BMJ Paediatrics Open

Transcutaneous Bilirubinometry during and after Phototherapy in Preterm Infants, Prospective Observational Study

Journal:	*BMJ Paediatrics Open*
Manuscript ID	bmjpo-2020-000681
Article Type:	Original research
Date Submitted by the Author:	08-Mar-2020
Complete List of Authors:	Raba, Ali ; Coombe Women and Infants University Hospital O'Sullivan, Anne; Coombe Women and Infants University Hospital, Miletin, Jan; Coombe Women and Infants University Hospital, Paediatric and Newborn Medicine
Keywords:	Jaundice, Neonatology, Monitoring

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Transcutaneous Bilirubinometry during and after Phototherapy in Preterm Infants, Prospective Observational Study

Ali Ahmed Raba[1,2], Anne O'Sullivan[2], Jan Miletin[1,2,3,4]

[1] UCD School of Medicine and Medical Sciences, Dublin, Ireland

[2] Coombe Women and Infants University Hospital, Dublin, Ireland

[3] Institute for the Care of Mother and Child, Prague, Czech Republic

[4] 3rd School of Medicine, Charles University, Prague, Czech Republic

Short running title: Transcutaneous Bilirubinometry in Preterm Infants

Corresponding author: Prof Jan Miletin, MD, FRCPI, FJFICMI

Address: Coombe Women and Infants University Hospital, Cork Street, Dublin 8, Dublin, Ireland

Email: miletinj@yahoo.com

Telephone: 00353 14085654

Fax: 00353 14085209

Abstract

Objective: To examine the accuracy of Transcutaneous Bilirubinometry (TCB) measurements during and after phototherapy (PTH) in preterm infants

Design: Prospective observational cohort study

Setting: Level III neonatal centre.

Patients: Preterm infants (23^{+0} to 36^{+6} weeks of gestation) born between June 2017 and May 2018 requiring phototherapy

Interventions: TCB was measured from an exposed area of skin, the sternum (TCBU) and covered area of skin under the nappy (TCBC) within an hour of obtaining Total Serum Bilirubin (TSB) samples.

Main outcome measures: Correlation and agreement between TCB (TCBU and TCBC) and TSB during and after phototherapy.

Result: 196 preterm infants were enrolled. There was a significant correlation between TSB and TCB during PTH (r =0.71, 95% CI 0.65 to 0.76 in covered, r= 0.75, 95% CI 0.70 to 0.79 in uncovered areas) and after PTH (r =0.87, 95% CI 0.82 to 0.90). TCB underestimated TSB level during PTH with a mean TCBC-TSB difference of -25 ±43.4, 95% agreement limits of 59 to -109, and a mean TCBU-TSB difference of -47.6 ±46.3, 95% agreement limits of 43 to -138. The agreement between TCB and TSB after cessation of PTH improved, TCB underestimating TSB by a mean TCB-TSB difference of -10.3 ±31.4 (95% agreement limits of 51.2 to -71.8).

Conclusion: TCB measurements showed a significant correlation with TSB levels in preterm infants during and after phototherapy. TCB was universally underestimating TSB (most significantly on phototherapy in uncovered areas).

Keywords: Hyperbilirubinemia, Jaundice, Preterm infants, Transcutaneous bilirubinometry

Abbreviations: B-A, Bland-Altman; CWIUH, Coombe Women and Infants University hospital; DCT, direct Coombs test; EOS, early onset sepsis; GIT, gastrointestinal tract; IQR, interquartile range; mVSD, muscular ventricular septal defect; NICE, National Institute for Health and Care Excellence; PDA, patent ductus arteriosus; PFO, persistent foramen ovale; PTH, phototherapy; RDS, respiratory distress syndrome; SD, standard deviation; TCB, transcutaneous bilirubinometry; TCBC, transcutaneous bilirubinometry reading from covered skin; TCBU, transcutaneous bilirubinometry reading from uncovered skin; TSB, total serum bilirubin

Introduction

Neonatal hyperbilirubinemia is a very common condition with approximately 50% of term and 80% of preterm infants developing jaundice in the first week of life (1). Hyperbilirubinemia in most cases is benign and self-limiting condition, however, occasionally, severe hyperbilirubinemia can occur and may be associated with irreversible brain damage, especially in preterm infants (2, 3).

Phototherapy (PTH) is considered to be safe and effective treatment for neonatal unconjugated hyperbilirubinemia. The indication to commence treatment is based on the level of serum bilirubin, the age of the baby in hours, and gestational age (4). While appropriate PTH based on the above criteria is safe, aggressive PTH should be ideally avoided as it could reduce the antioxidant effects of moderate bilirubin levels leading to oxidative injury at cell membranes (5, 6).

Measurement of total serum bilirubin (TSB) remains the gold standard for monitoring bilirubin levels during and after PTH in term and preterm infants. However, obtaining heel stick or venous blood samples is painful, time-consuming, and increases the risk of local and systemic infection especially in preterm infants (8). Transcutaneous bilirubinometry (TCB) device works by directing light into the skin of the infant and measuring and analysing the intensity of the returned wavelengths to estimate a TSB (9). TCB has been recommended as a non-invasive, painless and time-saving test for bilirubin estimation in term and late preterm infants prior to the commencement of PTH (10-12).

The use of TCB for infants undergoing PTH is still controversial, some studies reported that PTH has an effect on the correlation between TCB and TSB. While others suggest that TCB readings from covered skin area could be safely used to guide PTH treatment (13, 14). We designed our study to examine the accuracy of TCB to estimate the TSB level in preterm infants undergoing PTH and its reliability after PTH.

Methods

A single centre prospective observational cohort study performed in the neonatal department of the Coombe Women and Infants University hospital (CWIUH), Dublin, Ireland (level III neonatal centre). All preterm infants (23+0 - 36+6 weeks of gestation) born between June 2017 and May 2018 in CWIUH, who developed significant jaundice requiring PTH, were elligible for enrollment to this study. During the study period, the protocols for screening, diagnosis and management of infants with jaundice were not changed. Infants with clinical or radiological evidence of major congenital anomalies (including those with gastrointestinal tract (GIT) deformities and congenital heart diseases apart from patent ductus arteriosus (PDA), persistent foramen ovale (PFO) and small (≤5 mm) muscular ventricular septal defect (mVSD)) were excluded from this study.

PTH was commenced based on TSB levels according to the hospital guidelines taking into account the infant's age in hours and gestation in weeks. Standard PTH units (Photo Therapy 4000, Draeger Medical, Germany) were used. Infants receiving PTH were completely exposed, except for their eyes (covered with a phototherapy goggles for protection) and the nappy area (covered with a disposable nappy). PTH was discontinued when the TSB fell below the relevant treatment threshold.

TCB was measured from uncovered/exposed (TCBU) and covered (TCBC) areas within an hour of obtaining TSB samples. The device was placed over an uncovered area (sternum) and pressed gently against the skin three times to obtain a reading. The process was repeated over the covered area, upper outer quadrant of the buttock (covered by the nappy). After cessation of PTH, TCB's were measured from the sternum. The measurements were obtained by experienced nurses trained and competent in the use of the Dräger Jaundice Meter (JM-105 or JM-103, Draeger Medical, Germany). The TCB devices were calibrated regularly according to the manufacturer's instructions and hospital guidelines. Blood samples for TSB were obtained

either by heel prick or venepuncture. The attending neonatologist directed the frequency of TSB measurements. TSB levels were measured in one clinical laboratory using direct spectrophotometry. Co-morbidities were recorded during the study period. Confirmed early onset sepsis (EOS) was defined according to National Institute for Health and Care Excellence (NICE) guidelines (15), as a positive blood culture bacterial infection within the first three days of life. PDA was diagnosed on the basis of echocardiographic findings, and it was considered haemodynamically significant in our study if the measured diameter was greater than 2mm (16).

Our main outcome was to examine the correlation and agreement between TCB (TCBU and TCBC) and TSB during and after PTH. Our secondary outcomes were to investigate the influence of gestational age, birth weight, sepsis, respiratory distress syndrome (RDS), PDA and positive Direct Coombs Test (DCT) on the correlation between TSB and TCB (TCBU and TCBC) readings during and after PTH.

Our prospective cohort study was approved by the Research Ethics Committee of the CWIUH (Study No.3-2017) and informed written consent was obtained from parents. Patients or the public were not involved in the design, conduct, or reporting plans of our research.

Data were entered into Microsoft Office Excel (MS Excel, Microsoft, USA) and analysed in SPSS version 24.0. Descriptive statistics were used for all demographic variables of interest using frequency distribution and percentage for categorical variables. Mean and standard deviation (SD) were used for parametric numeric data, while non-parametric numeric data were summarised using median and interquartile range (IQR). Paired Student t-test was used to compare means. Correlation between TCB (TCBU and TCBC) and TSB was calculated using Pearson's correlation coefficient during and after phototherapy. Bland-Altman (B-A) analysis was used to calculate and visualize the agreement between TSB and TCB. We used t-test to study the influence of gestational age, birth weight, sepsis, RDS, PDA and DCT positivity on

the difference between TSB and TCB during and post-phototherapy. Our results were summarized using p-values and 95% confidence intervals. P-values <0.05 were considered to be statistically significant.

Results

One hundred and ninety six jaundiced preterm infants, who received phototherapy, were enrolled to the study. The mean (±SD) gestational age and birth weight of our cohort were 30.4 weeks of gestation (±3.2) and 1605g (±638), respectively. The demographic description of our cohort is presented in the Table 1.

There were 328 simultaneous measurements (TSB and TCB) during PTH phase, and 137 pairs of readings after discontinuation of PTH. The PTH was commenced at mean (±SD) 32.5 (±20) hours of life, and the median duration of PTH exposure was 24 hours (IQR 24-32).

During PTH phase, the mean TSB ±SD (127 ±50 μmol/L) and mean TCBC ±SD (102 ±61.6) were statistically significantly different (p<0.0001) (Table 2). Similarly, the difference between the mean TSB ±SD (127 ± 50.5 μmol/L) and mean TCBU ±SD (79.3 ±70.4) was statistically significantly different during PTH (p <0.0001) (Table 2). Although, there was a significant correlation between TSB and TCB measurements during PTH (r =0.71, 95% CI 0.65 to 0.76 from covered, r= 0.75, 95% CI 0.70 to 0.79 from uncovered areas, p <0.0001), B-A plots showed significant bias and imprecisions in the TCB readings. TCB underestimated TSB level with a mean TCB-TSB difference of -25 ±43.4 from covered area (95% agreement limits of 59.2 to -109, p <0.0001) and of -47.6 ±46.3 from uncovered area (95% agreement limits of 43 to -138, p <0.0001) (Figure 1, Figure 2).

During post-phototherapy phase, TSB ±SD (152 ±51.2 μmol/L) and TCB ±SD (142 ±62.8) measurements were statistically significantly different (p<0.0001) (Table 3). After cessation of PTH, the correlation between TCB and TSB further improved (r =0.87, 95% CI 0.82-0.9, p

<0.0001) (Figure 3A). B-A plot showed also an improvement in the agreement between TCB and TSB, but TCB continued to underestimate of TSB level by -10.3 ±31.4 (95% agreement limits of 51.2 to -71.8, p <0.0001) (Figure 3B).

We determined that gestational age, birth weight, sepsis, RDS, PDA and DCT positivity had no influence on the mean difference between the TSB and TCB (TCBU and TCBC) readings during the PTH (Table 4). We found also that the difference between the TSB and TCB measurements after the phototherapy were not affected by gestational age, birth weight, PDA, DCT positivity. However, infants with a diagnosis of RDS had a statistically significant reduction in the difference between TCB and TSB, in comparison to those infants without RDS, after cessation the PTH (Table 5).

Discussion

Our study revealed a significant correlation between the TCB and TSB during and after phototherapy in preterm infants. However, our findings showed a significant wide disagreement between TCB and TSB measurements during PTH phase. Although, TCB readings from the covered skin had better agreement than those from the exposed skin, TCB measurements were associated with a large bias during PTH. We noted that the TCB device could underestimate serum bilirubin level by up to 109 and 138 μmol/L, from covered and uncovered area, respectively, during phototherapy.

In general, the significant correlation between TCB and TSB during PTH is consistent with the findings in some previous studies in preterm infants. Cucuy (17) et al conducted a study of 86 preterm infants with a mean gestational age of 32 weeks and a mean birth weight of 1637g. Although they found a good correlation between TSB and TCB during PTH (r =0.8), it was not clear if their TCB readings were measured from the exposed or covered skin during

phototherapy. In addition to this, they did not provide information about the level of agreement between the TCB and TSB measurements.

There are only a few studies that examined 95% agreement limits between the TCB and TSB in preterm infants during phototherapy. Nagar et al (18) performed a smaller study on 90 preterm infants with a mean gestational age of 32.4 weeks and a mean birth weight of 1847g. They found that TCB cannot be recommended for the bilirubin measurement during PTH in preterm infants due to the high risk of underestimation of TSB by up to 132 and 157 μmol/L from covered and uncovered skin, respectively. Although, their sample was smaller and infants older than our cohort, their results were quite comparable to our findings.

