Clinical Biochemistry 63 (2019) 148–151

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Short Communication

Implementation of a reformulated Roche® bilirubin Gen.3 reagent did not affect the relationship between BiliChek transcutaneous and Roche total serum bilirubin

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ARTICLE INFO

Keywords:
Hyperbilirubinemia
Transcutaneous bilirubin
Total serum bilirubin
Neonates

ABSTRACT

Objective: Determine whether introduction of a reformulated bilirubin reagent, the Roche bilirubin Gen.3 assay, changed the relationship between BiliChek transcutaneous bilirubin (TcB) and total serum bilirubin (TSB).
Design and methods: TcB results from term infants in the level 1 nursery obtained within one hour of a TSB were reviewed over two periods, six months before and after the conversion from the previous generation Roche bilirubin reagent to the new Roche Gen.3 bilirubin assay. TcB measurements were performed using BiliChek transcutaneous devices (Respironics, Marietta GA). Distribution of TSB results, and TcB minus TSB bias, were compared before and after introduction of the reformulated Roche bilirubin Gen.3 assay. Median and interquartile range (IQR) TSB values and bias were calculated. A statistical difference between median TSB values and bias were assessed using Man-Whitney test.
Results: A total of 301 paired TcB and TSB results were obtained, 172 before and 129 after implementation of the reformulated Roche bilirubin Gen.3 reagent. Median (IQR) TSB was 7.8 (6.8–8.7)mg/dL (133.3 (116.3–148.8) μmol/L) before and 7.6 (6.7–8.4)mg/dL (130 (114.6–143.6)μmol/L) after implementation of the reformulated reagent (p = .1373). Median (IQR) bias between TcB and TSB was 2.9 (2.2–3.7) mg/dL (49.6 (37.6–63.3)μmol/L) before the reformulated reagent was implemented; and did not change at 2.9 (2.1–3.9) mg/dL (49.6 (35.9–66.7)μmol/L) after implementation (p = .8242).
Conclusion: Implementation of the reformulated Roche bilirubin Gen.3 reagent did not affect the relationship between BiliChek transcutaneous and total serum bilirubin; thus no changes were needed to the neonatal TcB screening protocol as a result of the new bilirubin reagent.

1. Introduction

Neonatal jaundice is a common and most often benign condition in newborns infants [1]. Despite recent advances in treatment and prevention, severe consequences of hyperbilirubinemia persist; including bilirubin-induced neurologic dysfunction such as acute or chronic bilirubin encephalopathy (kernicterus) [1,2]. As a result, members of the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia recommend that all term newborns have either total serum bilirubin (TSB) or transcutaneous bilirubin (TcB) measurement prior to dismissal from the birth hospitalization to assess the risk of subsequent severe hyperbilirubinemia [2]. A similar guideline was published in 2007 by the Canadian Pediatric Society stating that all infants should receive a TcB or TSB measurement by 72 h of age or hospital discharge [3].

TcB screening is appealing because it provides rapid results, does not require a blood draw and provides a reasonable estimate of TSB in healthy newborns [4–7]. However, studies of TcB accuracy have demonstrated both systematic underestimation and overestimation of TSB. As a result there are concerns about the potential for missing infants with significant hyperbilirubinemia (underestimation) and creating unnecessary blood draws for a TSB measurement (overestimation) [4,7,8]. Poor standardization of commercial diagnostic bilirubin methods is one reason for differing conclusions about the relationship between TcB and TSB [9]. During the last 10 years, several commercial manufacturers recalibrated bilirubin assays to address the systematic overestimation of serum bilirubin values at higher

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https://doi.org/10.1016/j.clinbiochem.2018.10.001
Received 13 July 2018; Received in revised form 20 September 2018; Accepted 2 October 2018
Available online 03 October 2018

