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Evaluation of bilirubin estimates in newborns from smartphone digital images in a population in Botswana

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1. Introduction

1.1. Background

Neonatal jaundice is a condition in newborns with elevated levels of the waste product bilirubin, also known as hyperbilirubinemia. It is primarily caused by a combination of the rapid turnover of red blood cells and the immature liver functions in the newborn. Neonatal jaundice affects approximately 60 to 80 % of newborns but is usually self-limiting and harmless. The condition is still potentially dangerous because bilirubin can accumulate in the basal ganglia of the brain, where it can cause permanent brain damage. Such brain damage, better known as kernicterus, can manifest as cerebral palsy, deafness, language difficulty, or in the worst cases, be fatal. [1]

Bhutani et al. estimated the incidence and impairment of neonatal jaundice for 2010 and concluded that failure to manage hyperbilirubinemia results in 114 100 avoidable neonatal deaths and many newborns who grow up with severe disabilities. [2] Three-quarters of these deaths are estimated to occur in the poorest regions of the world; sub-Saharan Africa and South Asia. Neonatal jaundice is one of the top three leading causes of death among newborns in sub-Saharan Africa. [1]

The main challenge in reducing the burden of neonatal jaundice is to identify children at risk at an early stage so that effective treatment can be given at an early stage. [3] Treatment of hyperbilirubinemia is usually done by phototherapy and, in some extreme cases, by blood transfusion. In some areas, sunlight is an optional treatment. [4]

Standard detection of neonatal jaundice varies in different countries. In Denmark, all infants are screened by transcutaneous bilirubinometers (TcB). The limit for when to measure total serum bilirubin (TSB) based on cutaneous bilirubin depends on the TSB standardization and which type of cutaneous bilirubinometer is used (BiliCheck/Minolta) in the individual department. [5,6] In African countries, however, detection of neonatal jaundice usually includes a clinical risk evaluation by visual assessment (VA) and sometimes a diagnostic blood sample to measure the level of total serum bilirubin (TSB).

In cases of high bilirubin levels, most countries apply subsequent capillary/venous samples for follow-up and to determine the need for therapy. Invasive blood sampling is linked to stress, pain, and substantial blood loss. Moreover, there is an increased risk of infections.

VA of neonatal jaundice is often based on the cephalocaudal progression of the yellowness using Kramer's scale, which assumes that a higher number corresponds to an increased level of serum bilirubin[7] (Figure 1). This is the first-step screening option where other types of equipment are lacking. [8] Several studies have shown that VA is not a sufficient indicator on its own and unreliable as a screening tool for further testing. [9-13]

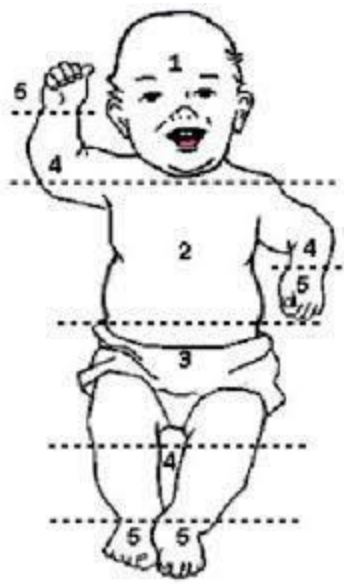

Fig. 1 *Kramer's scale*

To reduce the need to draw blood from the newborn, non-invasive devices that measure bilirubin concentration by shining light through the skin, such as transcutaneous bilirubinometers (TcB), have been developed. [14] TcB is one of the methods recommended by the American Academy of Pediatrics for the assessment of the risk of hyperbilirubinemia of newborns before discharge. [15] It is a safe, non-invasive method, and some studies have shown that its use can reduce the number of blood draws by 38,5%. [16] TcB shows a good correlation with blood samples, also in different ethnicities. [17] However, this is only true for the *BiliCheck* bilirubinometer. The *Minolta* bilirubinometer shows a higher transcutaneous bilirubin estimation in dark-skinned infants than in light-skinned infants. Additionally, it underestimates transcutaneous bilirubin when higher TSB levels are present. [5]

Lab equipment for TSB measurements and TcB devices is expensive, with a price range from 6.000 to 10.000 US dollars, thus making them practically unavailable in low-income countries. Because most deaths due to neonatal jaundice occur in low-income countries, there is a significant unmet need for simple, reliable, and affordable technologies to identify at-risk newborns. Mobile health, or mHealth, is an area with increased focus, as cell phone technology and smartphones are found worldwide, also in regions with scarce resources. This has given hope for new affordable solutions for global health. [18]

Picterus AS is a private limited liability company founded in Trondheim, Norway. They have developed a new smartphone app, Picterus Jaundice Pro (Picterus JP), that combines previous research on the bio-optics of jaundice in newborn skin with a mHealth approach. [19] Picterus JP uses an algorithm based on color analysis of a digital image to estimate the bilirubin levels in the blood, together with a unique color calibration card to adjust for different light conditions and the automatic adjustments in the phone (figure 2).

