

Relationship between carboxyhemoglobin levels and indirect hyperbilirubinemia in preterm newborns

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ABSTRACT

Objective: Neonatal jaundice, caused by elevated bilirubin levels, may lead to severe neurological complications, particularly in preterm infants. The aim of this study was to evaluate the predictive value of early carboxyhemoglobin (COHb) levels for identifying indirect hyperbilirubinemia and the need for phototherapy in preterm neonates.

Materials and Methods: This retrospective study included 297 preterm infants admitted to the neonatal intensive care unit (NICU). COHb, bilirubin, and related laboratory parameters were evaluated at 0–6 hours, 24 hours, and 48–96 hours after birth. Statistical analyses included the Mann–Whitney U test, Spearman correlation analysis, receiver operating characteristic (ROC) curve analysis, and logistic regression.

Results: Among 297 preterm newborns, 151 (50.8%) required phototherapy. Early COHb levels showed only very weak correlations with subsequent bilirubin concentrations. COHb levels at 0–6 hours and 24 hours demonstrated poor discriminatory performance for phototherapy requirement, with AUC values of 0.560 and 0.583, respectively. Neither measurement was independently associated with phototherapy requirement in multivariable analyses, whereas birth weight <2000 g remained an independent determinant in both models.

Conclusion: Although early COHb levels showed weak correlations with indirect bilirubin levels in preterm neonates, they were not reliable independent predictors of phototherapy requirement. These findings suggest that COHb has limited clinical utility as a standalone biomarker for guiding treatment decisions.

Keywords: Carboxyhemoglobin, hyperbilirubinemia, neonate, phototherapy, preterm infant

Introduction

Jaundice is characterized by the yellow discoloration of the skin and sclera due to the accumulation of bilirubin in the skin and mucosal membranes. In newborns, jaundice typically occurs when total bilirubin levels exceed 5 mg/dL (1). At least two-thirds of newborns develop clinical jaundice during the first week of life (2). When undiagnosed and untreated, high bilirubin levels can lead to bilirubin encephalopathy and serious neurological sequelae such as athetoid cerebral palsy, also known as kernicterus (3,4). Therefore, neonatal jaundice should be carefully evaluated and monitored to prevent excessive bilirubin levels and eliminate the risk of neurological damage (5). However, the extent to which total serum bilirubin (TSB) levels will rise in a specific newborn and the extent to which they may cause neurotoxicity remain unclear, which ongoing clinical studies aim to predict more accurately.

Jaundice first appears as yellowing of the skin, starting from the face and spreading down the body. If the yellowing is

above the nipple, bilirubin levels are likely below 12 mg/dL; if it is below the nipple, the relationship between bilirubin levels and physical signs becomes weaker. If there is any doubt, bilirubin levels should be measured. Visual assessment alone is insufficient to predict bilirubin levels (2,6). Transcutaneous bilirubin (TcB) measurement is a non-invasive screening test for hyperbilirubinemia that can reduce the frequency of blood tests. However, its effectiveness is limited in infants with darker skin tones and after phototherapy requirement (7). Measuring carbon monoxide (CO) levels, either as end-tidal CO (ETCO) or carboxyhemoglobin (COHb), is considered a potential method for predicting bilirubin levels and assessing the risk of hyperbilirubinemia in newborns. However, further research is needed in this area.

The term “acute bilirubin encephalopathy” refers to the symptoms of acute bilirubin toxicity observed during the first postnatal weeks, while the term “kernicterus” describes the chronic and permanent consequences of bilirubin toxicity (8). Thus, acute bilirubin encephalopathy corresponds to acute clinical signs of kernicterus, whereas chronic bilirubin

encephalopathy corresponds to kernicterus, the chronic and permanent clinical manifestation of the pathological condition caused by bilirubin neurotoxicity.

Approximately 80% of bilirubin production results from the breakdown of hemoglobin in senescent erythrocytes and prematurely destroyed erythroid cells in the bone marrow (9). Hemoglobin from erythrocytes collected and broken down in the RES is catabolized into heme and globin.

