Transcutaneous Bilirubin Measurement: A Multicenter Evaluation of a New Device

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ABSTRACT

Objectives. The early discharge of neonates from hospitals makes transcutaneous measurement of total bilirubin concentration a useful tool to monitor neonatal jaundice. The objectives of this study were to determine whether 1) transcutaneous bilirubin (TcB) measurement, as performed using BiliCheck (BC), correlates with total serum bilirubin (TSB) levels, measured with standard laboratory methods and with high-pressure liquid chromatography (HPLC-B); 2) infant race, gestational age, postnatal age, or body weight interferes with the measurement of TcB levels in newborn infants; 3) the variability of the TcB measurement is comparable to the variability of TSB measurements; and 4) TcB measurements obtained from the forehead (BCF) and sternum (BCS) generate comparable results.

Study Design. Newborn infants who were <28 days and >30 weeks' gestational age and who underwent tests for TSB as part of their normal care in 6 different European hospitals were studied. A total of 210 infants were enrolled in the study, 35 at each site. Near simultaneous (within ± 30 minutes) blood collection for TSB and BCF and BCS measurements were performed. TSB levels were determined by the serum bilirubin method in use at each site, and all HPLC-B determinations were made at the same, independent laboratory.

Results. The study group consisted of 140 white, 31 Asian, 14 Hispanic, 9 African, and another 16 newborns of different races. The correlation coefficient (r) between BCF and HPLC-B was 0.890 (95% confidence interval = 0.858-0.915). BCF and BCS generated similar results (r value = 0.890 for BCF and 0.881 for BCS), even if BCS slightly overestimated (mean error = -0.04 mg/dL) and BCF slightly underestimated (mean error = 0.96 mg/dL) in comparison with HPLC-B. Analysis of covariance demonstrated that BC accuracy was independent of race, birth weight, gestational age, and postnatal age of the newborn. Receiver operating characteristic curves were evaluated for BCF and TSB, each compared with HPLC-B. With the use of a cutoff point for HPLC-B of 13 mg/dL (222 µmol/L) and a cutoff of 11 mg/dL on the BCF and TSB, similar sensitivity/specificity (93%/73% for BCF, 95%/76% for TSB) were observed. The use of a cutoff point for HPLC-B of 17 mg/dL (290 µmol/L) and 14 mg/dL (240 µmol/L) for BCF and TSB also produced similar sensitivity/specificity (90%/87% for the BC and 87%/83% for TSB).

Conclusions. Because the correlation coefficient for HPLC-B and BCF is very similar to that found for HPLC-B and laboratory TSB, BC could be used not only as a screening device but also as a reliable substitute of TSB determination. At higher levels of TSB, in which phototherapy and/or exchange transfusion might be considered, BC performed slightly better than the laboratory. The accuracy and precision of the TcB measurement in this study was observed to be comparable to the standard of care laboratory test. Key words: jaundice, newborn, bilirubin, kernicterus, transcutaneous bilirubin measurement, laboratory bilirubin determination.

Within the past 2 years, there have been at least 3 publications that addressed the care and management of neonatal jaundice. In the first, Gartner et al1 reported the results of a survey of American neonatologists and
pediatricians in which they found striking differences in the practice patterns of these practitioners regarding the level of monitoring and therapy of newborn jaundice. Later, Bhutani et al reported on the hour-specific bilirubin nomogram that has been proposed as a more rigorous approach to predischarge screening than the day-specific values outlined by the American Academy of Pediatrics. Finally, Newman and Maisels recently discussed the theoretical and practical issues of developing and implementing practice guidelines specifically as they pertain to newborn jaundice. The recent attention given to this topic has, no doubt, emerged from the earlier reports that jaundice is the most common diagnosis that leads to hospital readmission of newborns within the first month of life and the anecdotal observation that there has been a reemergence of kernicterus in this population.