Zecca et al (19) conducted a study on 364 preterm and term infants requiring phototherapy. The mean gestational age and the mean birth weight of their sample were of 34.6 weeks of gestation and 2371g, respectively, which were more than the mean gestational age and the mean birth weight of our cohort. They reported a smaller bias between TCB readings from covered skin and TSB compared to our results. Their results demonstrated that TCB from exposed skin underestimated TSB by 54 ±51μmol/L, while TCB from covered skin underestimated TSB by 3.1 ±53μmol/L. However, B-A plots showed a wide TCB-TSB disagreement with a risk of underestimation of TSB by up to 106 μmol/L from covered skin and 153 μmol/L from exposed skin.

We have shown that the TCB readings from both covered and uncovered area were lower than TSB levels. Immaturity of the skin and the absence of subcutaneous fat in preterm infants which leads to rapid clearance of bilirubin levels from the skin following initiation of PTH may be an explanation (10, 20). This however would contravene the findings of DeLuca et al who reported that TCB reading from covered skin and TSB correlated strongly ($r = 0.84$, $p < 0.001$) in their study of 60 extremely preterm infants undergoing phototherapy and, unlike our finding, TCB overestimated TSB with a mean TCB-TSB difference of 47.8 ±41μmol/L (21).

During the post-phototherapy phase, our data revealed a better correlation between TCB and TSB reading (r =0.87) as compared to that during PTH phase (r=0.7). Moreover, there was a significant improvement in the agreement between TCB and TSB after cessation of the PTH. TCB underestimated of TSB only by -10.3 ±31.4 with 95% agreement limits of 51.2 to -71.8. The level of underestimation of TCB observed here are similar to those observed in Nagar et al study (18).

The strength of our study is that it is a large prospective observational study that enrolled not only healthy preterm infants, but also sick and ventilated premature infants. Another notable strength is that the number of paired TCB-TSB measurements was large in comparison to previous studies. Additionally, the possible risk factors that could affect the correlation between TCB-TSB readings were examined. Finally, we provided recent data for the agreement between TCB and TSB which is more helpful in clinical practice than correlation coefficient. Thus, our study added significant findings to the limited literature on the use of the TCB device in preterm infants during and after phototherapy.

The present study has some limitations. Firstly, we did not examine the effect of the duration and recommencement of PTH on the TSB-TCB correlation. Also, TCB was only measured from exposed skin (sternum) after PTH was discontinued, and the TCB measurements from covered area (nappy area) could have different correlation and agreement with TSB.

In conclusion, TCB measurements correlate strongly with TSB levels during and after phototherapy. However, as result of the wide disagreement between TCB and TSB measurements during PTH phase, TCB device cannot be recommended for monitoring bilirubin level during phototherapy. However, TCB should be used to estimate the bilirubin level post-phototherapy.

Acknowledgement: The authors would like to thank all babies and families who

participated in this study along with medical and midwifery staff at the CWIUH.

Contributors: Dr. Raba designed the study, contributed substantially to the data collection and analysis and drafted the initial manuscript. Ms. O'Sullivan contributed substantially to the data collection and analysis and reviewed and revised the manuscript. Prof Miletin conceptualised and designed the study, supervised the conduct of the study and coordinated the data analysis. He reviewed and revised the manuscript critically for important intellectual content. All the authors approved the final manuscript as submitted. They agree to be accountable for all aspects of the work.

Compliance with Ethical Statements:

Conflict of Interest: The authors declare that they have no conflict of interest

Ethical approval: The Research Ethics Committee of the Coombe Women and Infants University Hospital approved the study (Study No.3-2017)

Informed consent: Informed written consent was obtained from all participants

Funding: Dr Raba work was supported by the grant from Department of Cultural Affairs, Libya (Ref: HG6-490-45693) (managed by University College Dublin)

What is already known on this topic?

- Transcutaneous Bilirubinometry (TCB) is a non-invasive test used as a validated screening tool for hyperbilirubinemia in term infants
- The accuracy of TCB measurement during phototherapy is still controversial in term and preterm infants
- Few studies reported that TCB measurement from covered skin during phototherapy could provide more accurate approximations of total serum bilirubin level in term infants

What this study adds?

- Transcutaneous Bilirubinometry (TCB) measurements correlates significantly with Total Serum Bilirubin (TSB) levels in preterm infants during and after phototherapy
- TCB underestimates TSB with a significant wide disagreement between TCB and TSB measurements during phototherapy in preterm infants making it unreliable despite significant correlation
- The TCB should be used for TSB estimation post-phototherapy due to significant correlation and improved and clinically acceptable agreement between TCB and TSB

References

1. Woodgate P, Jardine LA. Neonatal jaundice. BMJ Clin Evid. 2011;15.

2. Watchko JF, Oski FA. Kernicterus in preterm newborns: past, present, and future. Pediatrics. 1992;90(5):707-15.

3. Cashore WJ. The neurotoxicity of bilirubin. Clin Perinatol. 1990;17(2):437-47.

4. Mitra S, Rennie J. Neonatal jaundice: aetiology, diagnosis and treatment. Br J Hosp Med (Lond). 2017;78(12):699-704.

5. Tozzi E, Tozzi-Ciancarelli MG, Di Giulio A, D'Alfonso A, Farello G, Spennati GF, et al. In vitro and in vivo effects of erythrocyte phototherapy on newborns. Biol Neonate. 1989;56(4):204-9.

6. Morris BH, Oh W, Tyson JE, Stevenson DK, Phelps DL, O'Shea TM, et al. Aggressive vs. conservative phototherapy for infants with extremely low birth weight. N Engl J Med. 2008;359(18):1885-96.

7. Tyson JE, Pedroza C, Langer J, Green C, Morris B, Stevenson D, et al. Does aggressive phototherapy increase mortality while decreasing profound impairment among the smallest and sickest newborns? J Perinatol. 2012;32(9):677-84.

8. Badiee Z, Mohammadizadeh M, Shamee M. Diagnostic usefulness of transcutaneous bilirubinometry in very preterm newborns. Int J Prev Med. 2012;3(4):262-5.

9. Ahmed M, Mostafa S, Fisher G, Reynolds TM. Comparison between transcutaneous bilirubinometry and total serum bilirubin measurements in preterm infants <35 weeks gestation. Ann Clin Biochem. 2010;47(Pt 1):72-7.

10. Ozkan H, Oren H, Duman N, Duman M. Dermal bilirubin kinetics during phototherapy in term neonates. Acta Paediatr. 2003;92(5):577-81.

11. Beck M, Kau N, Schlebusch H. Transcutaneous bilirubin measurement in newborn infants: evaluation of a new spectrophotometric method. Arch Dis Child Fetal Neonatal Ed. 2003;88(4):F350-1.

12. Nagar G, Vandermeer B, Campbell S, Kumar M. Effect of Phototherapy on the Reliability of Transcutaneous Bilirubin Devices in Term and Near-Term Infants: A Systematic Review and Meta-Analysis. Neonatology. 2016;109(3):203-12.

13. Tan KL, Dong F. Transcutaneous bilirubinometry during and after phototherapy. Acta Paediatr. 2003;92(3):327-31.

14. Rylance S, Yan J, Molyneux E. Can transcutaneous bilirubinometry safely guide phototherapy treatment of neonatal jaundice in Malawi? Paediatr Int Child Health. 2014;34(2):101-7.

15. Caffrey Osvald E PP. NICE clinical guideline: antibiotics for the prevention and treatment of early-onset neonatal infection. 2014.

16. Evans N. Diagnosis of patent ductus arteriosus in the preterm newborn. Arch Dis Child. 1993;68(1 Spec No):58-61.

17. Cucuy M, Juster-Reicher A, Flidel O, Shinwell E. Correlation between transcutaneous and serum bilirubin in preterm infants before, during, and after phototherapy. J Matern Fetal Neonatal Med. 2018;31(10):1323-6.

18. Nagar G, Kumar M. Effect of phototherapy on the diagnostic accuracy of transcutaneous bilirubin in preterm infants. Journal of Clinical Neonatology. 2017;6(3):148-53.

19. Zecca E, Barone G, De Luca D, Marra R, Tiberi E, Romagnoli C. Skin bilirubin measurement during phototherapy in preterm and term newborn infants. Early Hum Dev. 2009;85(8):537-40.

20. Kanti V, Bonzel A, Stroux A, Proquitte H, Buhrer C, Blume-Peytavi U, et al. Postnatal maturation of skin barrier function in premature infants. Skin Pharmacol Physiol. 2014;27(5):234-41.

21. De Luca D, Dell'Orto V. Patched Skin Bilirubin Assay to Monitor Neonates Born Extremely Preterm Undergoing Phototherapy. J Pediatr. 2017;188:122-7.

Table 1
Baseline population Characteristics

Variable	Number
Male Sex n (%)	105 (53.6%)
Birth Weight (grams) Mean ±SD	1605 ±638
Gestational age (weeks) Mean ±SD	30.4 ±3.2
Mode of Delivery n (%) NVD Instrumental Delivery Elective LSCS Emergency LSCS	 56 (28.6%) 3 (1.5%) 59 (30.1%) 78 (39.8%)
Apgar score at 1st minute Median (IQR)	7 (5-9)
Apgar score at 5th minute Median (IQR)	9 (8-10)
Blood group infants (when done) n (%) A B AB O Rhesus +	175 (89%) 48 (27.4%) 18 (10.2%) 2 (1%) 108 (61.7%) 150/175 (85.7%)
Maternal group *n (%)* A B AB O Rhesus +	 60 (30.6%) 24 (12.2%) 5 (2.6%) 104 (53.1%) 176 (89.9%)
Positive DCT n (%)	7 (3.6%)
Maternal age (years) Mean ± SD	32 ±6.2
Proven EOS n (%)	5 (2%)
Antibiotics treatment n (%)	145 (74%)

NVD, normal vaginal delivery; DCT, direct Coombs test; LSCS, lower segment Caesarean section; EOS, early onset sepsis

Table 2
Paired samples of TSB and TCB from covered and uncovered skin during phototherapy

		µmol/l, mean (SD)	N	P-value
During PTH	TCBC	102 (61.6)	299	<0.0001
	TSB	127 (50.5)	299	
During PTH	TCBU	79.3 (70.4)	309	<0.0001
	TSB	126.9 (50.9)	309	

TSB, total serum bilirubin; TCBC, Transcutaneous bilirubinometry readings from covered skin; TCBU, Transcutaneous bilirubinometry readings from exposed skin; PTH, phototherapy

Table 3
Paired samples of TSB and TCB from uncovered skin post-phototherapy

		µmol/l, mean (SD)	N	P-value
After PTH	TCB	142 (62.8)	137	<0.0001
	TSB	153 (51.2)	137	

TSB, total serum bilirubin; TCB, Transcutaneous bilirubinometry; PTH, phototherapy

Table 4
The influence of EOS, PDA, RDS and positive DCT on the difference between TSB and TCB during phototherapy

	Difference between mean TSB and mean TCBC ±SD (µmol/l)	P-value	Difference between mean TSB and mean TCBU ±SD (µmol/l)	P-value
EOS				
Yes	21.9 ±39.5	0.258	49.2 ±42.4	0.064
No	18 ±31.8		58.6 ± 30.7	
PDA				
Yes	32.6 ±34.4	0.642	67.1 ±34.1	0.28
No	19.16 ±37.8		49 ± 40.2	
RDS				
Yes	22.3 ±37.9	0.627	48.2 ±38.1	0.246
No	16.5 ±36.1		65.2 ±43.4	
DCT				
Positive	3.5 ±20	0.181	27 ±43	0.645
Negative	21 ±38.8		52.6 ±40.3	

EOS, early onset sepsis; DCT, direct coombs test; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; TSB, total serum bilirubin; TCB, Transcutaneous bilirubinometry; TCBC, Transcutaneous bilirubinometry from covered area when on phototherapy; TCBU, Transcutaneous bilirubinometry from exposed area when on phototherapy

Table 5
The influence of EOS, PDA, RDS and positive DCT on the difference between TSB and TCB post-phototherapy

	Difference between mean TSB and mean TCB ±SD (µmol/l)	P-value
EOS		
Yes	7.9 ±32.2	0.637
No	16.3 ±31.2	
PDA		
Yes	13.8 ±34.3	0.623
No	9.4 ±31.6	
RDS		
Yes	9 ±33.8	0.024
No	15.9 ±22.4	
DCT		
positive	5.3 ±43.7	0.349
negative	10.9 ±31.5	

EOS, early onset sepsis; DCT, direct coombs test; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; TSB, total serum bilirubin; TCB, Transcutaneous bilirubinometry

Legends

Figure 1A

Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) from covered skin during phototherapy

Figure 1B

B–A plot showing the 95% limits of agreement between TCB from covered skin and TSB during phototherapy

Figure 2A

Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) from uncovered skin during phototherapy

