CORRELATION BETWEEN TRANSCUTANEOUS BILIRUBIN AND TOTAL SERUM BILIRUBIN LEVELS AMONG PRETERM NEONATES AT GROOTE SCHUUR HOSPITAL

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DECLARATION

I hereby declare that this work is original. This work has not been presented to any other university for award of any degree and has not been submitted anywhere for publication.

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A dissertation submitted in partial fulfilment of the requirement for award of the degree of master of philosophy in neonatology of University of Cape Town
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Dedication

This work is dedicated to my family who have been patient and endured my absence for long periods during my studies.

Acknowledgement

This work could only be accomplished with the love and patience demonstrated by my dear wife and sons who endured my absence for long hours during this course.

The guidance demonstrated by my supervisors to which there is no yard stick to measure, for every step I took they made sure I did not stumble. I highly appreciate and cannot thank them enough.

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To the Almighty, you enabled me and my family members stay healthy and safe during my training, you enable me and all the different parties (family, supervisors, consultants, nurses, and administrative staff) interact cordially and accomplish this piece of work.

Table of Contents

List of abbreviations

ABO	Blood groups A, B and O
APGAR	Appearance, Pulse, Grimace, Activity and Respiration
GSH	Groote Schuur Hospital
HPLC	High Performance Liquid Chromatography
ICU	Intensive Care Unit
RBC	Red Blood Cell
TcB	Transcutaneous Bilirubin
TSB	Total Serum Bilirubin
UDPGT	Uridine Diphosphoglucoronyl Transferase

Definition of terms

Neonate: An infant from birth to 28 days of age.

Preterm Neonate: Neonate delivered before 37 completed weeks of gestation.

Neonatal jaundice: Yellow discoloration of the skin of a neonate.

Total Serum Bilirubin: Bilirubin level determined by performing a blood test

Transcutaneous Bilirubin: Bilirubin level determined by transcutaneous bilirubinometer.

PART A (THE PROTOCOL)

CHAPTER ONE

Introduction

Neonatal jaundice is one of the most common clinical signs encountered among newborn babies. It results from deposition of bilirubin (yellow pigment) in the skin and mucus membranes. The main source of bilirubin is the breakdown of red blood cells in the reticuloendothelial system. Bilirubin is transported to the liver where it is conjugated to a water soluble product that is easily excreted. Immaturity of the neonatal liver enzyme system is the main factor placing them at risk of developing jaundice [1-4].

Unconjugated bilirubin has been noted to cross the blood-brain barrier, causing encephalopathy in the immediate period, and potential for long term choreoarthetoid cerebral palsy and other complications. [4-7]. The mainstays in preventing and managing bilirubin encephalopathy are the use of phototherapy and exchange transfusion which have been subjects of rigorous investigation over the last 60-70years [8,9].

The need to prevent bilirubin induced neurologic damage necessitates repeated blood withdrawal in order to ascertain bilirubin levels. Non-invasive, painless screening by use of transcutaneous bilirubinometry is becoming more acceptable especially for term and near term neonates [10,11]. However, for preterm neonates the use of transcutaneous bilirubinometry is still under scrutiny.

Transcutaneous bilirubin measuring devices have undergone modifications to enable them to overcome previous inconsistency of results in lower gestational ages and different skin pigmentations. Studies have shown the results from newer generation devices correlate well with serum bilirubin irrespective of skin pigmentation [12-14] but with respect to gestational age the findings are divergent [15-17].

Some studies among preterm neonates show acceptable correlation between transcutaneous and serum bilirubin levels [18-20]but no such study has been conducted in South Africa. The purpose of this study is to establish the correlation between transcutaneous and serum

bilirubin levels among preterm neonates <35weeks gestational age and its utility as a screening tool in the South African preterm population.

Literature review

Epidemiology of neonatal jaundice

Neonatal jaundice is a frequently observed sign. It occurs in 60% of term newborn infants and 80% of preterm infants during the first week of life [11].

Pathogenesis of neonatal jaundice

When red blood cells breakdown; the haemoglobin released is split into heme and globulin. The heme molecule is oxidised to iron and biliverdin by the enzyme heme oxygenase in the reticuloendothelial system. Biliverdin is then reduced to bilirubin by the enzyme biliverdin reductase. The bilirubin produced is transported to the liver tightly but reversibly bound to albumin [3,21,22].

At the hepatocyte, bilirubin is released from the albumin and is taken up by ligandin. Bilirubin is then conjugated to bilirubin mono- then diglucoronide by the enzyme uridine diphosphoglucoronyl transferase (UDPGT). This product is water soluble, and is secreted into bile [1,3,23].

Conjugated bilirubin in the gut is reduced by intestinal flora to urobilinogen and a small amount is hydrolysed by intestinal β-glucoronidase to unconjugated bilirubin which may get reabsorbed. Neonates especially those exclusively breast feeding have increased activity of β-glucoronidase [24].

There are many factors that predispose newborn infants to neonatal jaundice. Firstly, neonates produce bilirubin at a rate of 8-10mg/kg/24hours compared to adults 3-4mg/kg/24hours yet the liver enzyme system necessary for conjugation of bilirubin in the newborn is only 1% of adult level [1,25]. Apart from newborn babies having very low levels of UDPGT and increased β glucoronidase activity, they also have higher haemoglobins. These red cells have a shorter half-life of 75 days compared to adult red blood cells which have a

half-life of 90 days [26]. There is also an increased entero-hepatic circulation as a result of low volume of feeds [27,28].

When the amount of bilirubin produced is greater than what the liver can conjugate and albumin can bind, the free lipid soluble unconjugated bilirubin easily crosses biological membranes and accumulates in the skin giving the yellow discoloration[4]. Unconjugated bilirubin also crosses the blood-brain barrier and at high levels is neurotoxic [4,7,29].

Although the majority of neonates develop physiological jaundice due to the reasons discussed above, some newborns develop pathological jaundice from secondary causes. These include haemolysis of red blood cells due to blood group incompatibility, sepsis, enzyme deficiency (G6PD, pyruvate kinase), RBC membranopathy, and hemoglobinopathies. Other conditions such as Crigler Najjar syndrome and, hypothyroidism, may impair conjugation whilst intrinsic liver pathology and obstructive conditions such as biliary atresia, would increase the level of congujated bilirubin.

Bilirubin measurement

Different methods of estimating the amount of bilirubin in the serum have been developed. The gold standard is the use of high performance liquid chromatography (HPLC), this method is not used routinely in laboratories but reserved mainly for research laboratories [3].

The traditional method used in laboratories is the diazo method. This involves reacting serum with diazo reagent and then through spectrophotometry the levels of direct and indirect bilirubin are determined [3].

Non-invasive methods

Visual assessment

This was the first non-invasive way of jaundice assessment to be described. It was noted as early as 1940 that the level of jaundice correlated with the serum bilirubin but it was easy to miss infants with high levels of bilirubin. Studies have noted that there is cephalo-caudal progression of jaundice with raising levels of bilirubin and a Kramer scale which maps out

estimated bilirubin levels for different body parts was developed in 1969. Although this scale was developed there were wide bilirubin ranges for each zone [30].

Colour scale (icterometer)

In the early 60's an icterometer which constituted a strip with different shades of yellow was developed to which the infants' skin colour was compared [31,32].

Transcutaneous bilirubinometry

Using the principle of reflectance of light, a handheld device that generates light, was developed. It transilluminates the subcutaneous tissue then absorbs the reflected light and transmits it through a spectrophotometric module to measure the intensity of yellowness. This intensity can be used to estimate the amount of bilirubin in the skin. These devices have undergone modifications to correct for skin pigmentation [33,34].

CHAPTER TWO

Problem Statement

Preterm neonates are at increased risk for bilirubin encephalopathy and kernicterus [2]. The need to prevent these necessitates repeated blood withdrawals to determine bilirubin level in order to monitor levels. This process involves inflicting pain and distress to the neonate, it also places the neonate at risk for cellulitis and osteomyelitis from heel pricks as well as anaemia from blood withdrawals.

In our institution, routine total serum bilirubin is ordered at six hours of age among all neonates born to rhesus negative and group O positive mothers. This is also done at any age if any neonate appears clinically jaundiced. This constitutes a large workload for the nursing, clinical and laboratory staff as well as being a considerable financial cost for the institution.

Repeated total serum bilirubin measurement is costly, sometimes unnecessary, and the results are only obtained several hours after taking the specimen. There is risk of mislabelling or misplacing the specimen. All the above might be solved by a non-invasive, point-of-care technique like the transcutaneous bilirubinometer if its performance correlates well with the standard serum bilirubin measurement.