The incidence of neonatal jaundice has been reported to be between 30% and 60% of full-term newborns and in nearly all premature infants. Full-term neonates who present significant hyperbilirubinemia (total serum bilirubin [TSB] >12.9 mg/dL) range from 3.5% to 12%, although this varies according to feeding regimens. In contrast, it was found in a large prospective study that infants who are breastfed on demand have an incidence of hyperbilirubinemia similar to that found in formula-fed neonates. The practice of early discharge (<72 hours of age) of healthy, term newborns is growing worldwide as health care systems look for ways to reduce costs. Because peak TSB levels typically occur on postnatal days 3 to 5, an effective means of screening for and monitoring the onset of hyperbilirubinemia should enhance the safety of this growing practice.

Another complicating factor in the management of newborn jaundice has been the widespread reports of high variability in the laboratory measurement of bilirubin. Studies from the United States, the United Kingdom, the Netherlands and Germany, and New Zealand all indicate that the TSB values from which management decisions are made must be considered only approximations with a wide range of uncertainty. Furthermore, significant differences in TSB levels from capillary blood obtained by the heel-stick method compared with blood obtained from a vein have been reported, although this varies according to the TSB level. This observation adds additional uncertainty to the interpretation of TSB measurements. One can only speculate as to the differences that might be seen between umbilical artery and vein samples and even between suprahepatic and infrahepatic venous samples.

Fortunately, the management of newborn jaundice typically requires that therapy begin at TSB levels that are significantly below the levels at which kernicterus is considered an immediate threat. This safety margin still allows for therapy to be given to a relatively small number of patients in whom hyperbilirubinemia would resolve spontaneously to protect those who truly benefit from it.

Transcutaneous estimation of serum bilirubin is a universal practice. The visual inspection of the skin, sclera, and mucous membranes and observation of the cephalocaudal progression of jaundice is commonplace as the first indication of hyperbilirubinemia. More objective methods, such as the icterometer, have attempted to standardize the subjective visual assessment with modest success. Hannemann et al reported the ability to measure the light reflected from the skin and mathematically convert the data to a bilirubin value. This technique has been implemented commercially in a 2-wavelength (460 and 520 nm) device that generates a jaundice index. Despite reports of high correlation to TSB levels in some populations, this device has been limited to a screening method by the bias that race, age, and weight have on the index. Tayaba et al reported good results with a new device (Chromatics ColorMate III, New York, NY) that compares the change in yellow coloration of the newborn skin to a baseline skin color measurement. The drawback to this method is that every infant born, regardless of his or her likelihood to develop subsequent hyperbilirubinemia, would require a baseline measurement. The additional complexity and cost of this practice is too prohibitive for widespread use.

The subject of this study is the BiliCheck ([BC], SpectRx Inc, Norcross, GA), a new transcutaneous bilirubin (TcB) measuring device that uses the entire spectrum of visible light (380-760 nm) reflected from the skin. White light is transmitted into the skin of the newborn, and the reflected light is collected for analysis. The mathematical isolation of the light absorption of certain interfering factors (hemoglobin, melanin, and dermal thickness) allows the absorption of light caused by the presence of bilirubin in the capillary beds and...
subcutaneous tissue to be isolated by spectral subtraction. In theory, this will allow an unbiased measurement that is independent of the race, age, and weight of the newborn. Early reports by Bhutani et al.\textsuperscript{21,22} suggested favorable results with the use of the device in a diverse population.

A direct comparison between the TcB measurement and a single laboratory method would be dependent on the accuracy of the laboratory method. Errors in the TSB would be interpreted as errors in the TcB. Therefore, use of the gold standard high-pressure liquid chromatography bilirubin (HPLC-B) is necessary to serve as the true reference value. Individual laboratory devices tend to be consistent (low coefficient of variation) but often are not in agreement with other instruments, because of high interlaboratory variability.