Before hemoglobin is broken down, the globin chains separate and degrade, contributing to the amino acid pool, while heme undergoes a series of enzymatic reactions (10). The rate-limiting step in the conversion of heme to bilirubin is catalyzed by the enzyme heme oxygenase. During the heme oxygenase-catalyzed reaction, the alpha-methane bridge in heme is cleaved releasing free iron and CO. CO is then excreted through the lungs, allowing for potential methods of evaluating bilirubin production rates, such as measuring ETCO or COHb (11). Given the monitorability and predictability of neonatal jaundice, the preventability of bilirubin encephalopathy underpins our investigation of CO production in this period. This concept has increased interest in monitoring jaundice and highlighted the importance of its effective clinical management. Therefore, this study aimed to evaluate the predictive value of early-life COHb levels for phototherapy requirement in preterm neonates.

Materials and Methods

This retrospective observational study included all newborns hospitalized in the Neonatal Intensive Care Unit at The Akdeniz University Faculty of Medicine Hospital between January 1, 2015, and January 1, 2021. Patients' electronic medical records were retrospectively reviewed.

Eligible participants were preterm newborns admitted to the hospital within the first 24 hours after birth and who had blood gas analysis performed during their initial hospitalization. The exclusion criteria were as follows: high direct bilirubin levels (defined as direct bilirubin >1 mg/dL when TSB is <5 mg/dL, or direct bilirubin exceeding 20% of total bilirubin when TSB is >5 mg/dL), respiratory acidosis (defined as arterial blood gas pH <7.30 and partial pressure of carbon dioxide (pCO₂) >45 mmHg for 30–36-week-old infants, and pH <7.27 and pCO₂ >50 mmHg for infants <30 weeks old), incomplete data records, and mothers who smoked.

COHb levels were measured using an ABL800 FLEX blood gas analyzer (Radiometer Medical ApS, Brønshøj, Denmark) equipped with a co-oximetry module. COHb values were obtained from cord blood gas samples or clinically indicated venous and arterial blood gas samples collected during admission to the neonatal intensive care unit. Measurements were categorized according to the postnatal sampling time as 0–6 hours, 24 hours, and 48–96 hours. Samples were collected in heparinized blood gas syringes and analyzed after collection. The analyzer was calibrated according to the manufacturer's recommendations, and routine internal quality-control procedures were performed. Because of the retrospective study design, not all newborns had available measurements at every time point.

Demographic variables, such as birth weight, delivery mode, and gestational age, and clinical features, such as phototherapy administration history of sepsis, and culture growth, were recorded for all participants. The threshold value for phototherapy requirement was based on the 2016 National Institute for Health and Care Excellence guideline values (12). Maternal characteristics, such as age and comorbidities, were also documented. All newborns underwent a Direct antiglobulin test, and maternal and newborn blood groups were identified. Rhesus (Rh) incompatibility was defined as a Rh-positive newborn born to an Rh-negative mother. ABO incompatibility was defined as a mother with blood group O and a newborn with blood group A or B.

The required sample size was calculated using G*Power and was determined to be 172, indicating that the final sample size of 297 newborns was adequate.

Statistical analysis

All statistical analyses were performed using SPSS Statistics (version 23.0; IBM Corp., Armonk, NY, USA), and p-values <0.050 were considered statistically significant. The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables are presented as means with standard deviations (SDs) if normally distributed or medians (interquartile ranges (IQR: 25–75th percentile) if non-normally distributed, along with ranges (minimum–maximum). Categorical variables are presented as frequencies (percentages). The relationships between categorical variables were evaluated using Pearson's chi-square test and Fisher's exact test. Continuous variables were compared between two groups using the Mann–Whitney U test and among three or more groups using the Kruskal–Wallis test, with Bonferroni corrections applied in post-hoc tests.

Correlations between blood parameters measured at different times were assessed using Spearman's correlation coefficient (r_s). The optimal thresholds for COHb levels distinguishing phototherapy need in newborns at 0–6 hours and 24 hours were evaluated using receiver operating characteristic (ROC) curves with results presented with cut-off, sensitivity, specificity, negative and positive predictive values, positive and negative likelihood ratio and 95% confidence intervals (CIs), along with the area under the ROC curve (AUC). The optimal thresholds for variables were determined using the Youden index. Positive and negative likelihood ratios were calculated as $LR+ = \text{sensitivity} / (1 - \text{specificity})$ and $LR- = (1 - \text{sensitivity}) / \text{specificity}$.