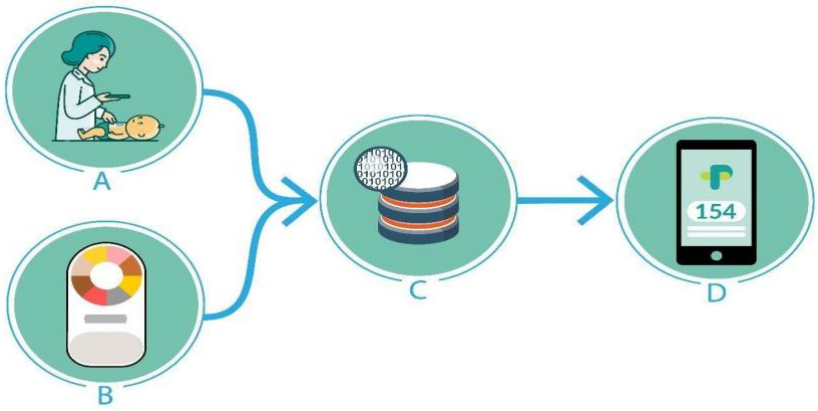

Fig. 2 *Workflow of Picterus JP system. A) A photo of the newborn is taken, B) colors from the calibration card are used to calibrate the image, C) calibrated colors are compared with an extensive database of color- and bilirubin pairs, and D) the matching bilirubin estimate is presented to the user.*

Picterus JP has been tested in 350 Caucasian and 170 Latin-American newborns, with mainly parents' skin types 1 to 4 according to the Fitzpatrick scale (Figure 3) and skin color 1 to 3 according to Neomar's neonatal skin color scale (Figure 4). [20,21] The studies showed a Pearson correlation index of 0.83 to 0.88 and sensitivity of 90 to 100% to detect newborns with severe jaundice, defined as TSB above 250 µmol/L and specificity of 70 to 80%.

However, there is still uncertainty about how Picterus JP works in newborns with high melanin content (Asian and African population / Neomar's scale 4). Melanin could potentially influence the results, as it is one of the essential absorbers of light in the skin. However, melanocytes are immature at birth, and melanin levels in the skin are lower in newborns compared to adults. [22]

Fig. 3 Fitzpatrick scale

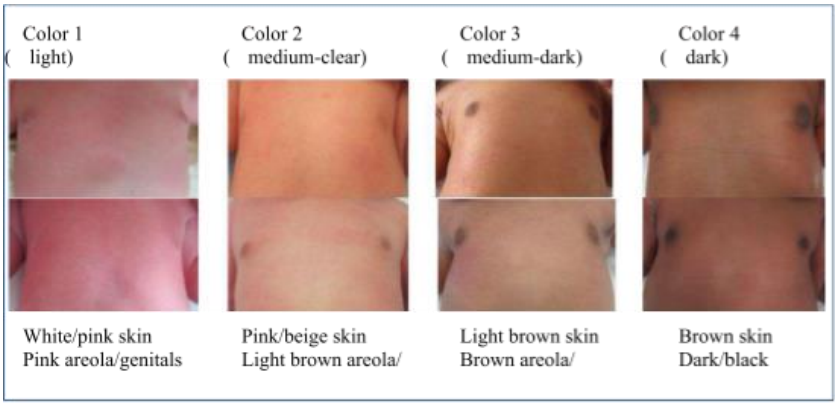

Fig. 4 Neomar's skin color scale for newborns [21]

1.2. Problem statement

Picterus performed a pilot study in Uganda from December 2019 to March 2020, which included 167 newborns. Results showed a Pearson correlation index of 0.59 between TSB and the Picterus image estimate, a sensitivity of 66%, and a specificity of 94%, when the cut-off was set to 250 µmol/L. Only six newborns had bilirubin levels higher than 250 µmol/L. It was concluded that more data is needed to optimize the performance of the tool for dark skin, including spectrophotometric measurements of skin color reflectance and newborns with higher bilirubin levels.

Currently, there is a running project in Uganda where data is collected from newborns with a broader range of bilirubin levels and high melanin content. Additional measurements of skin color reflectance with a spectrophotometer are made to adjust the Picterus JP algorithm and optimize the app performance. However, more data is needed to determine the adequacy of the app. Therefore, collecting data from different populations, such as in Botswana, will be valuable.

1.3. Purpose of study

This study will be conducted at a hospital in Botswana, where we aim to collect data from newborns with a broader range of bilirubin levels and high melanin content in a different population than in the Uganda study.

1.4. General Objective

The general objective is to investigate the use of Picterus JP in a population with high melanin content in the skin.

1.5. Specific Objectives

The specific objectives of this study are:

i. To assess the correlation between estimations of bilirubin obtained by Picterus JP with TSB, and TcB, in newborns with high melanin content in the skin.
ii. To determine the accuracy of Picterus JP used as a screening tool to identify high levels of bilirubin in newborns with melanin content in the skin.

1.6. Scope of study

The study will be conducted in the neonatal unit (NNU) and the postnatal ward (4G) at Princess Marina Hospital in Gaborone, the capital of Botswana.

