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Carboxyhemoglobin – the forgotten parameter of neonatal hyperbilirubinemia

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Abstract

Background: Neonatal hyperbilirubinemia is influenced by a wide variety of factors, one of which is hemolysis. Serious hyperbilirubinemia may lead to a kernicterus with detrimental neurologic sequelae. Patients suffering from hemolytic disease have a higher risk of developing kernicterus. Carbon monoxide (CO), a byproduct of hemolysis or heme degradation, was described by Sjöstrand in the 1960s. It is transported as carboxyhemoglobin (COHb) and exhaled through the lungs. We were interested in a potential correlation between COHb and total serum bilirubin (TSB) and the time course of both parameters.

Materials and methods: We used a point of care (POC) blood gas analyzer and did a retrospective analysis of bilirubin and COHb data collected over a 60-day period.

Results: An arbitrary cut-off point set at 2% COHb identified four patients with hemolytic disease of different origins who required phototherapy. In one patient with atypical hemolytic uremic syndrome (aHUS), COHb preceded the rise in bilirubin by about 2 days. Despite this displacement, there was a moderately good correlation of COHb with TSB levels <15 mg/dL (257 μmol/L) (r^2: 0.80) and direct bilirubin (r^2: 0.78) in the first patient. For all the four patients and all time points the correlation was slightly lower (r^2: 0.59).

Conclusions: COHb might be useful as a marker for high hemoglobin turnover to allow an earlier identification of newborns at risk to a rapid rise in bilirubin.

Keywords: Carbon monoxide; carboxyhemoglobin; hemolysis; neonatal hyperbilirubinemia; point of care testing.

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Introduction

Term neonates generally present physiologic jaundice within the first 3–5 days of life caused by deposits of bilirubin in the skin. Physiologic jaundice is caused by degradation of senescent fetal erythrocytes as well as by immature UDP-glucuronyl-transferase function. Insoluble bilirubin is bound by albumin and glucuronized in the liver to be excreted into the intestinal lumen. Neonatal intestinal transformation of bilirubin to urobilinogen may be impaired due to immature intestinal flora, leading to increased enterohepatic circulation of unconjugated bilirubin. Reabsorption of unconjugated bilirubin may be partially elevated in breast-fed infants due to β-glucuronidase in breast milk, which detaches glucuronic acid from conjugated bilirubin [1]. Jaundice is classified as physiological up to a total serum bilirubin (TSB) level of 17 mg/dL (291 μmol/L), above which it is classified as pathologic [2]. Rise of serum bilirubin levels is caused by the equilibrium of increased production and reduced excretion of bilirubin. Various factors disturbing this fine balance may lead to a significant increase in serum bilirubin levels [3, 4].

Hemolytic disease of the newborn (HDN) is the main cause of pathologic jaundice. However, increased bilirubin production of various origins may also lead to serious illness.

Above a certain individual TSB level, the newborn may show signs of neurological dysfunction, either as acute or chronic bilirubin encephalopathy [5–8]. First signs of impaired neurological function are lethargy and impaired feeding [7]. Neurologic sequelae, such as varying muscle tone, athetosis and impaired hearing are other symptoms and may be partially irreversible if adequate treatment is not initiated immediately [9]. At worst neurologic dysfunction may result in seizures, apnea and death, or in case of survival, deafness, spasticity and impaired neuromotor development are a significant threat to these patients. Post-mortem autopsy regularly shows yellow staining of the basal ganglia resulting in the colorful term kernicterus, as described by Schmorl in 1904 [3, 10].

The armamentarium of therapies for severe hyperbilirubinemia comprises phototherapy, administration of immunoglobulins [2, 11], inhibition of heme oxygenase (HO) with metalloporphyrins [12] or blood exchange transfusion.

It has been recognized that bilirubin toxicity is not well reflected by TSB, as this figure does not accurately represent the amount of free bilirubin responsible for the toxicity [3]. Other factors, such as sepsis, acidosis, prematurity, lack of albumin and increased hemolysis play an important role in the diffusion of unbound bilirubin across the blood-brain-barrier to accumulate in basal ganglia [3, 9, 13, 14]. A rapid rise in bilirubin production due to hemolysis and degradation of hemoglobin may be an additional risk factor leading to an overload of albumin binding capacity for bilirubin.

One method of determining hemolytic disease described by Sjöstrand in 1952 relates to the formation of carbon monoxide (CO) due to hemolysis and the degradation of hematoma in humans [15]. Later, these data were confirmed by demonstrating an increase in carboxyhemoglobin (COHb) in neonates with hemolytic disease [16–19].