Multivariable logistic regression analyses were performed to identify factors independently associated with phototherapy requirement. Covariates included in the multivariable models were selected a priori based on their clinical relevance and their potential association with neonatal hyperbilirubinemia and phototherapy requirement. Phototherapy requirement was entered as the dependent variable. Two separate models were constructed for COHb measurements obtained at different postnatal time points. Model 1 included birth weight category, gestational age category, maternal age, maternal thyroid disease, and COHb measured at 0–6 hours. Model 2 included the same covariates and COHb measured

at 24 hours instead of the 0–6-hours measurement. The results were presented as adjusted odds ratios with 95% confidence intervals and p values.

Results

A total of 297 newborns met the eligibility criteria and were included in this study. Regarding gestational age, 117 newborns (39.4%) were born before 29 weeks, 72 (24.2%) were born between 29 and 33 weeks plus 6 days, and 108 (36.4%) were born between 34 and 36 weeks plus 6 days. Most newborns ($n = 271$, 91.2%) were delivered via cesarean section (Table I).

Regarding sepsis, 238 newborns (80.1%) did not develop sepsis, 38 (12.8%) developed clinical sepsis, and 21 (7.1%) developed culture-positive sepsis (Table I). The most frequently identified microorganism in culture-positive sepsis was *Acinetobacter* spp. ($n=11$, 3.7%). Phototherapy was administered to 151 newborns (50.8%). Regarding blood groups 263 newborns (88.6%) had compatible blood groups, 20 (6.7%) had ABO incompatibility, and 14 (4.7%) had Rh incompatibility. Only 3 newborns (1.0%) had positive Direct antiglobulin test result. The demographic and clinical characteristics of newborns according to their need for phototherapy are presented in Table I.

Regarding maternal characteristics, 16 mothers (5.4%) were aged 20 years or younger, 126 (42.4%) were age 20–30 years, 142 (47.8%) were age 31–40 years, 12 (4%) were age 41–50 years, and 1 (0.3%) was aged over 50 years. The most common maternal comorbidities were placental disease ($n = 50$, 16.8%), preeclampsia ($n=41$, 13.8%), and gestational diabetes ($n=15$, 5.1%). Mode of delivery was not significantly associated with phototherapy requirement ($p=0.928$).

The newborns' blood parameters were evaluated at 0–6 hours, 24 hours, and 48–96 hours after birth (Table II). A very weak

Table II: Descriptive statistics of blood parameters measured at 0–6 hours, 24 hours, and 48–96 hours after birth

Variables	First 6 hours	24 th Hour	48 th –96 th Hour
COHb*	0.9 (0.5–1.2)	0.7 (0.3–1)	0.7 (0.3–1.1)
pH*	7.36 (7.31–7.4)	7.36 (7.31–7.42)	7.32 (7.26–7.38)
PCO2*	36.4 (31–41.1)	33.8 (27.8–39.6)	37 (31.5–42.3)
Hemoglobin*	16.3 (14.6–17.9)	15.5 (13.8–17.2)	14.2 (12.3–16.2)
Total bilirubin	3.29 (1.49–6.46)	6.06 (4.9–7.2)	6.25 (3.92–8.71)
Indirect bilirubin*	2.97 (1–5.8)	5.5 (4.3–6.5)	5.59 (3.11–8.12)
Direct bilirubin*	0.43 (0.29–0.9)	0.53 (0.44–0.66)	0.72 (0.59–0.89)
Albumin*	2.98 (2.41–3.26)	2.9 (2.59–3.2)	3.09 (2.68–3.4)
Hemolysis†			
Negative	294 (99)	294 (99)	292 (98.3)
Positive	3 (1)	3 (1)	5 (1.7)
Reticulocyte (%)	0.83 (0.79–30.9)	5.17 (0.4–53)	9.52 (2.99–11.5)