1.7. Justification

We hypothesize that the Picterus JP will offer bilirubin level estimates that are highly correlated to bilirubin levels measured in blood samples in a population in Botswana. Following our hypothesis, the short-term goal for this project is to demonstrate that this screening method can be used to identify children with severe neonatal jaundice independent of skin color. Thus, providing a cheaper, patient-friendly, and more readily available means of neonatal jaundice detection.

1.8. Significance of study

The long-term goal is that with the implementation of the app, the identification of newborns with jaundice will be improved and, thus, referred at an earlier stage for diagnostics and treatment. This would lead to reduced neonatal morbidity, mortality, and fewer children with permanent disabilities following hyperbilirubinemia, also in patients with high melanin content, such as the population in Botswana. The clinicians at Princess Marina Hospital will benefit from this as they will be equipped with a cheap, readily available application for detecting neonatal jaundice.

2. Methodology

2.1. Study design

The study will be a diagnostic accuracy cross-sectional study with quantitative methods of data collection.

2.2. Study setting

The study will be conducted in the neonatal unit (NNU) and the postnatal ward (4G) at the Princess Marina Hospital (PMH) in Gaborone, Botswana. PMH is a provincial, government-funded hospital and the largest referral hospital in Botswana.

The hospital offers preventive, curative, and rehabilitation services and serves as a primary, district, and tertiary hospital. The hospital has an in-patient capacity of more than 500 beds and handles a minimum of 2500 outpatients daily.

The NNU has 38 beds distributed in 5 cubicles, is often overcrowded, and receives neonates from the on-site labor ward and babies referred from the surrounding clinics and district hospitals. On estimations from prof. Britt Nakstad, who is employed at the department, there are approximately 100 neonatal admissions per month (ranging from 82-127 monthly in 2017-2022), including patients who need intensive care on ventilators. Of these are 47% girls and 53% boys. The total mortality is 18% of the admitted infants. The estimated gestational age and weight of the patients at the NNU can be found in Table 2 and Table 3, respectively, and are based on the admitted infants in May 2022. In the postnatal ward, there are approximately 500 mothers admitted monthly (ranging from 498-508 monthly in May-July 2022), including normal and c-section deliveries. [23]

Gestational age	n	%
< 28 weeks	13	14.1
28-29 weeks	8	8.7
30-31 weeks	8	8.7
32-34 weeks	14	15.2
35-36 weeks	14	15.2
37-40 weeks	29	31.5
> 40 weeks	6	6.5
Total	**92**	**100**

***Table 2**: Estimated gestational age at NNU*

Weight	%
<1000 g	10
1000-1500 g	20
1500-2500 g	25
2500-4000 g	40
> 4000 g	5
Total	100

***Table 3**: Estimated weight at NNU*

2.3. Study population and sample size calculations

The study population will comprise 150 newborns from the Neonatal Unit and the postnatal ward at Princess Marina Hospital, a number determined by a sample size calculation based on standard errors of the 95% limit agreement, as described by Bland and Alman. [19]

The standard error of the 95% limit of agreement is given by $\sqrt{\frac{3 \times s^2}{n}}$, where s is the standard deviation of the differences between measurements by the two methods, and n is the sample size.

The study performed at St. Olav Hospital showed a standard deviation (s) of the difference between bilirubin estimates from digital images to blood samples of 35 µmol/L. [20] A sample size (n) of 150 participants will then give the confidence interval of the limits of 9,8 µmol/L, a relevant size for clinical use.

The study objective of this study is two-folded. First, we wish to determine the correlation between TSB measurement and Picterus image estimate of serum-bilirubin. Second, we wish to determine the accuracy of the Picterus App used as a screening tool.

The study conducted by Aune et al. found an AUC of 0.925. To calculate the sample size for this study we used the used the EasyROC tool, by setting an AUC of 0.9, a significance level of 0.5 and a power to 0.8. This gave the suggested sample size of 12 infants. In order to get a sufficient result for all bilirubin levels, we decided to apply our pilot data to see how many subjects were needed to receive a sample of 12 in three different groups; <100 µmol/L, 100-200 µmol/L and >200 µmol/L. Through this calculation we came to the number of 115 infants.

2.4. Participants and sampling procedure

Participants will be recruited from the NNU and postnatal ward at the Princess Marina Hospital. The parents of newborns with and without signs of jaundice will be asked to participate.

2.5. Selection criteria

150 newborns will be included in this study. Participants will be recruited from the NNU and the postnatal ward.

2.5.1. Inclusion criteria

- Gestational age $\geq$ 37 weeks
- Birth weight $\geq$ 2000 g and $\leq$ 4500 g
- Age > 24 hours and < 14 days
- Infants requiring a blood sample for clinically suspected jaundice or other screening

2.5.2. Exclusion criteria

- Infants transferred to the pediatric ward for advanced treatment
- Infants with a skin rash or other disease that affects the skin where measurements are performed
- Infants that receive or have received phototherapy in the last 24 hours
- Infants with an inborn disease

Newborns that have received phototherapy are excluded, as phototherapy will influence the result of transcutaneous measurements.

