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End-tidal carbon monoxide as an indicator of the hemolytic rate

R.D. Christensen [a,*], D.K. Lambert [a], E. Henry [a,b], H.M. Yaish [c], J.T. Prchal [d]

[a] Women and Newborn's Program, Intermountain Healthcare, Salt Lake City, UT, USA
[b] Institute for Healthcare Delivery Research, Salt Lake City, UT, USA
[c] Department of Pediatrics, Division of Hematology/Oncology, University of Utah School of Medicine, Salt Lake City, UT, USA
[d] Departments of Pathology and Internal Medicine, University of Utah School of Medicine, The Salt Lake VA Hospital, ARUP Laboratories, Salt Lake City, UT, USA

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ABSTRACT

In the first days of life, low grade jaundice is essentially universal. The source of the elevated bilirubin level giving rise to "physiological jaundice of the newborn" is only partly known. We hypothesized that it is, at least in part, the result of active and specific hemolysis involving a physiological mechanism to lower the high fetal hematocrit, appropriate for the relatively low oxygen environment *in utero*, to a lower level appropriate for the state of oxygen abundance after birth. We tested this by quantifying end tidal carbon monoxide (ETCO) as a marker of the rate of heme metabolism to bilirubin. We found that ETCO values of 20 neonates and children with known hemolytic disorders were higher than 20 age-matched healthy controls ($p < 0.0001$), indicating that this instrumentation recognizes hemolysis in neonates and children. We also found that ETCO reference intervals were indeed higher in healthy neonates during the first three days after birth (5th to 95th percentile reference range, 1.4 to 1.7 ppm) than after 1 month of age (all ≤1.0 ppm, $p < 0.0001$). These results suggest to us that hemolysis is physiological during the first days after birth. The cellular and molecular mechanisms responsible for transient hemolysis after birth are topics of current investigation.

Introduction

Carbon monoxide (CO) is generated stoichiometrically as heme is metabolized to bilirubin [1]. Thus, end tidal CO (minus ambient CO) can be used to quantify the hemolytic rate [2]. Maisels and Kring reported end tidal CO values from neonates during the first four days, showing that neonates who subsequently developed a bilirubin >75th percentile reference range had higher CO values than those with lower bilirubin levels [3]. Kaplan et al. used the same methodology to evaluate hemolysis among G6PD deficient neonates [4,5]. Javier et al. [6] and Barak et al. [7] correlated end tidal CO with reticulocyte counts and with hematocrit. These cited studies utilized the Natus end-tidal breath analyzer (Natus Medical, San Carlos, CA), which has not been available for approximately ten years [1,2].

Neonates with pathological hemolytic jaundice, such as that which can occur with hereditary spherocytosis, pyruvate kinase deficiency, or ABO or anti-D hemolytic disease, appear to be at higher risk for bilirubin-induced neurological dysfunction than neonates where jaundice is not due to hemolysis [8–11]. Indeed, we recently reported two neonates with hemolytic disorders who developed kernicterus, where the hemolytic disorders were not recognized during the birth hospitalization [12,13]. Upon their hospital readmission for treatment of hazardous hyperbilirubinemia we discovered that one had hereditary spherocytosis in addition to a known ABO incompatibility [12] and the other had G6PD deficiency with the Mahidol mutation [13]. We speculated that if their hemolytic disorders had been recognized during the birth-hospitalization, follow-up and treatment for hyperbilirubinemia might have been more timely and effective, preventing their poor outcomes.

Bhutani and Stevenson recently urged the development of new technologies to identify the subset of neonates who are likely to develop hazardous hyperbilirubinemia [14]. Here we tested a newly developed instrument (CoSense) for exhaled CO quantification. We studied 30 healthy term neonates, twice each between birth and hospital discharge, and also 20 neonates and children with known hemolytic disorders, compared with 20 age-matched healthy controls.

Materials and methods

Study design and populations

The study protocol was approved by the Intermountain Healthcare Institutional Review Board. Parents of participating neonates and children provided written informed consent and participants over 7 years-old also provided written assent.

This was a prospective study using convenience samples. Patients with hemolytic disorders were either in the McKay-Dee NICU, Ogden, UT or the Primary Children's Hospital Hematology Clinic, Salt Lake City

* Corresponding author at: Intermountain Healthcare, McKay-Dee Hospital Center, 4403 Harrison Blvd, Ogden, UT 84403, USA. Fax: +1 801 387 4316.
E-mail address: Robert.christensen@imail.org (R.D. Christensen).

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UT. Well children for the age-matched control group were recruited for this study from the McKay-Dee Pediatric Clinics or the McKay-Dee Perinatal Research Outpatient Service, Ogden, UT. Neonates were not enrolled in the study if the mother smoked tobacco, or if there were smokers in the mother's home, because of the potential effect on the neonate's exhaled CO.

The program used for data collection was a modified subsystem of "clinical workstation". Clinical workstation is a web-based electronic medical record application that stores demographic and clinical information, such as history, physical examination results, laboratory data, problem lists, and discharge summaries. 3M Company (Minneapolis, MN) approved the structure and definitions of all data points for use within the program. Data were managed and accessed by authorized data analysts.

Instrumentation for ETCO quantification

All 100 ETCO measurements in this study were performed using a newly-developed instrument CoSense (Capnia Inc., Palo Alto, CA). The device is compact and portable, and utilizes a one-time-use single nasal cannula for quantifying end tidal breath for CO and also samples ambient CO which is subtracted from the value in exhaled breath. CoSense has obtained FDA 510(k) clearance. This device also counts the breath rate, analyzes CO in individual breaths, and provides CO measurements in parts per million in a matter of minutes.