Figure 2B

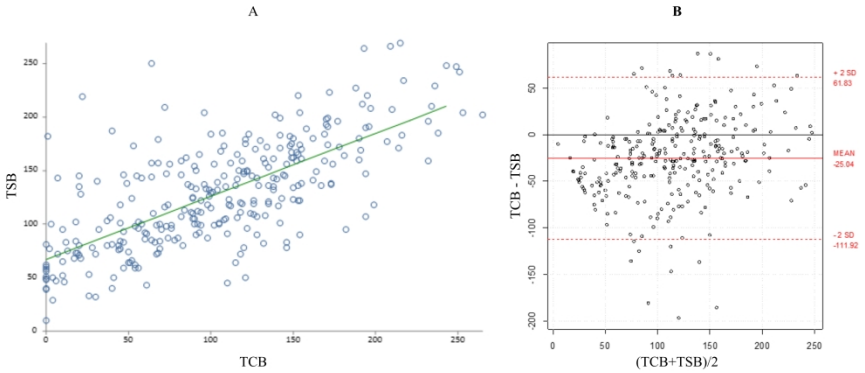
B–A plot showing the 95% limits of agreement between TCB from uncovered skin and TSB during phototherapy

Figure 3A

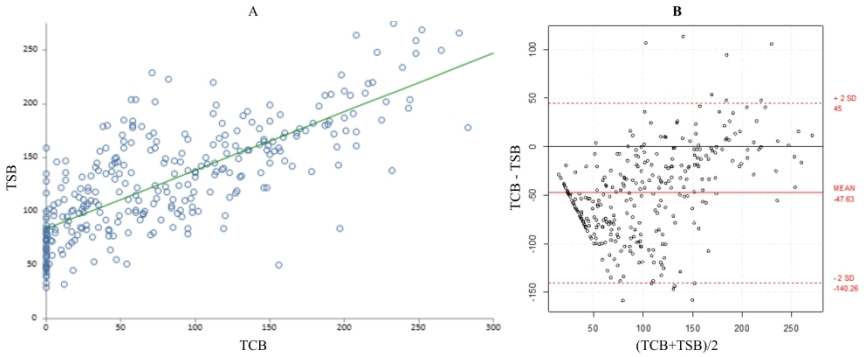
Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) after phototherapy

Figure 3B

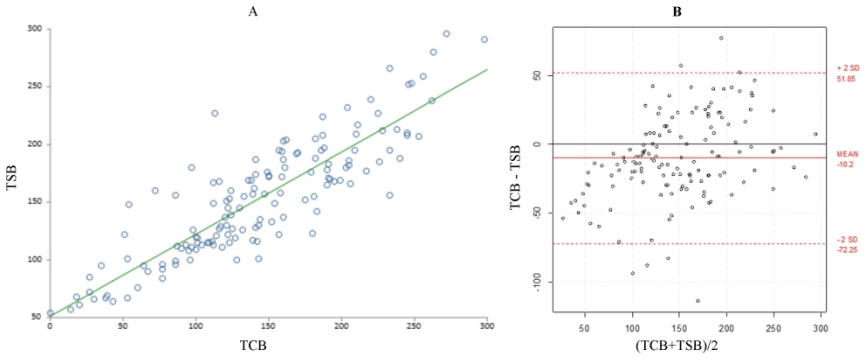
B-A plot showing the 95% limits of agreement between TCB and TSB after phototherapy



297x210mm (600 x 600 DPI)



297x210mm (600 x 600 DPI)



297x209mm (300 x 300 DPI)

BMJ Paediatrics Open

Transcutaneous Bilirubinometry during and after Phototherapy in Preterm Infants, Prospective Observational Study

Journal:	*BMJ Paediatrics Open*
Manuscript ID	bmjpo-2020-000681.R1
Article Type:	Original research
Date Submitted by the Author:	01-May-2020
Complete List of Authors:	Raba, Ali ; Coombe Women and Infants University Hospital O'Sullivan, Anne; Coombe Women and Infants University Hospital, Miletin, Jan; Coombe Women and Infants University Hospital, Paediatric and Newborn Medicine
Keywords:	Jaundice, Neonatology, Monitoring

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Transcutaneous Bilirubinometry during and after Phototherapy in Preterm Infants, Prospective Observational Study

Ali Ahmed Raba[1,2], Anne O'Sullivan[2], Jan Miletin[1,2,3,4]

[1] UCD School of Medicine and Medical Sciences, Dublin, Ireland

[2] Coombe Women and Infants University Hospital, Dublin, Ireland

[3] Institute for the Care of Mother and Child, Prague, Czech Republic

[4] 3rd School of Medicine, Charles University, Prague, Czech Republic

Short running title: Transcutaneous Bilirubinometry in Preterm Infants

Corresponding author: Prof Jan Miletin, MD, FRCPI, FJFICMI

Address: Coombe Women and Infants University Hospital, Cork Street, Dublin 8, Dublin, Ireland

Email: miletinj@yahoo.com

Telephone: 00353 14085654

Fax: 00353 14085209

Abstract

Objective: To examine the accuracy of Transcutaneous Bilirubinometry (TCB) measurements during and after phototherapy (PT) in preterm infants

Design: Prospective observational cohort study

Setting: Level III neonatal centre.

Patients: Preterm infants (23^{+0} to 36^{+6} weeks of gestation) born between June 2017 and May 2018 requiring PT

Interventions: TCB was measured from an exposed area of skin (the sternum, (TCBU)) and covered area of skin under the nappy (the bony part of the upper outer quadrant of the buttock (TCBC)) within an hour of obtaining Total Serum Bilirubin (TSB)

Main outcome measures: Correlation and agreement between TCB (TCBU and TCBC) and TSB during and after phototherapy

Result: We have enrolled 196 preterm infants. There was a significant correlation between TSB and TCB during PT (r =0.72, 95% CI 0.66 to 0.77 in covered, r =0.75, 95% CI 0.70 to 0.80 in uncovered areas) and after PT (r =0.87, 95% CI 0.83 to 0.91). TCB underestimated TSB level during PT with a mean TCBC-TSB difference of -25 ±43, 95% agreement limits of 62 to -112, and a mean TCBU-TSB difference of -48 ±46, 95% agreement limits of 45 to -140. The agreement between TCB and TSB after cessation of PT improved, TCB underestimating TSB by a mean TCB-TSB difference of -10 ±31 (95% agreement limits of 52 to -72).

Conclusion: TCB measurements correlated strongly with TSB levels during and after phototherapy. However, there was a wide and clinically relevant disagreement between TCB and TSB measurements during PT phase, improving significantly post PT.

Keywords: Hyperbilirubinemia, Jaundice, Preterm infants, Transcutaneous bilirubinometry

Abbreviations: B-A, Bland-Altman; CWIUH, Coombe Women and Infants University hospital; DCT, direct Coombs test; EOS, early onset sepsis; GIT, gastrointestinal tract; IQR, interquartile range; mVSD, muscular ventricular septal defect; NICE, National Institute for Health and Care Excellence; PDA, patent ductus arteriosus; PFO, persistent foramen ovale; PT, phototherapy; RDS, respiratory distress syndrome; SD, standard deviation; TCB, transcutaneous bilirubinometry; TCBC, transcutaneous bilirubinometry reading from covered skin; TCBU, transcutaneous bilirubinometry reading from uncovered skin; TSB, total serum bilirubin

Introduction

Neonatal hyperbilirubinemia is a very common condition with approximately 50% of term and 80% of preterm infants developing jaundice in the first week of life (1). Hyperbilirubinemia in most cases is a benign and self-limiting condition, however severe hyperbilirubinemia can occasionally occur and may be associated with irreversible brain damage, especially in preterm infants (2, 3).

Phototherapy (PT) is considered to be a safe and effective treatment for neonatal unconjugated hyperbilirubinemia. The indication to commence treatment is based on the level of serum bilirubin, the age of the baby in hours, and gestational age (4). Evidence is conflicting regarding the best therapeutic approach to hyperbilirubinemia, especially in extremely low birth weight (ELBW) infants. A randomised clinical trial (RCT) performed by the Neonatal Research Network found no significant difference in the rate of death or neurodevelopmental impairment at 18 to 22 months corrected age in ELBW infants who received aggressive PT versus those who received conservative PT. However, aggressive PT was associated with a reduction in the rate of neurodevelopmental impairment alone.(5) However, the post hoc analysis showed that in the smallest and sickest subgroup (mechanically ventilated infants with birth weight less than 750g), aggressive PT may increase mortality while reducing neurodevelopmental impairment.(6)

Measurement of total serum bilirubin (TSB) remains the gold standard for monitoring bilirubin levels during and after PT in term and preterm infants. However, obtaining heel stick or venous blood samples is painful, time-consuming, and increases the risk of local and systemic infection especially in preterm infants (7). A transcutaneous bilirubinometry (TCB) device works by directing light into the skin of the infant and measuring and analysing the intensity of the returned wavelengths to estimate a TSB (8). TCB has been recommended as a non-invasive, painless and time-saving test for bilirubin estimation in term and late preterm infants prior to

the commencement of PT (9-11). However, TCB measurements are not recommended in the first 24 hours of life or in preterm infants below 35 weeks of gestation according to the National Institute for Clinical Excellence (NICE) guidelines. (12) (https://www.nice.org.uk/guidance/cg98).

Despite the NICE guidelines, it seems that TCB has strong correlation and acceptable agreement in preterm infants before phototherapy.(13, 14) The use of TCB for infants during and after PT is still controversial, as some studies reported that PT blanches the skin thereby affecting the correlation between TCB and TSB during and after phototherapy(15) while others suggest that TCB readings from covered skin area could be safely used to guide treatment during and post PT.(16-18). We designed our study to examine the accuracy of TCB to estimate the TSB level in preterm infants undergoing PT and its reliability after PT.

Methods

A single centre prospective observational cohort study performed in the neonatal department of the Coombe Women and Infants University hospital (CWIUH), Dublin, Ireland (level III neonatal centre). All preterm infants (23+0 - 36+6 weeks of gestation) born between June 2017 and May 2018 in CWIUH who developed significant jaundice requiring PT were eligible for enrollment to this study. During the study period, the protocols for screening, diagnosis and management of infants with jaundice were not changed. Infants with clinical or radiological evidence of major congenital anomalies (including those with gastrointestinal tract (GIT) deformities and congenital heart diseases apart from patent ductus arteriosus (PDA), persistent foramen ovale (PFO) and small (≤5 mm) muscular ventricular septal defect (mVSD)) were excluded from this study.

PT was commenced based on TSB levels according to the hospital guidelines, taking into account the infant's age in hours and gestation in weeks. The NICE treatment charts were used for preterm infants below 32 weeks of gestation (Appendix 1). In infants ≥32 weeks of gestation, a chart adapted from the National Health Service (NHS), Glasgow, UK was used (Appendix 2). Standard PT units (Photo Therapy 4000, Draeger Medical, Germany) were used (overhead PT microlight units deliver ≥10 µW/cm2/nm and Halogen spotlights which can deliver 20-25uW/cm2/nm). Infants receiving PT were completely exposed, except for their eyes (covered with phototherapy goggles for protection) and the nappy area (covered with a disposable nappy). PT was discontinued when the TSB fell below the relevant treatment threshold.

TCB was measured from uncovered/exposed (TCBU) and covered (TCBC) areas within an hour of obtaining TSB samples. The device was placed over an uncovered area (sternum) and pressed gently against the skin three times to obtain one reading (the average of the three measured values). The process was repeated over the covered area, the bony part of the upper

outer quadrant of the buttock (covered by the nappy). After cessation of PT, TCB's were measured from the sternum. The measurements were obtained by experienced nurses trained and competent in the use of the Dräger Jaundice Meter (JM-105 or JM-103, Draeger Medical, Germany). The TCB devices were calibrated regularly according to the manufacturer's instructions and hospital guidelines. Blood samples for TSB were obtained either by heel prick or venepuncture. The attending neonatologist directed the frequency of TSB measurements. TSB levels were measured in one clinical laboratory using direct spectrophotometry (Abbot Architect C8000, Abbott, USA). Co-morbidities were recorded during the study period. Confirmed early onset sepsis (EOS) was defined according to National Institute for Health and Care Excellence (NICE) guidelines (19) as a positive blood culture bacterial infection within the first three days of life.

Our primary outcome was the correlation and agreement between TCB (TCBU and TCBC) and TSB during and after PT.

Our prospective cohort study was approved by the Research Ethics Committee of the CWIUH (Study No.3-2017) and informed written consent was obtained from parents. Patients or the public were not involved in the design, conduct, or reporting plans of our research.

