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Bilistick: A Low-Cost Point-of-Care System to Measure Total Plasma Bilirubin

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Key Words
Bilistick · Bilirubin · Point of care · Neonatal jaundice

Abstract
Background: Severe neonatal hyperbilirubinemia, with consequent encephalopathy, remains a common cause of morbidity and death in many regions of the world. Poor access to clinical laboratory resources and screening programs to measure plasma bilirubin levels is a major contributor to delayed treatment in developing countries, and the cost of existing point-of-care screening instruments precludes their dissemination. ***Objectives:*** We are evaluating the accuracy of a low-cost, minimally invasive point-of-care system (Bilistick) requiring a 25-μl blood sample that could be used in low-resource environments to evaluate patients with neonatal jaundice. ***Methods:*** We compared plasma bilirubin levels in divided blood samples by clinical laboratories and by Bilistick at two medical centers serving term and near-term newborns from ethnically different populations. ***Results:*** 118 neonates with bilirubin levels ranging from 24.8 to 501.0 μmol/l were analyzed. The mean bilirubin concentration (±SD) was 215.6 ± 85.5 μmol/l for Bilistick and 226.1 ± 86.4 μmol/l by laboratory determination. Pearson's correlation coefficient between all paired results was 0.961, and the Bland-Altman analysis showed a mean difference of 10.3 μmol/l with a 95% interval of agreement of –38.0 to 58.7 μmol/l. ***Conclusion:*** Bilistick is a minimally invasive method for measuring total bilirubin concentration over a wide range of values and should provide an affordable and accurate system for pre-discharge and follow-up screening of jaundiced infants, particularly in low-resource environments.

Introduction

Neonatal jaundice occurs in over 50% of term newborns, due to a transient increase in plasma unconjugated bilirubin concentration during the first week of life [1–3]. In some cases, particularly in babies with hemolytic diseases, hyperbilirubinemia may be severe, producing irreversible brain injury (kernicterus). While rather uncommon in the West, kernicterus is still a major cause of neurological injury, deafness and neonatal death in many regions of the world [4] with critical importance in developing countries [5–7].

Pre-discharge screening of serum bilirubin levels has become routine in many hospitals [8]. Methods currently used for bilirubin assay and screening can be grouped as:

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(1) non-invasive point-of-care transcutaneous reflectance instruments (TcB) (e.g. Minolta JM-103 device: Drager Medical Inc., Telford, Pa., USA; BiliCheck™ device: SpectRx, Inc., Norcross, Ga., USA); (2) non-chemical photometric measurement in blood samples (e.g. blood gas analyzer like ABL 735; Radiometer), and (3) laboratory analyzers for photometric measurement of total bilirubin (TB) levels in serum or plasma based on a diazo chemical reaction (e.g. Cobas Modular Instrument, Roche; Synchron CX PRU 16360, Beckman-Coulter) [9]. The lack of affordable phototherapy units in public hospitals (especially in remote areas), inadequate or inappropriate education and training of healthcare workers and limited access to bilirubin screening resources are the major factors contributing to the high prevalence of severe hyperbilirubinemia in developing countries [10–12]. Clinical laboratories are often poorly equipped to provide these services and are frequently not available at all in remote areas. A point-of-care instrument that could be used both in hospitals and in the field is sorely needed. Unfortunately, the cost and maintenance of transcutaneous monitors are currently prohibitive.

A new point-of-care system, Bilistick (Bilimetrix, Trieste, Italy), is currently under development with the primary goal of providing a low-cost minimally invasive method to screen bilirubin levels in babies born in low-resource environments. The hand-held Bilistick reader provides plasma TB estimates over a wide concentration range, has a long-lasting rechargeable battery energy source and requires only a small drop of blood. In the present study, we validate the accuracy of the Bilistick system by comparing bilirubin levels determined simultaneously by the instrument and by clinical laboratories in two hospital settings.