The present multicenter study is an attempt to evaluate the pooled performance of multiple TcB devices in a diverse international population, with multiple users, and to compare the performance with multiple laboratory methods. The objectives of our study were to determine whether 1) TcB measurement, as performed with the use of BC, correlates with the TSB levels, measured with standard laboratory methods and with HPLC-B; 2) infant race, gestational age, postnatal age, or birth weight interferes with the measurement of TcB levels in newborn infants; 3) the variability of the TcB measurement is comparable to the variability of TSB measurements; and 4) TcB measurements obtained from the forehead (BCF) and sternum (BCS) generate comparable performances.

**METHODS**

This study was performed on newborn infants who underwent tests for TSB as part of their normal care in 6 different European hospitals (Queen Charlotte and Chelsea Hospital, London, England; Maternité Regionale A. Pinard, Nancy, France; University Hospital, Zürich, Switzerland; University Hospital, Florence, Italy; Evangelisches Waldkrankenhaus Spandau, Berlin, Germany; and Hôpital Saint Antoine, Paris, France). A total of 210 infants were enrolled in the study, 35 at each site. Within 30 minutes before or after blood collection for TSB assay, TcB measurements were performed. The blood samples were collected by heel stick or by venous sampling as medically indicated. Standard precautions were used to protect the sample from exposure to light to prevent photoconversion of bilirubin in the blood. TSB levels were determined by the laboratory method in normal use at each site (Dade Dimension [E.I. duPont de Nemours and Company, Wilmington, DE], Ektachem DT-60 [Eastman Kodak Company, Rochester, NY], Ginevri Microbilimeter [Rome, Italy], Ohara Bilirubinometer, Olympus AV600 [Olympus America, Inc, Melville, NY], Ortho Diagnostic Systems Bilimeter [Johnson & Johnson Corporation, New Brunswick, NJ], and Pfaff Bilimeter II [Pfaff Technik & Medizin, Neuburg/Donau, Germany]).

**HPLC**

A 50-µL aliquot of the serum sample was frozen at less than −20°C and shipped on dry ice in an insulated shipping container to the laboratory of Dr Glenn Gourley at the University of Wisconsin School of Medicine for HPLC-B determination according to the method described by Bhutani et al.\textsuperscript{22} The HPLC technician was blinded to the results of the laboratory TSB and the TcB measurements.

**TcB**

For each patient, at least 1 TcB measurement was performed on the forehead and at least 1 on the sternum/thoracic region. Repeated measurements were taken from each anatomic site in 111 patients to estimate instrument precision.

The device was calibrated before each measurement according to the manufacturer’s instructions to ensure the accuracy of the measurements. This requires that a reference measurement be taken from a calibration standard (called BiliCal), which automatically adjusts for any changes in the performance of device components over time.
To take a measurement, the probe is positioned on the infant's skin and 5 individual scans are taken to produce 1 measurement that is displayed in mg/dL or µmol/L. If an erroneous measurement is taken, then an error message is displayed and the scan must be repeated.

Infants of both sexes and of any race were included in the study, provided that they were not more than 28 days old and were at least 30 weeks of gestational age. Patients who had known skin disorders or patients who were receiving phototherapy or exchange transfusions were excluded from the study.

This study was approved by the Ethics Committee of each participating hospital, and informed consent was obtained from a parent or guardian of each patient.

Statistics

Correlation coefficients (Pearson product moment) were calculated with the use of linear regression techniques between BC and laboratory TSB, BC and HPLC-B, and laboratory TSB and HPLC-B, with all sample pairs included in the analysis.

Variability and bias of laboratory TSB values were estimated for each laboratory and among laboratories by standard parametric techniques, with the use of the HPLC-B value as the standardized reference value for each sample. The same calculations were performed for TcB values with the use of the HPLC-B and the laboratory TSB as the reference values.

A small sample size would not find a large difference in the accuracy of the TcB significant. Conversely, a large sample size might find a clinically insignificant difference in the accuracy of the TcB to be statistically significant. We hypothesized that the accuracy of the TcB measurement after pooling the data from all sites would be comparable to the accuracy of the pooled TSB values when each is compared with the HPLC-B values.