*: median(IQR); †: n(%)

positive correlation was observed between 0–6-hours COHb levels and total bilirubin levels at 24-hours ($r=0.167$, $p=0.008$), as well as indirect bilirubin levels ($r=0.173$, $p=0.006$). A weak positive correlation was found between 24-hours COHb levels and total bilirubin levels ($r=0.237$, $p=0.037$), but the positive correlation between 24-hours COHb levels and indirect bilirubin levels was only marginally significant ($p=0.052$). At 0–6 hours, the median COHb level was 0.9 (IQR: 0.6–1.3) in newborns who required phototherapy and 0.8 (IQR: 0.4–1.1) in those who did not ($p=0.075$). At 24 hours, the corresponding median COHb levels were 0.7 (IQR: 0.4–1.1) and 0.6 (IQR: 0.3–0.9), respectively ($p=0.099$). Among newborns without blood group incompatibility, a weak positive correlation was observed between 24-hour COHb levels and total bilirubin levels measured at 48–96 hours ($r=0.239$, $p=0.048$). No statistically significant associations were observed between early COHb measurements and subsequent bilirubin levels in the ABO- or Rh-incompatibility subgroups.

The ability of 0–6-hours and 24-hours COHb levels to predict the need for phototherapy was analyzed using ROC analysis. ROC analysis demonstrated poor and statistically non-significant discriminatory performance for both early and 24-hours COHb measurements. The AUC was 0.560 (95% CI: 0.501–0.617; $p=0.076$) for COHb levels measured at 0–6 hours (Figure I) and 0.583 (95% CI: 0.494–0.668; $p=0.098$) for COHb levels measured at 24-hs (Figure II). Both values were close to 0.5 and were not statistically significant, indicating that COHb levels could not reliably distinguish newborns who subsequently required phototherapy from those who did not. A COHb threshold of $>0.6\%$ at 0–6 hours predicted phototherapy requirement with a sensitivity of 72.19%, specificity of 41.78%, positive predictive value of 56.2%, and negative predictive value of 59.2%, positive likelihood ratio of 1.24, and negative likelihood ratio of 0.67. At 24 hours, a COHb threshold of $>0.6\%$ yielded a sensitivity of 63.38% and specificity of 54.1%, positive predictive value of 61.6%, negative predictive value of 55.9%, positive likelihood ratio of 1.38, and negative likelihood ratio of 0.68. Despite moderate sensitivity, the low specificity and likelihood ratios close to 1 indicated that neither measurement provided clinically meaningful discrimination.

Multivariable logistic regression analyses were performed to identify factors independently associated with phototherapy requirement. Two separate models were constructed for

Table I: Demographic characteristics and clinical characteristics of the babies included in the study according to their need for phototherapy

