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Utility of measuring direct bilirubin at first 72h of age in neonates admitted to the neonatal intensive care unit

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Abstract

OBJECTIVE—To assess the utility of measuring direct bilirubin (DB) during the first 72 h of life in infants admitted to the neonatal intensive care unit (NICU).

STUDY DESIGN—Infants born between May 2006 and June 2013, and admitted to the NICU were included. Abnormal DB was defined as: DB level ≥ 1 mg dl⁻¹ with a corresponding TB of ≥ 5 mg dl⁻¹ or DB level $>20\%$ of the corresponding TB >5 mg dl⁻¹.

RESULTS—The DB levels were measured in 3715 infants during the first 72 h of life. Sixty-three infants (1.7%) had abnormal DB. In a number of infants with potentially treatable diseases (biliary atresia and choledochal cyst), the only abnormal finding was a mildly elevated DB (1 to 2 mg dl⁻¹) during the first 3 days of life. In 22 infants (35%), the cause for high DB was unknown (16%) or not investigated (19%).

CONCLUSIONS—Routine measurement of DB in neonates admitted to NICU may be helpful in identifying potentially treatable causes of cholestasis.

INTRODUCTION

Jaundice in neonates is very common, with about two-thirds of all newborns developing jaundice.¹ Most often, neonatal jaundice is due to indirect (unconjugated) hyperbilirubinemia, and resolves spontaneously.¹ In 2004, the American Academy of Pediatrics published a practice guideline for the universal screening of total bilirubin (TB) in term and late-preterm (≥ 35 weeks gestation) neonates.² These guidelines recommend the measurement of direct bilirubin (DB) only in infants receiving phototherapy for jaundice, ill-appearing neonates, and cases of prolonged jaundiced (at or beyond 3 weeks of life). In infants with an elevated DB, additional evaluation to identify causes of cholestasis is recommended.^{2,3} Direct hyperbilirubinemia can signify infection, hepatobiliary dysfunction, or metabolic dysfunction. Early detection is critical in identifying potentially treatable conditions (sepsis, congenital infections, hypothyroidism, metabolic disorders, biliary atresia and choledochal cysts). Even for disorders without curative treatments (cystic fibrosis,

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CONFLICT OF INTEREST

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alpha-1 antitrypsin deficiency), early medical management can optimize quality of life and prevent further complications.

It is routine practice at our institution to measure the TB and DB levels during the first 72 h of age in all infants admitted to the neonatal intensive care unit (NICU). However, the usefulness of measuring DB in all neonates admitted to the NICU is currently unknown. The objective of this study was to assess the utility of measuring DB within the first 3 days of life in all infants admitted to the NICU and to ascertain normal ranges of DB in the subset of preterm and term neonates who required admission the NICU.

MATERIALS AND METHODS

This is a retrospective study of term and preterm neonates born between May 2006 and June 2013, and admitted to the level III NICU at Thomas Jefferson University Hospital in Philadelphia, PA, USA. Criteria for admission to the NICU were: all preterm neonates born <35 weeks gestation, all symptomatic late-preterm and term neonates (respiratory distress, transient tachypnea of newborn, meconium aspiration syndrome, hypoxic ischemic encephalopathy (HIE), possible and proven sepsis, hypoglycemia, neonatal abstinence syndrome, and major congenital anomaly). The Institutional Review Committee at Thomas Jefferson University Hospital approved this study. The infants were identified from a neonatal database (Neodata, Isoprime Corporation, Lisle, IL, USA). Relevant demographic, clinical and laboratory data were collected from the database and medical records. At our institution, TB/DB is measured on all neonates admitted to the NICU during the first 3 days of life regardless of clinical signs of jaundice when blood is drawn for other laboratory tests. For our analysis, we used the first DB measured in neonates during the first 72 h of life. DB was measured by the quantitative diazo colorimetric method (Cobas C501, Roche Diagnostics, Indianapolis, IN, USA). An elevated DB was defined as per the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) guidelines (DB 1.0 mg dl^{-1} when the TB is 5 mg dl^{-1} and DB >20% of the TB value when TB is $>5 \text{ mg dl}^{-1}$).³ Descriptive statistical analysis was performed using the Sigma Stat 3.1 for Windows statistical package (Systat Software, Inc., Point Richmond, CA, USA).

