true positive), specificity (true negative), positive and negative predictive values (PPV and NPV), and positive and negative likelihood ratios (LR+ and LR-) were computed using an online research calculator (VassarStats).

Every sample participant had at least two TcB determinations performed during their postnatal hospitalization. For each participant, TcB values included in the analysis met the following criteria: (a) no more than four TcB values were used for a single
participant; (b) TcB values could be no less than 12 hours apart; and (c) if two values from the same participant were obtained in the same epoch, the value obtained at the oldest postnatal age was used. Transcutaneous bilirubin values obtained ± 6 hours of the following postnatal age were included in the analysis: 12, 24, 36, and 60 hours. For example, TcB values obtained at 12 postnatal hours included determinations between six and 18 postnatal hours. First, values obtained at 12, 24, or 36 postnatal hours (± 6 hours) were identified as being > or ≤ the 50th and > or ≤ the 75th percentile values of the nomogram. These values represent the “test result” in a 2 x 2 classification table. Then, TcB values obtained at 12 postnatal hours were examined with regard to a subsequent value at 36 postnatal hours (± 6 hours) was either > or ≤ the 95th percentile value of the nomogram. Next, TcB values at 24 and 36 postnatal hours were examined with regard to a subsequent value at 60 postnatal hours (± 6 hours) was either > or ≤ the 95th percentile value of the nomogram. The subsequent values represent the presence or absence of the “target disease” (i.e., significant hyperbilirubinemia) in a 2 x 2 classification table. Thus, the following periods or epochs of postnatal age were examined: 12 and 36, 24 and 60, and 36 and 60 hours.

The following example using a binary classification table will help illustrate how the analysis was performed (Table 3.5). One sample participant, Baby X had TcB measurements at 12 and 36 postnatal hours, with the following values of 3.0 and 10.0 mg/dL, respectively. First, the 12-hour TcB value (3.0 mg/dL) was identified as greater than both the 50th (2.0 mg/dL) and the 75th (2.8 mg/dL) nomogram percentile values at 12 postnatal hours. This TcB value represents the “test.” When this TcB value is compared to both the 50th and 75th nomogram hour-specific (i.e., 12-hours) percentile values, the test result is “positive.” Then, the subsequent 36-hour TcB value (10.0 mg/dL) was identified as greater than the 95th nomogram hour-specific percentile value (9.1 mg/dL).
Thus, this infant was accurately identified as having significant hyperbilirubinemia, i.e., a true positive, and the appropriate box of the 2x2 table would be in the shaded “box a.”

This exercise was repeated for each sample participant until the TcB results for each infant was correctly located in the appropriate box of the 2x2 table. The number of infants in each box is tabulated, which allows the sensitivity, specificity, predictive values, likelihood ratios, and positive and negative predictive values to be calculated using the formulas presented in Table 3.5.

Table 3.5 Analysis for Appraising the Accuracy of the Parkland Hispanic TcB Nomogram

<table>
<thead>
<tr>
<th>Target Disease</th>
<th>Significant Hyperbilirubinemia (TcB &gt; 95th Percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>&gt;95th Nomogram percentile value</td>
</tr>
<tr>
<td>Absent</td>
<td>≤ 95th Nomogram percentile value</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test</th>
<th>Baby X’s TcB Value at 36 postnatal hours</th>
<th>Totals</th>
</tr>
</thead>
</table>
| Baby X’s TcB value at 12 postnatal hours | Positive: | Negati

- **Positive:**
  - > 50th Nomogram percentile values
  - > 75th Nomogram percentile values

- **Negative:**
  - ≤ 50th Nomogram percentile values
  - ≤ 75th Nomogram percentile values

- **Baby X’s TcB Value at 36 postnatal hours**
  - a: True positive
  - b: False positive
  - c: False negative
  - d: True negative

- **Totals**
  - a + c
  - b + d
  - a + b + c + d

Sensitivity (%) = \( \frac{a}{a + c} \times 100 \)
Specificity (%) = \( \frac{d}{b + d} \times 100 \)
Positive Likelihood Ratio (LR+) = sensitivity/100% - specificity
Negative Likelihood Ratio (LR-) = 100% - sensitivity/specificity
Positive Predictive Value (PPV) = \( \frac{a}{a + b} \)
Negative Predictive Value (NPV) = \( \frac{d}{c + d} \)
The second research purpose of this study was to compare TcB values between two groups of neonates: early term and term. Descriptive statistics were computed for both groups on the categories of gender, mode of delivery, method of infant feeding, gestation, birth weight and maternal race. To answer the second research question, hour-specific TcB values between early term and term neonates were compared using an independent t-test. The significance level was set at a level of 0.05.

Delimitations

The delimitations of this study were that the study sample included healthy neonates, ≥ 35 weeks’ gestation and ≥ 2,500 g birth weight, and only those who were admitted to a single hospital newborn nursery and who were included in a clinical database. Thus, the findings of this study cannot be generalized to all newborn infants, such as those delivered in other hospital settings.

Summary

A secondary analysis of an available clinical database was used to validate the ability of the Parkland Hispanic TcB nomogram to identify Hispanic newborn patients at risk for the subsequent development of significant hyperbilirubinemia. In addition, a secondary analysis was used to examine differences in TcB values between early term and term neonates. This chapter described the methods, data collection procedures and statistical analysis used in this study. In addition, ethical considerations for the protection of human subjects and delimitations were presented.
Chapter 4

Findings

Introduction

The results of a secondary analysis of a clinical database are presented in this chapter. This analysis examined the accuracy of a transcutaneous bilirubin (TcB) nomogram for identifying subsequent significant hyperbilirubinemia in Hispanic newborns. In addition, a comparison of TcB values between early term neonates (birth from 37\textsuperscript{0/7} through 38\textsuperscript{6/7} weeks) and term neonates (birth from 39\textsuperscript{0/7} through 41\textsuperscript{6/7} weeks) was performed. Description of the sample and results of the analyses are presented for each of the research questions.

Results

Research Question # 1

Sample Description

Study participants were selected from the Early TcB database. The sample included 404 Hispanic newborns who admitted to the newborn nursery (NBN) of a large, university-affiliated public hospital from June 1-30, 2011 or May 1-31, 2012, and who had two or more TcB determinations during their postnatal hospital stay. As noted in Table 4.1, there were more males than females in the sample and the majority of infants were term. Mean gestational age (± SD) was 39.2 ± 1.3 weeks (median = 39.0; range = 35-42 weeks) and the mean birth weight was 3409 ± 464 g (median = 3380 g; range = 2155-4940 g).
Table 4.1 Demographic Characteristics of Sample for Research Question #1

<table>
<thead>
<tr>
<th>Demographic Characteristic</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>219</td>
<td>54</td>
</tr>
<tr>
<td>Female</td>
<td>185</td>
<td>46</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>281</td>
<td>70</td>
</tr>
<tr>
<td>Cesarean</td>
<td>123</td>
<td>30</td>
</tr>
<tr>
<td>Method of infant feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>181</td>
<td>45</td>
</tr>
<tr>
<td>Both</td>
<td>171</td>
<td>42</td>
</tr>
<tr>
<td>Formula</td>
<td>52</td>
<td>13</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-36</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>37-38</td>
<td>97</td>
<td>24.0</td>
</tr>
<tr>
<td>39-41</td>
<td>286</td>
<td>69.8</td>
</tr>
<tr>
<td>≥ 42</td>
<td>11</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Note: Breast refers to exclusive breastfeeding or breastfeeding with < 30 ml formula supplementation, both refers to breastfeeding with > 30 ml formula supplementation, and formula-fed refers to exclusive formula feeding.