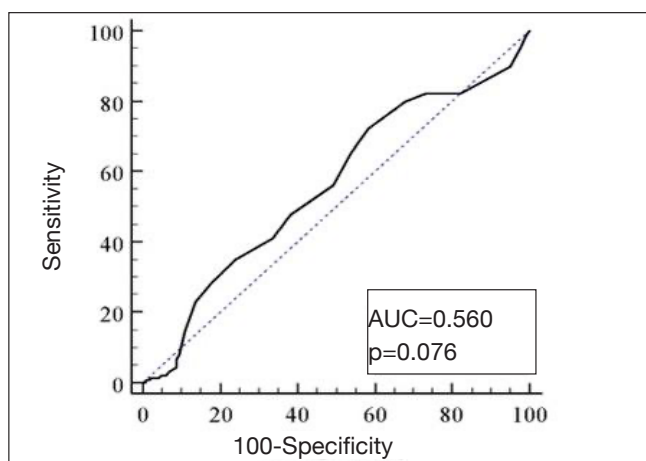
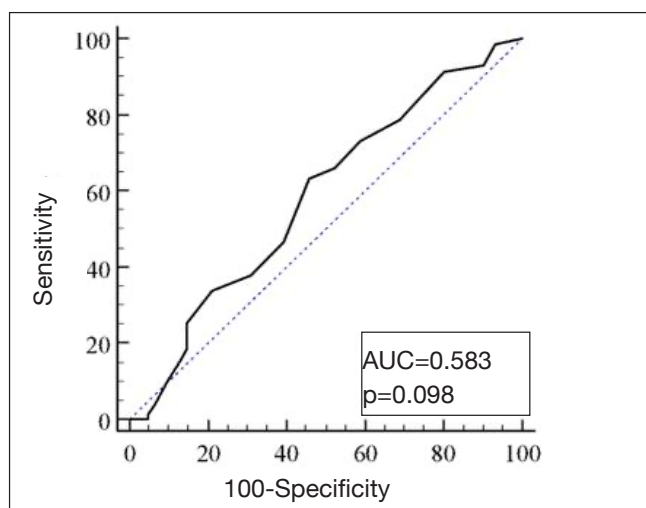
Variables	No*	Yes*	p
Total number of patients	146	151	-
Birth weight			
<2000 grams	80(54.8)	117(77.5)	<0.001
≥2000 grams	66(45.2)	34(22.5)	
Gestational age			
<29 weeks	52(35.6)	65(43)	<0.001
29–33 6/7 weeks	24(16.4)	48(31.8)	
34–36 6/7 weeks	70(47.9)	38(25.2)	
Mode of delivery			
Birth by cesarean section	133(91.1)	138(91.4)	0.928
Vaginal delivery	13(8.9)	13(8.6)	
Sepsis			
Negative	121(82.9)	117(77.5)	0.147
Clinical sepsis	19(13)	19(12.6)	
Culture positive sepsis	6(4.1)	15(9.9)	
Blood group incompatibility			
No	131(89.7)	132(87.4)	0.260
ABO	11(7.5)	9(6)	
Rh	4(2.7)	10(6.6)	
Direct antiglobulin test			
Negative	145(99.3)	149(98.7)	0.999
Positive	1(0.7)	2(1.3)	

*: n(%)

Table III: Multivariable logistic regression analyses of factors associated with phototherapy requirement

Variables	Model 1					Model 2				
	Univariable OR (%95 CI)	p	B	Adjusted OR (%95 CI)	p	Univariable OR (%95 CI)	p	B	Adjusted OR (%95 CI)	p
Birth weight										
<2000 g	2.839 (1.718–4.690)	0.001	0.857	2.356 (1.170–4.741)	0.016	3.441 (1.473–8.040)	0.004	1.218	3.381 (0.990–11.542)	0.048
≥2000 g (reference)	-	-	-	-	-	-	-	-	-	-
Gestational age										
<29w	2.303 (1.345–3.942)	0.002	0.269	1.309 (0.622–2.757)	0.478	2.885 (1.165–7.145)	0.022	0.234	1.264 (0.338–4.729)	0.728
29w–33w+6	3.684 (1.963–6.913)	<0.001	0.871	2.389 (1.162–4.911)	0.018	2.533 (0.916–7.005)	0.073	0.322	1.380 (0.401–4.748)	0.609
34w–36w+6 (reference)	-	-	-	-	-	-	-	-	-	-
Maternal age	1.237 (0.882–1.736)	0.218	0.203	1.225 (0.858–1.748)	0.265	1.314 (0.806–2.142)	0.273	0.222	1.248 (0.742–2.099)	0.403
Maternal thyroid disease	3.500 (0.715–17.135)	0.122	1.323	3.754 (0.705–19.986)	0.121	1.761 (0.311–9.965)	0.522	0.905	2.472 (0.372–16.442)	0.349
COHb at 0–6 hours	1.144 (0.796–1.644)	0.514	0.128	1.137 (0.774–1.670)	0.514	-	-	-	-	-
COHb at 24 hours	-	-	-	-	-	1.505 (0.700–3.236)	0.295	0.431	1.539 (0.682–3.472)	0.299

CI: confidence interval, **COHb:** carboxyhemoglobin, **OR:** odds ratio. The multivariable Model 1 was statistically significant, $\chi^2(6)=29.585$, $p<0.001$; Nagelkerke $R^2=0.126$; Hosmer–Lemeshow $p=0.906$; overall correct classification=64.3%. The multivariable Model 2 was statistically significant, $\chi^2(6)=12.549$, $p=0.048$; Nagelkerke $R^2=0.121$; Hosmer–Lemeshow $p=0.653$; overall correct classification=64.4%.

