Heme Catabolism and Bilirubin Production in Readmitted Jaundiced Newborns

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We measured end-tidal CO levels in 50 jaundiced newborns readmitted for phototherapy at age 54-244 hours. The median end-tidal CO level was 1.55 ppm, suggesting that hemolysis is not the primary contributor to the hyperbilirubinemia in many readmitted newborns. *(J Pediatr 2020;226:285-8).*

Neonatal hyperbilirubinemia is the result of an imbalance between bilirubin production and elimination.[1] Because the catabolism of heme produces equimolar quantities of CO, bilirubin, and iron, and because heme catabolism is the major contributor to the endogenous production of CO, the measurement of end-tidal CO corrected for ambient CO (ETCOc) is a direct measurement of heme catabolism and bilirubin production.[2] Elevated ETCOc measurements can provide direct evidence,[2-7] and studies of rebound following phototherapy[8-10] provide indirect evidence, that an increase in heme catabolism and bilirubin production is the primary cause of hyperbilirubinemia within 72 hours of birth. We studied the potential pathogenesis of hyperbilirubinemia in infants who had been discharged following birth but later readmitted for phototherapy.

Methods

Between July 2017 and December 2018, we used the Co-Sense ETCOc monitor (Capnia, Redwood City, California) to measure ETCOc in a convenience sample of 70 newborn infants at ≥35 weeks of gestation with hyperbilirubinemia who were readmitted and treated with phototherapy. To be included in the study population, infants had to be less than 14 days old and otherwise healthy, with hyperbilirubinemia as the sole reason for admission. We excluded infants who had received phototherapy during the birth admission or whose ETCOc was measured more than 24 hours after admission. A direct antibody test (DAT) using the "gel" method was administered to all ABO- or Rh-incompatible infants.[11]

The study was approved by the hospital's Human Investigation Committee. Because the measurement of ETCOc is a completely noninvasive diagnostic tool in routine use at our institution, the need for informed consent was waived. However, because we planned to use the data in a study, parents could refuse the use of their infant's data, although none did so. Attending pediatricians, who were not aware of the study, made the decisions regarding the need for admission and phototherapy. Hospital care was provided by a full-time hospitalist group.

AAP	American Academy of Pediatrics
DAT	Direct antibody test
ETCOc	End-tidal CO corrected for ambient CO
RBC	Red blood cell

Results

The **Figure** and **Table** show the relevant results. We could not measure the ETCOc in 11 infants (despite at least 1 repeat measurement in each case) because of elevated H_2 in the expired gas or an "error" reading on the instrument. Three infants were >14 days old, 2 infants had ETCOc measured more than 24 hours after admission, and 4 infants had received phototherapy during their birth admission, leaving 50 infants in the final study population. Among these 50 infants, 43 (86%) were inborn, and the racial distribution was 36 (72%) white, 2 black, 2 Asian, 7 Middle Eastern, and 3 other or unknown racial origins. Thirty-five infants (70%) were exclusively breastfed, 2 were formula fed, and 13 were breastfed supplemented with formula.

Although not specifically provided with guidelines for this study, attending pediatricians on the staff of Beaumont Hospitals are familiar with the 2004 American Academy of Pediatrics (AAP) guidelines for phototherapy[12] and the 2009 update.[13] Both of these guidelines are an integral part of the newborn record and available to pediatricians who use our electronic medical record system (Epic). Notwithstanding this availability and access to the AAP guidelines, 46 of the 50 infants (92%) were admitted with a serum bilirubin level below the AAP threshold, although in 37 of these infants (80%), the level was <2 mg/dL below the threshold.

ETCOc levels were below the median (1.55 ppm) in 25 infants (50%) and at or exceeding the 75th percentile (1.9 ppm)