The main source of endogenous CO is the degradation of senescent erythrocytes and oxygenation of heme to biliverdin by HO [20, 21]. One mole of CO is produced for every mole of biliverdin [21, 22]. Endogenous CO production can be measured by two means. First, by measuring end tidal CO (ETCO) or ETCO corrected for inspired CO ($ETCO_c$), as has been demonstrated repeatedly in neonates [22–24]. Second, by gas chromatographic analysis of COHb or COHb corrected for inspired CO ($COHb_c$), as has been described by Kaplan et al. repeatedly [25, 26]. $ETCO_c$ has been validated as an index for the rate of bilirubin production due to hemolysis [22, 25] and has gained clinical importance with newly developed screening devices. As yet COHb has not been studied extensively. This may be due to lack of reference ranges as well as due to methodological difficulties. The aim of this study was to analyze the correlation between COHb and total bilirubin and the time course of both parameters in individual patients.

Materials and methods

In a retrospective analysis of data collected on our point of care (POC) blood gas analyzer (Roche cobas b221 V4, Roche cobas, Rotkreuz, Switzerland) from the preceding 98 days, we screened 1911 data sets. These were filtered for COHb levels with an arbitrary cut-off value set at 2% COHb. All data above 2% COHb were analyzed further by connecting these data with TSB levels measured in parallel in each individual patient. COHb data were displayed on a chronological axis together with TSB levels gained in parallel. Finally, TSB and COHb were plotted against each other on a quasi-linear scale.

TSB and direct serum bilirubin were measured in serum with a modified diazo method developed by Jendrassik and Grof [27].

Standard therapy of hyperbilirubinemia in our unit is as follows: phototherapy was initiated in a closed incubator with a Draeger Heraeus Phototherapie 800 unit (Draeger, Lübeck, Germany) or a VARIOTHERM Bilicompact (Weyer GmbH, Kürten, Germany) lamp for irradiation from above. Intensification steps were initially a second phototherapy lamp from above, followed by either the OHMEDA Biliblanket (Datex-Ohmeda/GE Healthcare, Chicago, IL, USA) or a third phototherapy lamp. Irradiation intensity was not measured. During phototherapy repeat measurements of TSB were carried out through co-oximetry.

Correlation was calculated using Excel Data Analysis ToolPak in MS Excel 2007 (Microsoft, Redmond, WA, USA).

Results

Five hundred and fifty three (28.9%) measurements had been performed without co-oximetry, so only blood gas analysis and electrolyte measurements were performed. We obtained 184 (9.6%) data sets with a COHb value ≥2% and a bilirubin value in parallel. Six of these (3.3%) could not be attributed to a certain patient. The remaining 178 data sets showed COHb levels ≥2% for 13 patients. Of these all patients were excluded with less than 5 measurements of 2% COHb or higher. Four of the five remaining patients showed an increased hemoglobin turnover of varying cause (Table 1). The fifth patient was a premature male neonate of African ethnicity without recognizable hemolytic disease born at 23+4/7 weeks. Data from this neonate were not analyzed further as no hemolytic disease could be ascertained. All COHb data obtained from the other four patients were processed. As most data sets were from Patient 1 we analyzed his specific dynamic pattern of COHb level, TSB and LDH during the course of disease (Figure 1). From a visual interpretation, there was a displacement of about 2 days between the peaks of COHb and TSB, with the COHb always preceding TSB.

Table 1: Patient data showing gestational age, birth weight, maximum TSB, maximum COHb, hemoglobin level and diagnosis.

Patient	Gestational age (weeks)	Birth weight (g)	Maximum TSB [mg/dL (µmol/L)]	Maximum COHb (%)	Minimum Hb (g/L)	Hemolytic disease
1 ♂	36+5/7	4470	32.0 (547)	8.0	46	aHUS
2 ♀	34+4/7	2350	15.2 (260)	5.0	81	HDN (Anti-c, Anti-s)
3 ♂	38+3/7	3360	20.3 (347)	2.3	156	B-O-incompatibility
4 ♂	25+5/7	850	6.8 (116)	2.5	106	Bilateral cephalhematoma

aHUS = atypical hemolytic uremic syndrome; HDN = hemolytic disease of the newborn.