Justification

Transcutaneous bilirubin measurement is a non-invasive, painless, and blood sparing procedure which yields results promptly.

Findings from different studies have revealed transcutaneous bilirubin measurement to be cheaper compared to serum bilirubin measurement and its use as a screening tool has been shown to reduce number of blood withdrawals for bilirubin level measurement [14,18,35]. This minimizes pain, risk of infection and blood loss for the preterm neonate.

Studies from different parts of the world have shown acceptable correlation between transcutaneous and serum bilirubin level among preterm neonates [18-20] but further studies for preterm infants have been recommended by organizations such as the American Academy of Paediatrics.

Furthermore, no study among preterm neonates has been conducted in South Africa, and it is hoped that this study will provide useful data for the South African preterm population.

CHAPTER THREE

Methods

Study design

This will be a cross sectional study among preterm neonates less than 35 weeks of gestational age admitted to the Nursery at Groote Schuur Hospital.

Study setting

This study will be conducted at Groote Schuur Hospital Nursery.

Groote Schuur Hospital is a tertiary level teaching hospital in Cape Town South Africa and a teaching hospital for the University of Cape Town. It is a referral hospital for the Western Cape Province.

The nursery is level III care unit with 8 ICU spaces, 12 high care spaces, 20 observational care spaces, 10 special care space, 15 pre-Kangaroo mother care spaces and 10 kangaroo mother care (KMC) spaces. The nursery on average admits 200 neonates per month with 10% of admission constituting neonates < 1500g.

Serum bilirubin is ordered at 6 hours of age among all neonates born to Rhesus negative mothers and blood group O positive mothers and also among all neonates who clinically appear jaundiced. From review of records 94% of neonates weighing <1500g admitted to the nursery will have their serum bilirubin determined for one of the above reasons.

A sample for serum bilirubin is obtained by heel prick. This is routinely done by the nurses who are mandated to order the test if the neonate fulfils any of the above criteria. The nursery has one transcutaneous bilirubinometer JM103® type which currently is only used on term neonates.

Study questions

i) How does transcutaneous bilirubin measurement compare to serum bilirubin measurement among preterm neonates < 35weeks admitted to Groote Schuur Hospital nursery?

ii) To what extent can transcutaneous bilirubin measurement among preterm neonates < 35 weeks gestational age be used to decide on initiating phototherapy?

Objectives of the study

Primary objective:

To determine how transcutaneous bilirubin (TcB) levels relate to total serum bilirubin (TSB) levels in preterm neonates not under phototherapy.

Secondary objectives

i) To determine how transcutaneous bilirubin levels would influence decision to start phototherapy.

ii) To determine how skin colour, site of transcutaneous bilirubin measurement and gestational age influence the correlation between the two methods.

Study population:

All preterm neonates < 35weeks by gestational age admitted to the nursery of Groote Schuur hospital.

Inclusion criteria:

i) Preterm neonate less than 35weeks gestational age and less than 7 days old.

ii) Preterm neonate less than 35weeks gestational age whose TSB has been ordered.

Exclusion criteria:

i) Preterm neonate who is oedematous

ii) Preterm neonate with poor perfusion (capillary refill >3seconds)

iii) Preterm neonate who has started phototherapy

Sample Size estimation:

Sample size needed to determine the correlation between TcB and TSB:

Hypothesis test:

Null hypothesis (H0): p = 0

Alternative Hypothesis: p< >0

Alpha (α): 0.05

Power (1-β): 0.9

Effect Size: As determined by Jacob Cohen in *A Power Primer*.

Results for an effect size using correlation obtained from earlier study of 0.79[36]

	RESULTS for double sided			
Alpha	Power			
	0.6	**0.7**	**0.8**	**0.9**
0.1	8	9	11	14
0.05	9	11	13	**17**
0.01	13	16	18	23
0.001	19	22	26	31

The sample size required is 17.

Sample size needed to plot Bland-Altman plot [37]

Bland-Altman plots are used to determine agreement between two measurement methods.

The difference between TcB and TSB *(di = TcB - TSB)* is plotted against the mean *(M = (TcB+ TSB)/2)*

If we assume the differences between TcB and TSB have a normal distribution, for this study the difference in upper and lower limit of TcB measured to which intervention will not defer is considered to be +/- 20µmol/l (1.17mg/dl), this is the limit of agreement (LOA) between the tests. This assumption is based on the fact that at GSH if TSB is > 20µmol/l below the photo-line then phototherapy is not initiated.

The tolerance interval factor k_2:

$$k_2 = \frac{LOA}{\hat{\sigma}_d} = C$$

LOA = 20µmol/l (1.17mg/dl)

Standard deviation $\hat{\sigma}_d$ = 1.9, 1.3 and 1.6 for 24-28weeks, 29-30weeks and 31-34weeks

Alpha 0.05

$$k_2 \text{ (24-28weeks)} = \frac{1.17}{1.9} = 0.67$$

$$k_2 \text{ (29-30weeks)} = \frac{1.17}{1.3} = 0.9$$

$$k_2 \text{ (30-34weeks)} = \frac{1.17}{1.6} = 0.73$$

With reference to table of tolerance factor for normal distribution the k_2 value yields sample size of > 50 for all.

Sample size estimation for ROC, positive and negative predictive values

According to ET Schmidt findings, the correlation between TcB and TSB ranged from 0.79 to 0.92, and sensitivity ranged from 0.67 to 1.0 [36] assuming a correlation between TcB and TSB among preterm neonates is 80%, assuming TcB has a sensitivity of 80% in predicting

need to start photo therapy with 95% confidence, the sample size need to demonstrate this with power of 90% is

		TsB		
	Outcome	Y	N	
TcB	Y	50	12	62
	N	12	48	60
		62	60	122

A total of 122 preterm neonates will have to be enrolled.

Considering the above three sample sizes, the largest with **122** preterm neonates will be used.

Data collection

Study instruments

Data capturing sheets will be used to capture baseline information including mode of delivery, cause for preterm delivery, gestational age, birth weight, skin colour and age when TSB and TcBs were determined. TcB and TSB levels, phototherapy and exchange transfusion line will also be entered into the data capturing sheet.

Study Procedure

Screening and enrolment: Written consent will be obtained from parents of preterm neonates who meet the inclusion criteria. Transcutaneous bilirubin will be measured within 30 minutes of blood collection for TSB using JM 103® equipment on forehead, chest (over the sternum) and interscapular region of the baby.

Neonates' gestational age as determined by early ultra sound (< 20 weeks) if available or by the modified Ballard score [38], the skin colour, TcB and site of measurement, TSB,

phototherapy and exchange transfusion line at time of transcutaneous measurement will be entered in a data capturing sheet.

Specimen collection and TSB measurement: Using aseptic technique, 0.5ml of blood will be collected via heel prick and sent to the laboratory promptly. Using the diazo method, the level of serum bilirubin will be determined.

Patient management: Study participants will be managed in accordance with the units' protocol. Phototherapy will be initiated based on total serum bilirubin levels.

Data management and analysis

Data collecting sheet will be checked for completeness and stored securely in a locker.

Data from the collecting sheet will be entered into a computer using Microsoft Excel 2012 package. It will then be exported into statistical programme STATA version 11 software package for analysis with the help of a statistician.

Continuous variables (age, sex, gestational age, birth weight e.t.c) will be summarized as mean, median and standard deviation. A table of baseline characteristics will be generated.

Data will be stratified by gestational age and to measure the relation between TcB and TSB. The correlation coefficient will be computed, a scatter plot for TcB from different sites will be plotted against TSB, and absolute difference between TcB and TSB will be computed.

The sensitivity, specificity, negative and positive predictive value, likelihood ratios, and area under the receiver operator curve of TcB will be computed utilising the photo-line and TSB result.

Quality Control

Data collecting sheet will be pretested prior to commencement of study. On commencement the data collecting sheet will be checked daily for completeness. Transcutaneous bilirubinometer equipment will be calibrated daily and used according to the manufacturer's

instructions. The principal investigator will ensure that TcB measurements are done within 30 minutes of blood collection for TSB, and will also ensure that blood collected for TSB is sent to the laboratory promptly and personally follow-up on the results.

A log book will be in place to enter all neonates enrolled. This will help prevent a neonate being tested more than once.

Ethical consideration

The findings of this study are aimed at improving neonatal care by minimizing pain and other complications of heel pricks. It would also hopefully, minimize the risks of needle stick injury to the health care workers and decrease the overall cost of neonatal care.

Approval to conduct this study will be sought from the institution Research and Ethics committees (Human Research and Ethics Committee HREC).