Comparison of Two Samples

A Mann-Whitney U-test was computed to compare birth weight and gestational age between the dissertation study sample (“study sample”) and that of the original nomogram study. There were no significance differences between birth weight and gestational age between the two samples, $z = -0.518$, $p = 0.605$; $z = -0.868$, $p = 0.385$, respectively. A chi-square test was computed on the categories of gender, mode of delivery, and method of infant feeding. There was no difference in gender between the two samples, $\chi^2 (1) = 2.436$, $p = 0.119$. However, there were significant differences in the mode of delivery and method of infant feeding between the two samples.

Participants in both studies were admitted to the same hospital NBN during different years. The sample in the original nomogram study was born either in 2006 or 2007, whereas the participants in the study sample were born either in 2011 or 2012. The majority of participants (77.8%) in both studies were delivered vaginally; however, the
proportion of infants in the study sample delivered by cesarean section was significantly higher, \( \chi^2 (1) = 19.361, p < .0001 \) (Figure 4.1).

At the hospital where the participants in both studies were born, the cesarean delivery rate varied significantly in the intervening years (D. McIntire, personal communication, July 19, 2013). From 2006 to 2007 (“earlier period”), the cesarean delivery rate remained unchanged at 27%; however, from 2011 to 2012 (“later period”) the cesarean delivery rate increased from 31 to 32% (Figure 4.2). The number of cesarean deliveries in the earlier period equaled 8,709 out of 32,430 total deliveries, while the number of cesarean deliveries in the later period equaled 7,242 out of 22,969 total deliveries. A chi-square test was computed on the mode of delivery between the two periods. Compared to the nomogram study period, there were significantly more cesarean deliveries during the years when the participants in this study were born, \( \chi^2 (1) = 143.08, p < .0001 \).
The following criteria were used to define the method of infant feeding: (a) *exclusively breast-fed*: either exclusively breast-fed or breast-fed with < 30 ml (< 1 ounce) formula supplementation following breastfeeding; (b) *both*: breast with > 30 ml formula supplementation; and (c) *exclusively formula-fed*. As shown in Figure 4.3, compared with the nomogram sample, the proportion of infants in the study sample who were exclusively breast-fed was higher and the proportion of infants who were exclusively formula-fed was less ($\chi^2 (3) = 41.75, p < .0001$). The lower proportion of formula-fed infants in the study sample represents a difference of almost 18% from the proportion observed in the nomogram sample. This change is believed to have resulted from the hospital’s initiative to increase the number of exclusively breast-fed newborns in the intervening years between the two studies (G.L. Jackson, personal communication, September 23, 2012).
As shown in Table 4.2, a total of 948 TcB determinations were performed ± 6 hours of the following postnatal ages: 12, 24, 36, 48, and 60 (e.g., TcB-12). Thirty-four of 404 neonates (8%) in the study sample had significant hyperbilirubinemia (TcB > 95\textsuperscript{th} percentile) compared with 64 (3%) infants in the nomogram sample.

![Figure 4.3 Comparison of method of infant feeding between the two samples](image_url)

**Table 4.2 Hour-Specific TcB Results for the Study Sample**

<table>
<thead>
<tr>
<th>TcB</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CI</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcB-12</td>
<td>236</td>
<td>2.9</td>
<td>1.4</td>
<td>2.71 - 3.12</td>
<td>2.8</td>
<td>0 - 7.9</td>
</tr>
<tr>
<td>TcB-24</td>
<td>184</td>
<td>4.6</td>
<td>1.6</td>
<td>4.35 - 4.81</td>
<td>4.5</td>
<td>0.2 - 9.0</td>
</tr>
<tr>
<td>TcB-36</td>
<td>220</td>
<td>6.6</td>
<td>2.0</td>
<td>6.27 - 6.81</td>
<td>6.5</td>
<td>0.8 - 13.6</td>
</tr>
<tr>
<td>TcB-48</td>
<td>201</td>
<td>7.8</td>
<td>2.2</td>
<td>7.47 - 8.09</td>
<td>7.7</td>
<td>1.9 - 13.8</td>
</tr>
<tr>
<td>TcB-60</td>
<td>105</td>
<td>8.7</td>
<td>2.6</td>
<td>8.16 - 9.15</td>
<td>8.8</td>
<td>1.1 - 14.6</td>
</tr>
</tbody>
</table>

*Note: TcB, transcutaneous bilirubin value refers to postnatal age in hours (± 6 hours) at time of TcB measurement; n, number of TcB determinations; Mean, median, and range TcB results in mg/dL; SD, standard deviation; CI, 95% confidence intervals.*

A Mann-Whitney *U*-test was computed to compare TcB values between the two samples at each of the five epochs of postnatal age in hours (Table 4.3). At every epoch, TcB values were significantly higher in the study sample. In addition, as the postnatal age at
the time of the TcB determination increased so did the disparity between the TcB values for the two samples (Figure 4.4).

Table 4.3 TcB Values (Mean [Median] ± Standard Deviation) for the Nomogram Sample and the Study Sample

<table>
<thead>
<tr>
<th>TcB</th>
<th>Nomogram Sample</th>
<th>Study Sample</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcB-12</td>
<td>2.2 [2.0] ± 1.4</td>
<td>2.9 [2.8] ± 1.4</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TcB-24</td>
<td>4.2 [4.0] ± 1.9</td>
<td>4.6 [4.5] ± 1.6</td>
<td>.001</td>
</tr>
<tr>
<td>TcB-36</td>
<td>5.6 [5.6] ± 2.1</td>
<td>6.6 [6.5] ± 2.0</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TcB-48</td>
<td>6.9 [7.0] ± 2.4</td>
<td>7.8 [7.7] ± 2.2</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TcB-60</td>
<td>7.3 [7.5] ± 2.7</td>
<td>8.7 [8.7] ± 2.6</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Note: TcB, transcutaneous bilirubin value refers to postnatal age in hours (± 6 hours) at time of TcB measurement; TcB result in mg/dl; p < .05

Figure 4.4 TcB values at 5 epochs of postnatal age (± 6 hours)
Validation of the Parkland Hispanic TcB Nomogram

The first research question asked: Is the 50th or the 75th percentile of the Parkland Hispanic TcB nomogram able to identify Hispanic neonates who develop subsequent significant hyperbilirubinemia (TcB > 95th percentile) during their birth hospitalization?