Data was entered into Microsoft Office Excel (MS Excel, Microsoft, USA) and analysed by the StatsDirect v.3.2.10 software (StatsDirect Ltd, UK). Descriptive statistics were used for all demographic variables of interest using frequency distribution and percentage for categorical variables. Mean and standard deviation (SD) were used for normally distributed data, while non normal distribution data was summarised using median and interquartile range (IQR). Paired-samples t-test was used to compare TCB and TSB paired measurements. When the differences between pairs were not normally distributed, we used the Wilcoxon signed-rank test for two sample comparisons. For non-dependent variables we have used an unpaired t-test or Mann-Whitney U test as appropriate. Correlation between TCB (TCBU and TCBC) and

TSB was calculated using Pearson's correlation coefficient during and after phototherapy. Bland-Altman (B-A) analysis was used to calculate and visualize the agreement between TSB and TCB. The agreement limits are demonstrated as a 95% confidence interval (95% CI = mean ± 1.96 standard deviations), where the ideal agreement difference between measurements is zero. Our results were summarized using p-values and 95% confidence intervals. P-values <0.05 were considered to be statistically significant. We have used a convenience sample for the study with planned one year enrolment.

Results

One hundred and ninety six jaundiced preterm infants who received phototherapy were enrolled to the study. The mean (±SD) gestational age and birth weight of our cohort were 30.4 weeks of gestation (±3.2) and 1605g (±638), respectively. The demographic description of our cohort is presented in the Table 1.

There were 328 simultaneous measurements (TSB and TCB) during the PT phase and 142 pairs of readings after discontinuation of PT. The PT was commenced at mean (±SD) 32.5 (±20) hours of life and the median duration of PT exposure was 24 hours (IQR 24-32).

During the PT phase, the mean TSB ±SD (127 ±51 μmol/L) and mean TCBC ±SD (102 ±62) were statistically significantly different (p<0.0001) (Table 2). Similarly, the difference between the mean TSB ±SD (127 ±51 μmol/L) and mean TCBU ±SD (79 ±70) was statistically significantly different during PT (p <0.0001) (Table 2). Although there was a significant correlation between TSB and TCB measurements during PT, (r =0.72, 95% CI 0.66 to 0.77 from covered, r =0.75, 95% CI 0.70 to 0.80 from uncovered areas, p <0.0001), B-A plots showed significant bias and imprecisions in the TCB readings. TCB underestimated TSB level with a mean TCB-TSB difference of -25 ±43 from covered area (95% agreement limits of 62

to -112, p <0.0001) and of -48 ±46 from uncovered area (95% agreement limits of 45 to -140, p <0.0001) (Figure 1, Figure 2).

During the post-phototherapy phase, TSB ±SD (153 ±51 μmol/L) and TCB ±SD (143 ±63) measurements were statistically significantly different (p=0.0001) (Table 2). These measurements were taken at median time of 12 hours (IQR 8 - 24) post PT. After cessation of PT, the correlation between TCB and TSB further improved (r =0.87, 95% CI 0.83-0.91, p <0.0001) (Figure 3A). The B-A plot also showed an improvement in the agreement between TCB and TSB, but TCB continued to underestimate TSB level by -10 ±31 (95% agreement limits of 52 to -72, p =0.0001) (Figure 3B). At 12 hours after cessation of PT, the correlation between TCB and TSB was improved compared to eight hours post phototherapy with statistically significantly improving mean difference between TCB and TSB (p <0.0001) (Table 3).

Discussion

Our study revealed a significant correlation between the TCB and TSB during and after phototherapy in preterm infants. However, our findings also showed a significant wide disagreement between TCB and TSB measurements during the PT phase. Although TCB readings from the covered skin had better agreement than those from the exposed skin, TCB measurements were associated with a large bias during PT. We noted that the TCB device could underestimate serum bilirubin level by up to 112 and 140 µmol/L, from covered and uncovered area, respectively, during PT.

In general, the significant correlation between TCB and TSB during PT is consistent with the findings in some previous studies in preterm infants.(20) Cucuy (21) et al conducted a study of 86 preterm infants with a mean gestational age of 32 weeks and a mean birth weight of 1637g.

Although they found a good correlation between TSB and TCB during PT (r =0.8), it was not clear if their TCB readings were measured from the exposed or covered skin during phototherapy. In addition to this, they did not provide information about the level of agreement between the TCB and TSB measurements.

There are only a few studies that examined 95% agreement limits between the TCB and TSB in preterm infants during phototherapy. Nagar et al (22) performed a smaller study on 90 preterm infants with a mean gestational age of 32.4 weeks and a mean birth weight of 1847g. They found that TCB cannot be recommended for the bilirubin measurement during PT in preterm infants due to the high risk of underestimation of TSB by up to 132 and 157 µmol/L from covered and uncovered skin, respectively. Although their sample was smaller and infants older than our cohort, their results were quite comparable to our findings.

Similarly, Hulzebos et al demonstrated that TCB underestimated TSB in very preterm infants during phototherapy when measured on covered skin.(18) The same research group proposed different cut-off rules to improve the prediction of phototherapy thresholds when TCB measured during phototherapy on covered skin.(18)

Zecca et al (23) conducted a study on 364 preterm and term infants requiring phototherapy. The mean gestational age and the mean birth weight of their sample were of 34.6 weeks of gestation and 2371g, respectively, which were higher than the mean gestational age and the mean birth weight of our cohort. They reported a smaller bias between TCB readings from covered skin and TSB compared to our results. Their results demonstrated that TCB from exposed skin underestimated TSB by 54 ±51μmol/L, while TCB from covered skin underestimated TSB by 3.1 ±53μmol/L. However, B-A plots showed a wide TCB-TSB disagreement with a risk of underestimation of TSB by up to 106 µmol/L from covered skin and 153 µmol/L from exposed skin.

We have shown that the TCB readings from both covered and uncovered area were lower than TSB levels. Immaturity of the skin and the absence of subcutaneous fat in preterm infants which leads to rapid clearance of extravascular bilirubin levels from the skin following initiation of PT may be an explanation (9, 24). This however would contravene the findings of De Luca et al who reported that TCB reading from covered skin and TSB correlated strongly ($r = 0.84$, $p < 0.001$) in their study of 60 extremely preterm infants undergoing phototherapy and, unlike our finding, TCB overestimated TSB with a mean TCB-TSB difference of 47.8 $\pm 41 \mu$mol/L (25).

During the post-phototherapy phase, our data revealed a better correlation between TCB and TSB reading as compared to that during the PT phase. More interestingly, the mean difference of TSB-TCB pairs was much lower than reported previously, even in paired measurements done in our study as early as eight hours post phototherapy.(18) We observed improved correlation and decreasing mean difference of TSB-TCB pairs with the increased time post phototherapy, which would be different to previous observation by Cucuy et al as they reported that time after PT did not have any significant effect on the correlation between TSB and TCB.(21)

Moreover, our results showed a significant improvement in the agreement between TCB and TSB after cessation of the PT. The level of underestimation of TSB in our study are similar to those observed in Nagar et al study (22).

The strength of our study is that it is a large prospective observational study that enrolled not only healthy preterm infants, but also sick and ventilated premature infants. Another notable strength is that the number of paired TCB-TSB measurements was large in comparison to previous studies. Finally, we provided recent data for the agreement between TCB and TSB which is more helpful in clinical practice than correlation coefficient. Thus, our study added

significant findings to the limited literature on the use of the TCB device in preterm infants during and after phototherapy.

The present study has some limitations. Firstly, we did not examine the effect of the duration and recommencement of PT on the TSB-TCB correlation. Also, TCB was only measured from exposed skin (sternum) after PT was discontinued, and the TCB measurements from the covered area (nappy area) could have different correlation and agreement with TSB.

In conclusion, TCB measurements correlate strongly with TSB levels during and after phototherapy. However, as a result of the wide and clinically relevant disagreement between TCB and TSB measurements during the PT phase, a TCB device cannot be recommended for monitoring bilirubin level during phototherapy in our opinion. However, based on our results, we would advocate for using TCB for 'rebound' measurements at 12 hours post phototherapy to avoid unnecessary serum sampling.

Acknowledgement: The authors would like to thank all babies and families who participated in this study along with medical and midwifery staff at the CWIUH.

Contributors: Dr. Raba designed the study, contributed substantially to the data collection and analysis and drafted the initial manuscript. Ms. O'Sullivan contributed substantially to the data collection and analysis and reviewed and revised the manuscript. Prof Miletin conceptualised and designed the study, supervised the conduct of the study and coordinated the data analysis. He reviewed and revised the manuscript critically for important intellectual content. All the authors approved the final manuscript as submitted. They agree to be accountable for all aspects of the work.

Compliance with Ethical Statements:

Conflict of Interest: The authors declare that they have no conflict of interest

Ethical approval: The Research Ethics Committee of the Coombe Women and Infants University Hospital approved the study (Study No.3-2017)

Informed consent: Informed written consent was obtained from all participants

Funding: Dr Raba work was supported by the grant from Department of Cultural Affairs, Libya (Ref: HG6-490-45693) (managed by University College Dublin)

What is already known on this topic?

- Transcutaneous Bilirubinometry (TCB) is a non-invasive test used as a validated screening tool for hyperbilirubinemia in term infants
- The accuracy of TCB measurement during phototherapy (PT) is still controversial in term and preterm infants
- A few studies reported that TCB measurement from covered skin during PT could provide more accurate approximations of Total Serum Bilirubin level in term infants

What this study adds?

- During and after PT, TCB measurements correlate significantly with Total Serum Bilirubin (TSB) levels in preterm infants
- During PT, TCB underestimates TSB with a significant wide disagreement between TCB and TSB measurements, making it unreliable despite significant correlation in preterm infants
- Post-phototherapy, there is a significant correlation and acceptable agreement between TCB and TSB measurements with improving performance up to 12 hours after cessation of PT

References

1. Woodgate P, Jardine LA. Neonatal jaundice. BMJ Clin Evid. 2011;15.

2. Watchko JF, Oski FA. Kernicterus in preterm newborns: past, present, and future. Pediatrics. 1992;90(5):707-15.

3. Cashore WJ. The neurotoxicity of bilirubin. Clin Perinatol. 1990;17(2):437-47.

4. Mitra S, Rennie J. Neonatal jaundice: aetiology, diagnosis and treatment. Br J Hosp Med (Lond). 2017;78(12):699-704.

5. Morris BH, Oh W, Tyson JE, Stevenson DK, Phelps DL, O'Shea TM, et al. Aggressive vs. conservative phototherapy for infants with extremely low birth weight. N Engl J Med. 2008;359(18):1885-96.

6. Tyson JE, Pedroza C, Langer J, Green C, Morris B, Stevenson D, et al. Does aggressive phototherapy increase mortality while decreasing profound impairment among the smallest and sickest newborns? Journal of perinatology : official journal of the California Perinatal Association. 2012;32(9):677-84. Epub 2012/06/02.

7. Badiee Z, Mohammadizadeh M, Shamee M. Diagnostic usefulness of transcutaneous bilirubinometry in very preterm newborns. Int J Prev Med. 2012;3(4):262-5.

8. Ahmed M, Mostafa S, Fisher G, Reynolds TM. Comparison between transcutaneous bilirubinometry and total serum bilirubin measurements in preterm infants <35 weeks gestation. Ann Clin Biochem. 2010;47(Pt 1):72-7. Epub 2009/11/25.

9. Ozkan H, Oren H, Duman N, Duman M. Dermal bilirubin kinetics during phototherapy in term neonates. Acta Paediatr. 2003;92(5):577-81.

10. Beck M, Kau N, Schlebusch H. Transcutaneous bilirubin measurement in newborn infants: evaluation of a new spectrophotometric method. Arch Dis Child Fetal Neonatal Ed. 2003;88(4):F350-1.

11. Nagar G, Vandermeer B, Campbell S, Kumar M. Effect of Phototherapy on the Reliability of Transcutaneous Bilirubin Devices in Term and Near-Term Infants: A Systematic Review and Meta-Analysis. Neonatology. 2016;109(3):203-12. Epub 2016/01/21.

12. National Institute for Health and Care Excellence. Jaundice in newborn babies under 28 days

Clinical guideline [CG98]. 2016.

13. Nagar G, Vandermeer B, Campbell S, Kumar M. Reliability of Transcutaneous Bilirubin Devices in Preterm Infants: A Systematic Review. Pediatrics. 2013;132(5):871-81.

14. Hassan Shabuj M, Hossain J, Dey S. Accuracy of transcutaneous bilirubinometry in the preterm infants: a comprehensive meta-analysis. The Journal of Maternal-Fetal & Neonatal Medicine. 2019;32(5):734-41.

15. Juster-Reicher A, Flidel-Rimon O, Rozin I, Shinwell ES. Correlation of transcutaneous bilirubinometry (TcB) and total serum bilirubin (TsB) levels after phototherapy. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2015;28(11):1329-31. Epub 2014/09/23.

16. Tan KL, Dong F. Transcutaneous bilirubinometry during and after phototherapy. Acta Paediatr. 2003;92(3):327-31.

17. Rylance S, Yan J, Molyneux E. Can transcutaneous bilirubinometry safely guide phototherapy treatment of neonatal jaundice in Malawi? Paediatr Int Child Health. 2014;34(2):101-7. Epub 2013/12/06.