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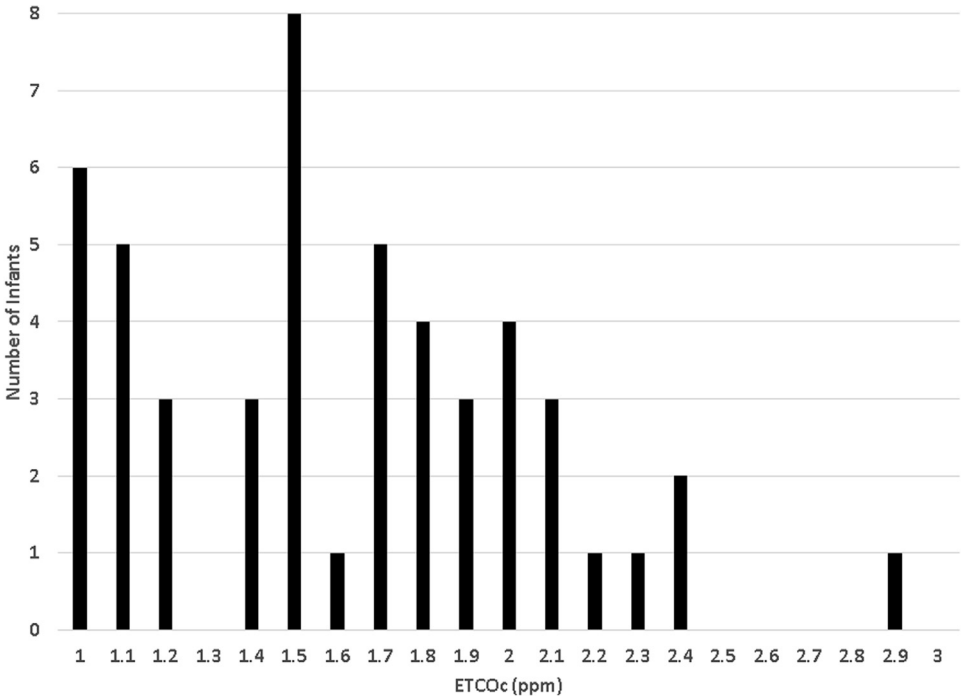

Figure. Distribution of ETCOc levels in the study population. The median ETCOc was 1.55 ppm, and the 25th, 50th, 75th, 90th, and 95th percentiles were 1.2, 1.5, 1.9, 2.1, and 2.4 ppm, respectively.

in 15 infants (30%). There was no apparent relationship between the ETCOc and the peak bilirubin level (data not shown), although the range of ages at which these variables were measured (Table) can affect these relationships.

Eighteen infants (36%) were at 35-37 weeks of gestation and 32 (64%) were at ≥38 weeks (Table). The median age at readmission was 108 hours (~4.5 days), with a range of 54-244 hours (2-10 days). Only 6 infants (12%) were admitted before 72 hours. The mean birth weight was 3423 ± 584 g, and the mean weight at readmission was 3201 g, representing a median weight loss of 6.8% (range, 1.8%-22.7%). One infant gained weight, and 1 infant's weight was unchanged. The mean direct bilirubin level was 0.6 ± 0.16 mg/dL (range, 0.3-1.3 mg/dL). Only 1 infant had a direct bilirubin level >1.0 mg/dL (95th percentile).[14] This infant's total/direct serum bilirubin level was 21.9/1.3 mg/dL, and direct bilirubin decreased to 0.5 mg/dL by the next day. Nine infants were ABO- incompatible with their blood group O mothers, and only 1 infant had a positive DAT. In this latter case, the mother was O, Rh(D) negative and the newborn was B, Rh(D) positive. The infant's ETCOc was 2.3 ppm, just below the 95th percentile of the study population. In the 8 DAT-negative ABO-incompatible infants, ETCOc levels ranged from 1.2 to 2.3 ppm, but only 1 infant had a value below the median, and 4 infants had levels >75th percentile, suggesting an increase in red blood cell (RBC) turnover in the absence of a positive DAT.

Glucose-6-phosphate dehydrogenase levels were measured in only 3 infants (all white), and were normal: 11.4, 12.6, and 14.4 U/g of hemoglobin, respectively. The infant with the highest ETCOc level (2.9 ppm) was a 40 2/7-week male admitted on day 4 with a total serum bilirubin concentration of 20.9 mg/dL. There was no ABO incompatibility, and laboratory tests showed hemoglobin of 21.4 g/dL, hematocrit of

Table. Additional data

Parameter	Age at admission, h	EGA, wk	Birth LOS, d	Birth weight, g	Readmission weight, g	% Weight loss, g*	Peak bilirubin, mg/dL	Phototherapy duration, h	Age at ETCOc, h	Time from admission to ETCOc, h	ETCOc, ppm
Number	50	50	48	49	50	47	50	31	50	50	50
Mean	117	38.6	2.1	3423	3201	7.5	18.7	20.7	128	10.97	1.61
Median	107.9	39	2	3315	3122	7.1	18.6	16.5	123	12.52	1.55
SD	44.5	1.52	0.9	584	575	4.2	1.7	11.6	44	4.9	0.45
Minimum	53.5	35	0.6	2280	2030	1.8	13.9	9.6	55	0.8	1
Maximum	244.3	41.7	4	4700	4515	22.7	21.9	66	245	18.5	2.9

EGA, estimated gestational age; *LOS*, length of stay.
*Only in infants who lost weight.