Statistical analysis

For the pilot reference range study 5th and 95th percentile limits were calculated for values obtained during the first 72 h of life. For the case–control study, each patient with hemolytic anemia (case) was matched with a healthy control neonate or child of similar age during a well-child visit to the Northern Utah Pediatrics Department at McKay-Dee Hospital (control). Cases and controls were age-matched ± 12 months for children >2 years old and ± 3 months for children ≤ 2 years. Whenever the ETCO reading was "<1.0 ppm", the number "0.8 ppm" was entered into the database in order to calculate group mean and 95% CI values. Student t-test was used to assess differences in groups of continuous variables. Means and 95% confidence intervals were used to express values in groups that were normally distributed. Correlation between ETCO and CBC parameters was assessed using Pearson product–moment correlation coefficient.

Results

The study of the 30 healthy babies occurred between 11/13/2013 and 12/18/2013. The 20 with hemolytic disorders and their 20 matched controls were studied between 3/13/2014 and 5/1/2014. For the first study, 34 sets of parents of healthy full-term neonates were approached about the study; four declined and 30 provided written consent. Two measurements were performed on each of the 30 neonates; one on the day of birth and a second sometime before discharge to home. These were obtained as soon as 70 min after birth and as late as 72 h after birth. Testing was not done if mothers were smokers or if the infant had a positive direct antibody test (DAT). Testing was always successful, whether neonates were awake or asleep. The values obtained are shown in Fig. 1. The 95% confidence intervals were 1.4 to 1.7 ppm. No differences were seen in ETCO values between neonates born vaginally ($n = 13$) *vs.* by Cesarean section ($n = 17$), nor between those whose mothers received Pitocin during labor ($n = 14$) *vs.* no Pitocin ($n = 16$) (these variables were examined because of the possibility that vaginal delivery, or Pitocin augmentation, might have resulted in some degree of fetal bruising). Values obtained during the first 24 h following birth ($n = 35$; mean 1.6 ppm) were not statistically different from those obtained in the period 25 to 72 h after birth ($n = 25$; mean 1.4 ppm, $p = 0.09$).

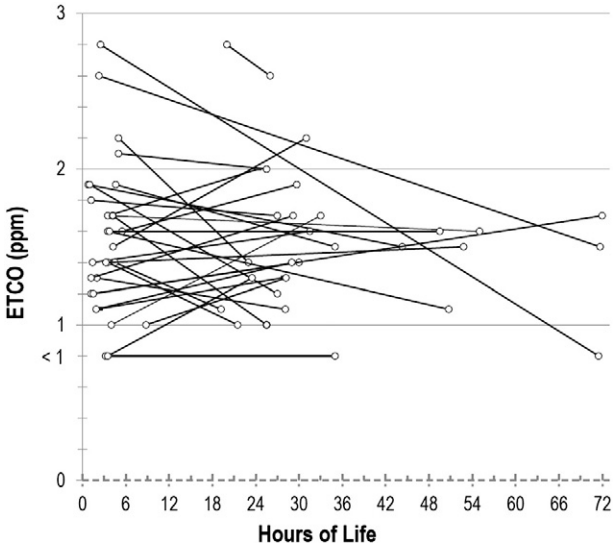

Fig. 1. Sixty end tidal CO measurements from 30 healthy term neonates; one measurement was obtained early and one later during the birth-hospitalization. Paired values are connected with a line.

For the study to compare end tidal CO of matched cases (hemolytic disorders) and controls (healthy neonates and children) 22 parents of neonates or children with hemolytic anemia/jaundice were approached about measuring end tidal CO; one refused and twenty-one provided written consent. The end tidal CO failed to register a value (for an undetermined reason) on one (a 3-day-old with Coombs positive ABO hemolytic jaundice). The other 20 measurements were successful. Table 1 shows the end tidal CO values, the presence and type of hemolytic disorders, hemoglobin and reticulocyte counts. End tidal CO values in this group correlated positively with reticulocyte count ($r = 0.588$, $p = 0.002$, Fig. 2) but not with hemoglobin or hematocrit (data not shown).

Twenty-four parents of healthy children were approached because their children were appropriately age-matched with the hemolytic cases; four refused and twenty provided written consent. Fourteen of the 20 healthy controls had end tidal CO values ≤ 1.0 ppm and all were less than their matched cases ($p < 0.00001$). The other six healthy control subjects were <1 month old and had values of 1.1 ($n = 2$), 1.2 ($n = 2$), 1.3, and 1.4 ppm (Fig. 3).

Discussion

Bilirubin-induced neurotoxicity remains a public health problem in the USA, and even more so in countries where conditions predisposing to neonatal hyperbilirubinemia, such as G6PD deficiency, hemoglobinopathies, and erythrocyte cytoskeletal mutations, are endemic, or where bilirubin monitoring and treatment programs are not well developed [14–16]. Neurodevelopmental deficiencies of children with bilirubin-induced toxicity range from mild to debilitating, and these can generate high financial and emotional costs to families and society [14–16]. In the Intermountain Healthcare system of hospitals, after we instituted pre-birth-hospital discharge bilirubin screening as recommended by the American Academy of Pediatrics subcommittee on hyperbilirubinemia [17], we had fewer neonates with a total serum bilirubin in the 20–25 mg/dL range, and fewer hospital readmissions for treatment of neonatal jaundice [18]. However, recent reports from our hospitals [12,13,19,20] and from Northern California [21] show that cases of hazardous hyperbilirubinemia and kernicterus continue to occur.

We recently reported a case-series where we identified a hemolytic disorder in 12 consecutive neonates who had a serum bilirubin ≥ 25 mg/dL [20]. We also reported that, unfortunately, the hemolytic