18. Hulzebos CV, Vader-van Imhoff DE, Bos AF, Dijk PH. Should transcutaneous bilirubin be measured in preterm infants receiving phototherapy? The relationship between transcutaneous and total serum bilirubin in preterm infants with and without phototherapy. PloS one. 2019;14(6):e0218131. Epub 2019/06/15.

19. Caffrey Osvald E PP. NICE clinical guideline: antibiotics for the prevention and treatment of early-onset neonatal infection. 2014.

20. Arman D, Topcuoglu S, Gursoy T, Ovali F, Karatekin G. The accuracy of transcutaneous bilirubinometry in preterm infants. Journal of perinatology : official journal of the California Perinatal Association. 2020;40(2):212-8. Epub 2019/08/01.

21. Cucuy M, Juster-Reicher A, Flidel O, Shinwell E. Correlation between transcutaneous and serum bilirubin in preterm infants before, during, and after phototherapy. J Matern Fetal Neonatal Med. 2018;31(10):1323-6. Epub 2017/04/24.

22. Nagar G, Kumar M. Effect of phototherapy on the diagnostic accuracy of transcutaneous bilirubin in preterm infants. Journal of Clinical Neonatology. 2017;6(3):148-53.

23. Zecca E, Barone G, De Luca D, Marra R, Tiberi E, Romagnoli C. Skin bilirubin measurement during phototherapy in preterm and term newborn infants. Early Hum Dev. 2009;85(8):537-40. Epub 2009/05/29.

24. Kanti V, Bonzel A, Stroux A, Proquitte H, Buhrer C, Blume-Peytavi U, et al. Postnatal maturation of skin barrier function in premature infants. Skin Pharmacol Physiol. 2014;27(5):234-41. Epub 2014/07/26.

25. De Luca D, Dell'Orto V. Patched Skin Bilirubin Assay to Monitor Neonates Born Extremely Preterm Undergoing Phototherapy. J Pediatr. 2017;188:122-7. Epub 2017/06/26.

Table 1
Baseline population Characteristics

Variable	Number
Male Sex n (%)	105 (53.6%)
Birth Weight (grams) Mean ±SD	1605 ±638
Gestational age (weeks) Mean ±SD	30.4 ±3.2
Mode of Delivery n (%) NVD Instrumental Delivery Elective LSCS Emergency LSCS	 56 (28.6%) 3 (1.5%) 59 (30.1%) 78 (39.8%)
Apgar score at 1st minute Median (IQR)	7 (5-9)
Apgar score at 5th minute Median (IQR)	9 (8-10)
Blood group infants (when done) n (%) A B AB O Rhesus +	175 (89%) 48 (27.4%) 18 (10.2%) 2 (1%) 108 (61.7%) 150/175 (85.7%)
Maternal group *n (%)* A B AB O Rhesus +	 60 (30.6%) 24 (12.2%) 5 (2.6%) 104 (53.1%) 176 (89.9%)
Positive DCT n (%)	7 (3.6%)
Maternal age (years) Mean ± SD	32 ±6.2
Proven EOS n (%)	5 (2%)
Antibiotics treatment n (%)	145 (74%)

NVD, normal vaginal delivery; DCT, direct Coombs test; LSCS, lower segment Caesarean section; EOS, early onset sepsis

Table 2
Paired samples of TSB and TCB from covered and uncovered skin during phototherapy and post phototherapy

		Mean ±SD µmol/l	Median (IQR) µmol/l	N	P-value
During PT	TCBC	102 ±62	102 (55, 146)	299	<0.0001
	TSB	127 ±51	124 (89, 162)		
	TCBC - TSB difference	-25 ±43	-25 (-49, 1)		
During PT	TCBU	79 ±70	61 (18, 127)	309	<0.0001
	TSB	127 ±51	122 (86, 162)		
	TCBU - TSB difference	-48 ±46	-48 (-79, -18)		
After PT	TCB	143 ±63	141 (100, 188)	142	0.0001
	TSB	153 ±51	153 (115, 187)		
	TCBC - TSB difference	-10 ±31	-13 (-28, 9)		

TSB, total serum bilirubin; TCBC, Transcutaneous bilirubinometry readings from covered skin; TCBU, Transcutaneous bilirubinometry readings from exposed skin; PT, phototherapy; TCB, Transcutaneous bilirubinometry

Table 3
TCB and TSB pairs 8 and 12 hour post phototherapy

Hours after PT	N	TSB (µmol/l) Mean ±SD Median (IQR)	TCB (µmol/l) Mean ±SD Median (IQR)	Mean difference (TCB – TSB) (µmol/l) Mean ±SD Median (IQR)	Correlation r (p value)
8 hours	40	133 ±51 124 (94, 168)	95 ±54 97 (53, 138)	-37 ±28 -32 (-49, -22)	0.86 (< 0.0001)
12 hours	36	147 ±52 135 (112, 173)	131 ±51 123 (95, 154)	-16 ±19 -17 (-23, -7)	0.93 (< 0.0001)

TSB, total serum bilirubin; TCB, Transcutaneous bilirubinometry; PT, phototherapy

Legends

Figure 1A

Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) from covered skin during phototherapy

Figure 1B

B–A plot showing the 95% limits of agreement between TCB from covered skin and TSB during phototherapy

Figure 2A

Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) from uncovered skin during phototherapy

Figure 2B

B–A plot showing the 95% limits of agreement between TCB from uncovered skin and TSB during phototherapy

Figure 3A

Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) after phototherapy

Figure 3B

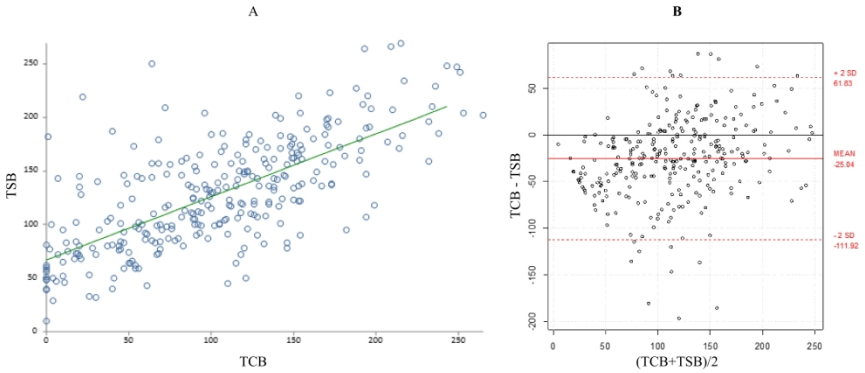
B-A plot showing the 95% limits of agreement between TCB and TSB after phototherapy

Appendix 1

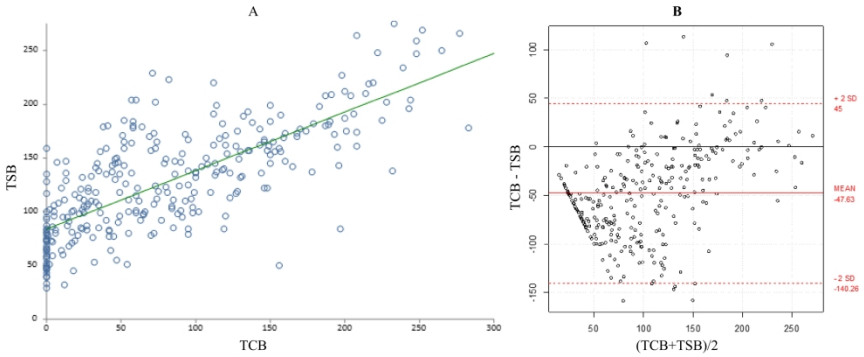
National Institute for Health and Care Excellence Guideline charts for phototherapy, used for infants less than 32 weeks of gestation

Appendix 2

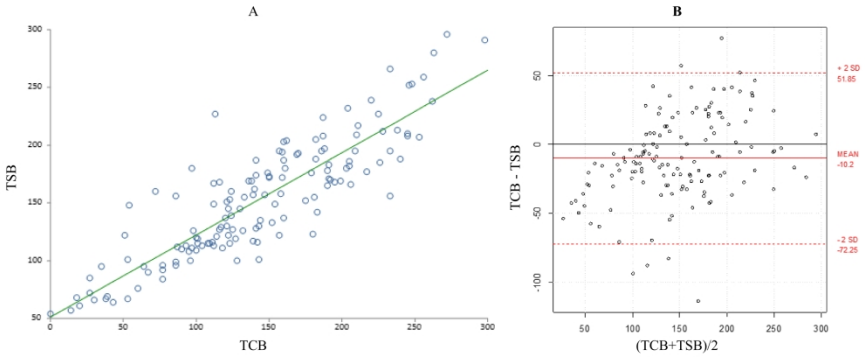
Phototherapy threshold chart used for infants ≥32 weeks of gestation (adapted from National Health Service, Glasgow, UK)



296x209mm (300 x 300 DPI)



296x209mm (300 x 300 DPI)



296x209mm (300 x 300 DPI)

NHS
National Institute for
Health and Clinical Excellence

Neonatal jaundice

Treatment threshold graphs

Graphs for assessing whether to treat neonatal jaundice by phototherapy or exchange transfusion

Clinical Guideline Implementation Tools

2010

NICE clinical guideline 98

National Institute for Health and Clinical Excellence

The NCC-WCH and the Guideline Development Group (GDG)would like to thank Dr Giles Kendall MBBS, BSc(hons), MRCPCH PhD Academic Clinical Lecturer Neonatal Medicine University College London / University College London Hospital NHS Foundation Trust, T J Cole, Professor of Medical Statistics, MRC Centre of Epidemiology for Child Health, UCL Institute of Child Health and Janet Rennie, Consultant and Senior Lecturer in Neonatal Medicine, Elizabeth Garrett Anderson Institute for Women's Health, University College London NHS Foundation Trust London for allowing the GDG to adapt their excel spreadsheet in developing the treatment threshold graphs included in this guideline.

National Institute for Health and Clinical Excellence

Treatment threshold graphs for neonatal jaundice - Instructions

These treatment threshold graphs will help healthcare professionals assess whether babies with jaundice should be given phototherapy or exchange transfusion. Please access the graphs directly from the NICE website to ensure that you are using the correct version of them.

Click on the 'Treatment threshold graphs' tab to access the graphs. The sheet contains a treatment graph for each gestational age. Before printing, use the drop-down menu that is marked in red to choose the graph for the correct gestational age for each baby with jaundice.

Print off the graph and keep it with the baby's notes. Plot the baby's bilirubin level on the graph each time it is measured, against the baby's age. Each line on the horizontal (x) axis is equal to 6 hours and each line on the vertical (y) axis is equal to 10 micromol/ litre. Assess whether the threshold for either phototherapy or exchange transfusion has been reached. Refer to the NICE neonatal jaundice guideline for detailed recommendations about the treatment of neonatal jaundice www.nice.org.uk/guidance/CG98/QuickRefGuide. Shade the 'single' or 'multiple' cells to show the type of phototherapy that the baby is receiving on each day.

Following a query to NICE about how the treatment threshold graphs for babies with jaundice should be used, please note: The graph that reflects the baby's actual gestational age should continue to be used until the baby is 14 days old. The baby's 'corrected' gestational age should not be taken into consideration, and you should not move up to the next graph when the baby is 7 days old. For example, for a baby of 35 weeks' gestation, the 35-week gestation graph should be used until the baby is aged 14 days. Please note that the NICE guideline does not cover treatment with phototherapy and exchange transfusion for babies older than 14 days. Trusts should therefore agree their own policy about when to treat babies over 14 days with phototherapy and exchange transfusion.