Table 4.4 shows the predictive ability of the 50th and the 75th percentile values of the Parkland Hispanic TcB nomogram to identify neonates with subsequent significant hyperbilirubinemia (TcB > 95th percentile). In every epoch examined, the sensitivity of the 50th and the 75th percentiles was 100%. This means that using the 50th and the 75th percentiles of the Parkland Hispanic TcB nomogram predicted all newborns with subsequent significant hyperbilirubinemia prior to hospital discharge. In addition, the NPV was always 100% when using the 50th and the 75th percentile values of the nomogram for subsequent TcB > 95th percentile. Thus, the use of both the 50th and the 75th percentile values of the nomogram had a high negative predictive value, which means that infants whose TcB values were < these two percentiles were extremely unlikely to have subsequent TcB values > 95th percentile (i.e., significant hyperbilirubinemia). The likelihood ratios associated with the 50th and the 75th TcB percentiles at 12, 24, and 36 postnatal hours to predict the outcome at 36 and 60 postnatal hours are presented in Table 4.5. The highest likelihood ratios were associated with the 75th percentile values at 24 and 36 postnatal age (6.8 and 6.3, respectively). These results indicate that for infants with TcB values > 75th percentile at 24 and 36 postnatal hours were six times more likely to have significant hyperbilirubinemia at 60 postnatal hours.
Table 4.4 Ability of TcB > 50th and > 75th Percentiles of the Parkland Hispanic TcB Nomogram to Identify Hispanic Neonates with Subsequent Significant Hyperbilirubinemia (TcB > 95th Percentile)

<table>
<thead>
<tr>
<th>Postnatal Age (hours)</th>
<th>Percentile</th>
<th>TP (n)</th>
<th>FN (n)</th>
<th>TN (n)</th>
<th>FP (n)</th>
<th>Sensitivity (CI)</th>
<th>Specificity (CI)</th>
<th>PPV (CI)</th>
<th>NPV (CI)</th>
<th>LR+ (CI)</th>
<th>LR– (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>&gt;50th</td>
<td>19</td>
<td>0</td>
<td>60</td>
<td>134</td>
<td>100% (79 - 100)</td>
<td>31% (25 - 38)</td>
<td>12% (8 - 19)</td>
<td>100%</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;75th</td>
<td>19</td>
<td>0</td>
<td>110</td>
<td>84</td>
<td>100% (79 - 100)</td>
<td>57% (50 - 64)</td>
<td>18% (12 - 28)</td>
<td>100%</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>&gt;50th</td>
<td>5</td>
<td>0</td>
<td>21</td>
<td>13</td>
<td>100% (47 - 100)</td>
<td>62% (44 - 77)</td>
<td>28% (11 - 54)</td>
<td>100%</td>
<td>2.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;75th</td>
<td>5</td>
<td>0</td>
<td>29</td>
<td>5</td>
<td>100% (66 - 100)</td>
<td>85% (68 - 94)</td>
<td>50% (20 - 80)</td>
<td>100%</td>
<td>6.8</td>
<td>0</td>
</tr>
<tr>
<td>36</td>
<td>&gt;50th</td>
<td>7</td>
<td>0</td>
<td>28</td>
<td>29</td>
<td>100% (57 - 100)</td>
<td>49% (36 - 63)</td>
<td>19% (9 - 37)</td>
<td>100%</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;75th</td>
<td>7</td>
<td>0</td>
<td>48</td>
<td>9</td>
<td>100% (57 - 100)</td>
<td>84% (72 - 92)</td>
<td>47% (21 - 70)</td>
<td>100%</td>
<td>6.3</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: Postnatal age refers to postnatal hours (± 6 hours) at time of transcutaneous bilirubin (TcB) measurement; Outcome represents significant hyperbilirubinemia at 36 and 60 postnatal hours (± 6 hours). TP, true positive; FN, false negative; TN, true negative; FP, false positive; PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio of a positive test result; LR–, likelihood ratio of a negative test result; CI, 95% confidence intervals.
Research Question #2

The second research question asked: Are there significant differences in hour-specific TcB values levels between early term and term neonates?

Sample Description

The sample included 513 neonates who were selected from the Early TcB database and met the criteria for the study. There were 131 infants in the early term group and 382 in the term group. Further description of the sample is presented in Table 4.5.

Table 4.5 Demographic Characteristics of the Sample for Research Question #2

<table>
<thead>
<tr>
<th></th>
<th>Early term n (%)</th>
<th>Term n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>68 (51.9)</td>
<td>200 (52.4)</td>
<td>ns</td>
</tr>
<tr>
<td>Female</td>
<td>63 (48.1)</td>
<td>182 (47.6)</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>87 (66.4)</td>
<td>281 (73.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Cesarean</td>
<td>44 (33.6)</td>
<td>101 (26.4)</td>
<td></td>
</tr>
<tr>
<td>Method of infant feeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>61 (47)</td>
<td>176 (46)</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>49 (37)</td>
<td>152 (40)</td>
<td></td>
</tr>
<tr>
<td>Formula</td>
<td>21 (16)</td>
<td>54 (14)</td>
<td>ns</td>
</tr>
<tr>
<td>Maternal race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>114 (87.0)</td>
<td>314 (82.2)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>10 (7.6)</td>
<td>39 (10.2)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>3 (2.3)</td>
<td>15 (3.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Asian</td>
<td>4 (3.1)</td>
<td>13 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Birth weight g, mean ± SD (median)</td>
<td>3158 ± 413 (3195)</td>
<td>3503 ± 440 (3478)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Gestational age (weeks) median</td>
<td>38</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>31 (23.7)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>100 (76.3)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>...</td>
<td>168 (44.0)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>...</td>
<td>137 (35.9)</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>...</td>
<td>41 (20.1)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Method of infant feeding categorized as breast refers to either exclusively breast-fed or breast-fed with < 30 ml (< 1 ounce) formula supplementation following breastfeeding; both refers to breast-fed with > 30 ml formula supplementation; and formula-fed refers to exclusively formula-fed; p < .05.
There was no difference in gender or mode of delivery between early term and term neonates, $\chi^2 (1) = .008, p = .93; \chi^2 (1) = 2.46, p = .12$, respectively. Likewise, the method of infant feeding did not differ significantly between the two groups, $\chi^2 (2) = .386, p = .82$. The majority of neonates in both groups were Hispanic, $\chi^2 (4) = 2.07, p = .722$. There was a significant difference in birth weight between the two groups ($z = -7.39, p < .0001$). Mean birth weight ($\pm SD$) in the early term group was 3158 g ($\pm 413$) and mean birth weight in the term group was 3503 ($\pm 440$). The median gestational age in the early term group was 38 weeks and the median gestational age in the term group was 40 weeks.