Materials and Methods

The Bilistick system consists of a hand-held rechargeable battery reflectance reader and test strips composed of a blood-plasma separator (filter) coupled with a nitrocellulose (NC) membrane, both encased in a plastic origami cassette. The size of the filter and volume of blood applied (25 μl) was dictated by the need to extract sufficient plasma from blood having a hematocrit up to 70%. After loading whole blood on the strip, the system requires only 100 s for plasma separation and NC membrane saturation. The reader measures reflectance of light emission (blue LED) from the plasma-saturated NC membrane (fig. 1). The LED has an emission peak between 470 and 530 nm with a spectrum similar to the absorbance spectrum of bilirubin. A second green LED detects whether hemoglobin contamination is present. The instrument is internally calibrated to optimize sensitivity at zero bilirubin concentration, and a standard curve (reflectance vs. TB concentration) was generated, using plasma spiked with purified bilirubin (B4126; Sigma-Aldrich, Milan, Italy), with TB ranging from 20.5 to 562.6 μmol/l (data not shown). A systematic relationship between dry and wet strip reflectance, as well as predictable relationships between dry strip reflectance and standard curve, permitted the development of an algorithm that corrects for variations in strip properties based on dry strip reflectance. No additional control standards were used in this study and no temporal drift in results was observed over the 5-month duration of data collection.

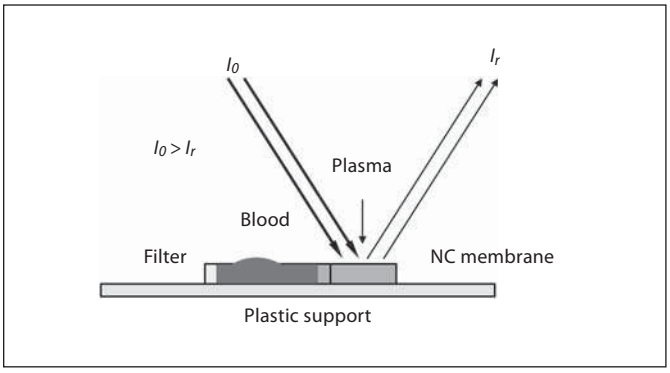

Fig. 1. Schematic diagram of the Bilistick system and its functionality. After 25-μl blood loading onto the filter, the plasma passes the capillary tube to the NC membrane. When the strip is inserted in the reader, the intensity of reflected light I_r is measured by the device to calculate the bilirubin concentration.

Operationally, test strips were inserted into the reader and reflectance of the dry strips determined. Blood samples were collected in 25-μl capillary tubes by nursing staff from heel pricks at the same time when duplicate samples were clinically required in each case for TB determination by the hospital clinical laboratories. The reflectance of the plasma-saturated NC membranes was measured 100 s after loading the blood onto the filter and the bilirubin level was determined using the algorithm-adjusted standard curve.

The system was validated by comparing laboratory and Bilistick assays on duplicate plasma blood samples obtained from 118 term and near-term neonates, with a mean age of 6.2 ± 3.6 days, born at IRCCS Burlo Garofolo Children's Hospital in Trieste, Italy (Burlo) or at Cairo University Children's Hospital in Cairo, Egypt (CUCH). Infants with hemolytic diseases were included in the study. Total serum bilirubin levels were determined using the Jendrassick-Grof method, analyzed with a Cobas modular instrument (Roche) at IRCCS Burlo Garofolo Children's Hospital in Trieste or a Synchron CX PRU 16360 (Beckman-Coulter) at CUCH.

Results obtained by Bilistick system were correlated with the corresponding values determined by the hospital laboratories. Pearson's correlation coefficient r was calculated to evaluate the accuracy of the system. A Bland-Altman analysis was carried out to evaluate the performance of the system. In this analysis, the difference between Bilistick bilirubin determination and TSB was plotted against the average of both determinations, and displayed with 95% confidence limits, to compare the variability across a range of bilirubin results.