RESULTS

A total of 4449 infants were admitted to the NICU during the study period. DB levels were measured in 3715 (84%) infants at first 72 h of life. DB was not performed in neonates who were admitted for observation in the NICU and transferred to the newborn nursery within 24 h of life, admitted to the NICU after 3 days of life, or who were critically ill and died within 24 h. First DB was measured in 2548 infants (69%) on day 1 (at or before 24 h), 973 infants (26%) on day 2 (25 to 48 h) and 194 infants (5%) on day 3 (49 to 72 h). The mean gestational age was 36.7 weeks (range 22 to 42 weeks) and the mean birth weight was 2839 grams (range 323 to 6115 g). Approximately 65% infants were born at term (≥ 37 weeks gestation), 12% late-preterm (34 to 36 weeks gestation) and 23% preterm (<34 weeks gestation). The clinical characteristics of the study population are further summarized in Table 1. Sixty-three (1.7%) infants had an abnormal DB during the first 72 h of life; in 15 of these infants, it was abnormal at or within 24 h. There were no significant differences in the

number of infants with an abnormal DB among preterm, near-term and full-term groups. An additional 31 infants (0.8%) developed an abnormal DB between days 4 and 7 (27 infants had a normal DB during first 72 h and 4 infants did not have DB measured during first 3 days). Table 2 and Figure 1 depict the DB levels on day 1, 2 and 3 of age in term, late-preterm and preterm neonates, respectively.

HIE was the most common (43%) identifiable cause of high DB. Biliary atresia was diagnosed in three infants (4.8%) (Table 3). Several neonates with potentially treatable conditions (biliary atresia, choledochal cyst, congenital syphilis and gestational alloimmune liver disease (GALD)) had an abnormal DB at 24 to 72 h of age. Mildly elevated DB (1 to 1.2 mg dl⁻¹) during the first 72 h of life was the only abnormal finding in two infants with biliary atresia and one infant with a choledochal cyst. One infant had possible GALD and was treated with intravenous immunoglobulin (IVIG) with markedly improved DB levels. One infant required transfer to a local children's hospital for a possible metabolic disease. About a third of neonates (35%) with an elevated DB had either an unknown etiology (16%) or no further investigation (19%). In 7 out of 10 infants with an elevated DB who were investigated but had no known cause, DB levels normalized before discharge or by follow-up (only three infants not in our health system had no follow-up data available). Six out of 12 infants with abnormal DB within 72 h were not investigated; however, DB levels normalized before discharge. The remaining six infants had a mildly elevated DB (1 to 1.8 mg dl⁻¹) and had no directed follow-up (these infants were not in our health system and no follow-up data are available).

Infants with an abnormal DB level as defined by the NASPGHAN were compared to 97.5th percentile from our cohort. More infants had an abnormal DB as defined by the 97.5th percentile than by the NASPGHAN definition (84 vs 63). No infant with known etiologies was missed if the NASPGHAN definition was used. An additional 21 infants had an abnormal DB as defined by 97.5th percentile (Table 3).

Table 4 depicts the causes of abnormal DB found at day 4 to 7. Thirty-one infants had abnormal DB levels at 4 to 7 days of life, and of these, 27 of them had normal DB levels on days 1 to 3. In 4 infants with abnormal DB at 4 to 7 days, no DB level was performed during days 1 to 3 (2 resolved cause unknown, and 1 each with extracorporeal membrane oxygenation (ECMO) and HIE).

During the study period, 291 infants were admitted with HIE, out of which 33 infants (11.3%) had abnormal DB levels during the first 7 days of life. Cholestasis resolved in 22 infants (66.6%) with HIE by 2 weeks and in 29 infants (87.9%) by 4 weeks. A total of 56 infants received ECMO during the study period, and 11 of these infants (19.6%) developed cholestasis. In these 11 infants on ECMO, DB remained elevated in 4 infants (36%) at 4 weeks of age and 1 infant (9%) at 6 weeks of age.