For preterm neonates who meet inclusion criteria, a detailed explanation of the procedure will be given to parents by the principal investigator and a translator in the event that parents do not understand English. Written consent will be sought from them.

Dissemination of results

Results of this study will be submitted to the School of Graduate Studies at University of Cape Town. Copies will be submitted to the School of Child and Adolescent Health (SCAH), the department of neonatology and efforts will be made to publish them in peer reviewed journal.

PART B (STRUCTURED LITERATURE REVIEW)

OBJECTIVES OF LITERATURE REVIEW

The objectives of this review were to examine the performance of transcutaneous bilirubinometry in preterm infants by:

1. Identifying literature on non-invasive bilirubinometry and the mechanisms by which they operate.
2. Identifying literature on transcutaneous bilirubinometry among preterm neonates.
3. Identifying knowledge gaps with respect to transcutaneous bilirubinometry in preterm neonates.

LITERATURE SEARCH STRATEGY

A comprehensive literature search was undertaken through PUBMED and the Cochrane library. The key search words used were: jaundice, transcutaneous bilirubin, preterm neonates and non-invasive bilirubin estimation.

For transcutaneous bilirubin estimation, the search was restricted to preterm neonates not under phototherapy, late preterm neonates were excluded. Studies that had a combination of children under phototherapy and those not under phototherapy were included only if during analysis they made this distinction.

Only literature where the two common transcutaneous bilirubinometers (Bilicheck and Minolta JM 103®) were used, was included. No year limit was set for the search.

Standard Neonatology text books and up-to-date information were used mainly for literature on pathogenesis of jaundice. The references from these sources were referred to for additional information.

Background

Description of the problem

Neonatal jaundice results from deposition of bilirubin (yellow pigment) to the skin and mucus membranes. The main source of bilirubin is the breakdown of red blood cells in the reticuloendothelial system. Bilirubin is transported to the liver where it is conjugated to a water soluble product that is easily excreted. The ability of the newborn to clear bilirubin is diminished due to a deficiency of the glucoronyl transferase enzyme which is only about 1% of the adult level on day 7 of life and even less before this [3,39,40].

Unconjugated bilirubin is known to cross the blood-brain barrier where it induces neurotoxicity and encephalopathy in the immediate period, and possible choreoarthetoid cerebral palsy or other complications in the long term [5-7,41].

Types of intervention

Bilirubin induced neurotoxicity can usually be prevented through the use of phototherapy which is known to convert unconjugated bilirubin by photo isomerization, photo oxidation and structural isomerization into more water soluble products [4,8,42].

At much higher serum bilirubin levels, exchange transfusion is the modality of choice. Exchange transfusion works by removing unconjugated bilirubin from circulation and also through the removal of maternal antibodies which may break down red blood cells [9,43,44].

Other modalities of treatment which include the use of intravenous immunoglobulin, tin protoporphyrin and clofibrate are not relevant for this review [45].

Diagnosis of high serum bilirubin levels

Phototherapy and exchange transfusion thresholds have evolved over time and include the Bhutani bilirubin centile charts and the South African modified chart [46-48]. However, the most crucial aspect to jaundice management is accurately measuring the bilirubin level.

The gold standard method of establishing total serum bilirubin level is through chromatographic analysis such as high performance liquid chromatography (HPLC) and reflectancy fluorometry [49,50]. These methods are usually reserved for research purposes. Under routine clinical conditions, the diazo method is used and results from this method have been noted to vary marginally from those of HPLC [51-53].

The quest for a non-invasive method of bilirubin measurement has been on going. The Kramers head to toe prediction using visual inspection [30] was among the first modalities to be developed but it was replaced by an icterometer method that uses a strip with different hues of yellow for comparison [32].

The visual methods have been discouraged [11] for their inaccuracies and have been overtaken by more objective methods that use skin reflectancy of light and analysis in a spectrophotometer [33].

How do the transcutaneous bilirubinometers work

One of the objectives of this literature search was to understand how transcutaneous bilirubinometers operate. The two commonly used transcutaneous bilirubinometer are the Bilicheck and the Minolta JM 103® bilirubinometer.

The concept behind these transcutaneous bilirubinometers is light reflectancy; the subcutaneous tissue is trans-illuminated and light is reflected back to the machine. The reflected light is carried by a fibre optic bundle to a spectrophotometer where it is split into a spectrum and absorption at different spectra correct for the haemoglobin level. The amount of yellow spectrum colour is processed electronically to estimate the bilirubin level in the skin [54].

Description of the studies

Eleven studies were identified; six of these met the inclusion criteria. Majority of the studies were conducted in European countries and one study was conducted in The United States of America. No study conducted in Africa on this subject was identified. The sample size in the included studies ranged from 24 to 340 preterm neonates.

Five studies were excluded; one used bilimed device[55], one assessed babies under phototherapy[56], one combined population of ill term and preterm neonates during analysis[57] and two used the Minolta JM 102 bilirubinometer [58,59]an older version of the JM 103® bilirubinometer.

Findings from the included studies

Table 1 shows summary of studies that were included in this literature review.

What all the included studies show is a statistically significant correlation coefficient between transcutaneous bilirubin and serum bilirubin among preterm neonates. Some studies found better correlation with lower gestational age [19,36] with one finding the opposite [15].

Three studies looked at the influence of postnatal age on correlation coefficient between transcutaneous and serum bilirubin. Knupfer and Schmidt et al [15,36] reported no effect while Ebbesen et al reported no difference with the Bilicheck but with the Minolta JM 103® postnatal age was found to be a confounder [60].

Two studies looked at the relation between correlation coefficient and ethnicity; Schmidt et al with 16 African American infants included found no association[36] and Ebbesen et al with only 6 Africans included in their study reported no association while using Bilicheck but significant differences when using the Minolta JM 103® (p 0.001)[60].

Table 1. Summary of studies included in literature review

Study Device (site)	Sample size	Results
Knupfer et al 2000[15] Bilicheck (forehead)	135	-r 0.73 ($p < 0.05$) among infants not on phototherapy. -Correlation was poor at low gestational age -Mean difference TSB and TcB: 14.4±49.1µmol/l - to start phototherapy sensitivity 88%, specificity 89%, NPV 97.4%, PPV 62.2% -Reliability- good among > 30weeks
Willems et al 2001[20] Bilicheck (forehead)	24	-r 0.86 ($p < 0.001$) -Mean difference TSB and TcB: Good skin: 2.42± 19.56µmol/l Poor skin: 12.25± 32.28µmol/l
Deluca et al 2006[17] Bilicheck (forehead)	340	-r 0.795 ($p <0.001$) - Mean difference: TSB and TCB:18.1 ± 34.2 µmol/l -TcB overestimated TSB in 61.5% -Negative predictive value ranged from 97.2% - 99.6%
Stillova et al 2008[19] Minolta103® (sternum, (forehead, abdomen)	32	-r head:0.9, sternum:0.84, abdomen:0.8 ($p<0.001$) -Forehead underestimates while abdomen overestimates serum bilirubin.
Schmidt et al 2009[36] Minolta103® (sternum)	90	r 0.88($p<0.001$) -Mean difference TSB and TcB:13.6 ±B32.3µmol/l
Ebbesen et al 2008[60] Bilicheck and Minolta 103® (forehead)	133	-r bilicheck: 0.83, Minolta: 0.86 -Minolta underestimated TSB for almost all cases while Bilicheck underestimated at high TSB levels

r: correlation coefficient
TSB: total serum bilirubin
TcB: Transcutaneous bilirubin
PPV: positive predictive value
NPV: Negative predictive value

Four of these studies compared how transcutaneous bilirubin measurements estimated serum bilirubin levels. Only one study reported a tendency of transcutaneous measurements to

overestimate serum levels [17] while the other three reported a tendency to underestimate [19,36,60].

Only one study looked at different body sites and found transcutaneous bilirubin from the forehead tends to underestimate, while sternum sites were closer to serum levels. Abdominal readings tended to overestimate serum bilirubin levels [19].

From these studies it seems that the correlation of transcutaneous and serum bilirubin vary depending on the site from which they are taken and the gestational age of the neonate. One possible explanation is the deposition of subcutaneous tissue.

A literature search on deposition of subcutaneous fat in the fetus elaborated on the interscapular site as one of the sites where there is early deposition [61]. No studies in my literature search had explored this site for transcutaneous bilirubinometry.

Also from my literature search I found no study has been conducted in South Africa with respect to transcutaneous bilirubinometry among preterm neonates.

