Patched Skin Bilirubin Assay to Monitor Neonates Born Extremely Preterm Undergoing Phototherapy

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Objective To verify the reliability and safety of transcutaneous bilirubin (TcB) measurements in patched skin areas in neonates born extremely preterm under phototherapy.

Study design Sixty neonates (<30 weeks' gestation) receiving phototherapy were enrolled and TcB was measured via a second-generation transcutaneous bilirubinometer in patched skin areas (of at least 2.5 cm diameter). Total serum bilirubin (TSB), lactate, pH, hemoglobin, and skin temperature were measured within 10 minutes of the TcB assay. Clinicians were blinded to TcB values, and clinical decisions about phototherapy were made with the TSB measurement only.

Results TcB and TSB significantly were correlated ($r = 0.84$; $P < .001$), even after adjustment for hemoglobin, pH, lactate, gestational and postnatal age (standardized $\beta = 0.8$; $P < .001$; adjusted $R^2 = 0.75$), or treatment duration (standardized $\beta = 0.8$; $P < .001$; adjusted $R^2 = 0.7$). When the Bland-Altman analysis was used, TcB overestimated TSB at high values (mean difference TSB – TcB: –2.8 [2.4] mg/dL). If clinicians used the TcB only, no neonate would have had phototherapy stopped prematurely, and 21 (35%) would have continued phototherapy when it could have been stopped.

Conclusions The correlation between TSB and TcB (measured in patched skin areas) was comparable with that obtained in more mature neonates, and it was not influenced by clinical variables or factors affecting skin bilirubin passage. TcB overestimated TSB, and this may expose infants born preterm to unnecessary phototherapy, although it could spare approximately 65% of TSB assays. *(J Pediatr 2017;188:122-7).*

The measurement of transcutaneous bilirubin (TcB) is gaining popularity, because it is an easy, pain-free, and blood-sparing technique.[1,2] Although TcB and total serum bilirubin (TSB) are different variables,[3] because they are highly correlated, modern transcutaneous bilirubinometers routinely are used to monitor jaundice in infants born full term and late preterm.[4] A recent meta-analysis pooled together data from infants born at <32 weeks' gestation and showed that TcB measurements also were reliable in neonates born premature.[5] However, few neonates born extremely premature were included in this analysis.[5]

Transcutaneous bilirubinometry would be advantageous for infants born extremely premature, as it might reduce the number of painful procedures and blood loss. Infants born extremely premature frequently require phototherapy,[6] and monitoring bilirubin levels requires additional blood sampling. Currently, no data are available about the reliability of the TcB assay in a homogeneous population of infants born extremely premature. Conversely, data about the safety and reliability of the TcB assay in infants born late preterm and term undergoing phototherapy are conflicting.[7,8]

The purpose of this study was to evaluate the reliability of the TcB assay (including the effect of variables possibly affecting skin bilirubin deposition) and the safety of monitoring bilirubin by TcB assay in patched skin areas of neonates born extremely preterm treated with phototherapy. Our hypothesis was that the TcB assay would be reliable and safe in this context.

Methods

This was a prospective, observational, blind, and pragmatic study. Neonates born at <30 weeks' gestation consecutively admitted to an academic level III neonatal intensive care unit (NICU) during 2016 were eligible if they developed jaundice requiring phototherapy according to the United Kingdom's National Institute for Clinical Excellence (NICE) guidelines.[9] Infants who had not been studied previously were recruited on day shifts if they did not meet any exclusion criteria. The exclusion criteria were (1) major congenital malformations or chromosomal abnormalities; (2) life-threatening situations; (3) need

LED	Light-emitting diode
NICE	National Institute for Clinical Excellence
NICU	Neonatal intensive care unit
TcB	Transcutaneous bilirubin
TSB	Total serum bilirubin

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for exchange-transfusion according to NICE guidelines[9]; or (4) evidence of cholestasis or any liver disease. Infants born small for gestational age were identified via the Fenton growth curves.[10] All patients were placed in incubators with temperature servo-control and humidity at 80%-85%; environmental light was constant during the day.

According to our routine clinical protocol, light-emitting diode (LED) continuous phototherapy was used for at least 24 hours; LED devices were preferred over conventional devices because they have comparable efficacy[11] and because LED devices reduce transepidermal water loss.[12] Phototherapy devices were placed on the incubators at about 40 cm from the neonates. Total irradiance reaching the newborn skin was 22-24 μW/cm^2/nm. Environmental lighting was constant during the study period. Infants were naked, but their eyes were protected and diapers were folded to allow maximum skin exposure to phototherapy. Infants were turned from the prone to supine position and vice versa every 6-8 hours.