The mean and median DB levels for various etiologies of cholestasis in neonates are described in Table 5.

DISCUSSION

The American Academy of Pediatrics recommends universal screening of TB in term and late-preterm neonates.² As a part of this clinical practice guideline, DB is obtained in sick, jaundiced infants. The usefulness of routinely measuring DB at the time of screening for TB is unknown. In the current study, we evaluated the utility of early routine measurement of DB in all neonates admitted to the NICU. We found that 1.7% of neonates admitted to the NICU had abnormal DB during the first 72 h of age. Several neonates with potentially treatable conditions had no abnormal clinical or laboratory findings other than a mildly elevated DB during the first 3 days of life. Routine measurement of DB in neonates admitted to NICU may be helpful in early identification of potentially reversible causes of cholestasis.

The practice guideline from the American Academy of Pediatrics recommends a measurement of DB in late-preterm and full-term infants receiving phototherapy for jaundice, jaundice presenting at or beyond 3 weeks of age, and sick infants.² Neonates admitted to the NICU are either sick or preterm and are likely to require phototherapy. At our institution, TB and DB are measured at first 72 h of age in all neonates admitted to the NICU. By routinely measuring DB in >3700 infants, we have identified 63 neonates (1.7%) with an abnormal DB during the first 3 days of life with an additional 31 infants (0.8%) detected between days 4 to 7. HIE was the most common cause of cholestasis in our cohort (43%). Transient cholestasis is common in neonates with HIE, affecting up to 8.5% of appropriate for gestational age infants and 33% of small for gestational age infants.⁴ The mechanism leading up to cholestasis in neonates with HIE is not well understood, but is thought to be due to reduced splanchnic blood flow and poor oxygenation of liver and bowel.⁴ HIE-associated hepatic dysfunction is likely to resolve over time without long-term impact on hepatic function.

Direct hyperbilirubinemia or neonatal cholestasis is found in 1 in 500 to 2500 infants.^{5,6} A multitude of illnesses can manifest as neonatal cholestasis. Early recognition of potentially treatable conditions improves outcomes in neonates with cholestasis. It is well known that early diagnosis of extrahepatic biliary atresia is advantageous because surgical correction with Kasai portoenterostomy is more successful under 60 days of age.^{7,8} In our cohort, three infants with biliary atresia and one infant with a choledocal cyst had abnormal DB during the first 72 h of age. In a retrospective review, Harpavat *et al.*⁹ described 34 infants with biliary atresia whose direct/conjugated bilirubin was measured at 0 to 96 h of age. They found that DB level was significantly higher at 24 to 48 h of age in neonates who developed BA compared with controls, and they concluded that BA can be detected earlier if DB is measured in all neonates.⁹ Our data also indicate that BA can be diagnosed early with routine measurement of DB during the first 72 h of age. Three infants in our cohort had a diagnosis of biliary atresia. This is higher than the reported incidence of 1 in 10 000 to 15 000.^{10,11} Our NICU is a regional referral center with a large number of outborn neonates admitted to the NICU. Only one of the three infants with biliary atresia was born at our hospital, whereas the remaining two were transferred in for a higher level care due to pulmonary hypertension. During the study period, ~ 15 000 infants were born at our center consistent with the incidence of BA, 1 in 15 000 live births. In two infants, biliary atresia was diagnosed at 3 weeks of age while in the NICU for increasing DB. One infant with

abnormal DB was discharged and readmitted at 6 weeks of age for increasing DB and was subsequently diagnosed with biliary atresia. Two of the infants with biliary atresia had mildly increased DB levels on day 1 (1 and 1.2 mg dl⁻¹). The third infant had a higher DB (level of 5.9 mg dl⁻¹) on the second day of life, likely to be due to embryonic form of biliary atresia.

GALD, previously known as neonatal hemochromatosis, is another important and potentially treatable cause of neonatal cholestasis.^{12,13} The new term reflects the alloimmune mechanism that is responsible for cholestasis in newborns.¹³ One infant in our cohort was suspected to have GALD and responded to treatment with IVIG. It is possible that GALD could be a potential cause of cholestasis in other neonates in our cohort who were not investigated or etiology of high DB could not be determined.