**Comparison of TcB Values**

A secondary purpose of this study was to compare TcB values between early term and term newborns to determine if, because of their relative physiologic immaturity, early term neonates have higher TcB values compared to infants born after 38 weeks of gestation. Transcutaneous bilirubin measurements performed at five epochs of postnatal age were compared. The results of the comparison in TcB values between the two groups of neonates are presented in Table 4.6. Early term neonates had higher TcB values in three of the five epochs (TcB-24, TcB-48, and TcB-60); however, the difference reached statistical significance only at the TcB-48 epoch, $t (208) = 2.294, p = .023$. Conversely, TcB values were higher in term neonates at two of the epochs (TcB-12 and TcB-36); however, these differences did not reach statistical significance.
Table 4.6 TcB Values (Mean ± Standard Deviation) for Early Term and Term Neonates

<table>
<thead>
<tr>
<th>TcB</th>
<th>Early term</th>
<th>TcB</th>
<th>Term</th>
<th>n</th>
<th>Value</th>
<th>n</th>
<th>Value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcB-12</td>
<td>65</td>
<td>2.6 ± 1.5</td>
<td>TcB-12</td>
<td>195</td>
<td>3.0 ± 1.4</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TcB-24</td>
<td>69</td>
<td>4.7 ± 1.6</td>
<td>TcB-24</td>
<td>194</td>
<td>4.6 ± 1.7</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TcB-36</td>
<td>68</td>
<td>6.3 ± 1.7</td>
<td>TcB-36</td>
<td>193</td>
<td>6.8 ± 3.1</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TcB-48</td>
<td>52</td>
<td>8.2 ± 2.5</td>
<td>TcB-48</td>
<td>158</td>
<td>7.3 ± 2.3</td>
<td>.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TcB-60</td>
<td>42</td>
<td>8.8 ± 2.2</td>
<td>TcB-60</td>
<td>86</td>
<td>8.3 ± 2.7</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: TcB, transcutaneous bilirubin refers to postnatal age in hours (± 6 hours) at time of TcB measurement; n, number of TcB determinations; TcB value in mg/dL and is reported as mean ± standard deviation; p < .05

Summary

This chapter describes the secondary data analyses of the Early TcB database. In the first analysis, the ability of the Parkland Hispanic TcB nomogram to accurately identify Hispanic neonates with subsequent hyperbilirubinemia (TcB > 95th percentile) was determined. In each of the postnatal periods examined, 100% sensitivity was reached for values greater than the 50th and the 75th percentiles of the nomogram. In addition, infants with TcB values > 75th percentile at 24 and 36 postnatal hours were six times more likely to have significant hyperbilirubinemia prior to 66 postnatal hours. These results provide evidence that the Parkland Hispanic TcB nomogram is valid to identify Hispanic neonates who subsequently develop significant hyperbilirubinemia. In the second analysis, a comparison of TcB values obtained at five epochs of postnatal age in early term and term neonates was performed. Although a significant difference in TcB values between early term and term neonates was found in only one of the epochs, no significant difference was observed for the other four epochs. These results suggest that there is no consistent pattern of differences in TcB values between early term and term neonates during the first 66 postnatal hours.
Chapter 5

Discussion

Transcutaneous bilirubin (TcB) nomograms have been proposed as potentially useful clinical tools in identifying neonates with hyperbilirubinemia; however, there are a limited number of studies in which the predictive accuracy of a TcB nomogram as a screening tool has been investigated. Therefore, the purpose of this study was to validate a previously published but unvalidated TcB nomogram to independently determine its accuracy in identifying Hispanic neonates at risk for the subsequent development of significant hyperbilirubinemia (TcB > 95th percentile). A secondary purpose of this study was to compare TcB values between early term (birth from 37\(^{0/7}\) through 38\(^{6/7}\) weeks of gestation) and term (birth from 39\(^{0/7}\) through 41\(^{6/7}\) weeks of gestation) neonates to determine if, because of their relative physiologic immaturity, early term neonates have higher TcB values compared to infants born after 38 weeks of gestation. This chapter discusses the study results obtained from the statistical analyses presented in chapter 4. Interpretations of the major findings for each of the research questions are described in conjunction with previously published studies. Limitations of the study are presented, and their relevance to interpretation of the study results is discussed. In addition, this chapter provides a discussion of the implications for nursing, recommendations for further research, and conclusions.
Interpretation of Major Findings

Research Question # 1

Question 1 asked: Is the 50th or the 75th percentile of the Parkland Hispanic TcB nomogram able to identify those Hispanic neonates who develop subsequent hyperbilirubinemia (TcB > 95th percentile) during their birth hospitalization? There has been limited research examining the validity of TcB nomograms in a second independent population. Similarly, no previous study has examined the ability of the previously published Parkland Hispanic TcB nomogram to accurately identify Hispanic neonates who subsequently develop significant hyperbilirubinemia prior to discharge from the birth hospital. This question provides an opportunity to independently assess a screening tool that may aid the healthcare professional in the identification and management of hyperbilirubinemia in this population.

Sample size

A pre-study power analysis was performed to ensure that an adequate sample size was selected to evaluate the accuracy of a screening tool (i.e., sensitivity, specificity, positive and negative predictive values, and likelihood ratios). This analysis indicated that a sample size of 73 subjects would be adequate. A convenience sample \((n = 404)\) was obtained from the Early TcB database, which provided a distinct and independent population of Hispanic neonates to externally validate the nomogram. Critical appraisal of a screening tool requires its evaluation in an independent group of patients (Sackett et al., 2000). In addition, the tool should be evaluated in an appropriate population of patients with the spectrum of the target condition or disease (i.e., neonatal hyperbilirubinemia) as would be seen in clinical practice (Haynes et al., 2006).
Interpretation of findings

The study sample was comprised of Hispanic neonates who were admitted to the same newborn nursery as participants in the original nomogram study. This nursery is located in a large public hospital in the Southwest region of the U.S. The two samples of neonates were similar with respect to ethnicity, gender, birth weight, and gestational age. The finding that there was no significance difference between the two populations for gestational age is important because gestational age is the single most important clinical factor associated with the risk of the subsequent development of neonatal hyperbilirubinemia (Keren et al., 2008; Kuzniewicz et al., 2008; Maisels & Kring, 1998; Newman et al., 2000; Newman et al., 2005). Conversely, there were significant differences between the nomogram sample and the study sample for the mode of delivery, the method of infant feeding, and hour-specific TcB values. At each of the five epochs of postnatal age examined, TcB values were significantly higher in the study sample. In addition, as the postnatal age at the time of TcB measurement increased so did the disparity between TcB values. The finding of significant differences in TcB values between the two samples may be partly explained by the higher proportion of cesarean births and exclusively breast-fed infants in the study sample.