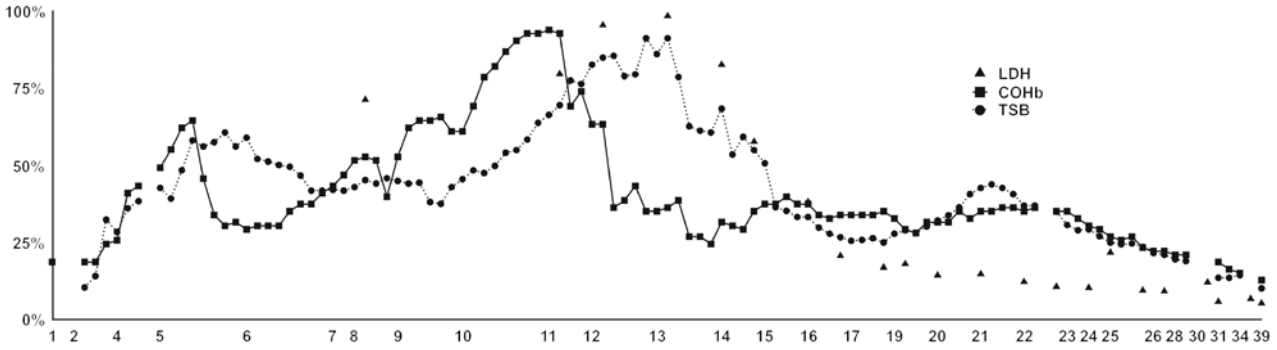

Figure 1: Course of COHb, TSB and LDH in Patient 1.
The measurements are listed as fractions of maximum measurement by days of life.

There was an almost linear correlation between COHb and TSB as well as between COHb and direct bilirubin for Patient 1 at all time points (r^2 0.53 and 0.58, respectively; Figure 2 – dotted line). The correlation below 15 mg/dL (257 µmol/L) TSB is even higher (r^2 0.80 and 0.78, respectively; Figure 2 – solid line). When calculating the correlation between COHb and TSB for all four patients the results were slightly lower [r^2 0.32 for all data and 0.59 for values below 15 mg/dL (257 µmol/L); Figure 3].

Discussion

Despite dedicated guidelines to screen for hyperbilirubinemia kernicterus, the most deleterious sequel of hyperbilirubinemia has not disappeared so far. Possibly, single serum bilirubin measurements in newborns may not sufficiently reflect the extent of heme turnover and ongoing bilirubin production as bilirubin, which has already diffused into tissue is under-represented. COHb measurements, therefore, may be advantageous, as they show good correlation with the turnover of heme molecules [22] and thus may identify newborns at increased risk for hyperbilirubinemia early.

Blood gas analyzers are part of POC(T) on each neonatal intensive care unit (NICU). Most of these analyzers nowadays offer the opportunity to measure COHb as part of the co-oximetry data set, so COHb can easily be used in clinical care without the need for expensive laboratory tests, additional venipuncture or additional equipment to measure exhaled CO.

A specific COHb level for the diagnosis of significantly elevated bilirubin production due to heme catabolism has not been defined so far, although a certain amount of evidence has been presented by various studies carried out on neonates [26, 28, 29]. Normal $COHb_c$ levels were demonstrated with a mean of 0.77% ± 0.19% in healthy male neonates [28] and 1.26% ± 036% in neonates with positive direct Coombs assay, having blood group A or B, while their mothers are blood group O [29]. Kaplan et al. have also described elevated levels of $COHb_c$ at 1.42% ± 0.39% in jaundiced neonates compared to 1.0% ± 0.25% in neonates without hemolytic disease [26]. These values were,

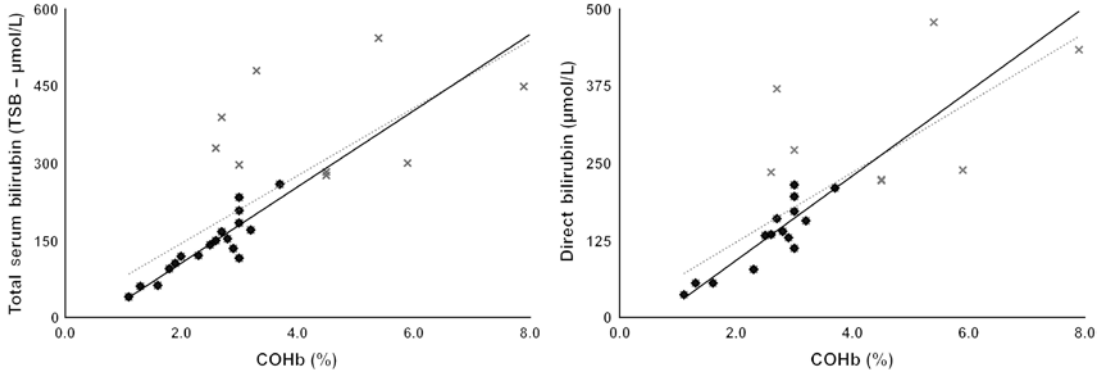

Figure 2: Association in Patient 1 between total serum bilirubin and COHb (left panel) and direct bilirubin with COHb (right panel).
The correlation of TSB values < 15 mg/dL (257 µmol/L) shows a higher linear relation (solid lines: r^2 0.80 TSB and 0.78 direct bilirubin) as opposed to the correlation for the whole range of TSB (dotted lines: r^2 0.53 TSB and 0.58 direct bilirubin).