The clinical protocol was that TSB was checked 4-6 hours after the beginning of phototherapy and then as ordered by the physicians (usually at least once per day). TSB was measured on whole blood collected into heparinized, light-shielded, capillary microtubes (190 μL) by heel prick, following nonpharmacologic sedation. Samples were assayed in a blood gas analyzer (ABL800, Radiometer Medical ApS, Copenhagen, Denmark), following the manufacturer's recommendations. This analyzer has been shown to measure TSB accurately in neonatal samples.[13] The analyzer is subjected to serial quality controls using bilirubin measured by direct spectrophotometry, which has good agreement with the gold standard technique,[14] and an analytical error of approximately ±10%.[15] Because the analyzer is located in the NICU, samples were analyzed immediately by nurses who performed the blood samples, as per the NICU operating procedures. The treatment was stopped when TSB levels decreased below the NICE threshold for starting phototherapy[9] but never before 24 hours of treatment. The analyzer automatically measures hemoglobin, lactate, and pH on the same microtubes via absorption spectroscopy, amperometry, and potentiometry, respectively.[15]

Within 10 minutes before blood sampling, when the infant was in a quiet state, investigators measured TcB using a multiwavelength transcutaneous bilirubinometer (Bilicheck, Philips Inc, Amsterdam, The Netherlands). Measurements were performed on the forehead of the newborn in a zone protected from the light. Because all patients were treated with continuous positive airway pressure, this was achieved by means of the white thick cotton cap used to keep in place continuous positive airway pressure circuit and interfaces. Particular care was taken to ensure that the cap was always in place during phototherapy and that TcB was measured in the middle of a patched area of at least 2.5 cm in diameter.[16] The Bilicheck was used following the manufacturer's recommendations and as previously published[17]; a calibration tip (BiliCal; Respironics, Murrysville, Pennsylvania) was used for each patient, and areas with skin injuries, nevi, hemangiomas, or hematomas were avoided.[17]

Clinical decisions regarding the discontinuation of phototherapy were made based on TSB levels only by the attending neonatologists, who were blinded to the TcB values. The TcB was recorded by nurses in an anonymized electronic sheet on a computer dedicated to research purposes immediately after the measurements. Basic clinical data, jaundice risk factors, hemoglobin, lactate, pH, and skin temperature also were recorded in real time. The ethical board approved the study, and oral informed consent was obtained from parents on their child's admission to the NICU.

Statistical Analyses

Two sample-size calculations were performed: one to detect a given TSB-TcB bias and another to detect a given correlation between TSB and TcB. Data from a previous study on transcutaneous bilirubinometry in infants born late preterm undergoing phototherapy were used to estimate the first sample size.[7] Assuming a power of 90% and an α-error of 0.05, a sample size of 49 would detect a clinically significant mean bias TSB-TcB of 1.75 mg/dL with an estimated SD of 3.1 mg/dL as previously published.[7] The null hypothesis was represented by a difference TSB-TcB ≤0.2 mg/dL.[7]

For the second calculation, considering previous data[18,19] about correlations between TcB and TSB in neonates born extremely preterm, Pearson coefficients of 0.74-0.86 were targeted.[18,19] For a power of 90% and an α-error of 0.05, the needed sample size was 8-14. Calculations were done with MedCalc 13.3 (MedCalc bvba, Ostend, Belgium).

Data were analyzed with the Shapiro-Wilk test to test their distribution and expressed as mean (SD) or median (IQR), accordingly. Because TSB and TcB were distributed normally, paired Student *t* tests were used to compare means. Pearson correlation analysis was performed. Multivariate linear regressions with backward stepwise models were performed to study the relationship between TSB and TcB, with adjustment for the following covariates: gestational and postnatal age, pH, lactate, hemoglobin, and skin temperature. In an alternative model, the time from the initiation of phototherapy was used instead of postnatal age. These covariates were chosen for their possible effect on the TcB reliability and for the possible influence on skin bilirubin deposition.[20] Model goodness-of-fit was evaluated with adjusted R^2, and the step with the greatest adjusted R^2 was chosen.

Bland-Altman analysis[21] also was performed, and local "smooth" regression was applied to study the trend of TSB-TcB bias with an Epanechnikov's kernel of 90%.[22] Taking into consideration the NICE threshold for phototherapy according to gestational and postnatal ages,[9] we calculated possible clinical errors that would have been made if decisions were based only on TcB levels. Statistics were performed with SPSS 15.0 (SPSS Inc, Chicago, Illinois) and *P* values <.05 were considered to be statistically significant.

Results

Clinical characteristics of the enrolled infants are shown in the **Table**; given the simplicity of study design, we continued the