While the majority of participants in both studies were delivered vaginally, the proportion of infants delivered by cesarean section was significantly higher in the study sample. At the hospital where the participants in both studies were born, the cesarean delivery rate varied significantly in the intervening years between the two study periods, increasing from 27% to 32%. Similar trends in cesarean delivery have been reported in national birth data. In the United States the cesarean delivery rate increased every year from 1996 to 2009, rising as much as 7% per year (Martin et al., 2012). From 2009 to 2010 the rate declined from 32.9 to 32.8% of all births, which was the first decline since

Findings from studies that have investigated the association between the mode of delivery and the neonatal hyperbilirubinemia have been mixed. Some investigators have reported that compared with vaginal birth, cesarean delivery decreases the risk of neonatal jaundice because there is less placental blood transfusion to the infant during the procedure (Bertini, Dani, Tronchin, & Rubaltelli, 2001; Osborn, Reiff, & Bolus, 1984; Yamauchi & Yamanouchi, 1989b). Yet, in large population-based studies vacuum extraction is the only method of delivery that has been found to be associated with neonatal hyperbilirubinemia (Keren et al., 2005; Keren et al., 2008; Newman et al., 2005). Vacuum extraction is used to facilitate a vaginal delivery by applying suction and traction to the fetal head during the pushing stage of labor. Infants delivered by this method are more likely to have a cephalhematoma (sub-periosteal hemorrhage) and/or bruising of the scalp, which increases the catabolism of red blood cells and yields more bilirubin (Cunningham et al., 2010). Thus, it seems unlikely that the increase in cesarean births in the study sample provides an explanation for the higher TcB values.

Compared with the nomogram sample, the proportion of infants in the study sample who were exclusively breast-fed was significantly higher. Many studies have found a strong association between exclusive breastfeeding and elevated bilirubin levels in the first postnatal days, as well as an increased risk of the subsequent development of significant hyperbilirubinemia (Maisels et al., 2009; Maisels & Kring, 1998; Newman et al., 2000; Stevenson, Fanaroff et al., 2001).

Some researchers believe that the neonatal hyperbilirubinemia is not associated with exclusive breastfeeding per se. Instead, they believe that hyperbilirubinemia results from a relative fasting or starvation state in the breast-fed neonate due to inadequate
caloric intake (Bertini et al., 2001; Gartner & Herschel, 2001). Typically, the amount of breast milk a woman produces is minimal for the first few days after delivery, but increases dramatically by 48 hours postpartum (Neville & Norton, 2001). Thus, caloric intake in the exclusively breast-fed newborn is limited until breast milk production is well established. Starvation in animals and human adults has been shown to enhance the enterohepatic circulation (i.e., shunting) of bilirubin, thus reducing its elimination (Bloomer, Barrett, Rodkey, & Berlin, 1971; Gartner, Goeser, & Wolkoff, 1997). The enterohepatic shunting of bilirubin is enhanced by any condition that delays or interrupts the passage of intestinal contents, as may be observed in an infant receiving inadequate nutrition due to difficulty in establishing lactation (Hansen, 2000). Thus, ineffective breastfeeding can enhance the enterohepatic circulation of bilirubin and reduce its elimination, resulting in increased bilirubin levels (Watchko, 2000).

The mode of delivery can have an indirect effect on optimal breastfeeding during the first few days postpartum. Breast-fed newborns delivered by cesarean section have been found to consume significantly fewer calories (i.e., less breast milk ingested) in the first six days compared with breast-fed infants who are delivered vaginally (Evans, Evans, Royal, Esterman, & James, 2002). Women delivered by cesarean section, especially emergent cesarean, often do not initiate breastfeeding as soon as women who deliver vaginally because of possible confinement to bed rest because of anesthesia and/or analgesia for pain (Zanardo et al., 2010). Likewise, cesarean delivery has been shown to have a negative effect on breastfeeding frequency, delayed milk production, and an increased incidence of providing supplemental non-breast milk fluids (e.g., dextrose water or formula) to the newborn (Bertini et al., 2001; Davanzo, Cannioto, Ronfani, Monasta, & Demarini, 2013; Dewey, Nommsen-Rivers, Heinig, & Cohen, 2003).
Despite the consistently higher TcB values in the sample, the findings in this study demonstrate the ability of the Parkland Hispanic TcB nomogram to identify Hispanic neonates at risk for the subsequent development of significant hyperbilirubinemia. Individual hour-specific TcB values > 50th and > 75th percentile at 12 ± 6, 24 ± 6, and 36 ± 6 postnatal hours were able to accurately identify all babies who subsequently developed hyperbilirubinemia (TcB > 95th percentile) at 36 ± 6 or 60 ± 6 postnatal hours and prior to discharge from the birth hospital. These results indicate that the Parkland Hispanic TcB nomogram is a valid and accurate clinical tool to identify Hispanic neonates who subsequently develop significant hyperbilirubinemia up to 66 postnatal hours. As shown in Table 4.7, TcB values obtained as early as 12 ± 6 hours generally were predictive of the development of subsequent significant hyperbilirubinemia.

Of all the various statistical measures of accuracy used in this study, the sensitivity of the nomogram percentiles to identify infants who subsequently develop significant hyperbilirubinemia is the most clinically relevant. When TcB is used to screen neonates to determine the need for a confirmatory serum bilirubin (TSB) measurement or the need for post-discharge follow-up care, false negative TcB values must be at a minimum or non-existent (Ebbesen, Vandborg, & Trydal, 2012). The medical risks associated with undetected hyperbilirubinemia (i.e., false negative result) far outweigh the risk associated with unwarranted serum bilirubin determination (i.e., false positive result). While blood sampling (heel-stick or venipuncture) is momentarily painful for the neonate, undetected hyperbilirubinemia can lead to serious consequences as described in first chapter.

Previously, only one study has examined the predictive accuracy of a TcB nomogram to identify the risk of neonatal hyperbilirubinemia. The European TcB
nomogram was developed in a predominately breast-fed population of Caucasian infants admitted to a hospital newborn nursery in Italy (DeLuca et al., 2008). The nomogram was validated in an independent population of neonates admitted to five hospital newborn nurseries in Italy (Romagnoli et al., 2012). Findings revealed that only after 48 postnatal hours the 75th percentile of nomogram accurately identified neonates who subsequently developed significant hyperbilirubinemia, defined as TSB > 17 mg/dL and/or the need for phototherapy. In the first 48 hours of age, 100% sensitivity was reached only with the 50th percentile of the TcB nomogram; however, there was a high false positive rate (71%). After 48 postnatal hours the 75th percentile of the nomogram was able to identify all infants (sensitivity 100%) at risk for the subsequent development of significant hyperbilirubinemia.

Research Question # 2

Question 2 asked: Are there significant differences in hour-specific TcB values between early term and term neonates? No known study has compared TcB values between early term and term neonates. This question provides an opportunity to examine dimensions of neonatal hyperbilirubinemia previously unexamined.