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PART C (RESULTS)

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1st November 2012

The Editor,

Archives of Diseases in Childhood,
Fetal and Neonatal Edition,

Dear Sir/ Madam

RE: SUBMISSION OF ORIGINAL ARTICLE FOR PUBLICATION

I hereby submit our original research report titled "Correlation between transcutaneous bilirubin and total serum bilirubin levels among preterm neonates at Groote Schuur Hospital" to be considered for publication. None of the authors have any conflict of interest to declare.

Thank you.

……..

Yours Faithfully,

Dr. Abdallah Yaser

CORRELATION BETWEEN TRANSCUTANEOUS BILIRUBIN AND TOTAL SERUM BILIRUBIN LEVELS AMONG PRETERM NEONATES AT GROOTE SCHUUR HOSPITAL

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KEY WORDS: Preterm neonates, transcutaneous bilirubin, interscapular transcutaneous bilirubin.

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ABSTRACT

Background: High levels of unconjugated bilirubin in the newborn can cause brain injury. To prevent this; repeated blood withdrawals are necessary to ascertain bilirubin levels and institute care. Non-invasive, painless and bloodless screening through transcutaneous bilirubinometry is the standard of care for term and near term neonates but among preterm neonates this is under scrutiny.

Objective: The aim of this study was to determine how transcutaneous bilirubin (TcB) level relates to total serum bilirubin (TSB) level among preterm neonates at Groote Schuur Hospital Nursery.

Design: This was a cross sectional study.

Methods: Over a 5 months period 122 consecutive preterm neonates < 35 weeks gestational age were enrolled. TcB's were measured over the forehead, sternum and interscapular area. Pearson correlation coefficients and differences between TSB and TcB's were computed. P value <0.05 was considered significant and confidence interval of 95% was used.

Results: The median gestational age of study participants was 31 weeks (range: 24-34 weeks), the median TSB level was 81.5µmol/l (range: 25-229µmol/l) and 45% had TSB at phototherapy threshold. The correlation coefficients for TcB's ranged from 0.859 to 0.929 ($p<0.001$). The difference between TSB and TcB's ranged from -86 to +51µmol/l. With respect to initiating phototherapy, the interscapular site had the highest sensitivity (93.9%) and lowest false negative rate (6.06%). Interscapular TcB cutoff value of 52umol/l could identify accurately neonates needing phototherapy with sensitivity of 95.5% and specificity of 35.7%.

Conclusion: TcB's from the sites assessed correlated significantly with TSB. Interscapular TcB screening identifies correctly the majority of preterm neonates who need phototherapy.

Background

Neonatal jaundice is one of the most common clinical signs encountered among newborn babies. Immaturity of the liver enzyme system among neonates is the main factor predisposing them to developing jaundice [1-4].

Unconjugated bilirubin can cross the blood-brain barrier and induce encephalopathy acutely and in the long term survivors' possible cerebral palsy [4-7]. The need to prevent this necessitates repeated blood withdrawal to ascertain bilirubin levels and institute early care.

Non-invasive, painless screening for jaundice by use of transcutaneous bilirubinometry is the standard of care for term and near term neonates [10,11]. Preterm neonates are susceptible to bilirubin encephalopathy at lower serum bilirubin level hence; despite some studies showing good correlation [18-20] the pursuit for more accurate screening test has continued to be explored.

The majority of studies using transcutaneous bilirubinometers have used the forehead and the sternum. We could find no literature on the use of the interscapular area.

The purpose of this study was to establish the correlation between transcutaneous and serum bilirubin levels among preterm neonates <35weeks gestational age and its utility as a screening tool in the South African preterm population.

Ethical approval: Ethical approval was obtained from the Human Research and Ethics Committee of the University of Cape Town and written consent was obtained from parents of study participants.

Methods

Setting: This study was conducted at Groote Schuur Hospital which is a level III neonatal care unit. The unit admits 200 neonates per month 10% weighing < 1500grams at birth.

Study population: Preterm neonates of gestational age < 35 weeks who were < 8 days old in whom serum bilirubin had been requested and phototherapy had not been initiated were

eligible. Eligible neonates who had poor perfusion (capillary refill >3 seconds) were excluded.

Sample size: Computed sample size for positive and negative predictive value assuming transcutaneous bilirubin had a sensitivity of 80% in predicting need to start photo therapy with 95% confidence, the sample size need to demonstrate this with power of 90% was 122.

Procedure: A cross sectional study was conducted between May 2012 and September 2012. Of the 306 preterm neonates <35 weeks gestational age admitted to the unit, 122 were enrolled for the study, see figure 1.

Transcutaneous bilirubin (TcB) was measured on the forehead, chest (sternum) and interscapular area by the principal investigator using a Minolta JM103® bilirubinometer. These measurements were obtained within 30 minutes of blood withdrawal to establish serum bilirubin (TSB) levels. The bilirubinometer was calibrated once daily and used according to manufacturer's instruction. An average of three measurements was recorded for each site.

Data management: Baseline characteristics of study participants (gestational age estimated by early antenatal ultrasound or Ballard score, birth weight, and gender) were captured. Time of blood drawing for measuring serum bilirubin level, transcutaneous bilirubin measurements, total serum bilirubin level and phototherapy line were entered into data capturing sheet. Data was entered into Microsoft excel 2010 package then transferred to STATA version 11 for analysis. P value <0.05 was considered significant and confidence interval of 95% was used.

Statistical methods: Shapirow wilk w test was used to classify distribution of data. Spearman's correlation was computed for the different TcB's, and using 2x2 tabulation with point of interest being initiation of phototherapy the sensitivity, specificity, positive and negative predictive values were computed. Receiver operating curve were also generated.

Results: The median gestational age of the study participants was 31 weeks (range: 24-34weeks), the median birth weight was 1242 grams (range: 608- 2560grams), 32 (26.2%) of study participants were small for gestational age (weight < 10th centile for gestational age), the median age when blood was drawn for serum bilirubin measurement was 21 hours (range: 4- 136hours) 65.5% of these were obtained within the first 24 hours, and the median serum bilirubin was 81.5µmol/l (range: 25- 229µmol/l). Ninety four percent (94%) of study participants' skin colour was described as pink and the rest as red. Table 1 shows baseline characteristics of study participants.

TcB from sites studied (forehead, chest and interscapular) had statistically significant correlation with total serum bilirubin. The site with highest correlation coefficient was the chest (Cr 0.929 p<0.001). Table 2 shows correlation and difference between TSB and TcB.

The correlation coefficients did not differ significantly between study participants of gestational age ≤ 30 weeks and those > 30 weeks. Table 3 show correlation and difference between TSB and TcB at different gestational ages.

The difference between TSB and TcB's ranged from -86µmol/l to +51µmol/l with one outlier in whom the interscapular TcB underestimated TSB by 118µmol/l. Interscapular TcB tended to overestimate TSB.

Graph 1 shows the correlation of transcutaneous and serum bilirubin at different TSB levels. From the graph it is evident that there was a tendency for TcB to underestimate TSB at low TSB levels and to overestimate at high levels.

With respect to initiating phototherapy, the interscapular site had the highest sensitivity (93.9%). Table 4 shows sensitivity, specificity and false negative rates of transcutaneous bilirubin. The chest had the highest specificity (94.6%) while the interscapular area had the lowest false negative rate (6.06%).

From the Receiver Operative Curve, the area under the curve for all the three sited were comparable. Transcutaneous bilirubin cut off values of 42, 39.5 and 52 for forehead, chest

and interscapular site had sensitivities of 97%, 95.5%, and 95.5% respectively and specificities of 39.3%, 41% and 35.7% respectively, see figure 2, table 5 and 6.

Discussion: The correlation coefficient of forehead TcB of 0.904 ($p<0.001$) found in this study was higher than that described by other studies [15,17,20,60] while the correlation coefficient of chest (sternum) TcB of 0.929 ($p<0.001$) is comparable to the finding of Schmidt et al over the sternum 0.79 to 0.92 ($p<0.001$)[36].

The correlation coefficients for forehead and chest TcB's were higher among neonates of gestational age ≤ 30 weeks compared to those > 30 weeks. Although Knupfer et al using Bilicheck [15] found an increased discrepancy between serum bilirubin and forehead TcB with reducing gestational age, Ebbesen et al [60] using Minolta JM 103® found no association. Schmidt et al demonstrated an increase correlation of sternal TcB with decreasing gestational age [36].

The discrepancy in correlation of forehead and total serum bilirubin may be due to the fact that the forehead is continuously exposed to ambient light hence results varying from infant to infant.

