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Interpreting conjugated bilirubin levels in newborns

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Abstract

Objective—To examine the clinical significance of elevated conjugated bilirubin (CB) levels in newborns.

Study design—This retrospective study evaluated a birth cohort of 271,186 full-term newborns born within a Northern California hospital network from 1995–2004. All CB and direct bilirubin (DB) levels were available in a database and were correlated with the patients' in and out patient ICD-9 diagnoses.

Results—The 99th percentile for CB is 0.5 mg/dL, and the 99th percentile for DB is 2.1 mg/dL. CB levels between 0.5–1.9 mg/dL can be associated with infection, but most often remain unexplained. Liver and biliary disease become increasingly likely as CB levels increase, for CB ≥5 mg/dL 47% have biliary disease and 43% have liver disease.

Conclusions—CB and DB levels are not interchangeable. In newborns with CB levels ≥0.5 mg/dL and <2 mg/dL, infection must be ruled out and the newborn should be followed. In newborns with levels ≥2 mg/dL, a more in-depth assessment of the hepatobiliary system is indicated.

Keywords

conjugated bilirubin; epidemiology; newborn; cholestasis; diagnostic test

Neonatal jaundice occurs in about two thirds of all newborns.(1) The vast majority of jaundiced newborns have elevated unconjugated bilirubin levels, most often due to hemolytic causes. A small minority have cholestasis with causes including congenital abnormalities and infectious, metabolic, iatrogenic and idiopathic disorders.(2) Identifying these newborns cholestasis from the masses of jaundiced newborns can be difficult but is important for early diagnosis and treatment. For this reason, conjugated (CB) and direct-reacting bilirubin levels (DB) are often measured.

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However, CB and DB are inherently different. Unconjugated bilirubin is the breakdown product of heme. In the liver, it is converted to CB by the conjugation of glucuronic acid. A small portion, termed delta bilirubin, is CB covalently bound to albumin. Direct bilirubin (DB) measurements estimate the total concentration of the conjugated and the delta bilirubin. The method used today in most laboratories takes advantage of unconjugated bilirubin being a poor reactant with a diazo reagent without an accelerant.(3) On the other hand, CB will react "directly" with the reagent without an accelerant. However, in high concentrations some unconjugated bilirubin will react with the diazo reagent without addition of the accelerant causing DB measurements to overestimate the concentration of CB. CB measurements are generally made using the BuBc slide method, originally developed by Kodak.(4,5) In this method unconjugated and CBs are simultaneously measured by direct spectrophotometry.(6)

In this study we evaluated CB and DB levels in newborns with three objectives: 1) to compare their distributions in the first two weeks after birth, 2) to estimate the incidence of cholestatic disease and 3) to describe the relationship of CB with various diseases.

METHODS

The Northern California Kaiser Permanente Medical Care Program (NC-KPMCP) is a group-model managed care organization that covers ~3.3 million members, 30% of the insured population in northern California. The cohort from which the subjects were identified included all newborns born alive in 12 NC-KPMCP hospitals from January 1, 1995, to December 31, 2004, whose birth weight was ≥ 2000 g and whose gestational age was ≥ 37 weeks (N = 271,186) who had at least one CB or DB measured within the first two weeks after birth (N = 66,431). We obtained laboratory tests, ICD-9 diagnostic codes from inpatient and outpatient visits, and basic demographic data on the entire cohort from NC-KPMCP electronic databases, as described previously.(7,8) This project was approved by the NC-KPMCP Institutional Review Board for the Protection of Human Subjects and by the University of California San Francisco Committee on Human Research.

Eleven of the 12 Northern California Kaiser Permanente Hospitals generally test for conjugated and unconjugated bilirubin (rather than direct and indirect bilirubin) on Vitros instruments using the BuBc slide method. One hospital generally used the DuPont Automated Chemical Analyzer, which measures direct and indirect bilirubin. This is significant for this study because when using the BuBc slide method both conjugated and unconjugated bilirubin are measured and reported regardless of whether the provider has a suspicion of cholestasis, whereas in the NC-KPMCP DB levels must be ordered separately.

Statistical Analysis

We described the distributive percentiles for maximum CB and DB in the first 2 weeks after birth. We grouped ICD-9 codes into the following categories: bacterial infections, biliary tract disorders, chromosomal abnormalities, fetal distress, fluid and electrolytes disorders, gastrointestinal abnormalities, liver diseases, metabolic disorders, severe bacterial infections, urinary tract infections, viral hepatitides and viral infections. For example, the biliary tract disorder category included 8 different groups of ICD-9 codes (Table I; available at www.jpeds.com). The incidence of each disease category was estimated and the occurrence of cholestasis given a disease category was calculated. We included only diagnoses for which the proportion with maximum CB ≥ 2 mg/dL or DB ≥ 5 mg/dL was at least 1%. The relationship between maximum CB level and the likelihood of a diagnostic category was investigated. Using various cutoffs for CB and DB we calculated likelihood ratios of hepatobiliary disease. We defined hepatobiliary disease as having a diagnosis in

either the liver or the biliary disease category. We used STATA 9.2 (Statacorp, College Station, TX) for all analyses.