2.6. Data collection

Following informed consent, background data such as birth weight, age on examination, gestational age, and type of feeding will be obtained. Gestational age will be based on ultrasound determination and the last normal menstruation period (LNMP). The skin type of the infant will be classified according to the newborn Neomar's scale score (Figure 4).

Transcutaneous bilirubin estimates will be performed over the sternum of the infant. A Dräger Jaundice Meter JM-105 will be used in this study.

Skin reflectance will be done using a CE-marked portable Konica Minolta spectrophotometer CM-700d. The spectrophotometer readings will be used to adjust the existing algorithm from Picterus to African skin and thereafter bilirubin estimates from the taken images will be determined.

The Picterus calibration card will be fixed in place with a disposable back sticker on the newborn's chest with the hole of the card placed over the infant's sternum. A validated smartphone with Picterus JP will then be used to collect digital images, as shown below. Once the phone is aligned with the card, two sets of 6 images of each newborn will be captured, 3 with flash and 3 without flash. After all the images are obtained, a unique ID will be displayed on the smartphone. This ID will be recorded on the case report forms and later used to pair clinical data and digital images.

Fig.5 Picterus JP image procedure

A blood sample to determine TSB will be obtained within 60 minutes of obtaining the images and processed at the Department of clinical biochemistry in the hospital laboratory. In addition, TSB will be measured with neonatal bilirubin analyzer Neo-Bil Plus from DAS to assess the quality of the equipment. For the hospital laboratory analysis, 1 ml of blood is required and will be obtained either by venous puncture or heel prick. An additional 10 µl volume of blood will be collected in a heparinized capillary tube for analysis in the Neo-Bil Plus equipment.

2.7. Data analysis

2.7.1. Laboratory analysis

Total bilirubin levels will be measured at the Department of clinical biochemistry. 1 ml of blood is required for the analysis and can be obtained through venous blood sampling. This will be carried out by the collaborators in the neonatal unit. The analysis is performed on CC- Beckman Coulter AU 680 Analyzer 01. Laboratory equipment is calibrated and maintained according to standard procedures.

2.7.2. Statistical analysis

The bilirubin estimates from the images taken will be determined. The images will be compared to the TSB, NeoBil and TcB measurements using the Pearson correlation coefficient. Sub-analysis for the different skin colors, gestational ages, days of life, and bilirubin levels will be performed for the TSB and image estimate. Sensitivity and specificity analysis will be calculated for different cut-off values using the ROC analysis. Systematic over- or under-estimation of bilirubin levels will be evaluated using Bland-Altman plots. To avoid bias, the developer of the product – Picterus AS – will not be involved in statistical work-up or even see the raw data.

2.7.3. Data handling

Only anonymized data will be used in this research project. Images are cropped, so it is impossible to identify the newborn from the images. Data will be stored on secure, password-protected computers, to which only leading investigators have access. ID key will be held at a safe location in Botswana and will not be transported from this location. Image analysis and statistical analysis will be performed in Denmark with the local principal investigator. Transfer from Botswana to Denmark will be performed following the rules at Princess Marina Hospital.

2.8. Risk assessment

The overall assessment of risk in this study is considered low.

The procedure of obtaining the smartphone images involves no risk of importance. Blood samples will be obtained by the standard method, and no additional risk is involved in this study.
All bilirubin estimates from the digital images will be performed after collecting data, as the smartphone application has not yet been validated for this population. This leaves no risk of presenting false bilirubin estimates to participants.

2.9.Research team

The principal investigator, Julie Zimmer, is a medical student in her fourth year at the University of Copenhagen in Denmark. She is responsible for the management of the study and will provide training for the personnel in order to start up the project in Botswana.

Co-investigator prof. Britt Nakstad (MD, Ph.D., MSc), is a medical doctor and neonatologist educated from the University of Oslo. In Botswana, she works as a neonatologist at the Princess Marina Hospital in Gaborone and as a professor at the University of Botswana. She will be inviting and enrolling patients in the study and collecting data.

Co-investigator dr. Lizzy M Pezwa is soon to be a resident in pediatrics at the University in Botswana and will be inviting and enrolling patients in the study and collecting data.

Co-investigator dr. Mammule Tau is a first-year resident that will be inviting and enrolling patients in the study and collecting data.

Co-investigator prof. Bo Mølholm Hansen (MD, Ph.D.) is a medical doctor educated at the University of Copenhagen and works as a neonatologist at Hillerød hospital in Denmark. He will supervise the principal investigator in the planning process, statistical analysis, and the writing of the final article.

Co-investigator dr. Anders Aune (MD) is a medical doctor educated at NTNU and works as a pediatrician at the Neonatal Intensive Care Unit at St. Olav Hospital. He holds a master's degree in Public Health from Yale University. He has a strong passion for this project, as it follows an idea of him dating back to 2011. He will represent the Picterus team in this study, which will provide the project with the Picterus JP equipment and Neo-bil Plus bilirubin analyzer.

2.10. Ethical consideration

Ethical clearance will be obtained from the National Committee on Health Research Ethics in Denmark, the Research Ethics Committee at Princess Marina Hospital, the Research Ethics Committee at the University of Botswana, and the Botswana Ministry of Health and Welfare.