The NICE neonatal jaundice guideline and all implementation tools can be found at www.nice.org.uk/guidance/CG98

Treatment threshold graph for babies with neonatal jaundice

NHS
National Institute for
Health and ClinicalExcellence

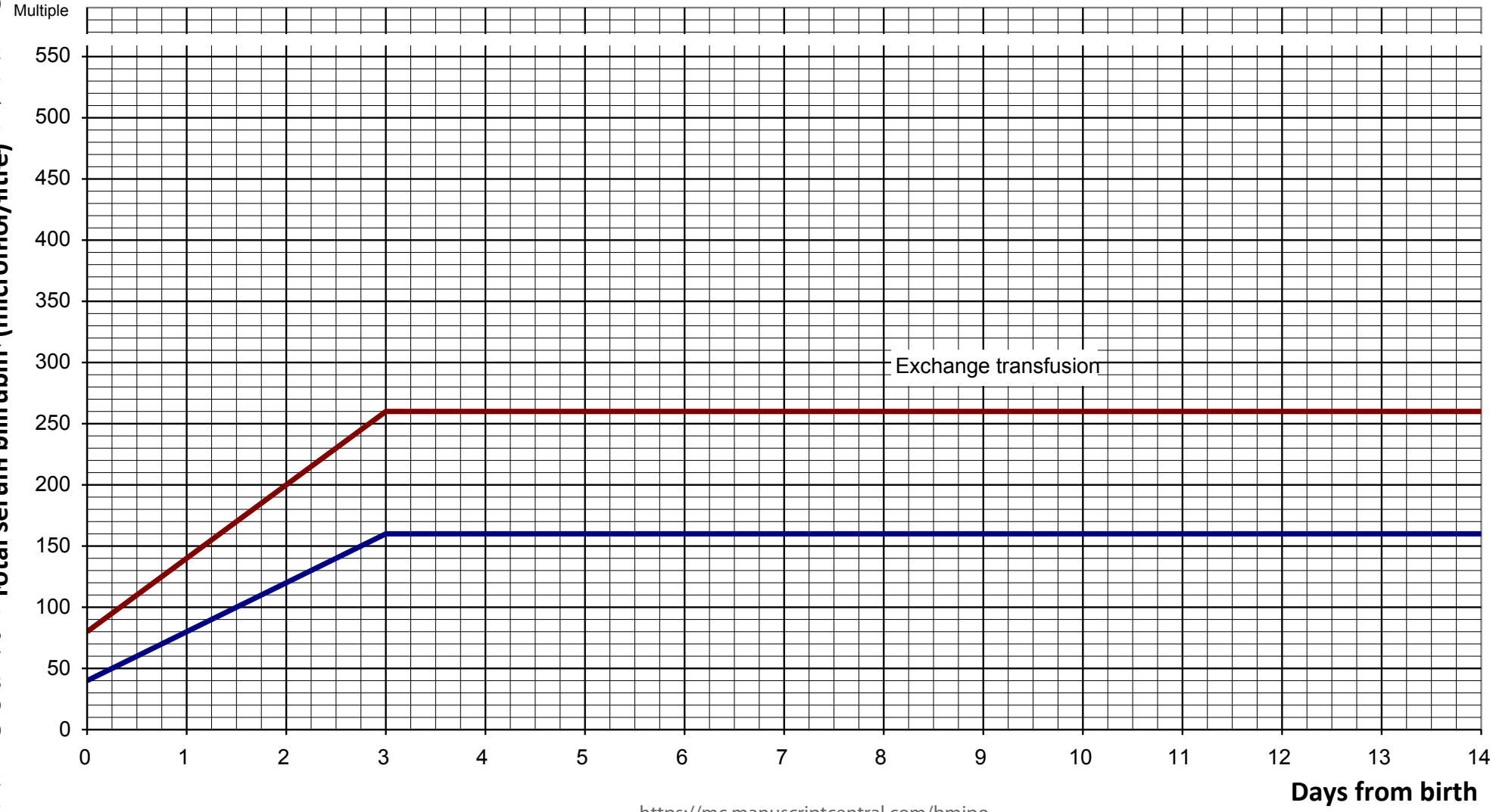
Baby's name _____ Date of birth _____

Hospital number _____ Time of birth _____ Direct Antiglobulin Test _____

Click below and choose gestation

26 **weeks gestation**

Shade for phototherapy Baby's blood group _____ Mother's blood group _____



NHS
National Institute for
Health and Clinical Excellence

Where to find the guidance

The NICE neonatal jaundice guideline contains recommendations about the recognition, assessment and treatment of neonatal jaundice.

You can download the following documents from www.nice.org.uk/ guidance/CG98.

- The NICE guideline – all the recommendations
- The full guideline – all the recommendations, details of how they were developed and summaries of the evidence they were based on
- The quick reference guide – a summary of the recommendations for healthcare professionals (www.nice.org.uk/guidance/CG98/QuickRefGuide)
- 'Understanding NICE guidance' – a version of the guideline for parents and carers

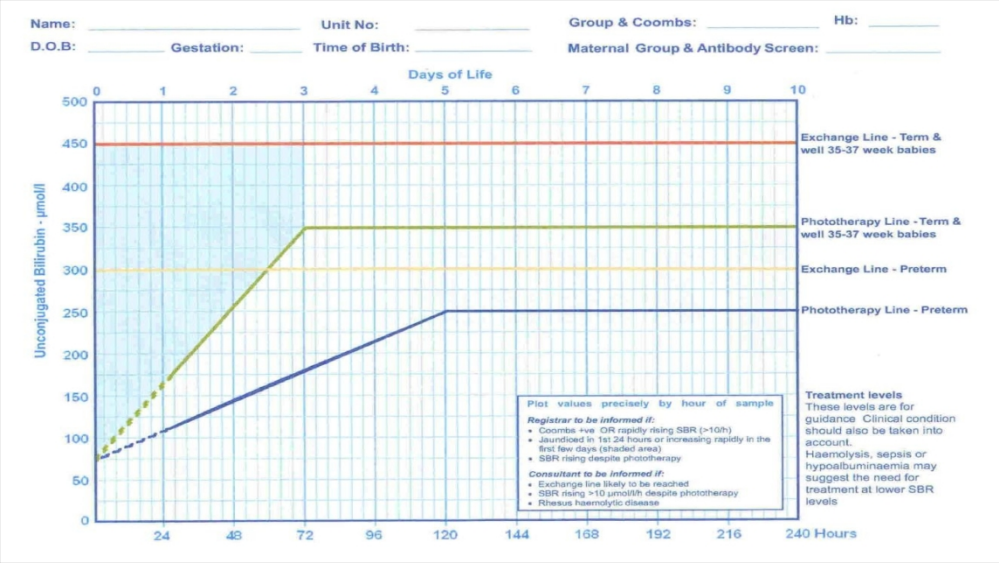
For printed copies of the quick reference guide or 'Understanding NICE guidance' phone NICE publications on 0845 003 7783 or email publications@nice.org.uk and quote:

- N2143 – (quick reference guide)
- N2144 – ('Understanding NICE guidance')

Other implementation tools are available from the NICE website:

- slide set
- parent information factsheet
- audit tools
- costing tools

National Institute for Health and Clinical Excellence
71 High Holborn
London
WC1V 6NA
www.nice.org.uk



338x190mm (300 x 300 DPI)

BMJ Paediatrics Open

Transcutaneous Bilirubinometry during and after Phototherapy in Preterm Infants, Prospective Observational Study

Journal:	*BMJ Paediatrics Open*
Manuscript ID	bmjpo-2020-000681.R2
Article Type:	Original research
Date Submitted by the Author:	03-Jun-2020
Complete List of Authors:	Raba, Ali ; Coombe Women and Infants University Hospital O'Sullivan, Anne; Coombe Women and Infants University Hospital, Miletin, Jan; Coombe Women and Infants University Hospital, Paediatric and Newborn Medicine
Keywords:	Jaundice, Neonatology, Monitoring

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Transcutaneous Bilirubinometry during and after Phototherapy in Preterm Infants, Prospective Observational Study

Ali Ahmed Raba[1,2], Anne O'Sullivan[2], Jan Miletin[1,2,3,4]

[1] UCD School of Medicine and Medical Sciences, Dublin, Ireland

[2] Coombe Women and Infants University Hospital, Dublin, Ireland

[3] Institute for the Care of Mother and Child, Prague, Czech Republic

[4] 3rd School of Medicine, Charles University, Prague, Czech Republic

Short running title: Transcutaneous Bilirubinometry in Preterm Infants

Corresponding author: Prof Jan Miletin, MD, FRCPI, FJFICMI

Address: Coombe Women and Infants University Hospital, Cork Street, Dublin 8, Dublin, Ireland

Email: miletinj@yahoo.com

Telephone: 00353 14085654

Fax: 00353 14085209

Abstract

Objective: To examine the accuracy of Transcutaneous Bilirubinometry (TCB) measurements during and after phototherapy (PT) in preterm infants

Design: Prospective observational cohort study

Setting: Level III neonatal centre.

Patients: Preterm infants (23^{+0} to 36^{+6} weeks of gestation) born between June 2017 and May 2018 requiring PT

Interventions: TCB was measured from an exposed area of skin (the sternum, (TCBU)) and covered area of skin under the nappy (the bony part of the upper outer quadrant of the buttock (TCBC)) within an hour of obtaining Total Serum Bilirubin (TSB)

Main outcome measures: Correlation and agreement between TCB (TCBU and TCBC) and TSB during and after phototherapy

Result: We have enrolled 196 preterm infants. There was a significant correlation between TSB and TCB during PT (r =0.72, 95% CI 0.66 to 0.77 in covered, r =0.75, 95% CI 0.70 to 0.80 in uncovered areas) and after PT (r =0.87, 95% CI 0.83 to 0.91). TCB underestimated TSB level during PT with a mean TCBC-TSB difference of -25 ±43, 95% agreement limits of 62 to -112, and a mean TCBU-TSB difference of -48 ±46, 95% agreement limits of 45 to -140. The agreement between TCB and TSB after cessation of PT improved, TCB underestimating TSB by a mean TCB-TSB difference of -10 ±31 (95% agreement limits of 52 to -72).

Conclusion: TCB measurements correlated strongly with TSB levels during and after phototherapy. However, there was a wide and clinically relevant disagreement between TCB and TSB measurements during PT phase, improving significantly post PT.

Keywords: Hyperbilirubinemia, Jaundice, Preterm infants, Transcutaneous bilirubinometry

Abbreviations: B-A, Bland-Altman; CWIUH, Coombe Women and Infants University hospital; DCT, direct Coombs test; GIT, gastrointestinal tract; IQR, interquartile range; mVSD, muscular ventricular septal defect; NICE, National Institute for Health and Care Excellence; PDA, patent ductus arteriosus; PFO, persistent foramen ovale; PT, phototherapy; SD, standard deviation; TCB, transcutaneous bilirubinometry; TCBC, transcutaneous bilirubinometry reading from covered skin; TCBU, transcutaneous bilirubinometry reading from uncovered skin; TSB, total serum bilirubin

Introduction

Neonatal hyperbilirubinemia is a very common condition with approximately 50% of term and 80% of preterm infants developing jaundice in the first week of life [1]. Hyperbilirubinemia in most cases is a benign and self-limiting condition, however severe hyperbilirubinemia can occasionally occur and may be associated with irreversible brain damage, especially in preterm infants [2 3].

Phototherapy (PT) is considered to be a safe and effective treatment for neonatal unconjugated hyperbilirubinemia. The indication to commence treatment is based on the level of serum bilirubin, the age of the baby in hours, and gestational age [4]. Evidence is conflicting regarding the best therapeutic approach to hyperbilirubinemia, especially in extremely low birth weight (ELBW) infants. A randomised clinical trial (RCT) performed by the Neonatal Research Network found no significant difference in the rate of death or neurodevelopmental impairment at 18 to 22 months corrected age in ELBW infants who received aggressive PT versus those who received conservative PT. However, aggressive PT was associated with a reduction in the rate of neurodevelopmental impairment alone.[5] However, the post hoc analysis showed that in the smallest and sickest subgroup (mechanically ventilated infants with birth weight less than 750g), aggressive PT may increase mortality while reducing neurodevelopmental impairment.[6]

Measurement of total serum bilirubin (TSB) remains the gold standard for monitoring bilirubin levels during and after PT in term and preterm infants. However, obtaining heel stick or venous blood samples is painful, time-consuming, and increases the risk of local and systemic infection especially in preterm infants [7]. A transcutaneous bilirubinometry (TCB) device works by directing light into the skin of the infant and measuring and analysing the intensity of the returned wavelengths to estimate a TSB [8]. TCB has been recommended as a non-invasive, painless and time-saving test for bilirubin estimation in term and late preterm infants prior to the commencement of PT [9-11]. However, TCB measurements are not recommended in the first

24 hours of life or in preterm infants below 35 weeks of gestation according to the National Institute for Clinical Excellence (NICE) guidelines.[12] (https://www.nice.org.uk/guidance/cg98).

It seems that TCB has strong correlation and acceptable agreement in preterm infants before phototherapy.[13 14] The use of TCB for infants during and after PT is still controversial, as some studies reported that PT blanches the skin thereby affecting the correlation between TCB and TSB during and after phototherapy[15] while others suggest that TCB readings from covered skin area could be safely used to guide treatment during and post PT.[16-18]. We designed our study to examine the accuracy of TCB to estimate the TSB level in preterm infants undergoing PT and its reliability after PT.

Methods

A single centre prospective observational cohort study performed in the neonatal department of the Coombe Women and Infants University hospital (CWIUH), Dublin, Ireland (level III neonatal centre). All preterm infants (23+0 - 36+6 weeks of gestation) born between June 2017 and May 2018 in CWIUH who developed significant jaundice requiring PT were eligible for enrollment to this study. During the study period, the protocols for screening, diagnosis and management of infants with jaundice were not changed. Infants with clinical or radiological evidence of major congenital anomalies (including those with gastrointestinal tract (GIT) deformities and congenital heart diseases apart from patent ductus arteriosus (PDA), persistent foramen ovale (PFO) and small (≤5 mm) muscular ventricular septal defect (mVSD)) were excluded from this study.