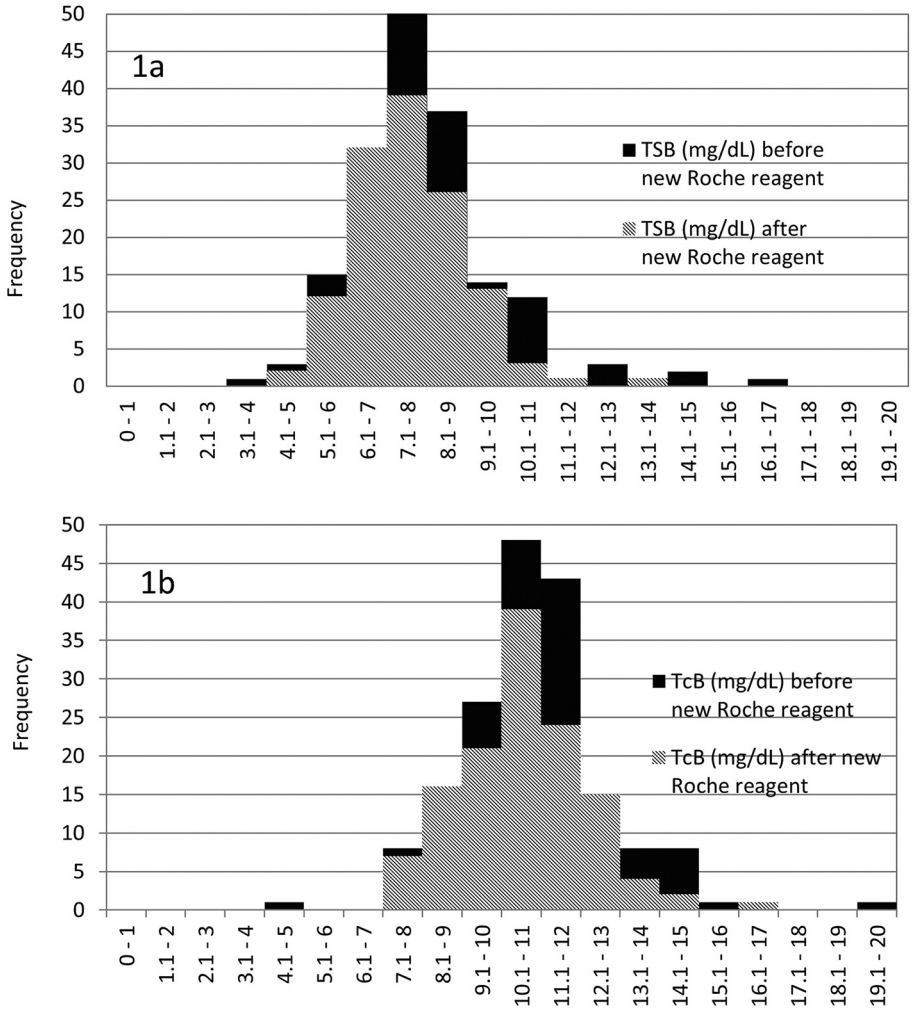

Fig. 1. (a) Distribution of total serum total bilirubin (TSB) and (b) transcutaneous bilirubin (TcB) values in the six months before and after implementation of the Roche bilirubin Gen.3 reagent.

concentrations [10,11]. Recalibration of the Vitros bilirubin assay lowered TSB values to the extent that phototherapy usage decreased in one large health system after introduction of the recalibrated reagent [11].

Roche diagnostics released a reformulated, bilirubin Gen.3 reagent in 2014. One study, using a regression equation provided by the manufacturer to predict Gen.3 results based upon infants' bilirubin results obtained using the previous generation reagent, concluded that the reagent reformulation would systematically lower bilirubin results such that fewer infants would meet phototherapy thresholds [12]. However no direct comparison of TSB values in infants before and after introduction of the Roche bilirubin Gen.3 diazo assay has been performed. In order to determine whether changes to our current TcB screening protocol were needed, we compared the relationship between BiliChek TcB and TSB before and after introduction of Roche bilirubin Gen.3 reagent.

2. Materials and methods

2.1. Comparison of neonatal transcutaneous and total serum bilirubin

Paired TcB and TSB results (TcB results obtained within one hour of a serum bilirubin) were reviewed from neonates in the level 1 newborn nursery at Rochester Hospital, Methodist Campus (Rochester, MN). We compared paired TcB and TSB in the 6 months prior (May to October 2014) and 6 months after introduction of the Roche bilirubin Gen.3 assay (December 2014 to May 2015), in order to determine whether introduction of the reformulated reagent changed the relationship between TcB and TSB.

2.2. BiliChek transcutaneous bilirubin (TcB) analyzer

Nurses performed transcutaneous bilirubin (TcB) measurements prior to discharge, using the BiliChek transcutaneous bilirubin monitor device (Respironics, Marietta GA). BiliChek was calibrated with a disposable tip (BiliCal) before each measurement, allowing measurement of cutaneous bilirubin by spectral subtraction of hemoglobin, melanin, and other skin components using a proprietary algorithm and according to the manufacturer's instructions. An adjusted TcB value, after subtraction of 1 mg/dL (17.1 μmol/L) to account for the observed bias between TcB and TSB [14], was used to determine risk for hyperbilirubinemia using *bilitool.org*. Infants with high-intermediate or high risk TcB values had TSB performed for confirmation.