The null hypothesis is that the standard deviation (SD) of the BC measurement is >1.2 times the SD (20% higher) of the laboratory TSB value. Rejection of the null hypothesis would allow us to accept the alternative hypothesis, which is that the error in the TcB measurement is within 120% of the error in the laboratory TSB measurement.

\[ H_0: \text{BC TcB SD >1.2 Times Laboratory TSB SD} \]

To prove that TcB measurement, as performed by BC, is as accurate, within 20%, as laboratory TSB concentration in estimating HPLC-B, the sample size was calculated with the power set at 80% and type I error (\( \alpha \)) at 0.05. With the use of a standard F test, a sample size of 200 would allow rejection of the null hypothesis.

The sensitivity and the specificity of BC and laboratory TSB to predict accurately HPLC-B was estimated at a range of values and plotted on receiver operator characteristic (ROC) curves. Such curves are useful in analyzing a test that is continuous but needs to be dichotomized.

To determine whether the patient characteristics of race, gestational age, postnatal age, and birth weight interfered with measurements of TcB levels, we categorized demographic values and performed an analysis of covariance using HPLC-B as the covariate. This method tests the null hypothesis that the mean of errors is the same for the different categories of the categorical variables included in the model given the adjustment of the covariate. All statistical analyses were performed with SAS software (SAS Institute, Cary, NC).
The demographic characteristics of the group of newborn infants studied are given in Table 1. This group consisted of 140 white, 31 Asian, 14 Hispanic, 9 African, and another 16 of different races. The relationship between BCF and BCS measurements to HPLC-B and to TSB is reported in Table 2. The correlation coefficient ($r$) for the combined BCF data was 0.890 (95% confidence interval [CI] = 0.858-0.915) compared with HPLC-B with an offset near 0 (0.17) and a slope near 1 (1.07). The combined BCS data also had a high correlation to HPLC-B ($R = 0.881$; 95% CI = 0.846-0.908). BCS very slightly overestimated (mean error = −0.04 mg/dL) and BCF slightly underestimated (mean error = 0.961 mg/dL) in comparison with HPLC-B.

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<td>Demographic Characteristics of Newborn Infants Studied</td>
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<td>Relationship Between Transcutaneous Bilirubin Measurements (Forehead and Sternum) and Serum Bilirubin Concentration, Measured With HPLC Method or With Standard Laboratory Methods ($N = 210$)</td>
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The correlation between BCF and laboratory TSB was very good ($r = 0.870$; 95% CI = 0.832-0.899), but in this case the offset was 1.3 with a slope near 0.89. The mean error (TSB − BCF) was 0.13 mg/dL; this means that BCF measurement insignificantly underestimated in comparison with laboratory TSB.

The correlations between BCS and HPLC-B and between BCS and laboratory TSB also were high. In both cases, the mean error was negative (−0.043 and −0.865), which means that BCS, in this study, overestimated in comparison with HPLC-B and laboratory TSB.

The intradevice coefficient of variation for the BCF was 6.69%. Because of the limitations of the serum sample size, repeated measurements for determination of intralaboratory and interlaboratory variability were not possible.

The correlation between HPLC-B and standard laboratory methods also was high ($r = 0.927$; 95% CI = 0.906-0.944) even when different methods were used in different hospitals. The mean error (HPLC-B − TSB) was positive, and with the exception of 1 site, TSB determined by the laboratory underestimated in comparison with HPLC-B. Regression plots of BCF, HPLC-B, and TSB are shown in Fig 1A-C. Error plots (according to Bland and Altman) for each pair are shown in Fig 1D-F.