Sample size

A pre-study power analysis was performed to ensure that an adequate sample size was selected to examine differences between two independent groups. This analysis indicated that a sample size of 250 subjects would be adequate. A convenience sample of neonates was obtained from the Early TcB database, which provided an adequate sample size to estimate differences in TcB values between early term and term neonates. The two groups were similar with respect to ethnicity, gender, method of infant feeding, and mode of delivery. In contrast, compared with term newborns, infants born at early term gestations had significantly lower birth weights. This was an anticipated finding.
since birth weight reflects the length of gestation as well as intrauterine growth (Allen, Alexander, Tompkins, & Hulsey, 2000).

Interpretation of findings

In the present study, no consistent pattern of differences in TcB values measured between six and 66 hours of life between early term and term neonates was identified. This is the first known study to compare TcB values between neonates born from 37\(^{0/7}\) through 38\(^{6/7}\) weeks of gestation (early term) with neonates born from 39\(^{0/7}\) through 41\(^{6/7}\) weeks of gestation (term). In previous investigations of TcB levels in newborn populations, researchers have grouped together infants from different categories of gestational age, rather than the mutually exclusive classifications recommended by expert groups (American College of Obstetricians and Gynecologists and Society for Maternal-Fetal Medicine, 2013).

In an investigation of TcB values from healthy newborns ≥ 35 weeks of gestation in the first 96 postnatal hours, Maisels and Kring (2006) grouped participants in the following categories of gestational age: 35-37, 38-39, and ≥ 40 weeks (referent group). In the first 24 postnatal hours TcB values were significantly \(p < .01\) higher in infants born at 35-37 weeks compared with infants born at ≥ 40 weeks of gestation. However, in the first 24 postnatal hours, differences in TcB values between infants born at 38-39 weeks and the referent group were not significant. Compared with infants born at ≥ 40 weeks of gestation, infants born at 35-37 weeks had consistently higher TcB levels at 48, 72, and 96 postnatal hours \(p < .00001\). In addition, compared with the referent group, infants born at 38-39 weeks had significantly \(p < .00001\) higher TcB levels from 48 to 96 postnatal hours. Compared with infants born at term gestations bilirubin levels in late preterm (birth from 34\(^{0/7}\) through 36\(^{6/7}\) weeks) infants have been found to be significantly higher and sustained over a longer period (Fouzas, Mantagou et al., 2010). Thus, it is
possible that grouping late preterm (35 and 36 weeks) infants with early term (37 weeks) infants could explain these findings.

Two groups of investigators found that Greek late preterm and term neonates who required phototherapy presented a different pattern of incremental TcB increase compared to their counterparts who did not require treatment (Fouzas, Karatza et al., 2010; Fouzas, Mantagou, et al., 2010). Between 24 and 48 postnatal hours the rate of increase in TcB between the two groups was similar. Thereafter, the rate of increase remained high in neonates who required phototherapy, whereas it decreased, to almost zero by the fourth day of life in neonates who did not develop significant hyperbilirubinemia.

Analysis in the current study also revealed that from 42 to 66 postnatal hours (TcB-48 and TcB-60 epochs), TcB values in early term infants were consistently higher compared with those in term infants; however, the differences reached the level of statistical significance only at the 48-hour epoch. These findings are consistent with the model of transitional neonatal hyperbilirubinemia (Figure 1.2), which illustrates the imbalance between bilirubin production and bilirubin elimination in the transition after birth. Following birth, when the placental passage of bilirubin is terminated, bilirubin levels are time-dependent and include the following dynamics: (a) an incremental rate of rise (mg/dL/hour), (b) a peak (maximum level), and (c) a plateau (rate of bilirubin production and excretion are equal) followed by a gradual resolution. It should be noted that expression of the enzyme that is related to bilirubin conjugation (and then excretion) is developmentally regulated and is low in both term and preterm infants (Kawade & Onishi, 1981).

The inability to show a consistent pattern of difference in TcB values between early term and term neonates may have been due to gestational ages of the infants in the
early term group. The majority (76.3%) of infants in the early term group were born at 38 weeks of gestation. These neonates were relatively more mature than those infants with gestations younger than 38 weeks, thus their TcB values may be more comparable to infants born at term. Moreover, the inability to show a consistent difference in TcB values between the two groups of neonates may have been due to the lack of available TcB data beyond three days of age. It is possible, that at older postnatal ages there would have been significant differences in TcB values between the two groups. Evidence has shown that numerous factors that can affect serum bilirubin levels in neonates, such as gestational age, exclusive breastfeeding, and ethnicity. There are other factors that may have an important influence on bilirubin levels, including intrapartum events (e.g., vacuum extraction delivery, cephalhematoma, significant infant bruising), maternal factors (e.g., smoking, diabetes), and other neonatal factors (e.g., blood group incompatibility, hemolytic disease, previous sibling with jaundice) (Maisels & Newman, 2012). Thus, the lack of consistent differences in TcB values between early term and term neonates may have been due to a confounder that was not identified in this study due to limited data availability.

Study Limitations and Strengths

The findings from this study should be considered in the context of its potential limitations. This study was limited by the research design, which was a secondary analysis of an existing clinical database. Data in the Early TcB database were abstracted from electronic medical records (EMR) and represents a retrospective review of the EMR. With retrospective studies the investigator has less control of the variables in the study. Although some data regarding variables that are known to influence bilirubin levels were available, other data were not.
Another limitation of this study is the way in which neonates were selected for inclusion in the Early TcB database. Transcutaneous bilirubin data performed prior to six hours of age (i.e., early) and at least once every 24 hours prior to hospital discharge were collected from a convenience sample of neonates who were admitted to the newborn nursery from June 1-30, 2011 or May 1-31, 2012. One of the inclusion criteria for the first research question was that an infant had two or more TcB measurements performed between six and 66 postnatal hours. Prior to data analysis, six periods of postnatal age at the time of TcB measurement were chosen for comparison with the Parkland Hispanic TcB nomogram percentile values. Thus, not every TcB value was included in the analysis since only values within those periods were used.

There is limited information concerning the infant's complete race or ethnicity, since the only information elicited from the infant's mother prior to delivery was her self-reported race/ethnicity, and there is no information available regarding the ethnicity of the father of the baby. W.D. Engle and associates (2009) reported that informal surveys of the population that delivers at the hospital where the nomogram was developed indicate that Mexico is the country of origin for the vast majority of their Hispanic families. Since there is considerable variability among various Hispanic populations, the results of this study cannot be generalized to Hispanic neonates with different countries of origin.

Another limitation of this study is that no information was available regarding TcB levels or the development of significant hyperbilirubinemia following hospital discharge. All of these factors limit the generalizability of the findings. Thus, the sample may not be representative of other newborn patients born in this medical center, such as those who require phototherapy or of the larger hypothetical population of neonates at risk for hyperbilirubinemia.
Despite these limitations, the study had notable strengths. This is the first known study to investigate the predictive accuracy of a TcB nomogram in a neonatal population. The study showed that the Parkland Hispanic TcB nomogram is a valid and accurate tool to screen infants for the risk of the development of significant hyperbilirubinemia prior to 66 postnatal hours and hospital discharge. In addition, the findings from this study provide insight into gestational age as a clinical risk factor for neonatal hyperbilirubinemia.