The interscapular site has previously not been studied. This study included this site for two reasons: firstly it is often shielded from ambient light and secondly the interscapular area is one of the areas where subcutaneous fat is first deposited [61]. The correlation coefficient of interscapular TcB was 0.859 ($p<0.001$),but for one of the study participants interscapular TcB significantly underestimated TSB by 118µmol/l. When this neonate was excluded from the analysis the correlation was 0.89 ($p<0.001$).

The correlation of interscapular TcB among neonates of gestational age ≤ 30 weeks was 0.892 ($p<0.001$) and 0.844 ($p<0.001$) in those > 30 weeks.

The difference between TSB and TcB's from forehead and chest in this study ranged from

- 86 to + 44 µmol/l, and - 52 to +48µmol/l respectively. These differences did not vary much when study population was stratified by gestational age of ≤ 30 or > 30 weeks. Twenty one 21 (17.2%) of forehead and 9 (7.3%) of chest TcB overestimated while 24 (19.7%) of forehead and 32 (26.2%) of chest TcB underestimated TSB by >20µmol/l.

Similar to findings from this study, other studies using different transcutaneous bilirubinometers have also reported wide differences between TSB and TcB's measured over forehead and chest [15-17,20] with an overall tendency of forehead and chest TcB to underestimate the TSB. The reason for this is not clear but if might have to do with light exposure to the forehead and scarcity of subcutaneous fat in the sternal area.

The difference between TSB and interscapular TcB in this study was -80 to +51µmol/l with one outlier in whom the TcB underestimated TSB by 118µmol/l. This difference did not vary much when study population was stratified by gestational age of ≤ 30 or > 30 weeks.

Twenty nine 46 (37.7%) of interscapular TcB overestimated while 11 (9.0%) underestimated TSB by >20µmol/l. No study has previously assessed this site. It appears that the interscapular TcB tends to overestimate TSB with fewer cases underestimated compared to the forehead and the chest sites. The reason might be that this site has more adipose tissue.

In accordance with Groote Schuur Hospital / South African Guidelines [48], phototherapy is commenced when serum bilirubin level is at phototherapy line or ≤ 20µmol/l below the line. During data analysis this was put into consideration. Total serum bilirubin ≤ 20µmol/l within phototherapy line, at phototherapy line or above phototherapy line was categorized as an indication for phototherapy.

Table 4 shown sensitivity, specificity and false negative rates for using transcutaneous bilirubin as a trigger to start phototherapy. The sensitivity of forehead transcutaneous bilirubin in identifying study participants in need of phototherapy was 80.8% and comparable to Knupfer et al who found a sensitivity of 86.8% using the Bilicheck on the forehead.

Knupfer et al found an increase in sensitivity with an increase in gestational age similarly our study found the same.

From this study it seems that the chest was the poorest site of choice as many participants would not receive phototherapy when they actually needed it (30.3%). No study that we came across looked at sensitivity and specificity of the chest (sternum) transcutaneous bilirubin in determining need to initiate phototherapy without setting a cut off value.

The interscapular TcB had the highest sensitivity in identifying study participants in need of phototherapy (93.9%) and it wrongly mislabelled 6.06% of those in need of phototherapy. At a relatively higher cutoff value of 52umol/l intescapular TcB had a high sensitivity of 95.5% but low specificity of 35.7% although at this same cut off value forehead and chest TcB's had lower sensitivities of 92.4 and 84.8% respectively. At lower cutoff values the interscapular TcB maintained a higher sensitivity compared to the forehead and chest. No study has previously had a look at the interscapular site. It is a highly vascular site and one of the first sites where adipose tissue is deposited [61]. It is also often shielded from ambient light. All these characteristic make this site a favourable one for transcutaneous bilirubinometry.

Study Limitations

1. Transcutaneous bilirubin measurements were obtained by the principal investigator who ensured that the Minolta JM 103® bilirubinometer was calibrated and followed manufacturers' instruction. Under daily clinical practice if these are not observed the results might be highly varied.

2. Highest total serum bilirubin obtained was 229µmol/l, so the performance of this device at much higher serum bilirubin levels is not known and cannot be extrapolated.

3. These results only reflect the Minolta JM 103® bilirubinometer and these results cannot be applied when using a different bilirubinometer type.

Conclusion: TcB's from the sites assessed correlated significantly with TSB. The sensitivity of interscapular TcB in identifying preterm neonates who need phototherapy increased with increasing birth weight. The interscapular site is safe for jaundice screening of preterm neonates using Minolta JM 103® bilirubinometer.

What is already known on this subject

Although transcutaneous bilirubin measured from the forehead and sternum significantly correlate with the total serum bilirubin level, they have a tendency to underestimate total serum bilirubin levels.

The use of transcutaneous bilirubin screening is currently not recommended for preterm neonates < 35 weeks gestational age.

What this study adds

This study has shown that the interscapular TcB tends to slightly overestimate total serum bilirubin and may be a useful site to screen for jaundice.

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PART D (SUPPORTING DOCUMENTS)

APPENDIX 1: Informed consent form

Title of study: Correlation between transcutaneous bilirubin and total serum bilirubin levels among preterm neonates at Groote Schuur hospital

Dr. Abdallah Yaser from the Department of Neonatology is conducting a study in babies born before 35 weeks of pregnancy. He wants to find out how the level of yellow pigment (bilirubin) in skin compares to that in blood among these babies. We are approaching you for your consent in order to include your baby in the study.

Introduction:

The majority of babies born prematurely (<37 weeks of gestation) are at risk of turning yellow. This yellow discoloration is from breakdown of red blood cells which can be normal in newborn babies. The yellow pigment is normally broken down in the liver which tends to be less prepared among premature babies and this places premature babies at risk of turning yellow. High levels of yellow pigment (bilirubin) in blood can easily reach the brain and is known to cause brain damage. In order to prevent this blood is usually taken from babies to measure the amount of bilirubin and if found to be high the babies are placed under special lights. In order to reduce taking blood from babies for this purpose a special device has been developed that measures the amount of bilirubin in the skin. In term babies this method has been proved to be good. Using this method has not been studied among premature babies in South Africa yet this might help reduce number of pricks our preterm neonates suffer and also reduce amount of blood they lose

Purpose: The aim of the study is to find out how yellow pigment (bilirubin) measured by skin device compares to that measured in blood among preterm neonates <35weeks gestational age.

Procedure: Only children born <35weeks gestational age in whom blood has been taken to measure yellow pigment (bilirubin) will be involved in this study. If your child is enrolled we will use the device for measuring yellow pigment in the skin on his/her forehead, chest and back. Your child will continue to receive care in line with hospital guidelines.

Risks: There are no risks associated with the procedure your child will undergo. The procedure compares to gentle press with a finger. There will be no blood loss during the procedure.

Benefits: Although this study might not benefit your child directly, we believe the findings of this study will throw more light on this method of measuring yellow pigment among

premature babies. If the method is established to be good, premature babies will be spared from pricks and blood loss.

Costs: You will not be paid any incentive for your child's participation. You will not be charged for the tests this study will be using.

Patients' right: Your child's participation will be voluntary, and you have the right to refuse participating in the study. Your refusal to participate will not affect the care your child will receive. You have the right to know results obtained from your child.

Confidentiality: All information from you and your child including laboratory results will be kept confidential and will be used for research purposes only. Only information that we think will improve the care your child receives will be released to the doctors attending to your child.

Contact persons: If you have more questions and information that you may need clarification on, contact the following at any time:

Principal investigator, Dr. Abdallah Yaser, Tel: +27797027500,

E-mail: yasam7862002@yahoo.com

Dr. Natasha Rhoda, Tel: +27837000423, E-mail: Natasha.rhoda@uct.ac.za

Dr. Lloyd Tooke, Tel: +27834562575, E-mail:Lloyd.tooke@uct.ac.za

Department of Neonatology, Groote Schuur Hospital.

If you want to know about your right and your child's rights, contact the Chairman Human Research and Ethics Committee;

Prof. M Blockman Tel: +27214066626.

Statement of consent: The purpose and nature of this study has been explained to me and I understand that my child's participation in this study is voluntary and that no consequences will result if I refuse to participate. I understand that some tests will be carried out; I have the rights to know the results. I……. (Mother) do hereby consent for my child to participate in the above study.

Sign/thumb (mother) ……Date……..

Witness (Not the person administering consent form)

Name……..

Signature……..Date…….

Consent administrator

Name of Investigator……..

Signature……..……..Date……

APPENDIX 2: Data Capturing Sheet

Patients Sticker

Date and time of birth…… Mode of delivery……

Reason for preterm birth……..

Steroid mature……

Birth weight…… Sex..... Skin colour..... Ethnicity……

Ballard score…….. Early U/S gestational age est……..