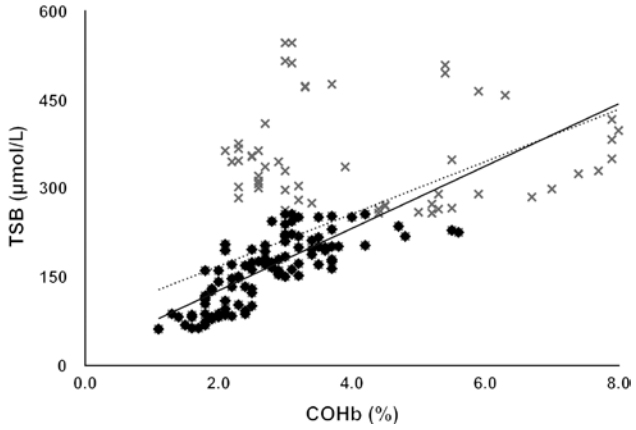

Figure 3: Association between total serum bilirubin and COHb for all the four patients.
The correlation of TSB values < 15 mg/dL (257 µmol/L) shows a higher linear relation (solid lines: r^2 0.59) as opposed to the correlation for the whole range of TSB (dotted lines: r^2 0.32).

however, corrected for inspired CO whereas our data were not corrected, as ambient CO was not measured in our unit. Maximum values in our study thus may be higher.

Our cut off was set at 2% COHb arbitrarily. Despite our supposedly high cut-off value, we did detect one patient with no apparent source of increased heme catabolism, so we cannot rule out that there might be an alternative source for production of COHb or a measuring error. However, that patient was not thoroughly analyzed for rare hemolytic diseases such as thalassemia or sickle cell disease or other reasons for increased COHb.

To our knowledge, this is the first study that presents the dynamic course of COHb and TSB. Correlation between the two parameters had been demonstrated in ABO incompatibility by Fällström and Bjure in the 1960s [16, 18]. It is interesting that we observed a displacement of the COHb and TSB course, with COHb preceding TSB by about 2 days (see Figure 1). This phenomenon may result from enterohepatic circulation or the latency between COHb production and increase in TSB. This might preclude a significant correlation between COHb against TSB (Figures 2 and 3). The fact that all the patients were treated with phototherapy may contribute as well. Direct bilirubin, in particular, may be confounded as the diazo method also measures one-half of bilirubin photoisomer (ZE-bilirubin) as direct bilirubin. Additionally, the amount of direct bilirubin also depends on liver function particularly the capacity for glucuronidation. Whether the correlation between COHb and direct bilirubin will prove to be significant is yet to be determined.

Finally, TSB levels will largely depend not only on hemoglobin degradation but also on bilirubin glucuronization and excretion from the body. There may be considerable interindividual differences in the bilirubin excretion, and exogenous factors like hydration status or type of nutrition may have additional impact [30]. Therefore, COHb may not always be associated with a uniform increase in TSB.

For this study, we have selected the differentiation between TSB levels above and below 15 mg/dL (257 µmol/L) as this is the recommended level to initiate phototherapy in infants at higher risk (35–37 6/7 weeks + risk factors) [2]. Bilirubin and COHb values in the upper range seem to show a certain variance, but we do not have enough values above 3% COHb to elucidate a significant correlation in this range as our data above this threshold was collected primarily from Patient 1. The other three patients were either of younger gestational age thereby resulting in lower phototherapy levels or experienced a rapid reduction of bilirubin due to adequate phototherapy.

Most importantly, the increase in COHb preceded the rise in TSB. Therefore, COHb may prove to be helpful in earlier identification of neonates at increased risk of developing significant hyperbilirubinemia, who may subsequently need closer monitoring of bilirubin values either transcutaneously or invasively or even might need earlier therapy. However, cut-off values, sensitivity and specificity of this parameter have to be substantiated in larger prospective trials before this parameter may be introduced into clinical praxis.

Conclusions

We demonstrated that analysis of COHb values may help to identify patients with increased heme catabolism, at risk of developing severe hyperbilirubinemia. As elevated COHb levels preceded the rise in serum bilirubin COHb might evolve as an additional early screening parameter in future. However, further investigation is necessary before this parameter might be used outside of clinical studies.

Author's Statement
Conflict of interest: Authors state no conflict of interest.
Material and methods: Informed consent: Not required as this is a retrospective data analysis.
Ethical approval: Not required.

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