**Fig. 1.** Regression and error plots for comparisons among each of the 3 methods: BC TcB, HPLC-B, and laboratory TSB.
In Fig 2A-C, the ROC curves are reported. As the sensitivity and the specificity of a test increases, the ROC curve will appear in the upper left-hand corner of the plot. Table 3 reports the sensitivity, specificity, positive predictive value, and negative predictive value of BCF and laboratory TSB methods in relationship with HPLC-B at various clinically relevant cutoff points. To assess the clinical utility of the BC compared with the laboratory methods, we examined the ROC curves to compare the ability of the 2 methods to identify accurately the patients with TSB levels of interest. The first cutoff point selected was an HPLC-B level of 13 mg/dL (222 µmol/L). At increments of 1 mg/dL, it can be seen that a cutoff of 11 mg/dL on the BCF has approximately the same sensitivity and specificity (93%, 73%) as the laboratory TSB (95%, 76%).

![Fig. 2. ROC curves for BC TcB and laboratory TSB compared with HPLC-B at 3 levels of TSB.](image)

### Table 3

The Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of BC and Laboratory Methods in Relationship to HPLC

At the higher levels of TSB, at which phototherapy and/or exchange transfusion might be considered, the BC performed slightly better than the laboratory. When the HPLC-B was set at 17 mg/dL (290 µmol/L), use of a cutoff point of 14 mg/dL (240 µmol/L) produced similar sensitivities and specificities: 90%, 87% for the BCF and 87%, 83% for the laboratory TSB.

The analysis of covariance (Table 4) indicates that none of the categorical variables tested are significant contributors to the BCF error when the HPLC-B is included in the model (gestational age: $P = .127$; birth weight: $P = .155$; postnatal age: $P = .208$; race: $P = .436$). Hence, the mean error of BCF compared with the mean HPLC-B level is independent of race, gestational age, postnatal age, and birth weight at measurement time. Although race was not a significant variable in the analysis of covariance, it must be pointed out that the majority of the patients (66.7%) were white and only 4.3% were of African descent. However, our finding of racial independence of the BC TcB measurement also has been demonstrated in another study.22
The SD of errors (compared with HPLC-B) for the BCF (SD = 2.22 mg/dL) and for the laboratory TSB (SD = 1.84 mg/dL) were tested for significance by an F test ($P = .53$). The nonsignificance of this test means that we cannot reject the null hypothesis; therefore, the difference between BCF variability and the TSB variability was not less than 20%. However, the clinical importance of the 0.3 mg/dL (5 µmol/L) difference between the 2 methods is subjective.

### DISCUSSION

Kernicterus, which was thought to have almost completely disappeared, is now of greater concern for neonatologists and pediatricians because the earlier discharge from the hospital of mothers and neonates prevents an adequate monitoring of jaundice. The possibility of using a noninvasive, painless, and reliable method to determine the bilirubin level and its increment in the first 36 to 48 hours after birth could be very important in prevention of kernicterus. Since the early 1980s, a device dedicated to the bilirubin measurement has been proposed. Unfortunately, this device seems to have various limitations. First, it gives an index of jaundice, not the value of serum bilirubin concentration in standard clinical units of measurement. Race, gestational age, and body weight are factors that interfere with the accuracy of the jaundice index. However, as a screening device, this apparatus still is used in some locations. Recently, a new computer-driven, handheld device to estimate serum bilirubin from skin color of neonates was proposed. Tayaba et al. reported good results with this new instrument, which estimates the bilirubin concentration with the use of a color discrimination algorithm. However, this instrument requires an initial set of skin color measurements within the first 30 hours after birth. Hence, to be successful with subsequent measurements, every newborn infant would require this transcutaneous measurement within the first 30 hours after birth.

BC, which measures the transcutaneous serum bilirubin by determining the intensity of specific wavelength bands that are reflected from the skin, acts independent of the age of the neonate. In addition, it does not seem to be influenced by the race, birth weight, gestational age, and postnatal age of the newborn for the range of patients included in this study.

The TcB determination with the use of BC seems to be more comparable to HPLC-B determination when performed on the forehead than on the sternum. In addition, the correlation between BCF and HPLC-B is slightly greater than with the laboratory TSB (0.890 vs 0.870), and it is comparable to the correlation between the laboratory TSB and the HPLC-B (0.890 vs 0.927).