**Figure 1:** ROC curve for COHb measured at 0–6 hours in predicting phototherapy requirement in preterm newborns.**Figure 2:** ROC curve for COHb measured at 24 hours in predicting phototherapy requirement in preterm newborns.

COHb measurements obtained at 0–6 hour and 24-hours. In Model 1, birth weight <2000 g was independently associated with an increased likelihood of phototherapy requirement (adjusted OR: 2.356; 95% CI: 1.170–4.741; $p=0.016$). Gestational age between 29 weeks and 33 weeks plus 6 days was also

independently associated with phototherapy requirement compared with gestational age between 34 weeks and 36 weeks plus 6 days (adjusted OR: 2.389; 95% CI: 1.162–4.911; $p=0.018$). Gestational age <29 weeks was not independently associated with phototherapy requirement (adjusted OR: 1.309; 95% CI: 0.622–2.757; $p=0.478$). COHb measured at 0–6 hours was not independently associated with phototherapy requirement (adjusted OR: 1.137; 95% CI: 0.774–1.670; $p=0.514$). In Model 2, birth weight <2000 g remained independently associated with phototherapy requirement (adjusted OR: 3.381; 95% CI: 1.030–11.542; $p=0.048$). Neither gestational age category nor COHb measured at 24-hours was independently associated with phototherapy requirement. The complete multivariable logistic regression results are presented in Table III.

Discussion

The preventability of bilirubin encephalopathy has increased the importance of jaundice monitoring and clinical management. Many studies have aimed to predict jaundice. Our study demonstrated very weak positive correlations between early COHb levels and subsequent bilirubin concentrations; however, these associations were not sufficiently strong to provide clinically meaningful predictive value.

In a prospective survey of preterm and small-for-gestational-age infants in the Netherlands, those with neurodevelopmental problems had significantly higher maximum TSB levels than those with normal neurodevelopment (13). Thus, this finding highlights the importance of determining predictive values for jaundice. Hyperbilirubinemia is known to be more common in preterm infants (14). In our study, newborns born between 29 0/7 and 33 6/7 weeks of gestation had a greater need for phototherapy, while those born between 34 0/7 and 36 6/7 weeks had the lowest need. Notably, bilirubin levels seem to decrease with gestational age (15,16).

When examining the relationship between delivery mode and hyperbilirubinemia, neither our study nor Phuapradit et al. (17) found a correlation between delivery mode and total bilirubin levels. However, Günel and Tan found that infants born via normal spontaneous vaginal delivery had significantly higher

mean total bilirubin levels than those born via cesarean section (16,18). The independent association between birth weight <2000 g and phototherapy requirement observed in our study is consistent with previous reports identifying low birth weight as a risk factor for neonatal hyperbilirubinemia (17,19).

During heme catabolism, the alpha-methene bridge is cleaved by the heme oxygenase enzyme, releasing free iron and CO. Therefore measuring CO levels, such as via ETCO or COHb, can be used to evaluate bilirubin production rates (10). Engel et al. (20) tested a portable prototype device that measured peak concentrations of CO, CO₂, and H₂ in breath samples from 50 infants with positive direct antiglobulin test results and blood group incompatibilities. The mean CO level was significantly higher among 18 antibody-positive, ABO-incompatible infants who received phototherapy than among 32 infants who did not ($p < 0.001$). A positive relationship was observed between CO levels and phototherapy duration. Thus, measuring inhaled gases as an indicator of heme catabolism and bilirubin production has been deemed clinically beneficial (20). These findings suggest that early COHb measurement alone may have limited clinical utility in predicting subsequent phototherapy requirement in preterm neonates.