No harm will be done to the subjects in this study. Verbal and written consent will be obtained from the parents of each study participant in English or Setswana if necessary. If the mother cannot read or write, they will be excluded from the study. Data collected will be handled with confidentiality, and the names of subjects will be coded.

The overall assessment of risk in this study is considered low. Obtaining the smartphone images involves no risk of importance, and the smartphone used will be disinfected between each baby. Blood samples will be obtained by the standard procedure, and no additional risk is involved in this study. For all studies with the goal of increasing the identification of disease, it is essential that treatment is available and that identified cases do not result untreated. In our study, the treatment consists of phototherapy, which is available at Princess Marina Hospital.

The benefit for the participants in this study will be that they will receive the results of the jaundice examination. Furthermore, if this reveals that treatment for jaundice is necessary, the costs related to this treatment will be covered by the project.

The ethical aspect of the potential unbalances between educated health professionals asking new parents, some with low education or illiterate, living in rural areas and not exposed to studies frequently. Asking them to accept that their children can participate in a study requires tact and concern. The parents must understand that participating is voluntary and that they can refuse with no negative consequences for their child’s care. Furthermore, they must be competent to make informed consent.

2.11. Limitations and mitigation

mHealth applications is a relatively new field with an increasing number of applications being launched. A concern regarding mHealth applications targeted toward the general public has been that many applications are unreliable and may lead to false results. [24] Many medical-related applications are developed entirely by non-medical people and made available without regulation or certification procedures. Picterus JP has been designed to fulfill all necessary criteria according to relevant ISO standards.

2.12. Plan for validation and dissemination

The results from this study will have relevance for the potential validation of the innovation. Studies demonstrating the tool's potential in a high-burden setting will be necessary for users of the device. This is the first validation study following the study in Uganda. The results of this study will result in a published paper in a peer-reviewed journal. Further, we will try to communicate the study's results through popular media and seminars in both countries.

Sources

1. Slusher TM, Zipursky A, Bhutani VK. A global need for affordable neonatal jaundice technologies. Semin Perinatol 2011;35(3):185-91. DOI: 10.1053/j.semperi.2011.02.014.

2. Bhutani VK, Zipursky A, Blencowe H, et al. Neonatal hyperbilirubinemia and Rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels. Pediatr Res 2013;74 Suppl 1(Suppl 1):86-100. (In eng). DOI: 10.1038/pr.2013.208.

3. Olusanya BO, Ogunlesi TA, Slusher TM. Why is kernicterus still a major cause of death and disability in low-income and middle-income countries? Arch Dis Child 2014;99(12):1117-21. DOI: 10.1136/archdischild-2013-305506.

4. Slusher TM, Olusanya BO, Vreman HJ, et al. A Randomized Trial of Phototherapy with Filtered Sunlight in African Neonates. N Engl J Med 2015;373(12):1115-24. DOI: 10.1056/NEJMoa1501074.

5. Ebbesen F, Vandborg PK, Trydal T. Comparison of the transcutaneous bilirubinometers BiliCheck and Minolta JM-103 in preterm neonates. Acta Paediatr 2012;101(11):1128-33. DOI: 10.1111/j.1651-2227.2012.02797.x.

6. Pernille Vandborg BMH, Anna Lewis, Jesper Padkær, Mette Line Roed, Hristo Stanchev, Finn Ebbesen. Behandlingsstandard for neonatal hyperbilirubinæmi. Danish Paediatric Society. (https://www.paediatri.dk/images/dokumenter/Retningslinjer_2019/Hyperbilirubinæmi_guideline.Juli_2019.pdf).

7. Kramer LI. Advancement of dermal icterus in the jaundiced newborn. Am J Dis Child 1969;118(3):454-8. DOI: 10.1001/archpedi.1969.02100040456007.

8. Hatzenbuehler L, Zaidi AK, Sundar S, et al. Validity of neonatal jaundice evaluation by primary health-care workers and physicians in Karachi, Pakistan. J Perinatol 2010;30(9):616-21. DOI: 10.1038/jp.2010.13.

9. Riskin A, Tamir A, Kugelman A, Hemo M, Bader D. Is visual assessment of jaundice reliable as a screening tool to detect significant neonatal hyperbilirubinemia? J Pediatr 2008;152(6):782-7, 787 e1-2. DOI: 10.1016/j.jpeds.2007.11.003.

10. Keren R, Tremont K, Luan X, Cnaan A. Visual assessment of jaundice in term and late preterm infants. Arch Dis Child Fetal Neonatal Ed 2009;94(5):F317-22. DOI: 10.1136/adc.2008.150714.

11. Varughese PM. Kramer's scale or transcutaneous bilirubinometry: the ideal choice of a pediatrician? can we trust our eyes? International Journal of Contemporary Pediatrics 2019. DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20193702.

12. Aprillia Z, Gayatri D, Waluyanti FT. Sensitivity, Specificity, and Accuracy of Kramer Examination of Neonatal Jaundice: Comparison with Total Bilirubin Serum. Compr Child Adolesc Nurs 2017;40(sup1):88-94. DOI: 10.1080/24694193.2017.1386975.