2.3. Total serum bilirubin (TSB) determination

Serum samples were obtained by venipuncture or capillary heelstick. TSB concentration was measured using the previous generation Roche diazo bilirubin assay, the TBILS reagent marketed by Roche, until November 2014. The reformulated Roche bilirubin Gen.3 diazo assay (also called BILT3) was used for all bilirubin measurements after November 2014. Both assays were performed on a Roche Cobas c501

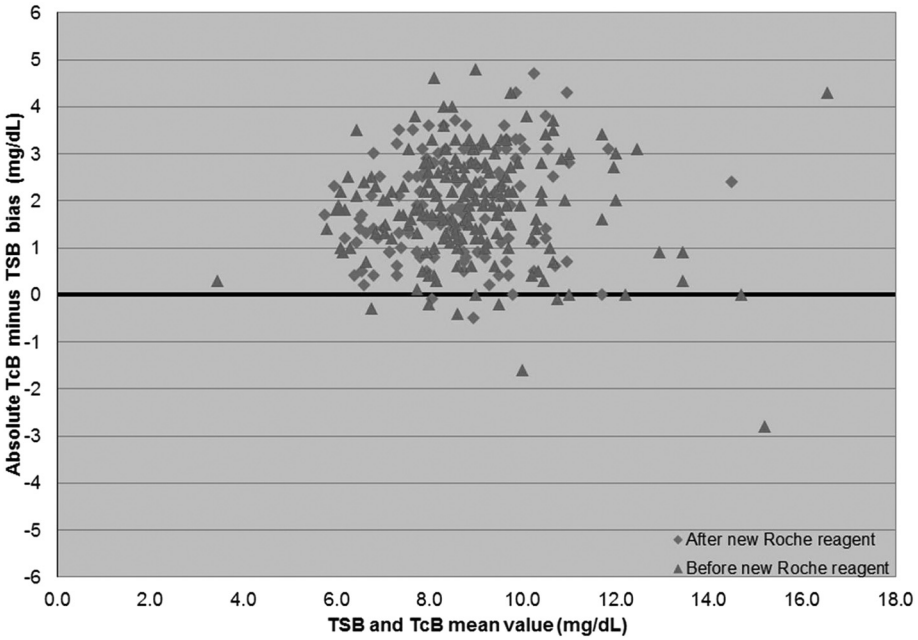

Fig. 2. Bland Altman plot of bias between transcutaneous (TcB) and laboratory total serum bilirubin (TSB) values (difference between TcB and TSB vs. mean of TcB and TSB values), in the six months before and after implementation of the Roche bilirubin Gen.3 reagent.

analyzer (Roche Diagnostics, Indianapolis, IN). The study was performed as a quality improvement project and deemed exempt from Mayo Clinic Institutional Review Board Approval.

2.4. Statistical analysis

The distributions of TSB results, and TcB minus TSB bias were compared before and after the introduction of the Roche bilirubin Gen.3 reagent. Median and interquartile range (IQR) TSB values, and median and IQR bias (TcB minus TSB) were calculated. A Bland-Altman plot was used to display the relationship of TcB to TSB before and after the introduction of the reformulated Roche bilirubin Gen.3 assay. Statistical differences between median values of TSB and median bias (TcB minus TSB) were assessed using the Man-Whitney test using GraphPad InStat version 3 (GraphPad Software, San Diego).