PT was commenced based on TSB levels according to the hospital guidelines, taking into account the infant's age in hours and gestation in weeks. The NICE treatment charts were used for preterm infants below 32 weeks of gestation (https://www.nice.org.uk/guidance/cg98/resources). In infants ≥32 weeks of gestation, a chart adapted from the National Health Service (NHS), Glasgow, UK was used (Appendix 1). Standard PT units (Photo Therapy 4000, Draeger Medical, Germany) were used (overhead PT microlight units deliver ≥10 µW/cm2/nm and Halogen spotlights which can deliver 20-25uW/cm2/nm). Infants receiving PT were completely exposed, except for their eyes (covered with phototherapy goggles for protection) and the nappy area (covered with a disposable nappy). PT was discontinued when the TSB fell below the relevant treatment threshold.

TCB was measured from uncovered/exposed (TCBU) and covered (TCBC) areas within an hour of obtaining TSB samples. The device was placed over an uncovered area (sternum) and pressed gently against the skin three times to obtain one reading (the average of the three measured values). The process was repeated over the covered area, the bony part of the upper

outer quadrant of the buttock (covered by the nappy). After cessation of PT, TCB's were measured from the sternum. The measurements were obtained by experienced nurses trained and competent in the use of the Dräger Jaundice Meter (JM-105 or JM-103, Draeger Medical, Germany). The TCB devices were calibrated regularly according to the manufacturer's instructions and hospital guidelines. Blood samples for TSB were obtained either by heel prick or venepuncture. The attending neonatologist directed the frequency of TSB measurements. TSB levels were measured in one clinical laboratory using direct spectrophotometry (Abbot Architect C8000, Abbott, USA).

Our primary outcome was the correlation and agreement between TCB (TCBU and TCBC) and TSB during and after PT.

Our prospective cohort study was approved by the Research Ethics Committee of the CWIUH (Study No.3-2017) and informed written consent was obtained from parents. Patients or the public were not involved in the design, conduct, or reporting plans of our research.

Data was entered into Microsoft Office Excel (MS Excel, Microsoft, USA) and analysed by the StatsDirect v.3.2.10 software (StatsDirect Ltd, UK). Descriptive statistics were used for all demographic variables of interest using frequency distribution and percentage for categorical variables. Mean and standard deviation (SD) were used for normally distributed data, while non normal distribution data was summarised using median and interquartile range (IQR). Paired-samples t-test was used to compare TCB and TSB paired measurements. When the differences between pairs were not normally distributed, we used the Wilcoxon signed-rank test for two sample comparisons. For independent variables we have used an unpaired t-test or Mann-Whitney U test as appropriate. Correlation between TCB (TCBU and TCBC) and TSB was calculated using Pearson's correlation coefficient during and after phototherapy. Bland-Altman (B-A) analysis was used to calculate and visualize the agreement between TSB and TCB. The agreement limits are demonstrated as a 95% confidence interval (95% CI = mean ±

1.96 standard deviations), where the ideal agreement difference between measurements is zero. Our results were summarized using p-values and 95% confidence intervals. P-values <0.05 were considered to be statistically significant. We have used a convenience sample for the study with planned one year enrolment.

Results

One hundred and ninety six jaundiced preterm infants who received phototherapy were enrolled to the study. The mean (±SD) gestational age and birth weight of our cohort were 30.4 weeks of gestation (±3.2) and 1605g (±638), respectively. The demographic description of our cohort is presented in the Table 1.

There were 328 simultaneous measurements (TSB and TCB) during the PT phase and 142 pairs of readings after discontinuation of PT. The PT was commenced at mean (±SD) 32.5 (±20) hours of life and the median duration of PT exposure was 24 hours (IQR 24-32).

During the PT phase, the mean TSB ±SD (127 ±51 μmol/L) and mean TCBC ±SD (102 ±62) were statistically significantly different (p<0.0001) (Table 2). Similarly, the difference between the mean TSB ±SD (127 ±51 μmol/L) and mean TCBU ±SD (79 ±70) was statistically significantly different during PT (p <0.0001) (Table 2). Although there was a significant correlation between TSB and TCB measurements during PT, (r =0.72, 95% CI 0.66 to 0.77 from covered, r =0.75, 95% CI 0.70 to 0.80 from uncovered areas, p <0.0001), B-A plots showed significant bias and imprecisions in the TCB readings. TCB underestimated TSB level with a mean TCB-TSB difference of -25 ±43 from covered area (95% agreement limits of 62 to -112, p <0.0001) and of -48 ±46 from uncovered area (95% agreement limits of 45 to -140, p <0.0001) (Figure 1, Figure 2).

During the post-phototherapy phase, TSB ±SD (153 ±51 μmol/L) and TCB ±SD (143 ±63) measurements were statistically significantly different (p=0.0001) (Table 2). These

measurements were taken at median time of 12 hours (IQR 8 - 24) post PT. After cessation of PT, the correlation between TCB and TSB further improved (r =0.87, 95% CI 0.83-0.91, p <0.0001) (Figure 3A). The B-A plot also showed an improvement in the agreement between TCB and TSB, but TCB continued to underestimate TSB level by -10 ±31 (95% agreement limits of 52 to -72, p =0.0001) (Figure 3B). At 12 hours after cessation of PT, the correlation between TCB and TSB was improved compared to eight hours post phototherapy with statistically significantly improving mean difference between TCB and TSB (p <0.0001) (Table 3).

Discussion

Our study revealed a significant correlation between the TCB and TSB during and after phototherapy in preterm infants. However, our findings also showed a significant wide disagreement between TCB and TSB measurements during the PT phase. Although TCB readings from the covered skin had better agreement than those from the exposed skin, TCB measurements were associated with a large bias during PT. We noted that the TCB device could underestimate serum bilirubin level by up to 112 and 140 µmol/L, from covered and uncovered area, respectively, during PT.

In general, the significant correlation between TCB and TSB during PT is consistent with the findings in some previous studies in preterm infants.[19] Cucuy [20] et al conducted a study of 86 preterm infants with a mean gestational age of 32 weeks and a mean birth weight of 1637g. Although they found a good correlation between TSB and TCB during PT (r =0.8), it was not clear if their TCB readings were measured from the exposed or covered skin during phototherapy. In addition to this, they did not provide information about the level of agreement between the TCB and TSB measurements.

There are only a few studies that examined 95% agreement limits between the TCB and TSB in preterm infants during phototherapy. Nagar et al [21] performed a smaller study on 90 preterm infants with a mean gestational age of 32.4 weeks and a mean birth weight of 1847g. They found that TCB cannot be recommended for the bilirubin measurement during PT in preterm infants due to the high risk of underestimation of TSB by up to 132 and 157 µmol/L from covered and uncovered skin, respectively. Although their sample was smaller and infants older than our cohort, their results were quite comparable to our findings.

Similarly, Hulzebos et al demonstrated that TCB underestimated TSB in very preterm infants during phototherapy when measured on covered skin.[18] The same research group proposed different cut-off rules to improve the prediction of phototherapy thresholds when TCB measured during phototherapy on covered skin.[18]

Zecca et al [22] conducted a study on 364 preterm and term infants requiring phototherapy. The mean gestational age and the mean birth weight of their sample were of 34.6 weeks of gestation and 2371g, respectively, which were higher than the mean gestational age and the mean birth weight of our cohort. They reported a smaller bias between TCB readings from covered skin and TSB compared to our results. Their results demonstrated that TCB from exposed skin underestimated TSB by 54 ±51μmol/L, while TCB from covered skin underestimated TSB by 3.1 ±53μmol/L. However, B-A plots showed a wide TCB-TSB disagreement with a risk of underestimation of TSB by up to 106 µmol/L from covered skin and 153 µmol/L from exposed skin.

We have shown that the TCB readings from both covered and uncovered area were lower than TSB levels. We speculate, that immaturity of the skin and the absence of subcutaneous fat in preterm infants may lead to rapid clearance of extravascular bilirubin levels from the skin following initiation of PT [9 23]. This however would contravene the findings of De Luca et al who reported that TCB reading from covered skin and TSB correlated strongly (r = 0.84, p

<0.001) in their study of 60 extremely preterm infants undergoing phototherapy and, unlike our finding, TCB overestimated TSB with a mean TCB-TSB difference of 47.8 ±41μmol/L [24]. During the post-phototherapy phase, our data revealed a better correlation between TCB and TSB reading as compared to that during the PT phase. More interestingly, the mean difference of TSB-TCB pairs was much lower than reported previously, even in paired measurements done in our study as early as eight hours post phototherapy.[18] We observed improved correlation and decreasing mean difference of TSB-TCB pairs with the increased time post phototherapy, which would be different to previous observation by Cucuy et al as they reported that time after PT did not have any significant effect on the correlation between TSB and TCB.[20]

Moreover, our results showed a significant improvement in the agreement between TCB and TSB after cessation of the PT. The level of underestimation of TSB in our study are similar to those observed in Nagar et al study [21].

The strength of our study is that it is a large prospective observational study with substantial number of paired TCB-TSB measurements in comparison to previous studies. We have also provided recent data for the agreement between TCB and TSB which is more helpful in clinical practice than correlation coefficient. Thus, our study added significant findings to the literature on the use of the TCB device in preterm infants during and after phototherapy.

The present study has some limitations. Firstly, we did not examine the effect of the duration and recommencement of PT on the TSB-TCB correlation. Also, TCB was only measured from exposed skin (sternum) after PT was discontinued, and the TCB measurements from the covered area (nappy area) could have different correlation and agreement with TSB.

In conclusion, TCB measurements correlate strongly with TSB levels during and after phototherapy. However, as a result of the wide and clinically relevant disagreement between TCB and TSB measurements during the PT phase, a TCB device cannot be recommended for

monitoring bilirubin level during phototherapy in our opinion. However, based on our results, we would advocate for using TCB for TSB 'rebound' measurements at 12 hours post phototherapy to avoid unnecessary serum sampling.

Acknowledgement: The authors would like to thank all babies and families who participated in this study along with medical and midwifery staff at the CWIUH.

Contributors: Dr. Raba designed the study, contributed substantially to the data collection and analysis and drafted the initial manuscript. Ms. O'Sullivan contributed substantially to the data collection and analysis and reviewed and revised the manuscript. Prof Miletin conceptualised and designed the study, supervised the conduct of the study and coordinated the data analysis. He reviewed and revised the manuscript critically for important intellectual content. All the authors approved the final manuscript as submitted. They agree to be accountable for all aspects of the work.

Compliance with Ethical Statements:

Conflict of Interest: The authors declare that they have no conflict of interest

Ethical approval: The Research Ethics Committee of the Coombe Women and Infants University Hospital approved the study (Study No.3-2017)

Informed consent: Informed written consent was obtained from all participants

Funding: Dr Raba work was supported by the grant from Department of Cultural Affairs, Libya (Ref: HG6-490-45693) (managed by University College Dublin)

What is already known on this topic?

- Transcutaneous Bilirubinometry (TCB) is a non-invasive test used as a validated screening tool for hyperbilirubinemia in term infants
- The accuracy of TCB measurement during phototherapy (PT) is still controversial in term and preterm infants
- A few studies reported that TCB measurement from covered skin during PT could provide accurate approximations of Total Serum Bilirubin level in term infants

What this study adds?

- During and after PT, TCB measurements correlate significantly with Total Serum Bilirubin (TSB) levels in preterm infants
- During PT, TCB underestimates TSB with a significant wide disagreement between TCB and TSB measurements, making it unreliable
- Post-phototherapy, there is a significant correlation and acceptable agreement between TCB and TSB measurements with improving performance up to 12 hours after cessation of PT

References

1. Woodgate P, Jardine LA. Neonatal jaundice. *BMJ Clin Evid* 2011;15
2. Watchko JF, Oski FA. Kernicterus in preterm newborns: past, present, and future. *Pediatrics* 1992;90(5):707-15.
3. Cashore WJ. The neurotoxicity of bilirubin. *Clin Perinatol* 1990;17(2):437-47.
4. Mitra S, Rennie J. Neonatal jaundice: aetiology, diagnosis and treatment. *Br J Hosp Med (Lond)* 2017;78(12):699-704. doi: 10.12968/hmed.2017.78.12.699
5. Morris BH, Oh W, Tyson JE, et al. Aggressive vs. conservative phototherapy for infants with extremely low birth weight. *N Engl J Med* 2008;359(18):1885-96. doi: 10.1056/NEJMoa0803024
6. Tyson JE, Pedroza C, Langer J, et al. Does aggressive phototherapy increase mortality while decreasing profound impairment among the smallest and sickest newborns? *Journal of perinatology : official journal of the California Perinatal Association* 2012;32(9):677-84. doi: 10.1038/jp.2012.64 [published Online First: 2012/06/02]
7. Badiee Z, Mohammadizadeh M, Shamee M. Diagnostic usefulness of transcutaneous bilirubinometry in very preterm newborns. *Int J Prev Med* 2012;3(4):262-5.
8. Ahmed M, Mostafa S, Fisher G, et al. Comparison between transcutaneous bilirubinometry and total serum bilirubin measurements in preterm infants <35 weeks gestation. *Ann Clin Biochem* 2010;47(Pt 1):72-7. doi: 10.1258/acb.2009.009072 [published Online First: 2009/11/25]
9. Ozkan H, Oren H, Duman N, et al. Dermal bilirubin kinetics during phototherapy in term neonates. *Acta Paediatr* 2003;92(5):577-81.
10. Beck M, Kau N, Schlebusch H. Transcutaneous bilirubin measurement in newborn infants: evaluation of a new spectrophotometric method. *Arch Dis Child Fetal Neonatal Ed* 2003;88(4):F350-1.