13. De Luca D, Zecca E, Zuppa AA, Romagnoli C. The joint use of human and electronic eye: visual assessment of jaundice and transcutaneous bilirubinometry. Turk J Pediatr 2008;50(5):456-61. (https://www.ncbi.nlm.nih.gov/pubmed/19102050).

14. Engle WD, Jackson GL, Engle NG. Transcutaneous bilirubinometry. Semin Perinatol 2014;38(7):438-51. DOI: 10.1053/j.semperi.2014.08.007.

15. American Academy of Pediatrics Subcommittee on H. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2004;114(1):297-316. DOI: 10.1542/peds.114.1.297.

16. van den Esker-Jonker B, den Boer L, Pepping RM, Bekhof J. Transcutaneous Bilirubinometry in Jaundiced Neonates: A Randomized Controlled Trial. Pediatrics 2016;138(6). DOI: 10.1542/peds.2016-2414.

17. Afanetti M, Eleni Dit Trolli S, Yousef N, Jrad I, Mokhtari M. Transcutaneous bilirubinometry is not influenced by term or skin color in neonates. Early Hum Dev 2014;90(8):417-20. DOI: 10.1016/j.earlhumdev.2014.05.009.

18. Kallander K, Tibenderana JK, Akpogheneta OJ, et al. Mobile health (mHealth) approaches and lessons for increased performance and retention of community health

workers in low- and middle-income countries: a review. J Med Internet Res 2013;15(1):e17. DOI: 10.2196/jmir.2130.

19. Vartdal G. Development of a Smartphone-based diagnostic Tool for Jaundice. Faculty of Natural Sciences and Technology; 2014.

20. Aune A, Vartdal G, Bergseng H, Randeberg LL, Darj E. Bilirubin estimates from smartphone images of newborn infants' skin correlated highly to serum bilirubin levels. Acta Paediatr 2020;109(12):2532-2538. DOI: 10.1111/apa.15287.

21. Maya-Enero S, Candel-Pau J, Garcia-Garcia J, Gimenez-Arnau AM, Lopez-Vilchez MA. Validation of a neonatal skin color scale. Eur J Pediatr 2020;179(9):1403-1411. DOI: 10.1007/s00431-020-03623-6.

22. Walsh RJ. Variation in the Melanin Content of the Skin of New Guinea Natives at Different Ages. J Invest Dermatol 1964;42:261-5. DOI: 10.1038/jid.1964.59.

23. Hospital PM. Press Release: Princess Marina Still Concerned About the Number of Visitors. (https://www.facebook.com/sepatela/posts/pfbid0RuWiu9Kf3Nfop23kKCjDbtqvVtHwa3cajsE6nxeDGmUzSubjwbj6yostZHXFhpyNl).

24. Boulos MN, Brewer AC, Karimkhani C, Buller DB, Dellavalle RP. Mobile medical and health apps: state of the art, concerns, regulatory control and certification. Online J Public Health Inform 2014;5(3):229. DOI: 10.5210/ojphi.v5i3.4814.

25. Lilian de Greef MG, Min Joon Seo, Eric C Larson, James W. Stout, James A. Taylor, Shwetak N. Patel. Bilicam: using mobile phones to monitor newborn jaundice. 2014 (https://dl.acm.org/doi/abs/10.1145/2632048.2632076 https://ubicomp.org/ubicomp2014/proceedings/ubicomp_adjunct/posters/p39-de%20greef.pdf).

26. Leung TS, Kapur K, Guilliam A, et al. Screening neonatal jaundice based on the sclera color of the eye using digital photography. Biomed Opt Express 2015;6(11):4529-38. DOI: 10.1364/BOE.6.004529.

27. Taylor JA, Stout JW, de Greef L, et al. Use of a Smartphone App to Assess Neonatal Jaundice. Pediatrics 2017;140(3). DOI: 10.1542/peds.2017-0312.

28. Aydin M, Hardalac F, Ural B, Karap S. Neonatal Jaundice Detection System. J Med Syst 2016;40(7):166. DOI: 10.1007/s10916-016-0523-4.

29. Xiaoyue Dong XS, Zhangbin Yu, Shuping Han. Image-Based Neonatal Hyperbilirubinemia Screening after Hospital Discharge. Tehran University of Medical Sciences 2020. DOI: https://doi.org/10.18502/ijph.v49i6.3359.

30. Rong ZH, Luo F, Ma LY, et al. [Evaluation of an automatic image-based screening technique for neonatal hyperbilirubinemia]. Zhonghua Er Ke Za Zhi 2016;54(8):597-600. DOI: 10.3760/cma.j.issn.0578-1310.2016.08.008.

31. ZHAO BYDHXGMSMLHLYRCSD. [Neonatal and early infantile jaundice: assessment by the use of the smarphone]. Chin J Neonatol 2018 (http://zhxsekzz.yiigle.com/CN101451201804/1049735.htm?utm_source=TrendMD&utm_medium=cpc&utm_campaign=Chinese_Journal_of_Neonatology_TrendMD_0).