RESULTS

Of the 271,186 subjects born within this cohort, 75,661 (27.9%) had at least one serum total bilirubin level, 64,095 (23.6%) had a CB and 3,245 (1.2%) had a DB measured in their first 14 days after birth. Of the 22,272 subjects who were born at the hospital that generally used the diazo method, 11,323 had at least one total bilirubin level measured, of which 707 (6.2%) had a DB measured and 2,177 (19.2%) had a CB measured. Of the 248,914 subjects born at the other 11 facilities, 64,338 had at least one total bilirubin level measured, of which 2,538 (3.9%) had a DB measured and 61,918 (96%) had a CB measured. Of those who had a CB measured in their first 14 days after birth, 43,240 (67.5%) had it measured at least once in their first 3 days and 40,761 (63.6%) had it measured at least once between day 3 and day 14. The demographics of the entire cohort and of the subjects who had either a CB or DB level measured are shown in Table II. There are significant differences between the group that was tested and the group that was not that likely reflects which patients have a higher risk of unconjugated hyperbilirubinemia. The subjects tested were smaller, gestationally younger and more likely to be Asian and male than the subjects who were not tested.

Table III demonstrates the distribution of maximum measured CB and DB in the first two weeks after birth. An undetectable amount of CB is the norm and anything above 0.3 mg/dL is 2 standard deviations above the mean. Because of the differences in how these two measurements are made, DB levels were considerably higher than CB levels, but even with DB levels 98.6% of measurements were 2 mg/dL or less.

In patients (n=19,906) who had CB levels measured both before 3 days and between 3 days and 14 days after birth there was a small, but significant, increase in the mean CB from 0.10 mg/dL to 0.15 mg/dL ($p<0.001$).

Biliary atresia occurred in 27 (1 in ~10,000) of our subjects which is consistent with previously published reports.(9–12) Other diagnoses were more common, but less often associated with cholestasis (Table IV). Liver and biliary tract diseases were the only conditions to commonly present with cholestasis. CB levels 0.5 to 1.9 mg/dL usually remain unexplained, and were more associated with severe infections than with hepatobiliary disease (Table V). The frequency of GI and liver diagnoses increased with CB over 2 mg/dL, but did not predominate until CB was > 5 mg/dL. It is important to note that many subjects had ICD-9 diagnoses in more than one disease category. The calculated likelihood ratios are presented in Table VI.

DISCUSSION

In this study we were able to examine the distribution of serum direct and CB levels in a large community health care population. We were able to examine what diagnoses were associated with elevated levels.

Our study did have some important limitations. We were unable to isolate parenteral nutrition through our databases. It is therefore unclear how many of our subjects had total parental nutrition (TPN) related cholestasis and were put into the “No diagnosis associated with cholestasis” category. We excluded premature newborns to minimize this problem, but there are certainly some term newborns who receive TPN. Many of them have other abnormalities such as intestinal atresias that we would have picked up with ICD-9 codes, but

some may have had heart conditions or lung conditions which in themselves are not associated with cholestasis.

Our study was also limited in that it was a database review and not a chart review. ICD-9 codes may be used inconsistently or inaccurately and this could make our conclusions less accurate.

It is clear from this study that CB and DB measurements cannot be interpreted interchangeably, especially when <2 mg dL. It is also clear that even mildly elevated CB levels are abnormal. Therefore, as more institutions switch to the BuBc slide method and more providers are being presented with CB levels we suggest that infection be considered in any patient with levels ≥0.5 mg/dL and <2 mg/dL, as severe infection occurs in 2% of these patients. We would also recommend following these patients closely over time and rechecking a CB level to assure it has returned to normal. Furthermore, our study suggests that levels ≥2 mg/dL indicate that a more in-depth assessment of the hepatobiliary system is warranted.

The cost-effectiveness of universal screening of CB in neonates or of routine use of BuBc slide method is not something that this study can address sufficiently. The incremental cost of obtaining a CB level with an unconjugated bilirubin level through the BuBc slide method is zero. However, it is unclear what the cost of further evaluation in patients with false positives would be. False positives are less likely using CB than DB because CB is more specific. Only 0.3% of newborns have a moderately elevated CB (≥2–4.9 mg/dL) and >30% of them have an associated disease. For this reason using BuBc slides to reduce false positives and direct further evaluations may be appropriate.