Age in hours when TSB taken……..

Reason for TSB request……

Photo line…….. Exchange line…….

TcB result Forehead……

Chest.....

Interscapular.....

TSB result…….

APPENDIX 3: Study Time Frame

Jan 2012	Feb 2012	March 2012	April 2012	May 2012	June 2012	July 2012	Aug 2012	Sept 2012

January and February 2012: Ethical approval

March 2012: Final touches on paper work

April to July 2012: Data collection

August 2012: Data entry and statistical analysis

September 2012: Final results and dissertation submission

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

14 May 2012

HREC REF: 195/2012

Dr A Yaser
Neonatology
H49, OMB

Dear Dr Yaser

PROJECT TITLE: CORRELATION BETWEEN TRANSCUTANEOUS BILIRUBIN AND TOTAL SERUM BILIRUBIN LEVELS AMONG PRETERM NEONATES AT GROOTE SCHUUR HOSPITAL.

Thank you for responding to the issues raised by the Faculty of Health Sciences Human Research Ethics Committee in your letter dated 13th May 2012.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year till the 30th May 2013.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
pp **CHAIRPERSON, HSF HUMAN ETHICS**
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

s.thomas

Figure 1. Study profile

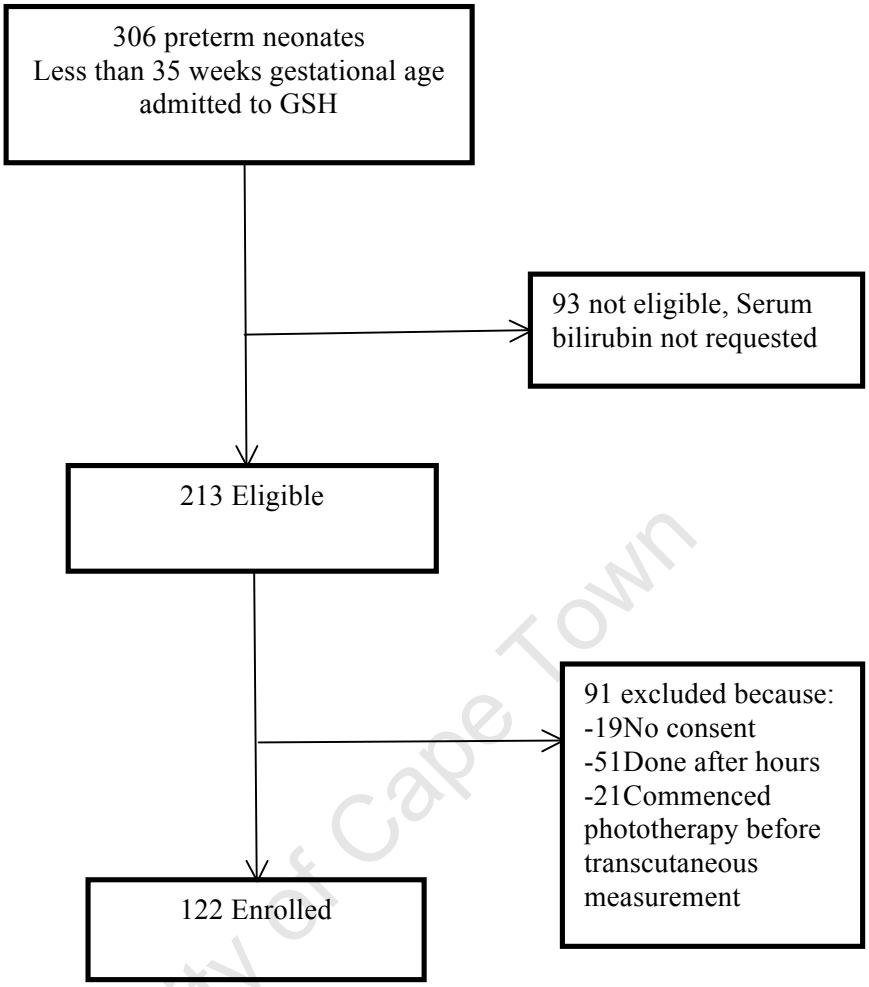

Table 1. Baseline Characteristics of study participants

Variables		Frequency (122)	percentage (%)
Sex	Female	66	54.0
	Male	56	46.0
Birth weight	≤1000grams	30	24.6
	1001-1499grams	62	50.8
	≥1500grams	30	24.6
Gestational age	≤30 weeks	51	41.8
	>30weeks	71	58.2
Steroid maturity	2doses	59	48.4
	<2doses	63	51.6
Age serum bilirubin done			
	≤ 12 hours	30	24.7
	13-24hours	50	41.0
	25-36hours	12	9.8
	35-48hours	9	7.3
	>48hours	21	17.2
Serum bilirubin levels*			
	<80micromoles/l	58	47.5
	80 – 150micromoles/l	51	41.8
	>150micromoles/l	13	10.6

*maximum serum bilirubin 229µmol/l

Table 2: Table of correlation and difference between total serum bilirubin and transcutaneous bilirubin from the three body sites

Test	Correlation co-efficient‡(Cr)	Difference (TSB - TcB)	Percentage over estimated/under estimated by >20µmol/l
TcB forehead	0.904 (p<0.001)	-86 – 44µmol/l	17.2/19.7
TcB Chest	0.929 (p<0.001)	-52 – 48µmol/l	7.3/26.2
TcB Interscapular	0.859 (p<0.001)	-80 – 118µmol/l	37.7/9.0

‡Spearman's correlation
TSB - Total serum bilirubin
TcB - Transcutaneous bilirubin

Table 3: Table of correlation and difference between total serum bilirubin and transcutaneous bilirubin from the three body sites with respect to gestational age.

Test	Correlation coefficient (Cr)	Difference (TSB-TcB)
TcB Forehead ≤ 30 weeks >30 weeks	0.923 (p<0.001) 0.894(p<0.001)	-86-43µmol/l -76-44µmol/l
TcB Chest ≤ 30 weeks > 30 weeks	0.965 (p<0.001) 0.908 (p<0.001)	-51-45µmol/l -52-48µmol/l
TcB Interscapular ≤ 30weeks >30weeks	0.892 (p<0.001) 0.844 (p<0.001)	-80-51µmol/l -76-37µmol/l (One outlier 118µmol/l)

Graph1: Correlation between transcutaneous bilirubin and total serum bilirubin.