32. Srujana Swarna SP, Balaji Chinnasami, Nirmala Manasa D., Balaji Ramraj. The smart phone study: assessing the reliability and accuracy of neonatal jaundice measurement using smart phone application. International Journal of Contemporary Pediatrics 2018. DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20175928.

33. Moyer VA, Ahn C, Sneed S. Accuracy of clinical judgment in neonatal jaundice. Arch Pediatr Adolesc Med 2000;154(4):391-4. DOI: 10.1001/archpedi.154.4.391.

34. Sara M. Acosta-Torres MTT-E, José A. Colina-Araujo, José A. Colina-Chourio. Utilidad diagnóstica del método de Kramer para la detección clínica de la hiperbilirrubinemia neonatal. Investigación Clínica 2012 (http://www.scielo.org.ve/scielo.php?script=sci_arttext&pid=S0535-51332012000200004).

35. Knudsen A, Brodersen R. Skin colour and bilirubin in neonates. Arch Dis Child 1989;64(4):605-9. DOI: 10.1136/adc.64.4.605.

36. Slusher TM, Zamora TG, Appiah D, et al. Burden of severe neonatal jaundice: a systematic review and meta-analysis. BMJ Paediatr Open 2017;1(1):e000105. DOI: 10.1136/bmjpo-2017-000105.

37. Olusanya BO, Kaplan M, Hansen TWR. Neonatal hyperbilirubinaemia: a global perspective. Lancet Child Adolesc Health 2018;2(8):610-620. DOI: 10.1016/S2352-4642(18)30139-1.

38. Donneborg ML, Hansen BM, Vandborg PK, Rodrigo-Domingo M, Ebbesen F. Extreme neonatal hyperbilirubinemia and kernicterus spectrum disorder in Denmark during the years 2000-2015. J Perinatol 2020;40(2):194-202. DOI: 10.1038/s41372-019-0566-8.

39. Olusanya BO, Teeple S, Kassebaum NJ. The Contribution of Neonatal Jaundice to Global Child Mortality: Findings From the GBD 2016 Study. Pediatrics 2018;141(2). DOI: 10.1542/peds.2017-1471.

40. Olusanya BO, Osibanjo FB, Mabogunje CA, Slusher TM, Olowe SA. The burden and management of neonatal jaundice in Nigeria: A scoping review of the literature. Niger J Clin Pract 2016;19(1):1-17. DOI: 10.4103/1119-3077.173703.

41. Simiyu DE. Morbidity and mortality of neonates admitted in general paediatric wards at Kenyatta National Hospital. East Afr Med J 2003;80(12):611-6. DOI: 10.4314/eamj.v80i12.8776.

42. Mwaniki MK, Gatakaa HW, Mturi FN, et al. An increase in the burden of neonatal admissions to a rural district hospital in Kenya over 19 years. BMC Public Health 2010;10:591. DOI: 10.1186/1471-2458-10-591.

43. Nyangabyaki-Twesigye C, Mworozi E, Namisi C, et al. Prevalence, factors associated and treatment outcome of hyperbilirubinaemia in neonates admitted to St Francis hospital, Nsambya, Uganda: a descriptive study. Afr Health Sci 2020;20(1):397-405. DOI: 10.4314/ahs.v20i1.46.

44. Donneborg ML, Hansen BM, Petersen JP, Vandborg PK, Ebbesen F. [An update on unconjugated neonatal hyperbilirubinaemia in Denmark]. Ugeskr Laeger 2020;182(14a) (In dan).

45. Qattea I, Farghaly MAA, Elgendy M, Mohamed MA, Aly H. Neonatal hyperbilirubinemia and bilirubin neurotoxicity in hospitalized neonates: analysis of the US Database. Pediatr Res 2022;91(7):1662-1668. DOI: 10.1038/s41390-021-01692-3.

46. Manning D, Todd P, Maxwell M, Jane Platt M. Prospective surveillance study of severe hyperbilirubinaemia in the newborn in the UK and Ireland. Arch Dis Child Fetal Neonatal Ed 2007;92(5):F342-6. DOI: 10.1136/adc.2006.105361.

47. McGillivray A, Polverino J, Badawi N, Evans N. Prospective Surveillance of Extreme Neonatal Hyperbilirubinemia in Australia. J Pediatr 2016;168:82-87 e3. DOI: 10.1016/j.jpeds.2015.08.048.

Appendices

Appendix I: Active timeline

Application for Ethical Review from Danish NVK	September 2022
Application for Ethical Review from Princess Marina Hospital REC and Ministry of Health and Welfare	September 2022
Data collection	March 2023 - May 2024
Data analysis	June 204
Report writing and dissemination of results	June - September 2024

Appendix II: Estimated budget (DKK)

Purpose	**Budget (DKK)**
Travel t/r Botswana (Julie Zimmer)	20 000
Residency in Botswana (Julie Zimmer, 3 weeks)	7 000
Expenses to personnel in Botswana	40 000
Expenses to blood analysis in Botswana	30 000
Other operating expenses in Botswana (contingency)	3 000
Total	100 000

Date of document: March 1, 2023

Appendix III: English version of the consent form

Primary consent form:

STUDY TITLE
Evaluation of bilirubin estimates in newborns from smartphone digital images in a population in Botswana.