Table 1. Summary of data collected at IRCCS Burlo Garofolo Children's Hospital and at CUCH

	IRCCS		CUCH		Total	
Measurements, n	31		87		118	
TB determination	hospital lab result	Bilistick results	hospital lab result	Bilistick results	hospital lab result	Bilistick results
Mean (± SD) bilirubin concentration, μmol/l	169.1 ± 68.4	167.8 ± 75.1	246.2 ± 83.3	232.7 ± 82.8	226.1 ± 86.4	215.6
Minimum value, μmol/l	24.8	39.3	61.6	56.4	24.8	39.3
Maximum, μmol/l	381.3	408.0	501.0	502.09	501.0	502.9
Pearson's correlation coefficient r	0.971		0.954		0.961	

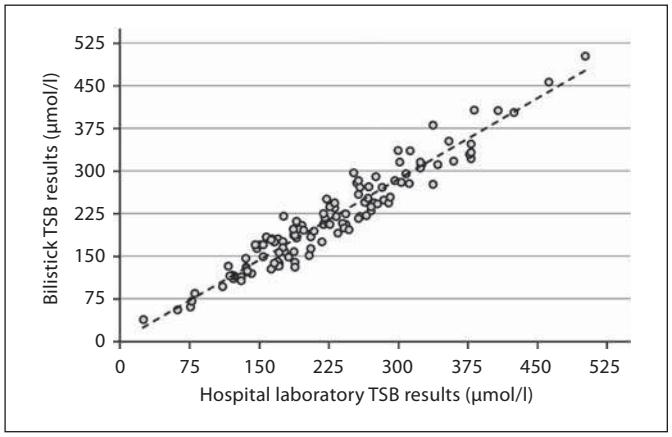

Fig. 2. Correlation of TB determinations obtained by Bilistick and hospital laboratories. The linear correlation (dashed line) is expressed by the equation y = 0.9509x + 0.0445.

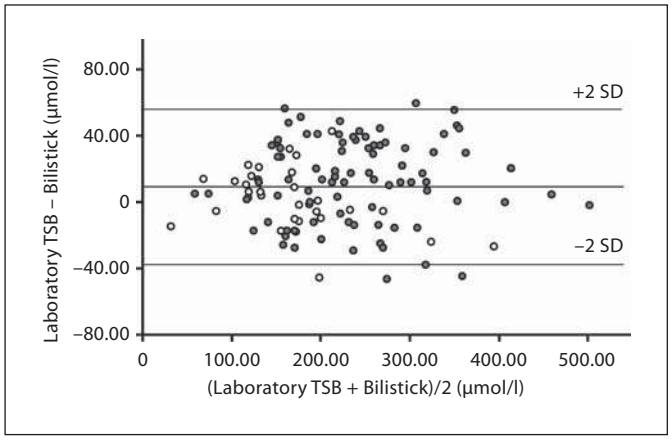

Fig. 3. Bland-Altman plot of the difference between laboratory TSB and Bilistick measurements performed at both IRCCS Burlo Garofolo Children's Hospital (empty circles) and CUCH (full circles), plotted against the mean bilirubin estimate. The lines represent mean difference (10.3 μmol/l) and ±2 SD of the difference (±24.1 μmol/l). All values are presented in μmol/l.

The research ethics boards at each hospital approved the study, and informed consent was obtained from a parent by nursing staff or the investigator responsible for the study before samples were obtained.

Results

In the present study, 31 tests were performed on newborns delivered at Burlo Garofolo Hospital while 87 tests were carried out at CUCH in Egypt (table 1). After each determination a visual analysis of the strip was performed to confirm successful corpuscular separation. Complete plasma separation was observed with hematocrit values ranging from 27.0 to 72.7%, with a mean value of 41.5%. The mean TB was 215.6 ± 85.5 μmol/l for Bilistick and 226.1 ± 86.4 μmol/l for the laboratory result. The minimum and maximum values obtained with Bilistick were, respectively, 39.3 and 502.9 μmol/l, compared with 24.8 and 501.0 μmol/l using chemical assays.