3. Results

A total of 301 paired (obtained within one hour of each other) BiliChek TcB and Roche TSB results were obtained, 172 infants in the 6 months before and 129 infants in the 6 months after implementation of the Roche bilirubin Gen.3 reagent. For the 172 samples analyzed by the previous generation Roche bilirubin assay, the median (IQR) TSB was 7.8 (6.8–8.7)mg/dL (133.3 (116.3–148.8)μmol/L); and for the 129 samples analyzed by Roche bilirubin Gen.3 assay, the median (IQR) TSB bilirubin was 7.6 (6.7–8.4)mg/dL (130 (114.6–143.6)μmol/L). While the median TSB value did not differ between periods (p = .1373), visually there appeared to be shift in the distribution of TSB values with fewer high (TSB 8–12 mg/dL) (136.8–205.2 μmol/L) results observed among infants studied in the six months after implementation of the Roche bilirubin Gen.3 reagent (Fig. 1a). This was accompanied by a similar shift towards fewer high (TcB 10–14 mg/dL) (171–239.4 μmol/L) transcutaneous bilirubin values observed among infants studied during the same period (Fig. 1b). The end result was no change in the median bias between TcB and TSB. The median (IQR) bias between TcB and TSB was 2.9 (2.2–3.7) mg/dL (49.6 (37.6–63.3)μmol/L) before the Roche bilirubin Gen.3 new generation reagent was implemented; and did not change at 2.9 (2.1–3.9) mg/dL (49.6 (35.9–66.7)μmol/L) after the new reagent was implemented (p = .8242). A Bland Altman plot demonstrates that over a wide range of TSB values (3–17 mg/dL) (51.3–290.7 μmol/L), there was no change in the distribution of bias observed with introduction of the Roche bilirubin Gen.3 new generation reagent (Fig. 2).

4. Discussion

One large (n = 8319) multi-center study demonstrated that transcutaneous bilirubin measurement provided reasonable estimates of serum bilirubin (mean bias 0.84 ± 1.78 mg/dL, correlation between paired measurements 0.78), but also found that TcB underestimated TSB by ≥3 mg/dL in approximately 2% of infants, resulting in some screening failures (failure to identify infant at risk for severe hyperbilirubinemia). The same study also showed that 10% of TcB measurements overestimated serum bilirubin (by up to 3 mg/dL), potentially resulting in unnecessary blood draws for TSB testing [4]. This study used two different transcutaneous devices and multiple laboratory serum bilirubin methods, confounding interpretation of pooled data on the relationship between TcB and TSB [4]. Single center studies of BiliChek TcB have reached variable conclusions; with some studies finding systematic underestimation of TSB [5,6] while others found systematic overestimation of TSB [7,14]. Two single center studies found that BiliChek overestimated TSB at lower bilirubin concentrations, but underestimated TSB at higher bilirubin concentrations [13]. Thus there remain concerns about the safety of TcB screening for subsequent severe hyperbiluribinemia, particularly when serum bilirubin concentrations are higher.

One reason for differing conclusions on the relationship between TcB and TSB is poor standardization of commercial serum bilirubin assays [9]. In the past many commercial serum bilirubin assays overestimated serum bilirubin at higher TSB concentrations [7,9,10,12]. This was due to a combination of factors including inappropriate calibration materials, and lack of an available reference plasma to ensure traceability in calibration schemes (9, 14). Development of a new reference measurement procedure, not requiring reference plasma for calibration [14], allowed one manufacturer to recalibrate their assay to improve accuracy of measurement at higher concentrations. The recalibration of the previous generation Roche bilirubin reagent lowered bilirubin values at higher concentrations and increased the systematic

bias between BiliChek TcB and TSB [10]. Other manufacturers have also recalibrated bilirubin assays over the last few years. In the case of the Vitros bilirubin assay, recalibration of the assay lowered TSB values to the extent that phototherapy usage decreased in one large health system after introduction of the recalibrated reagent [11].

Recently Roche diagnostics released a reformulated bilirubin Gen.3 reagent. One study, using a regression equation provided by Roche diagnostics to predict bilirubin Gen.3 results from bilirubin results obtained from infants using the older reagent, predicted that use of Gen.3 reagent would systematically decrease serum bilirubin results. This study predicted that this could reduce the number of infants meeting phototherapy thresholds by up to 7% [12]. In our practice, we perform universal BiliChek TcB screening of infants using a protocol based upon the observed relationship between BiliChek TcB and total serum bilirubin. We previously demonstrated that our protocol not only safely and effectively detected infants at risk for subsequent severe hyperbilirubinemia [15], but also led to decreased phototherapy usage without increasing the number of serum bilirubin measurements performed before universal TcB screening was implemented [16].

Because one study predicted that reformulation of the Roche bilirubin would systematically decrease neonatal bilirubin values, the reagent change had potential to affect the safety and efficacy of our TcB screening protocol. In this study, we assessed the relationship between BiliChek TcB and TSB in the six months before and after implementation of the Roche Gen.3 bilirubin reagent. Reformulation of the Roche bilirubin reagent (implementation of the Gen.3 assay in our lab) did not affect the relationship between BiliChek transcutaneous and total serum bilirubin; therefore no changes were needed to the neonatal TcB screening protocol as a result of the modified bilirubin reagent.

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