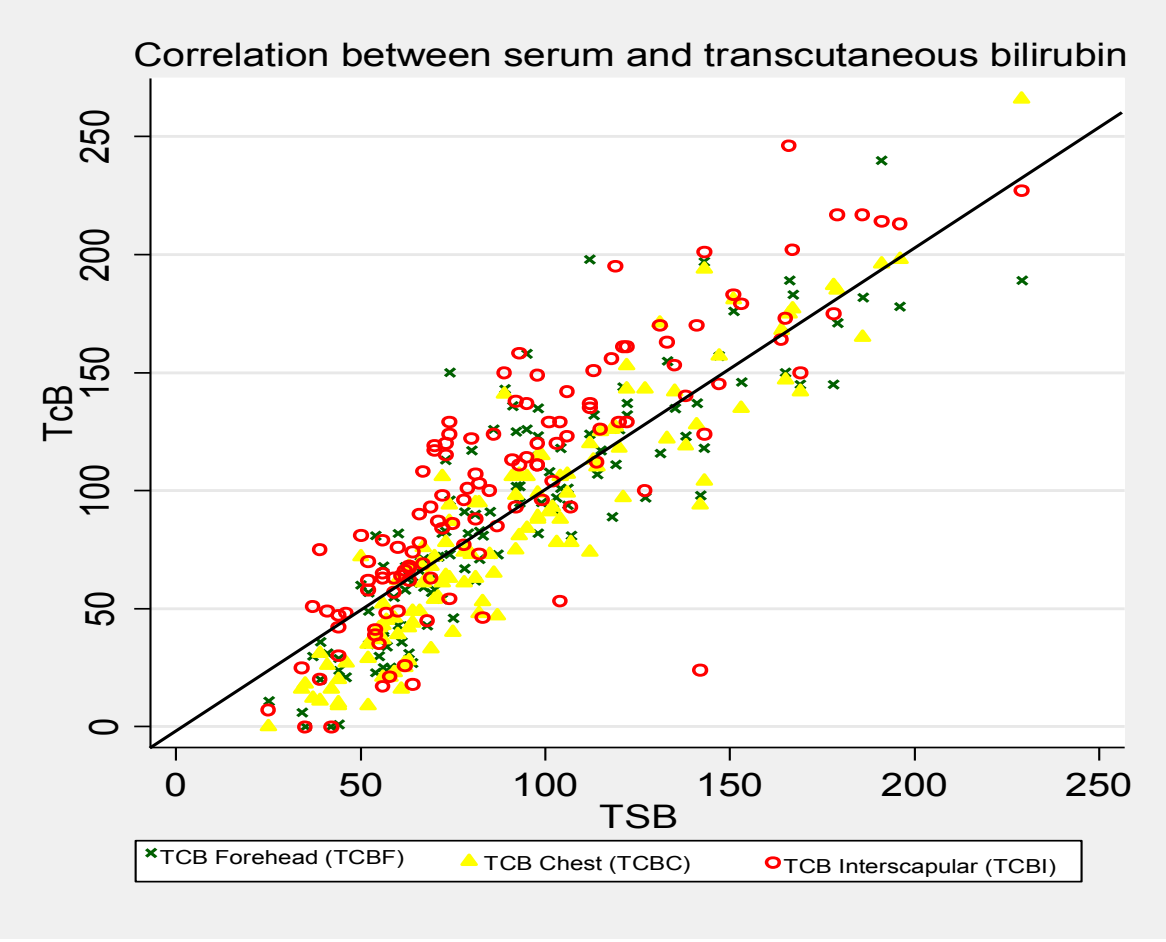

Table 4: Table of sensitivity, specificity and false negative rate of transcutaneous bilirubin from the three body site with respect to initiation of phototherapy

Test	≤ 30 weeks	>30weeks	Overall
Sensitivity			
TcBf	69.2%	87.5%	80.3%
TcBc	69.2%	70.0%	69.7%
TcBi	92.3%	95.0%	**93.9%**
Specificity			
TcBf	83.3%	87.5%	85.7%
TcBc	95.8%	93.7%	94.6%
TcBi	83.3%	62.5%	71.4%
False Negative rate			
TcBf	30.7%	12.5%	19.7%
TcBc	30.7%	30.0%	30.3%
TcBi	7.6%	5.0%	**6.06%**

95% confidence interval.
TcBf- Transcutaneous bilirubin forehead
TcBc- Transcutaneous bilirubin chest
TcBi- transcutaneous bilirubin interscapular

Fig 2 ROC

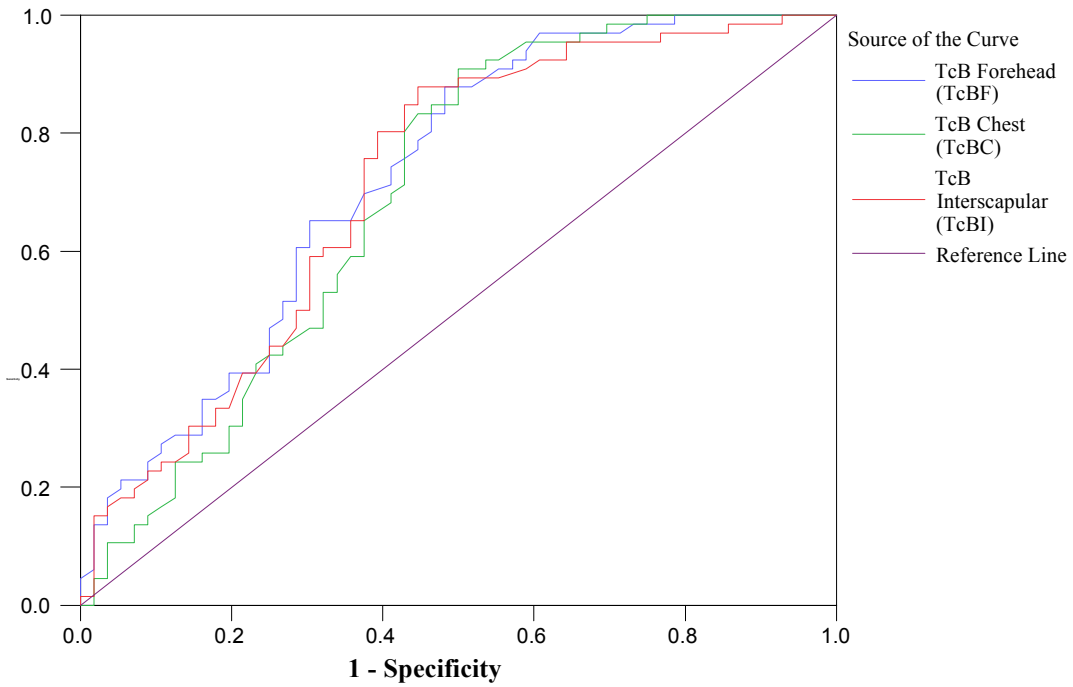

Table 5. Area under the Curve

Test Result Variables	Area	Std. Error[a]	Asymptomatic Sig.[b]	Asymptomatic 95% Confidence interval Lower Bound/ Upper Bound	
TcB Forehead (TCBF)	.726	.047	.000	.635	.817
TcB Chest (TCBC)	.697	.050	.000	.600	.795
TcB Interscapular (TCBI)	.714	.048	.000	.620	.808

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Table 6. Sensitivities and specificities of TcB's at best cutoff values for each site

Cutoff	**TcB Forehead**		**TcB Chest**		**TcB Interscapular**	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
52.0umol/l	92.4%	53.0%	84.4%	47.2%	**95.5%**	35.7%
42.0umol/l	**97.0%**	39.3%	92.4%	45.7%	97.0%	33.0%
39.5umol/l	97.0%	36.0%	**95.5%**	41.0%	97.0%	19.0%

APPENDIX 5:

The Group

Manuscript format

Cover letter

Title page

Manuscript format

Statistics

Style

Figures/illustrations

Tables

References

Supplementary files

All material submitted is assumed to be submitted exclusively to the journal unless the contrary is stated. Submissions may be returned to the author for amendment if presented in the incorrect format. It should be in both the manuscript and the details page during submission.

Please note that only the article text (from first word of main text to the last word in reference list) will be used to typeset your article.

All other data (known as the metadata), such as article title, author names and addresses, abstract, funding (etc) statements will be taken from the fields you have filled in at submission, so you must ensure that these are up to date and accurate.

Cover letter

Your cover letter should inform the Editor of any special considerations regarding your submission, including but not limited to:

1. Details of related papers published or submitted for publication.

Copies of related papers should be submitted as "Supplementary files not for review" to help the Editor decide how to handle the matter.

2. Details of previous reviews of the submitted article.

The previous Editor's and reviewers' comments should be submitted as Supplementary material along with your responses to those comments. Editors encourage authors to submit these previous communications - doing so may expedite the review process.

3. Indication as to whether any of your article (for example, appendices, large tables) could be published as Web only files rather than in the print version of the article. Please label any files for online publication only with this designation.

Title page

The title page **must** contain the following information:

1. Title of the article.
2. Full name, postal address, e-mail, telephone and fax numbers of the corresponding author.
3. Full names, departments, institutions, city and country of all co-authors.
4. Up to five keywords or phrases suitable for use in an index (it is recommended to use MeSH terms).
5. Word count - excluding title page, abstract, references, figures and tables.

Acceptance

Please note: If any of this information is repeated in the final Word document it will be removed by the typesetters and replaced with the information from the submission system. Therefore please check the metadata on ScholarOne Manuscripts carefully and make any changes before submitting the final version of your Word document.

List of the information taken from submission system only:

• Article type

• Title

• Author names

• Author affiliations, and corresponding author's full details

• Abstract (where applicable)

• Keywords

• Study approval

• Patient consent

• Funding statement

• Competing interests

• Contributor statement

• Trial Registration number (for clinical trials)

Manuscript format

Please note, this instruction is for submission only.

The manuscript must be submitted in Word. PDF format is not accepted.

The manuscript must be presented in the following order:

1. **Title page**.

2. **Abstract** (or summary for case reports) (note: references not allowed in abstracts or summaries).

3. **Main text** (provide appropriate headings and subheadings as in the journal. We use the following hierarchy: **BOLD CAPS**, **bold lower case**, Plain text, *Italics*).

4. **Tables** should be in the same format as your article (ie Word) and not another format embedded into the document. They should be placed where the table is cited and they must be cited in the main text in numerical order.

5. **Acknowledgments, Competing interests, Funding**.

6. **Reference list**.

Appendices (these should be Web only files to save space in the print journal; if so, please ensure you upload appendices as Web Only files and ensure they are cited in the main text as such.)

Images must be uploaded as separate files (view further details in Figures/illustrations) All images must be cited within the main text in numerical order.