62.9%, reticulocytes 43/b RBCs (0.7%), nucleated RBCs 2/200 WBCs, and an unremarkable peripheral smear. His serum bilirubin concentration declined to 11.5 mg/dL after 19 hours of phototherapy. There was no subsequent hyperbilirubinemia but, unfortunately, no further hematologic evaluation was performed.

Discussion

Our data suggest that in many infants readmitted for hyperbilirubinemia who had not received phototherapy during their birth admission, the most common cause of the hyperbilirubinemia is a decrease in bilirubin clearance rather than an increase in bilirubin production. However, without the ability to measure clearance, it is not possible to calculate the contribution of elimination to the ultimate total bilirubin level.

In our study cohort, the distribution of ETCOc levels suggests that most of the infants readmitted with hyperbilirubinemia at around age 4.5 days did not have a significant increase in heme catabolism and bilirubin production. Nonetheless, the hyperbilirubinemia in those infants with an ETCOc level between the median and 75th percentile could certainly be the result of a combination of decreased clearance and a modest increase in bilirubin production, and those with ETCOc in the upper 25% are likely primarily hemolyzing.[5] The ETCOc levels in the group of ABO-incompatible newborns are also of interest. Although 8 of these 9 infants were DAT-negative, only 1 infant had an ETCOc level below the median, and 4 (50%) had levels >75th percentile, suggesting that an increase in heme catabolism is not uncommon in such infants, consistent with similar observations by Stevenson et al[3] and Kaplan et al.[15]

Limitations of this study include the relatively small sample, the absence of subjects with extreme hyperbilirubinemia (total serum bilirubin level >25 mg/dL), and the lack of a control group of infants of similar age without hyperbilirubinemia from whom percentiles can be calculated. We previously measured ETCOc (with a different instrument)[4] in 108 infants with total serum bilirubin level before discharge exceeding the 75th percentile on the Bhutani nomogram and 164 control newborns on days 1-5. In the control group, aged 4-5 days, with total serum bilirubin levels below the 75th percentile (n = 11), the mean ETCOc was 1.5 ± 0.33 ppm, very close to the value found in the present study (1.6 ± 0.45 ppm).

Bhutani et al measured ETCOc with the Co-Sense device in 283 well newborn infants with a mean gestational age of 39.1 weeks (range, 35.4-42.1 weeks) at a mean age of 32 hours (range, 13-61 hours).[5] Their study population comprised infants with ABO and Rh incompatibility and a mean bilirubin level (serum or transcutaneous) at age 32 hours of 6.9 ± 2.5 mg/dL (range, 0.3-20.8 mg/dL). Our cohort had a median gestation of 39 weeks, a median age at ETCOc of 121 hours (range, 55-245 hours), and a mean peak serum bilirubin level of 18.7 mg/dL (range, 13.9-21.9 mg/dL). Despite these differences, the 50th, 75th, and 90th percentiles for ETCOc in the population studied by Bhutani et al were 1.6, 2.0, and 2.3 ppm respectively, almost identical to the values in our significantly older and more jaundiced population of 1.5, 1.9, and 2.1 ppm, respectively, providing modest justification for the use of percentiles in the absence of an appropriate control population. Bhutani et al also suggested that their infants with ETCOc levels ≥75th percentile "likely represented a group of infants with excessive heme catabolism from red blood cell breakdown," and it is plausible that the same conclusion could be reached at the 75th percentile ETCOc level of ≥1.9 ppm in our cohort. In our previous study noted above,[4] the mean ETCOc levels in the infants with higher bilirubin levels were consistently and statistically significantly greater on every day compared with those in the control infants. The ETCOc levels increased daily over the first 4 days in the jaundiced newborns but decreased in the control infants. These data suggest that an increase in heme catabolism and bilirubin production was the primary mechanism responsible for hyperbilirubinemia in infants at 1-4 days of age and before their discharge, a conclusion supported by other studies of ETCOc levels[2-7] and the need for phototherapy in the first few days of life.[8-10]

An additional limitation could be the delay between admission, institution of intensive phototherapy, and measurement of ETCOc (median, 12.5 hours). In a study of 27 newborns at ≥35 weeks of gestation who received intensive phototherapy,[16] we documented an average decrease of 1% in the ETCOc level (measured with a different instrument) after 8 hours of phototherapy and a 9.2% decrease after 22 hours of phototherapy. Thus, in some infants, it is possible that the measured ETCOc could have underestimated the rate of RBC turnover.