In 2016, Bhutani et al. (21) investigated the relationship between TSB and ETCO concentrations in healthy late preterm and term newborns. They included 79 infants over 35 weeks of gestation and weighing >2000 grams, excluding those requiring respiratory support, those with severe life-threatening congenital anomalies, those weighing <2000 grams, and those with mothers who smoked in the past three months, but including those who were ABO-incompatible. They concluded that the risk of hyperbilirubinemia was not independent of ETCO concentration (21). Another study published in 2016 suggested that COHb could serve as a marker for increased hemoglobin turnover and help identify newborns at risk for rapid bilirubin increases earlier (22). A further study published in 2022 created an ETCO concentration normogram for term and late preterm infants without hemolytic disease, suggesting it as a complement to TSB/TcB measurements rather than a standalone screening tool (23). The American Academy of Pediatrics and the Canadian Pediatric Society recommend screening all newborns for jaundice before discharge. However, a 2024 review on TcB as a non-invasive screening method for term infants did not find strong evidence for reducing the occurrence of acute bilirubin encephalopathy (24). Although some studies suggest that CoHb levels rise before jaundice onset, further research is needed to explore its utility in screening.

In 2017, Bailey et al. (22) studied the relationship between COHb and TSB in Germany, independent of gestational age, disease subgroup classification, and need for phototherapy. A delay of up to two days was observed between peak COHb and TSB values, with COHb always peaking first (22).

The diagnostic performance of CO-related measurements has varied considerably across previous studies. These discrepancies may be explained by differences in study

populations, study designs, measurement methods, sampling times, and outcome definitions. Studies including term or late-preterm newborns, direct antiglobulin test-positive infants, or newborns with confirmed blood group incompatibility may include a higher proportion of infants with increased hemolysis and may therefore demonstrate a stronger association between CO production and hyperbilirubinemia. In contrast, our study included a heterogeneous population of preterm newborns, most of whom had neither blood group incompatibility nor a positive direct antiglobulin test. Differences between ETCO and blood COHb measurements may also contribute to the inconsistent findings. ETCO reflects exhaled endogenous CO, whereas COHb represents the proportion of circulating hemoglobin bound to CO and may be influenced by the timing and method of blood sampling. Furthermore, differences in gestational-age distributions, phototherapy thresholds, sampling intervals, and definitions of clinically significant hyperbilirubinemia may limit direct comparisons across studies.

In our cohort of preterm newborns observed a very weak but significant positive correlation between 0–6-hours COHb levels and 24-hours total bilirubin levels, as well as indirect bilirubin levels. It also observed a weak positive correlation between 24-hours COHb levels and total bilirubin levels, whereas the positive correlation with indirect bilirubin levels was only marginally significant.

COHb was found to be a sensitive and specific method for detecting hemolysis and could be used for the early diagnosis of hemolytic diseases leading to early and severe hyperbilirubinemia (25). Neonatal hemolytic disease due to blood group incompatibilities occurs when maternal antibodies target antigens on neonatal erythrocytes. It can be caused by ABO, Rh, and subgroup incompatibilities (26). Blood group incompatibilities are the most common causes of neonatal jaundice, with ABO and Rh incompatibilities being the most frequently observed. In our study, 88.6% of newborns had no blood group incompatibility ($n = 263$), while 6.7% had ABO incompatibility ($n = 20$) and 4.7% had Rh incompatibility ($n = 14$). The weak association between 24-hour COHb and subsequent total bilirubin observed in newborns without blood group incompatibility suggests a possible relationship between early bilirubin production and later bilirubin levels. However, the absence of significant associations in the ABO- and Rh-incompatibility subgroups should be interpreted cautiously because of the small sample sizes. However, because these subgroups included only 20 and 14 newborns, respectively, and the number of available observations was even lower at some measurement time points, these analyses should be considered exploratory and interpreted cautiously. Therefore, the nonsignificant findings should not be interpreted as evidence of the absence of an association. Consistent with the limited discriminatory value observed in our study, a 2022 study reported that COHb was not superior to the direct antiglobulin test for identifying severe hyperbilirubinemia (26).

In our study, COHb measurements obtained at 0–6 hours and 24-hours showed poor and statistically non-significant discriminatory performance for identifying newborns who

required phototherapy, with AUC values of 0.560 and 0.583, respectively. Although the $>0.6\%$ thresholds provided moderate sensitivity, their low specificity and likelihood ratios close to 1 limited their clinical usefulness. In contrast, a study conducted in Türkiye involving 221 preterm newborns reported that a COHb threshold of $\geq 0.95\%$ predicted significant hyperbilirubinemia with a sensitivity of 90% and a specificity of 88% (27). Differences in study populations, gestational-age distributions, the prevalence of hemolytic conditions, the timing and method of COHb measurement, outcome definitions, and phototherapy thresholds may account for the discrepancy between these findings.