Do not use the automatic formatting features of your word processor such as endnotes, footnotes, headers, footers, boxes etc. Please remove any hidden text.

Statistics

Statistical analyses must explain the methods used.

Guidelines on presenting statistics.

Guidelines on RCTs: CONSORT, QUORUM, MOOSE, STARD, and Economic submissions.

Style

Abbreviations and symbols must be standard and SI units used throughout except for blood pressure values which are reported in mm Hg.

Whenever possible, drugs should be given their approved generic name. Where a proprietary (brand) name is used, it should begin with a capital letter.

Acronyms should be used sparingly and fully explained when first used.

Figures/illustrations

Colour images and charges

If you wish to publish colour figures in print you will be charged a fee that will cover the cost of printing. The journal charges authors for the cost of reproducing colour images on all unsolicited articles, see the journal web pages for cost information. Alternatively, authors are encouraged to supply colour illustrations for online colour publication and black and white publication in the print. This is offered at no charge.

File type

Ideally, submit your figures in TIFF or EPS format. We can also accept figure files of the following types: BMP, EPI, GIF, JPEG, PDF, PNG,PNG8, PNG24, PNG32, PS, PSD, SVG, WMF.

Resolution requirements apply (9cm across for single column, 18cm for double column):

1. For B/W, the format should be either TIFF or EPS. The resolution should be in 300 DPI.

2. For 4-colour, the format should be either tiff or eps in CMYK. The resolution should be 300 DPI.

3. For line-art, vector format is preferable. Otherwise, the resolution should be 1200 DPI.

During submission, when you upload the figure files label them with the correct **File Designation**: for example Mono Image,for black and white figures, and Colour Image for colour figures.

Histograms should be presented in a simple, two-dimensional format, with no background grid.

Figures are checked using automated quality control and if they are below standard you will be alerted and provided with suggestions in order to improve the quality.

All images should be mentioned in the text in **numerical order** and figure legends should be listed at the end of the manuscript.

Please ensure that any specific patient/hospital details are removed or blacked out.

NOTE: we do NOT accept figures which use a black bar to obscure a patient's identity.

Online only material

Additional figures and tables, methodology, references, raw data, etc may be published online only to link with the printed article. If your paper exceeds the word count you should consider if any of the article could be published online only as a "data supplement". These files will not be copyedited or typeset.

All data supplement files should be uploaded using the File Designation: "Web only files".

Please ensure any data supplement files are cited within the text of the article.

Multimedia files

You may submit video and other files to enhance your article (video files should be supplied as .FLV, .F4V, .Mov, .WMV, .AVI, .MP4, .MPG). When submitting video files, ensure you upload them using the File Designation "Video Files".

Using material already published elsewhere

If you are using any figures, tables or videos that have already been published elsewhere you must obtain permission from the rightsholder (this is usually the publisher and not the author) to use them and add any required permission statements to the legends.

Tables

Tables should be submitted in the same format as your article (Word) and not another format embedded into the document. They should appear where the table should be cited, cited in the main text and in numerical order. Please note: we **cannot** accept tables as Excel files within the manuscript.

If your table(s) is/are in Excel, copy and paste them into the manuscript file.

Tables should be self-explanatory and the data they contain must not be duplicated in the text or figures - we will request that any tables that are longer/larger than 2 pages be uploaded as web only data.

References

Authors are responsible for the accuracy of cited references: these should be checked against the original documents before the paper is submitted. It is vital that the references are styled correctly so that they may be hyperlinked.

Citing in the text

References must be numbered sequentially as they appear in the text. References cited in figures or tables (or in their legends and footnotes) should be numbered according to the place in the text where that table or figure is first cited. Reference numbers in the text must be inserted immediately after punctuation (with no word spacing)—for example,[6] not [6].

Where more than one reference is cited, separate by a comma—for example, [1, 4, 39]. For sequences of consecutive numbers, give the first and last number of the sequence separated by a hyphen—for example, [22-25]. References provided in this format are translated during the production process to superscript type, which act as hyperlinks from the text to the quoted references in electronic forms of the article.

Please note, if your references are not cited in order your article will be returned to you before acceptance for correct ordering.

Preparing the reference list

References must be double spaced (numbered consecutively in the order in which they are mentioned in the text) in the [slightly modified] Vancouver style (see example below). Only papers published or in press should be included in the reference list. (Personal communications or unpublished data must

be cited in parentheses in the text with the name(s) of the source(s) and the year. Authors should get permission from the source to cite unpublished data.).

References must follow the [slightly modified] Vancouver style:

12 Surname AB, Surname CD. Article title. Journal abbreviation Year;**Vol**:Start page–End page.

Use one space only between words up to the year and then no spaces. The journal title should be in italic and abbreviated according to the style of Medline. If the journal is not listed in Medline then it should be written out in full.

Check journal abbreviations using PubMed.

List the names and initials of all authors if there are 3 or fewer; otherwise list the first 3 and add et al. (The exception is the Journal of Medical Genetics, which lists all authors.)

Example references: **Journal article**

13 Koziol-Mclain J, Brand D, Morgan D, et al. Measuring injury risk factors: question reliability in a statewide sample. Inj Prev 2000;**6**:148–50.

Chapter in book

14 Nagin D. General deterrence: a review of the empirical evidence. In: Blumstein A, Cohen J, Nagin D, eds. Deterrence and Incapacitation: Estimating the Effects of Criminal Sanctions on Crime Rates. Washington, DC: National Academy of Sciences 1978:95–139.

Book

15 Howland J. Preventing Automobile Injury: New Findings From Evaluative Research. Dover, MA: Auburn House Publishing Company 1988:163–96.

Abstract/supplement

16 Roxburgh J, Cooke RA, Deverall P, et al. Haemodynamic function of the carbomedics bileaflet prosthesis [abstract]. Br Heart J 1995;73(Suppl 2):P37.

Electronic citations

Websites are referenced with their URL and access date, and as much other information as is available. Access date is important as websites can be updated and URLs change. The "date accessed" can be later than the acceptance date of the paper, and it can be just the month accessed. See the 9th edition of the AMA Manual of Style for further examples.

Electronic journal articles

Morse SS. Factors in the emergency of infectious diseases. Emerg Infect Dis 1995 Jan-Mar;1(1). www.cdc.gov/nciod/EID/vol1no1/morse.htm (accessed 5 Jun 1998).

Electronic letters

Bloggs J. Title of letter. Journal name Online [eLetter] Date of publication. url

eg: Krishnamoorthy KM, Dash PK. Novel approach to transseptal puncture. Heart Online [eLetter] 18 September 2001. http://heart.bmj.com/cgi/eletters/86/5/e11#EL1

Check your citation information using PubMed.

Digital Object Identifiers (DOIs)

DOIs are a unique string created to identify a piece of intellectual property in an online environment; particularly useful for articles which have been published online before appearing in print (and therefore the article has not yet been assigned the traditional volume, issue and page number reference). The DOI is a permanent identifier of all versions of an article, whether raw manuscript or edited proof, online or in print. Thus the DOI should ideally be included in the citation even if you want to cite a print version of an article.

How to cite articles before they have appeared in print

1. Alwick K, Vronken M, de Mos T, et al. Cardiac risk factors: prospective cohort study. Ann Rheum Dis Published Online First: 5 February 2004. doi:10.1136/ard.2003.001234

How to cite articles once they have appeared in print

Vole P, Smith H, Brown N, et al. Treatments for malaria: randomised controlled trial. Ann Rheum Dis 2003;**327**:765–8 doi:10.1136/ard.2003.001234 [published Online First: 5 February 2002.

More comprehensive guidance about DOIs.

PLEASE NOTE: RESPONSIBILITY FOR THE ACCURACY AND COMPLETENESS OF REFERENCES RESTS ENTIRELY WITH THE AUTHORS.

Supplementary files

Supplementary material

You may submit supplementary material which may support the submission and review of your article. This could include papers in press elsewhere, published articles, appendices, video clips (please see Multimedia files instructions), etc.

All supplementary material files should be uploaded using the File Designation: Supplementary material

Online only material

Additional figures and tables, methodology, references, raw data, etc may be published online only to link with the printed article. If your paper exceeds the word count you should consider if any of the article could be published online only as a "data supplement". These files will not be copyedited or typeset.

All Appendices should be considered Online only material.

All data supplement files should be uploaded using the File Designation: Web Only files.

Please ensure any data supplement files are cited within the text of the article.

Multimedia files

You may submit video and other files to enhance your article (video files should be supplied as .avi, .wmv, .mov .mp4 or .H264). When submitting video files, ensure you upload them using the File Designation “Video Files”.