The correlation between TB values measured at both hospitals by Bilistick and clinical laboratories is shown in figure 2. Pearson's correlation coefficient (r) was 0.961 ($p < 0.0001$) over the wide range of TB analyzed. Comparison of methods by Bland-Altman analysis showed that the difference between laboratory results and Bilistick determinations can be underestimated by up to 38.0 μmol/l or overestimated by as much as 58.7 μmol/l in 95% of cases, with a mean difference value of 10.3 μmol/l (fig. 3). The underestimation was observed more frequently at values >342.0 μmol/l.

A hospital-specific analysis was also performed (table 1) showing that at IRCCS Burlo Garofolo Children's Hospital in Trieste, the mean of the TB measurements for over 31 neonates was 167.8 ± 75.1 μmol/l for Bilistick and 169.1 ± 68.4 μmol/l for the hospital laboratory results. The minimum and maximum values obtained were 39.3–408.0 and 24.8–381.3 μmol/l for Bilistick and clini-

cal laboratory, respectively; Pearson's correlation coefficient was r = 0.971 ($p < 0.0001$). At CUCH the mean TB for over 87 neonates was 232.7 ± 82.8 μmol/l for Bilistick and 246.2 ± 83.3 μmol/l for the clinical laboratory. The minimum and maximum values were 56.4–502.9 and 31.6–501.0 μmol/l for Bilistick and clinical laboratory, respectively. Pearson's correlation coefficient for Cairo results was r = 0.954 ($p < 0.0001$).

Discussion

The results obtained in this study support Bilistick as a promising tool to evaluate the severity of newborn hyperbilirubinemia and prevent the associated neurological damage. Pearson's correlation coefficient obtained with Bilistick (r = 0.961) was higher than those reported for any transcutaneous POC instrument that ranged from 0.70 to 0.87 [13–21]. The Bland-Altman analysis showed a mean difference between hospital laboratory results and Bilistick determinations of 10.3 μmol/l and a 95% confidence interval of –38.0 to –58.7 μmol/l. The best results reported using a transcutaneous device were obtained by Romagnoli et al. [20] using a Konica-Minolta Air Shield JM-103 (Drager Medical, Inc.) where the mean difference value was 5.1 μmol/l and the confidence interval –44.5 to –54.7 μmol/l. In that study the device was tested in 630 neonates with TB ranging from 8.6 to 307.8 μmol/l and Pearson's coefficient was r = 0.87. Additional subjects will be needed to confirm the accuracy of Bilistick values, particularly within the critical TSB range (e.g. 256.5–427.5 μmol/l) that might require test confirmation and/or medical intervention.

The main advantages of Bilistick when compared with other technologies are the low cost of the system and the ability to conduct assays on whole blood under field conditions. The estimated cost of materials for the prototype reader is less than EUR 150, plus a few cents for each test strip. Newer 'on-site' systems utilizing blood gas analyzers [22] or spectrophotometry [23] are more costly, immobile (and hence not truly point-of-care) and may require blood centrifugation.

The present study confirms that a comparatively simple system, Bilistick, can estimate plasma TB concentration with equal or greater accuracy than transcutaneous instruments and over a wider range of TB concentrations. While the Bilistick was designed to meet a critical need in low-resource nations, it could also be used for rapid screening of jaundiced infants in outpatient environments in affluent countries. In birthing centers, the burden of discomfort and cost of obtaining blood samples for pre-discharge screening [24] can be mitigated by performing the test together with obligatory metabolic screening.

In conclusion, the Bilistick POC system is an effective low-cost method to screen bilirubin levels in jaundiced newborns both in hospital and field environments. It can be used for newborn discharge screening, follow-up evaluations, and, because of its wide sensitivity range, identification of infants at risk for kernicterus.

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