Infants requiring phototherapy had significantly different birth-weight and gestational-age distributions compared with those who did not require phototherapy, indicating that the degree of prematurity was an important potential confounder. After adjustment for birth weight, gestational age, maternal age, and maternal thyroid disease, neither COHb measured at 0–6 hours nor COHb measured at 24 hours was independently associated with phototherapy requirement. In contrast, birth weight <2000 g remained independently associated with phototherapy requirement in both models, suggesting that prematurity-related characteristics were more important determinants of treatment requirement than early COHb levels. Gestational age between 29 weeks and 33 weeks plus 6 days was independently associated with phototherapy requirement only in Model 1, whereas gestational age <29 weeks was not significant. Therefore, these findings should not be interpreted as demonstrating a linear association between decreasing gestational age and increasing phototherapy requirement.

Our study has several strengths, including an adequate sample size determined by G*Power, assessment of COHb levels at different postnatal time points, and evaluation of its predictive performance using ROC analysis with sensitivity, specificity, and positive and negative predictive values. However, the retrospective design resulted in missing COHb measurements at some time points, and the small number of newborns with ABO or Rh incompatibility limited the subgroup analyses. In addition, the correlations between COHb and bilirubin levels were weak, and the ability of COHb to discriminate phototherapy requirement was limited. Therefore, these findings should be interpreted with caution.

Limitations

This study has several limitations. The retrospective and single-center design may have increased the risk of selection and information bias, limiting causal inference and generalizability of the findings. Inclusion of only newborns with available blood gas measurements may also have contributed to selection bias. While birth weight and gestational age were considered in multivariable analyses, residual confounding due to prematurity and unmeasured clinical variables could not be entirely ruled out. Furthermore, the timing of COHb measurements and the types of blood samples used were not fully standardized, and data for all measurement times were not available in some newborns. The limited number of cases with ABO and Rh incompatibility reduced the statistical power of subgroup analyses; therefore, these findings should be considered exploratory in nature and interpreted with caution. Finally, the

COHb cut-off values were derived and evaluated in the same cohort without external validation.

Overall, COHb had limited clinical value for predicting phototherapy requirement in preterm newborns. The low AUC values and the absence of an independent association between either 0–6-hours or 24-hours COHb levels and phototherapy requirement in the multivariable analyses do not support the use of COHb as a reliable standalone screening or predictive marker. Although COHb primarily reflects heme degradation and bilirubin production, clinically significant hyperbilirubinemia is determined by several physiological processes, including hepatic uptake and conjugation, intestinal elimination, and enterohepatic circulation. Its discriminatory performance may also have been affected by gestational age, postnatal age, bilirubin concentration, accompanying clinical risk factors, variability in sampling time, and the low prevalence of immune-mediated hemolysis in our cohort. Nevertheless, prospective studies are needed to determine whether COHb provides complementary predictive information when incorporated into multivariable models that include gestational age, birth weight, postnatal age-specific TSB or TcB values, the rate of bilirubin increase, and other markers of hemolysis.

Conclusion

Although early postnatal COHb levels were weakly associated with subsequent bilirubin concentrations, their ability to predict phototherapy requirement in preterm newborns was poor. The low AUC values, likelihood ratios close to 1, and lack of an independent association in the multivariable analyses do not support the use of COHb as a standalone screening or predictive marker. Prematurity-related characteristics, particularly low birth weight, appeared to be more important determinants of phototherapy requirement. Further prospective studies are needed to determine whether COHb provides incremental predictive value when added to established clinical and laboratory parameters.

Ethics committee approval

This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by Akdeniz University Faculty of Medicine (13.01.2021, reference number: 34).

Contribution of the authors

Conception and design: YFB, HO, Data collection: YFB, ZK, Review analysis: YFB, KÇ, SA, Manuscript writing: YFB, Final approval: All authors

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Conflict of interest

The authors declare that there is no conflict of interest.

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