Routine measurement of DB in all neonates may also be useful in identifying other treatable diseases like congenital infections (for example, cytomegalovirus, toxoplasmosis and syphilis), early onset bacterial infections, metabolic conditions (for example, galactosemia, tyrosenemia, cystic fibrosis and bile acid synthetic defects), and endocrine disorders (for example, hypothyroidism and hypopituitarism). In our cohort, one infant had congenital syphilis, while another infant had a possible metabolic disorder. In addition, two infants with culture proven sepsis had abnormal DB between 4 and 7 days.

As this is a retrospective study, we can only speculate from reviewing the chart that the routine measurement of DB was useful in identifying treatable diseases. Two of the three infants with BA and one infant with choledochal cyst had only mildly elevated DB levels. They would have been discharged without follow-up or workup if DB was not measured during the first 3 days of life. The third infant with BA was sick and required prolonged total parenteral nutrition. This infant would have been investigated and diagnosed with BA during the hospital course. One infant with GALD was investigated and treated only because of abnormal DB found during the routine screening. The infant with possible metabolic disease would have been investigated due to other laboratory abnormalities (hypoglycemia and lactic acidosis) and infant with congenital syphilis had abnormal maternal serological tests.

We found several infants requiring ECMO had abnormal DB in the first 72 h with spontaneous resolution by discharge. Cholestasis occurs frequently (14 to 39%) in infants who are managed on ECMO.^{14,15} Severe hemolysis during ECMO support produces a large bilirubin load whose excretion is inhibited by mechanisms similar to inspissated bile syndrome and/or exposure to plasticizer di-(2-ethylhexyl) phthalate.¹⁶ Cholestasis induced by ECMO resolves over time without hepatic sequelae.^{14,15}

Despite elevated DB levels, a significant number of infants in our cohort were neither investigated nor followed up after discharge. Similarly, Harpavat *et al.*⁹ reported that 16 of 34 infants who were diagnosed late with BA had abnormal DB at 24 to 72 h of age but caregivers failed to direct them for follow-up after discharge from the nursery. In our cohort, 6 out of 12 infants with abnormal DB who were not investigated also did not have follow-up arranged at the time of discharge. Unfortunately, we do not have follow-up data on infants

with early abnormal DB who were not investigated or nor followed up after discharge from the hospital.

Currently, literature on the normal range of DB in term, late-preterm and preterm neonates during the first 72 h of life is limited. From this large cohort of neonates, we can provide normative data of DB levels in the first 72 h of life for preterm infants (<34 weeks gestation) and a subset of term and late-preterm infants who require NICU care. In a retrospective database study, Davis *et al.*¹⁷ measured conjugated and DB levels in a large cohort of term neonates during first 14 days of life. The 96th percentile DB level (1.0 mg dl⁻¹) was similar to the 96th and 97.5th percentile DB levels (0.976 and 1.0 mg dl⁻¹, respectively) in our cohort. The neonates in the study by Davis *et al.* were born at term and a majority of them were admitted to the regular newborn nursery. The infants in our cohort were sick preterm and term neonates admitted to the NICU, but the 96th percentile DB levels were similar to the cohort of infants from the study by Davis *et al.* More infants had abnormal DB defined by the 97.5th percentile as compared with the NASPGHAN definition (84 vs 63). If the NASPGHAN definition is used, all infants with known etiologies are identified. An additional 21 infants had abnormal DB based on 97.5th percentile. The abnormal DB resolved in 16 infants but 5 additional infants had abnormal DB without directed follow-up. It is possible that some of these infants with unresolved abnormal DB may have organic etiology for high DB.

Using the NASPGHAN definition of abnormal DB, only 21% of infants with BA would be detected in the study by Harpravat *et al.*⁹ In their cohort, the lowest DB in the BA group was 0.8 mg dl⁻¹ and the highest DB in the control group was 0.7 mg dl⁻¹. Applying our normogram to the cohort of infants from Harvprat's study, we would detect all infants with BA. We suggest that any infant with DB levels of >95th percentile in our normogram should be closely followed; and if DB is persistently high or increasing, further investigation should be required.