Conclusion

In the current healthcare environment of early hospital discharge following childbirth, newborns with unrecognized or unmonitored jaundice represent a vulnerable population as they transition from the hospital to home. These concerns have led to the recommendation of predischarge risk assessment for the subsequent development of significant hyperbilirubinemia. In 2004, the American Academy of Pediatrics (AAP) recommended the development of TcB nomograms specifically designed for diverse populations of neonates. Thereafter, approximately 10 TcB nomograms were developed and published; however, only one has been validated in an independent population of neonates. Findings from this study demonstrate that the Parkland Hispanic TcB nomogram is an accurate and discriminatory tool for predicting the risk of the subsequent development of hyperbilirubinemia in Hispanic neonates prior to discharge from the birth hospitalization.

This is also the first study to examine differences in TcB values between early term and term neonates. Although observed trends indicated somewhat higher values in early term neonates, in summary there was a varying pattern of differences in TcB values between these two groups during the first 66 postnatal hours. Of all the risk factors for hyperbilirubinemia that have been reported, decreased gestational age is the factor that
has been identified most consistently. In part, this observation reflects a maturational-dependent effect on bilirubin production and the ability to metabolize and eliminate bilirubin in neonates. In light of other studies indicating “relative immaturity” of early-term neonates, the results of this study were supportive of increased vigilance for this group of infants during the postnatal period.

Implications for Nursing

Current evidence-based clinical practice guidelines recommend secondary prevention to promptly diagnose and treat neonatal hyperbilirubinemia before it can cause significant morbidity (AAP, 2004). All newborns should be routinely monitored during hospitalization and prior to discharge to identify elevated bilirubin levels, and to determine the risk for the development of significant hyperbilirubinemia (AAP, 2004). The key to secondary prevention is vigilance on the part of the healthcare team. Nurses play a vital role in the implementation of universal screening using TcB determinations to identify and monitor infants at risk for hyperbilirubinemia (Association of Women’s Health, Obstetric, and Neonatal Nurses [AWHONN], 2010). The measurement and interpretation of a predischarge TcB level can help determine the need for additional assessment, such as a confirmatory TSB determination, the need for treatment using phototherapy, and to help determine the timing of outpatient follow-up evaluations.

Nursing leaders should help create and implement standardized, system-based policies and procedures for the identification and evaluation of neonatal hyperbilirubinemia (National Association of Neonatal Nurses [NANN], 2010). This would include policies that require all newborns to be assessed regularly (e.g., every eight to 12 hours). In addition, the creation and implementation of nursery protocols that provides nurses with independent authority to order TcB or TSB measurements based on identified risk factors for jaundice rather than create potential delays by waiting for a
medical order should be established (Association of Women’s Health, Obstetric, and Neonatal Nurses, 2010).

Evidence-based practice in neonatal nursing includes the interpretation of bilirubin levels according to the neonate’s age in postnatal hours. An hour-specific TcB nomogram is a tool for interpreting TcB levels according to the neonate’s age in postnatal hours with resultant percentile zones; however, it is important that the predictive accuracy of a nomogram has been externally validated. If an infant’s hour-specific TcB value falls below a certain percentile (e.g., 50th percentile), the nurse can conclude that the infant is at lower risk for developing significant hyperbilirubinemia. If the infant’s hour-specific TcB value falls above a higher percentile (e.g., 75th), the risk of significant hyperbilirubinemia is higher, and thus more vigilance and closer follow-up of the infant is warranted.

Findings from this study provide validation of a TcB nomogram for use in Hispanic neonates at a Southwestern public medical institution. In the future, nursing professionals at this institution will be able to use this nomogram to assist in their clinical decision-making with confidence that they are using a validated tool. A unified and evidence-based approach to the assessment of neonatal hyperbilirubinemia using a validated TcB nomogram will enable the professional nurse to think critically and intervene when necessary to ensure that the patient experiences the best care and outcome.

Recommendations for Additional Research

Several areas for further research were identified. This study examined the predictive accuracy of the Parkland Hispanic TcB nomogram, which was developed from TcB data obtained in Hispanic neonates between 10 to 74 postnatal hours. Compared with neonates of other ethnicities and races, peak TcB levels occurred the latest (≥ 96 postnatal hours) in Hispanic neonates (DeLuca, Jackson, et al., 2009). There is limited
research regarding the accuracy of TcB measurements in older neonates, such as those seen in outpatient settings, when bilirubin reaches maximum levels. Further studies are needed to determine the relative roles of increasing age, higher bilirubin values, and environmental factors on the accuracy of TcB measurement in the outpatient setting.

Additional research is needed to explore the variables that may have an important influence on neonatal bilirubin levels, such as maternal, intrapartum, and neonatal factors. This study was a secondary analysis of a clinical database. Data contained in the database was collected from the EMR according to those variables that were deemed important by the database administrator. Conducting a prospective study could be useful to add insight into this topic. Unlike a retrospective review, a prospective design would provide the investigator more control over the study variables. The contribution of maternal factors, such as maternal age, smoking, history of previous infants with hyperbilirubinemia, intrapartum factors, such as vacuum extraction delivery, and additional neonatal factors, such as blood group incompatibilities have been shown previously. These known factors could be acknowledged and controlled for in a prospective study.

The inability to demonstrate a consistent pattern of difference in TcB values between early term and term neonates may have been related to the research design and sample size. Findings demonstrated that from 42 to 66 postnatal hours early term neonates had higher TcB values compared values obtained in term neonates; however, the differences were significant only from 42 to 54 postnatal hours. Research is needed using a larger sample of early term neonates and an equal representation of infants born at 37 weeks of gestation. In addition, further research that examines TcB values between the two groups beyond 66 postnatal hours and beyond hospital discharge could possibly add meaningful information to the subject.
Summary

The findings from this study on the validation of the Parkland Hispanic TcB nomogram clearly provide evidence of its accuracy as a screening tool for the identification of significant hyperbilirubinemia in Hispanic neonates. The findings from this study on differences in TcB values between early term and term neonates support the need for further research in this area. Study limitations include the secondary data analysis design, especially regarding variables that may influence bilirubin levels; however, data were not available. Another limitation was the lack of TcB data beyond 66 postnatal hours. In light of the fact that TcB levels in Hispanic neonates have been shown to peak at 96 postnatal hours, this represents both a limitation and an area for additional research. Implications for nursing practice were described with the recognition that professional nurses play a primary role in the implementation of evidence-based practice guidelines. The chapter concluded with specific recommendations for additional research, including research using prospective designs to investigate TcB values in outpatient settings.
Appendix A