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ABBREVIATIONS

CB	Conjugated bilirubin
DB	Direct bilirubin
ICD-9	International Classification of Diseases, 9th Revision
NC-KPMCP	The Northern California Kaiser Permanente Medical Care Program
GI	Gastrointestinal
TPN	Total parental nutrition

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TABLE 1

ICD-9 codes included in biliary tract disorder category

277.4 Disorders of bilirubin excretion
571.6 Biliary cirrhosis
574.xx Cholelithiasis
575.xx Disorders of gallbladder
576.xx Disorders of biliary tract
751.6x Anomalies of gallbladder, bile ducts, and liver
751.61 Biliary atresia
779.3 Nonspecific abnormal findings on examination of biliary tract

TABLE 2

Demographics of study subjects

	CB or DB measurements in 1st 2 weeks after birth		
Subject characteristics	**No (N=204,755)**	**Yes (N=66,431)**	**P-value**
Female	(50.4)	(45.0)	<0.001
Ethnicity			<0.001
White	(53.5)	(48.4)	
Asian	(15.9)	(23.8)	
Black	(8.8)	(6.6)	
Latino	(3.9)	(3.9)	
Other/Unknown	(17.9)	(17.2)	
Gestation age (weeks)			<0.001
37	(5.2)	(10.5)	
38	(13.9)	(18.7)	
39	(28.1)	(29.0)	
40	(34.8)	(28.6)	
≥41	(18.0)	(13.2)	
Birthweight (grams)	3510 ± 475	3447 ± 506	<0.001

TABLE 3

Distribution of maximum CB and DB in 1st 2 weeks after birth

Serum Concentration (mg/dL)	Percentile	
	Conjugated	Direct
	(N=64,095)	(N=2,898)
≤0	73.8%	0.03%
≤0.1	81.2%	0.16%
≤0.3	94.4%	56.0%
≤1	98.6%	96.0%
≤2	99.7%	98.6%

TABLE 4

Cumulative incidence of diseases associated with >1% cholestasis in infancy

Diagnosis	N	per 10,000	1 in "x"	Cholestatic†
Bliary tract disorders	81	3.0	1 in 3348	63.0%
Biliary atresia	27	1.0	1 in 10040	100.0%
Liver diseases	142	5.2	1 in 1910	35.2%
Viral hepatitides	26	1.0	1 in 10428	30.8%
Gastrointestinal disorders	706	26.0	1 in 384	5.1%
Intestinal atresia	143	5.3	1 in 1896	4.9%
Hirschsprung's disease	76	2.8	1 in 3568	1.3%
Metabolic disorders	334	12.3	1 in 812	3.6%
Down's Syndrome	259	9.6	1 in 1047	3.5%
Severe bacterial infections	2152	79.4	1 in 126	1.3%

† Cholestatic refers to subjects with a maximum CB ≥2 mg/dL or DB ≥5 mg/dL in their 1st year after birth

TABLE 5

Relation between maximum conjugated bilirubin level in the first 14 days after birth and ICD-9 diagnostic categories*

Conjugated Bilirubin (mg/dL) 0.5–1.9 (N=4535)		Conjugated Bilirubin (mg/dL) 2–4.9 (N=167)		Conjugated Bilirubin (mg/dL) ≥ 5 (N=73)	
Diagnostic Categories	**Percent**	**Diagnostic Categories**	**Percent**	**Diagnostic Categories**	**Percent**
No dx associated with cholestasis†	96%	No dx associated with cholestasis†	69%	Biliary disorder	47%
Severe bacterial infection†	2%	Liver disease	11%	Liver disease	42%
GI abnormality	1%	Biliary disorder	10%	GI abnormality	27%
Liver disease	0.3%	GI abnormality	10%	No dx associated with cholestasis†	27%
Biliary disorder	0.2%	Severe bacterial infection†	10%	Severe bacterial infection‡	18%
Viral hepatitis	0.04%	Viral hepatitis	2%	Viral hepatitis	6%

*A single subject may have more than one ICD-9 diagnosis associated with cholestasis

†Signifies that there was no ICD-9 code in disease categories associated with cholestasis

‡Includes codes for diseases such as septicemia, meningitis and bacteremia

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Table 6

Likelihood ratios (LR) for hepatobiliary disease by maximum CB/DB levels in 1st 2 weeks after birth

CB level (mg/dL)	LR	95% CI	DB level (mg/dL)	LR	95% CI
0	0.36	(0.26–0.50)	0	-	-
0.1 – 0.5	1.07	(0.72–1.60)	0.1 – 0.5	0.19	(0.05–0.70)
0.5 – 1.9	2.77	(1.85–4.15)	0.5 – 1.9	1.14	(0.50–2.62)
2 – 4.9	80.6	(49.9–130)	2 – 4.9	17.6	(6.15–50.3)
≥ 5	495	(267–919)	≥ 5	223	(72.5–685)