As demonstrated previously, the measurement of ETCOc in jaundiced newborns can provide helpful information on the potential cause of the hyperbilirubinemia. At the serum bilirubin levels encountered in this study and in the population studied, we conclude that in many infants who have not received phototherapy during their birth hospitalization but subsequently develop hyperbilirubinemia, decreased bilirubin elimination rather than increased bilirubin production is likely the most common mechanism responsible for their hyperbilirubinemia. Nevertheless, some degree of hemolysis is probably still present in a significant proportion of this readmitted population. ■

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Data Statement

Data sharing statement available at www.jpeds.com.

References

1. Kaplan M, Muraca M, Hammerman C, Rubaltelli FF, Vilei MT, Vreman HJ, et al. Imbalance between production and conjugation of bilirubin: a fundamental concept in the mechanism of neonatal jaundice. Pediatrics 2002;110:e47.
2. Stevenson DK, Vreman HJ, Oh W, Fanaroff AA, Wright LL, Lemons JA, et al. Bilirubin production in healthy term infants as measured by carbon monoxide in breath. Clin Chem 1994;40:1934-9.
3. Stevenson DK, Fanaroff AA, Maisels MJ, Young BW, Wong RJ, Vreman HJ, et al. Prediction of hyperbilirubinemia in near-term and term infants. Pediatrics 2001;108:31-9.
4. Maisels MJ, Kring E. The contribution of hemolysis to early jaundice in normal newborns. Pediatrics 2006;118:276-9.
5. Bhutani VK, Maisels MJ, Schutzman DL, Castillo Cuadrado ME, Aby JL, Bogen DL, et al. Identification of risk for neonatal haemolysis. Acta Paediatr 2018;107:1350-6.
6. Bhutani VK, Srinivas S, Castillo Cuadrado ME, Aby JL, Wong RJ, Stevenson DK. Identification of neontal haemolysis: an approach to predischarge management of neonatal hyperbilirubinemia. Acta Paediatr 2016;105:e189-94.
7. Bhutani VK, Wong RJ, Vreman HJ, Stevenson DK. Bilirubin production and hour-specific bilirubin levels. J Perinatol 2015;2015:735-8.
8. Chang PW, Kuzniewicz MW, McCulloch CE, Newman TB. A clinical prediction rule for rebound hyperbilirubinemia following inpatient phototherapy. Pediatrics 2017;139:e20162896.
9. Maisels MJ, Kring E. Rebound in serum bilirubin level following intensive phototherapy. Arch Pediatr Adolesc Med 2002;156:669-72.
10. Kaplan M, Kaplan E, Hammerman C, Algur N, Bromiker R, Schimmel MS, et al. Post-phototherapy neonatal bilirubin rebound: a potential cause of significant hyperbilirubinaemia. Arch Dis Child 2006;91:31-4.
11. Parker V, Tormey CA. The direct antiglobulin test: indications, interpretation, and pitfalls. Arch Pathol Lab Med 2017;141:305-10.
12. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114:297-316.
13. Maisels MJ, Bhutani VK, Bogen D, Newman TB, Stark AR, Watchko JF. Hyperbilirubinemia in the newborn infant ≥35 weeks' gestation: an update with clarifications. Pediatrics 2009;124:1193-8.
14. Noorulla F, Dedon R, Maisels MJ. Association of early direct bilirubin levels and biliary atresia among neonates. JAMA Netw Open 2019;2:e1913321.
15. Kaplan M, Hammerman C, Renbaum P, Klein G, Levy-Lahad E. Gilbert's syndrome and hyperbilirubinaemia in ABO-incompatible neonates. Lancet 2000;356:652-3.
16. Maisels MJ, Kring EA. Does intensive phototherapy produce hemolysis in newborns of 35 or more weeks gestation? J Perinatol 2006;26:498-500.