Although HPLC is considered the gold standard and these results are comparable to previous reports, a number of factors may have contributed to degradation in both the BC and the laboratory performance in this study. Different laboratory methods were used in each hospital that participated in this study. In addition, in some hospitals, TSB was determined in the central laboratory by automated methods, whereas in others it was performed in the nursery by neonatologists or nurses. The additional handling of the serum samples, extended storage periods, and transatlantic transport of the samples could alter significantly the integrity of some samples.

In this study, both the BCF measurement and the laboratory TSB underestimated slightly the HPLC-B. Because photo or thermal degradation of a sample would normally produce a reduction in the HPLC-B determination, an alternative explanation is a concentrating effect as a result of evaporation or sublimation.
However, one would not expect to see a 10% reduction in the volume of a frozen serum sample during this interval.

Conversely, BCS overestimated in comparison with HPLC-B, but the differences are very modest. In fact, the sternum measured 0.8 to 0.9 mg/dL (on average) higher than the forehead. This is counterintuitive on the basis of the observation of cephalocaudal progression of jaundice. A possible explanation for this observation is the effect of natural phototherapy on the forehead, which typically is exposed to ambient light more than the sternum is.

The correlation coefficient for HPLC-B and BCF (0.890) is very similar to that found for HPLC and laboratory (0.927), with a slope of the regression line of 1.07 and y intercept of 0.167. This implies that BC could be used not only as a screening device but also as a reliable substitute of TSB determination in the serum. Moreover, BC seems to have a good coefficient of variation and an acceptable accuracy.

The TSB level at which therapeutic decisions would be made depends on many factors, most notably the gestational and postnatal ages. To assess the clinical utility of the BC compared with the laboratory methods, we examined the ROC curves to compare the ability of the 2 methods to identify accurately the patients with TSB levels of interest. The first cutoff point selected was an HPLC-B level of 13 mg/dL (222 µmol/L). At increments of 1 mg/dL, it can be seen that a cutoff of 11 mg/dL on the BC has approximately the same sensitivity and specificity (93%, 73%) as the laboratory TSB (95%, 76%). Because TSB levels of <13 mg/dL account for a high percentage of the serum bilirubin analyses, the vast majority of invasive blood tests can be avoided depending on the population and the clinical situation (intensive care, normal newborn nursery, or outpatient facility).

At the higher levels of TSB, at which phototherapy and/or exchange transfusion might be considered, the BC performed slightly better than the laboratory. When the HPLC-B was set at 17 mg/dL (290 µmol/L), use of a cutoff point of 14 mg/dL (240 µmol/L) produced similar sensitivities and specificities: 90%, 87% for the BC and 87%, 83% for the laboratory.

**CONCLUSION**

TSB remains the standard of care for assessing newborn jaundice, and substitution of a new method requires substantial investigation and evidence of its superiority. The accuracy and the precision of the TcB measurement in this study was observed to be comparable to the standard of care laboratory test. Because the correlation coefficient for HPLC-B and BCF is very similar to that found for HPLC-B and laboratory TSB, BC could be used not only as a screening device but also as a reliable substitute of TSB determination.

The 2 methods do not, in fact, measure the same parameter. Although the laboratory method measures only the bilirubin that is circulating in the blood, the TcB measures the amount of bilirubin that has moved from the serum into the tissue. If this were indicative of the serum bilirubin levels that also were available to move into the brain tissue, which is our real concern, then it may offer additional information from which clinical management decisions can be made. These questions, as well as the effects of phototherapy and exchange transfusion on the TcB measurement, the effects of drugs, and the accuracy in very low birth weigh neonates and neonates <30 weeks' gestational age, all need additional study.

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FOOTNOTES

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ABBREVIATIONS

TSB, total serum bilirubin; BC, BiliCheck; TcB, transcutaneous bilirubin; HPLC-B, high-pressure liquid chromatography bilirubin; BCF, BiliCheck forehead; BCS, BiliCheck sternum; SD, standard deviation; ROC, receiver operating characteristic; CI, 95% confidence interval.

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