The cost effectiveness of measuring DB levels during the first 3 days of life in all neonates admitted to the NICU can not be accurately determined from this study. The charge to the patient for measuring DB at our institution is \$16. However, the actual cost of measuring DB level is much lower. The total charge to patients for measuring DB in our cohort is approximately \$60 000 with an additional cost of repeating levels and evaluating neonates with false-positive results. Yet, several infants with potential treatable diseases can be diagnosed early with this screening test. The early diagnosis of biliary atresia, GALD, infections and metabolic disease can not only improve outcomes but also reduce the cost related to complications of delayed diagnosis.

The major strength of the current study is the large sample size with over 3700 neonates. It is the first to compile normative DB values in neonates admitted the intensive care unit. We recognize some important limitations of this study. This is a retrospective study from a single center. Our unit is a level III NICU and a center for ECMO and therapeutic hypothermia. HIE and ECMO may not be common causes of high DB in NICUs without ECMO and therapeutic hypothermia. We are unable to present follow-up data on infants who were discharged with abnormal DB. Last, DB, not conjugated bilirubin, was measured

in our cohort. In high concentrations, unconjugated bilirubin may react with diazo reagent falsely elevating the DB.¹⁷

In conclusion, our findings suggest that a large number of infants admitted to the NICU have abnormal DB during the first 3 days of life. Routine measurement of DB in neonates admitted to NICU may be helpful in early identification of potential treatable causes of cholestasis. With current practices, a number of neonates with abnormal DB levels are either not investigated or directed follow-up at discharge. Neonates with abnormal DB should be investigated and followed closely to ensure resolution. Further studies are needed to assess the utility of routine screening for DB in all neonates.

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Figure 1. Direct bilirubin concentrations in mg dl^{-1} (5th, 50th, 95th and 97.5th percentile) on day 1, 2 and 3 of age in preterm, late-preterm and full-term neonates.

Table 1

The demographics and baseline clinical characteristics of the study population ($n=3715$; mean $\pm$ s.d.)

	All neonates ($n = 3715$)	Term (37–42 weeks) ($n = 2406$)	Late preterm (34–36 weeks) ($n = 461$)	Preterm (<34 weeks) ($n = 848$)
Birth weight (g)	2839 $\pm$ 940	3349 $\pm$ 590	2511 $\pm$ 460	1573 $\pm$ 604
Gestational age (weeks)	36.7 $\pm$ 13.9	39.1 $\pm$ 1.2	35.5 $\pm$ 0.5	30.7 $\pm$ 3.2
Male gender (%)	2000 (54)	1325 (55)	244 (53)	431 (50)
Race, Black (%)	1450 (39)	898 (37)	172 (37)	380 (45)
Apgar score, <5 at 5 min (%)	289 (7.8)	199 (8.3)	37 (8.0)	53 (6.3)
Abnormal DB during the first 72 h (%)	63 (1.7)	44 (1.8)	10 (2.2)	9 (1.1)
Abnormal DB during the first 7 days (%)	94 (2.5)	56 (2.3)	18 (3.9)	20 (2.4)

Abbreviation: DB, direct bilirubin.

Table 2
Direct bilirubin concentrations (mg dl⁻¹) on day 1, 2 and 3 of age in the study population

	50th	2.5th	5th	95th	97.5th
<i>Day 1</i>					
All	0.4	0.1	0.2	0.6	0.8
Full term	0.4	0.2	0.2	0.7	0.8
Late preterm	0.4	0.1	0.2	0.6	0.8
Preterm <34 weeks	0.3	0.1	0.1	0.6	0.7
<i>Day 2</i>					
All	0.4	0.2	0.2	0.8	0.9
Full term	0.4	0.2	0.2	0.8	0.9
Late preterm	0.4	0.2	0.2	0.8	0.9
Preterm <34 weeks	0.4	0.1	0.2	0.7	0.8
<i>Day 3</i>					
All	0.4	0.1	0.2	0.9	1.0
Full term	0.5	0.2	0.2	1.0	1.0
Late preterm	0.4	0.2	0.2	0.9	1.0
Preterm <34 weeks	0.4	0.1	0.1	0.8	0.9