11. Nagar G, Vandermeer B, Campbell S, et al. Effect of Phototherapy on the Reliability of Transcutaneous Bilirubin Devices in Term and Near-Term Infants: A Systematic Review and Meta-Analysis. *Neonatology* 2016;109(3):203-12. doi: 10.1159/000442195 [published Online First: 2016/01/21]

12. National Institute for Health and Care Excellence. Jaundice in newborn babies under 28 days Clinical guideline [CG98], 2016.

13. Nagar G, Vandermeer B, Campbell S, et al. Reliability of Transcutaneous Bilirubin Devices in Preterm Infants: A Systematic Review. *Pediatrics* 2013;132(5):871-81. doi: 10.1542/peds.2013-1713

14. Hassan Shabuj M, Hossain J, Dey S. Accuracy of transcutaneous bilirubinometry in the preterm infants: a comprehensive meta-analysis. *The Journal of Maternal-Fetal & Neonatal Medicine* 2019;32(5):734-41. doi: 10.1080/14767058.2017.1390561

15. Juster-Reicher A, Flidel-Rimon O, Rozin I, et al. Correlation of transcutaneous bilirubinometry (TcB) and total serum bilirubin (TsB) levels after phototherapy. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet* 2015;28(11):1329-31. doi: 10.3109/14767058.2014.953923 [published Online First: 2014/09/23]

16. Tan KL, Dong F. Transcutaneous bilirubinometry during and after phototherapy. *Acta Paediatr* 2003;92(3):327-31.

17. Rylance S, Yan J, Molyneux E. Can transcutaneous bilirubinometry safely guide phototherapy treatment of neonatal jaundice in Malawi? *Paediatr Int Child Health* 2014;34(2):101-7. doi: 10.1179/2046905513Y.0000000050 [published Online First: 2013/12/06]

18. Hulzebos CV, Vader-van Imhoff DE, Bos AF, et al. Should transcutaneous bilirubin be measured in preterm infants receiving phototherapy? The relationship between transcutaneous and total serum bilirubin in preterm infants with and without phototherapy. *PloS one* 2019;14(6):e0218131. doi: 10.1371/journal.pone.0218131 [published Online First: 2019/06/15]

19. Arman D, Topcuoglu S, Gursoy T, et al. The accuracy of transcutaneous bilirubinometry in preterm infants. *Journal of perinatology : official journal of the California Perinatal Association* 2020;40(2):212-18. doi: 10.1038/s41372-019-0445-3 [published Online First: 2019/08/01]

20. Cucuy M, Juster-Reicher A, Flidel O, et al. Correlation between transcutaneous and serum bilirubin in preterm infants before, during, and after phototherapy. *J Matern Fetal Neonatal Med* 2018;31(10):1323-26. doi: 10.1080/14767058.2017.1315662 [published Online First: 2017/04/24]

21. Nagar G, Kumar M. Effect of phototherapy on the diagnostic accuracy of transcutaneous bilirubin in preterm infants. *Journal of Clinical Neonatology* 2017;6(3):148-53. doi: 10.4103/jcn.JCN_94_16

22. Zecca E, Barone G, De Luca D, et al. Skin bilirubin measurement during phototherapy in preterm and term newborn infants. *Early Hum Dev* 2009;85(8):537-40. doi: 10.1016/j.earlhumdev.2009.05.010 [published Online First: 2009/05/29]

23. Kanti V, Bonzel A, Stroux A, et al. Postnatal maturation of skin barrier function in premature infants. *Skin Pharmacol Physiol* 2014;27(5):234-41. doi: 10.1159/000354923 [published Online First: 2014/07/26]

24. De Luca D, Dell'Orto V. Patched Skin Bilirubin Assay to Monitor Neonates Born Extremely Preterm Undergoing Phototherapy. *J Pediatr* 2017;188:122-27. doi: 10.1016/j.jpeds.2017.05.080 [published Online First: 2017/06/26]

Table 1

Baseline population Characteristics

Variable	Number (n =196)
Male Sex n (%)	105 (53.6%)
Birth Weight (grams) Mean ±SD	1605 ±638
Gestational age (weeks) Mean ±SD	30.4 ±3.2
Mode of Delivery n (%) NVD Instrumental Delivery Elective LSCS Emergency LSCS	 56 (28.6%) 3 (1.5%) 59 (30.1%) 78 (39.8%)
Apgar score at 1st minute Median (IQR)	7 (5-9)
Apgar score at 5th minute Median (IQR)	9 (8-10)
Blood group infants (when done) n (%) A B AB O Rhesus +	175 (89%) 48 (27.4%) 18 (10.2%) 2 (1%) 108 (61.7%) 150/175 (85.7%)
Maternal group *n (%)* A B AB O Rhesus +	 60 (30.6%) 24 (12.2%) 5 (2.6%) 104 (53.1%) 176 (89.9%)
Positive DCT n (%)	7 (3.6%)
Maternal age (years) Mean ± SD	32 ±6.2

NVD, normal vaginal delivery; DCT, direct Coombs test; LSCS, lower segment Caesarean section

Table 2
Paired samples of TSB and TCB from covered and uncovered skin during phototherapy and post phototherapy

		Mean ±SD µmol/l	Median (IQR) µmol/l	N	P-value
During PT	TCBC	102 ±62	102 (55, 146)	299	<0.0001
	TSB	127 ±51	124 (89, 162)		
	TCBC - TSB difference	-25 ±43	-25 (-49, 1)		
During PT	TCBU	79 ±70	61 (18, 127)	309	<0.0001
	TSB	127 ±51	122 (86, 162)		
	TCBU - TSB difference	-48 ±46	-48 (-79, -18)		
After PT	TCB	143 ±63	141 (100, 188)	142	0.0001
	TSB	153 ±51	153 (115, 187)		
	TCBC - TSB difference	-10 ±31	-13 (-28, 9)		

TSB, total serum bilirubin; TCBC, Transcutaneous bilirubinometry readings from covered skin; TCBU, Transcutaneous bilirubinometry readings from exposed skin; PT, phototherapy; TCB, Transcutaneous bilirubinometry

Table 3
TCB and TSB pairs 8 and 12 hour post phototherapy

Hours after PT	N	TSB (µmol/l) Mean ±SD Median (IQR)	TCB (µmol/l) Mean ±SD Median (IQR)	Mean difference (TCB – TSB) (µmol/l) Mean ±SD Median (IQR)	Correlation r (p value)
8 hours	40	133 ±51 124 (94, 168)	95 ±54 97 (53, 138)	-37 ±28 -32 (-49, -22)	0.86 (< 0.0001)
12 hours	36	147 ±52 135 (112, 173)	131 ±51 123 (95, 154)	-16 ±19 -17 (-23, -7)	0.93 (< 0.0001)

TSB, total serum bilirubin; TCB, Transcutaneous bilirubinometry; PT, phototherapy

Legends

Figure 1A

Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) from covered skin during phototherapy

Figure 1B

B–A plot showing the 95% limits of agreement between TCB from covered skin and TSB during phototherapy

Figure 2A

Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) from uncovered skin during phototherapy

Figure 2B

B–A plot showing the 95% limits of agreement between TCB from uncovered skin and TSB during phototherapy

Figure 3A

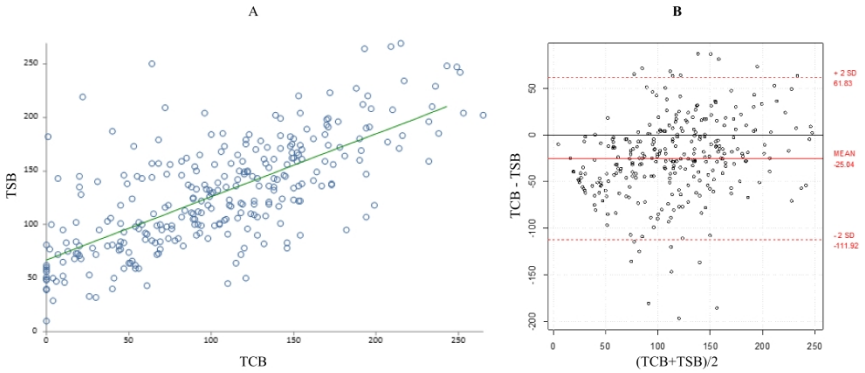
Correlation between Total Serum Bilirubin (TSB) and Transcutaneous Bilirubinometry (TCB) after phototherapy

Figure 3B

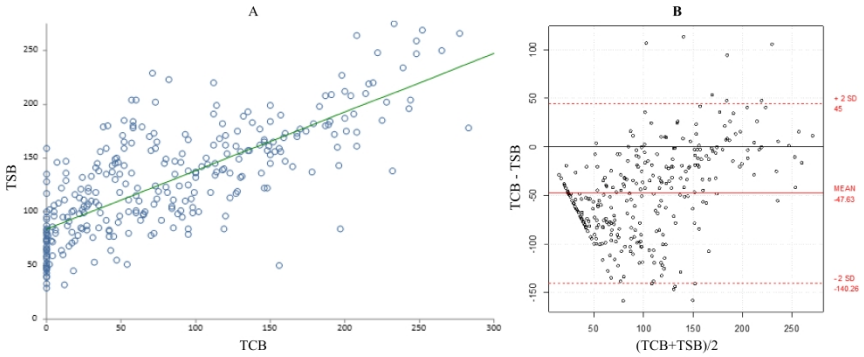
B-A plot showing the 95% limits of agreement between TCB and TSB after phototherapy

Appendix 1

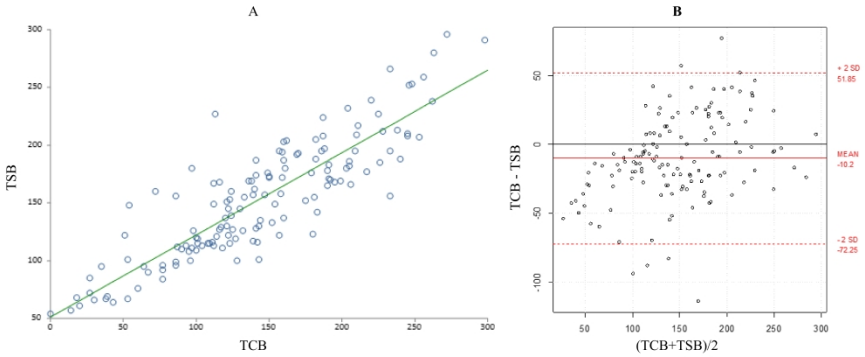
Phototherapy threshold chart used for infants ≥32 weeks of gestation (adapted from National Health Service, Glasgow, UK)



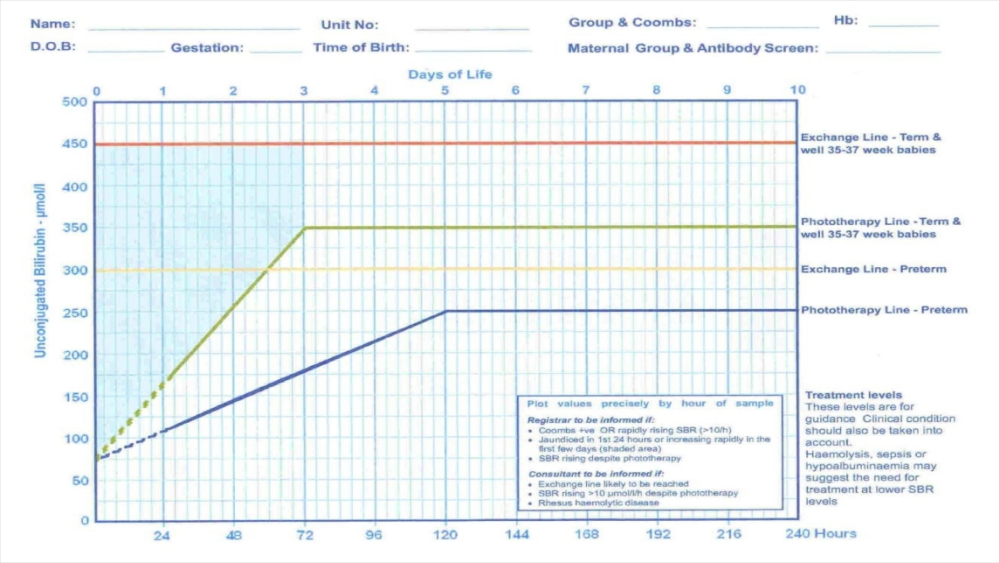
297x210mm (600 x 600 DPI)



297x210mm (600 x 600 DPI)



297x210mm (600 x 600 DPI)



338x190mm (300 x 300 DPI)