Data Collection Tool
Appendix A
Data Collection Tool

Demographic Data

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<th>Study ID#</th>
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<th>Birth Weight (grams)</th>
<th>Gestational Age (weeks)</th>
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<th>Method of Infant Feeding</th>
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Note: Gender: Male = 1, Female = 2; Mode of delivery: Vaginal = 1, Cesarean section = 2 Method of infant feeding: Exclusively breast-fed or breast-fed with < 30 ml formula supplementation = 1; Breast-fed with > 30 ml formula supplementation = 2; Exclusively formula-fed = 3

TcB Determinations at Selected Postnatal Ages & Results

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Note: TcB, transcutaneous bilirubin value refers to postnatal age in hours (± 6 hours) at time of TcB measurement; result in mg/dL
Appendix B

University of Texas Southwestern Medical Center IRB Approval Letter
Appendix B

The UT Southwestern Institutional Review Board (IRB) reviewed the above-referenced research study via an expedited review procedure on January 16, 2013 in accordance with 45 CFR 46.110(a)(1)(ii). Having met all applicable requirements, the research study is approved. The approval period for this research study begins on January 17, 2013 and lasts until January 15, 2014.

The requirement to obtain informed consent is waived in accordance with 45 CFR 46.116(d).

The research study cannot continue beyond the approval period without continuing review and approval by the IRB. In order to avoid a lapse in IRB approval, the Principal Investigator must apply for continuing review of the protocol and related documents before the expiration date. A reminder will be sent to you approximately 30 days prior to expiration of research study approval.

The approved number of subjects to be enrolled is 600. If additional subjects are needed, you first must obtain permission from the IRB to increase the sample size.

If you have any questions related to this approval letter or about IRB policies and procedures, please telephone the IRB office at 214-648-3660.

General Instructions

To maintain IRB approval in good standing, please observe the following requirements:

1. Obtain prior IRB approval for any modifications including addition of new recruiting materials, changes in research personnel or site location, sponsor amendments or other changes to the protocol or associated documents. Only those changes that are necessary to avoid an unacceptable risk to a subject may be implemented without prior IRB approval.
2. Report all adverse events, protocol violations, and study closures promptly to the IRB.
3. Make study records available for inspection. All research-related records and documentation may be inspected by the IRB for the purpose of ensuring compliance with UT Southwestern policies and procedures and federal regulations governing the protection of human subjects. The IRB has authority to suspend or terminate its approval if applicable requirements are not strictly adhered to by all research study personnel.

This is a private message for authorized UT Southwestern employees only. If the content of this message is not intended for the addressee, you are hereby notified that any dissemination, distribution or copying of this information is STRICTLY PROHIBITED.
Appendix C

University of Texas at Arlington IRB Approval Letter
Appendix C

Institutional Review Board
Acknowledgment of Approved Research Activity

January 28, 2013

Nancy Engle
Dr. Judy Leflore
College of Nursing
The University of Texas at Arlington
Box 19407

UTA Protocol No.: 2013-0345
Protocol Title: Validation of Transcutaneous Bilirubin (TcB) Nomogram in Identifying Hispanic Neonates at Risk for Hyperbilirubinemia

The UT Arlington Office of Research Administration - Regulatory Services and Institutional Review Board (IRB) are pleased to acknowledge your engagement in this research protocol involving human subjects which has been approved by the IRB at UT Southwestern Medical Center at Dallas (UTSW). The UTSW IRB is noted as the “IRB of record” for this protocol. An IRB of record assumes IRB responsibilities for another institution as specified in each institution’s Federalwide Assurance (FWA), and has an agreement of reliability on file. Having met the conditions for approval set forth by the IRB at UTSW, and in compliance with applicable regulations, acknowledgment of such approval has been granted by the UTA IRB or designee.

UTSW IRB No: STU 122012-023
Review Level: Expedited
Approval Date: January 17, 2013

Please note that you are responsible for providing UT Arlington’s IRB with a copies of official notifications or approvals from the IRB of record, including but not limited to: approval letters for continuing reviews, approval letters for protocol modifications, incident or adverse event reports, audit or monitoring reports, or study closures.

The UT Arlington IRB and the Office of Research Administration - Regulatory Services appreciate your continuing commitment to the protection of human subjects engaged in research and wish you all the best in your research endeavors. Should you require further assistance, please contact Robin Dickey at robind@uta.edu or you may contact the office of Regulatory Services at regulatoryservices@uta.edu or 817-272-3723.
Appendix D

Study Site Approval Letter for Parkland Health & Hospital Systems
From: Susan Partridge  
Vice President, Clinical Research  
Christopher Madden, M.D.  
Interim Chief Medical Officer

RE: Parkland Health & Hospital System Study Site Approval

IRB Number: 122012-023

Principal Investigator: Gregory Jackson

Study Title: Validation of Transcutaneous Bilirubin (TcB) Nomogram in Identifying Hispanic Neonates at Risk for Hyperbilirubinemia

Dear Investigator:

Your study, as approved by the University of Texas Southwestern (UTSW) Medical Center Institutional Review Board (IRB), has received site approval from Parkland Health & Hospital System (PHHS). This approval is contingent upon compliance with UTSW IRB rules and regulations, with PHHS Institutional policies and any of the requirements that apply to your study covered in the general instructions included with this letter.

If there are research charges related to this study, they are documented in the Special Accounts Receivable (SAR) document which will be sent to you separately.

If you have any questions related to this approval letter, PHHS research policies and procedures or compliance requirements, please contact the Clinical Research Department Office at researchdepartmentparkland@phhs.org or by telephone at 214-590-1170.

Sincerely,

Susan Partridge, BSN, MBA  
Vice President, Clinical Research  
Parkland Health and Hospital System

cc: Anna Barden

Attachments: Executed Waiver
References


doi: 10.1007/s12098-008-0017-6


doi:10.1007/BF02723615

doi: 10.1038/jp.2008.213


doi: 10.1542/peds.103.1.6


Biographical Information

Nancy Engle completed her undergraduate nursing education at Texas Woman’s University in 1977. Following graduation she began a long career in the neonatal intensive care unit at several Dallas hospitals. In 2003 she graduated from the University of Texas at Arlington with a Master of Science in Nursing as a pediatric nurse practitioner (PNP). Engle was the recipient of Outstanding UTA PNP student and was chosen by the graduate pediatric faculty to give the 2003 commencement address. She is a member of Sigma Theta Tau International (STTI) Honor Society for Nurses and Golden Key International Honour Society. Following several years as a PNP in inpatient newborn care, outpatient neonatal and pediatric developmental care, and program manager in fetal diagnostic and family care coordination, Nancy began her doctoral studies at the University of Texas at Arlington. While pursuing her doctorate, she received a Delta Theta Chapter Sigma Theta Tau International Research Grant and the M.L. Bond Endowed Fellowship to support her dissertation research. Beginning in 2011, Nancy was an adjunct faculty member in the UTA PNP program. Future plans include employment as an Assistant Clinical Professor and continued research with a focus on the newborn.