Table 3

Possible etiologies of abnormal direct bilirubin during the first 3 days of life using NASPGHAN criteria and 97.5th percentile from our cohort

Etiology	Abnormal DB using NASPGHAN criteria (n = 63)	Abnormal DB using 97.5th percentile (N = 84)
HIE (%)	27 (43)	27 (35)
ECMO (%)	4 (6.3)	4 (4.8)
Biliary atresia	3 (4.8)	3 (3.8)
Possible metabolic disease (%)	1 (1.6)	1 (1.2)
GALD (%)	1 (1.6)	1 (1.2)
Congenital syphilis (%)	1 (1.6)	1 (1.2)
Choledochal cyst (%)	1 (1.6)	1 (1.2)
Neonatal hepatitis (%)	1 (1.6)	1 (1.2)
Severe hemolysis (Hb H, ABO incompatibility) (%)	2 (3.2)	2 (2.4)
<i>Unknown investigated (%)</i>	10 (15.8)	10 (11.9)
Resolved	3	3
Unresolved	7 (follow-up directed 6)	7 (follow-up directed 6)
<i>Unknown not investigated (%)</i>	12 (19.0)	33 (39.3)
Resolved	6	22
Unresolved	6 (no directed follow-up)	11 (no directed follow-up)

Abbreviations: DB, direct bilirubin; ECMO, extracorporeal membrane oxygenation; NASPGHAN, North American Society for Pediatric Gastroenterology, Hepatology and Nutrition.

Table 4

Possible etiologies of abnormal direct bilirubin found at days 4–7

Etiology	Abnormal DB (<i>n</i> = 31)	Abnormal 4–7 days (normal at 1–3 days) (<i>N</i> = 27)
HIE (%)	6 (19)	5 (18)
ECMO (%)	7 (23)	6 (26)
Sepsis (%)	2 (6)	2 (7)
<i>Unknown investigated (%)</i>	8 (26)	8 (30)
Resolved	6	6
Unresolved	2 (directed follow-up 2)	2 (directed follow-up 2)
<i>Unknown not investigated (%)</i>	8 (26)	6 (22)
Resolved	6	4
Unresolved	2 (died 1, no directed follow-up 1)	2 (died 1, no directed follow-up 1)

Abbreviations: DB, direct bilirubin; ECMO, extracorporeal membrane oxygenation.

Table 5

The mean and median direct bilirubin levels during first 3 days of life for various etiologies of cholestasis in neonates (first abnormal DB level is used if multiple levels are available)

Etiology	Mean $\pm$ s.d.	Median (range)
HIE (27)	1.4 $\pm$ 0.4	1.3 (1–2.3)
ECMO (4)	1.2 $\pm$ 0.3	1.1 (1–1.8)
Biliary atresia (3)	2.7 $\pm$ 2.8	1.2 (1–6)
Possible metabolic disease (1)	5.6	5.6
GALD (1)	10.6	10.6
Congenital syphilis (1)	10.9	10.9
Choledochal cyst (1)	1.2	1.2
Neonatal hepatitis (1)	1.2	1.2
Severe hemolysis (Hb H, ABO incompatibility) (2)	4.8 $\pm$ 4.2	4.8 (1.8–7.8)
<i>Unknown investigated (10)</i>	1.6 $\pm$ 0.9	1.3 (1–4.1)
Resolved (3)	1.3 $\pm$ 0.3	1.5 (1.2–2.3)
Unresolved (7)	1.6 $\pm$ 1.1	1.2 (1–4.1)
<i>Unknown not investigated (12)</i>	1.3 $\pm$ 0.3	1.2 (1–1.8)
Resolved (6)	1.4 $\pm$ 0.05	1.4 (1.4–1.5)
Unresolved (6)	1.2 $\pm$ 0.3	1.1 (1–1.8)

Abbreviations: DB, direct bilirubin; ECMO, extracorporeal membrane oxygenation; GALD, gestational alloimmune liver disease.