Lead investigator
Julie Zimmer, University of Copenhagen, juzim@online.no, +47 95102393

Supervisor
Britt Nakstad, professor and head of neonatology, tel 76682945
Lizzy Pezwa, resident in paediatrics, tel 74608559
Mammule Tau, resident in paediatrics, tel 75355407

Background and rationale for the study
This research project is about a smartphone application that can be used to identify Jaundice, a potentially dangerous condition caused by too much of a yellowish waste product called bilirubin that causes the skin to appear yellow. Jaundice is common among newborns and is in most cases self-limiting and harmless. However, when severe and unrecognized it can be fatal or cause serious brain injury. The measurement of bilirubin is normally done with blood samples. We want to try out a model that estimates bilirubin using smartphones. This may potentially save many lives and reduce the number of children with disabilities

Purpose of this research study
The purpose of this research study is to see how effective the smartphone application is in identifying neonatal jaundice. To find out if the mobile app is reliable, we need to test many newborns, so we invite you to participate.

Procedure
The study is being conducted at Princess Marina Hospital. Before you take part in this research study, the study must be explained to you, and you must be given the chance to ask questions. You must read and sign this informed consent form.
To check if your baby is jaundiced, we ask to take a photo of your baby's breast using a smartphone and analyze the skin color in the picture. We will take a picture of the skin of the chest while your baby is lying on its back on the examination table. The face of your child will not be visible in the picture This will be done while collecting blood obtained by a puncture in the baby's skin. Lastly, we compare the 2 methods for measuring bilirubin.

Length of your participation
The photo and sampling for bilirubin measurement will last a maximum of an hour. If jaundice is identified, treatment will be started.

Cost and compensation
By signing this consent form, you will not waive any of your legal rights or release the parties involved in this study from liability for negligence.

Possible risks or discomforts by taking part in this study
The risks and discomforts brought about by this study are minimal. Taking smartphone images involves no risk or pain and blood sampling is routine when jaundice is suspected.

Possible benefits to you for taking part in this study
As a participant in this study, we may get results from photographing indicating that your child will need treatment for jaundice.

Voluntary participation
Your participation in this study is voluntary. If you decide to take part in this study, we ask for your signature on the consent form. You are free to withdraw from this study at any time. Withdrawing from this study will not affect your relationship with the researcher, if any. If you withdraw from the study before data collection is completed, your data will be returned to you or destroyed.

Confidentiality
The results related to your child will be anonymous. Participant data will be kept confidential except in cases where the researcher is legally obligated to report specific incidents.

Contact information
If you have any questions relating to the study or your participation you may contact dr. Lizzy Pezwa or dr. Mammule Tau, or prof. Britt Nakstad, or nurses and doctors in the ward, or the lead investigator Julie Zimmer. Their details are noted on the first page.

Consent

I certify that I have had the study form explained to me, have read or have had read to me the information describing the procedures, benefits, and risks of the study, as well as this consent form. I have been given an opportunity to ask questions about the study, which have been answered to my satisfaction. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without cost. I understand that I will be given a copy of this consent form. I voluntarily agree to allow my child to take part in this study.

Participant's signature _____ Date _____

Investigator's signature _____ Date _____

Date of document: March 1, 2023

Secondary consent form:

Title of Project:

Evaluation of bilirubin estimates in newborns from smartphone digital images in a population in Botswana.

Name of researchers responsible for the project:

Julie Zimmer and Britt Nakstad

Statement	**Please initial each box**
I confirm that I have read the information sheet version 1.1 for the above-named study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason.	
I agree to be recorded during the interviews and understand the original recording will be destroyed once the interview is transcribed and verified.	
I agree for quotations to be used in any report or paper. I understand that the use of my quotations in any report or paper will be anonymous and not attributable to me.	
I agree for anonymized transcripts to be used for future research outputs.	
I agree to take part in the above-named study	

Printed name of participant	Signature of participant	Date

Printed name of person obtaining consent	Signature of person obtaining consent	Date

Appendix iV: Standard operating procedure

1. Obtain written informed consent from the participants.
2. Collect background data such as: birth weight, gender, age on examination, gestational age, type of feeding, and Fitzpatrick scale score, newborn Neomar's scale score.
3. Using a transcutaneous bilirubinometer Dräger Jaundice Meter JM-105 obtain and record bilirubin estimates.
4. Take three measurements of skin reflectance using a CE-marked portable Konica Minolta spectrophotometer CM-700d by placing it on the newborn's chest.
5. Place the Picterus Calibration Card on the chest of the newborn with the sticky side facing down and with its hole over the infant's sternum.
6. Collect digital images using a validated smartphone with Picterus JP. Once the phone is aligned with the card, capture two sets of 6 images, 3 without flash and 3 with flash.
7. Within 60 minutes of obtaining the images, collect a blood sample for TSB estimation and send to the hospital lab for processing.
8. Carryout a second TSB measurement with the neonatal bilirubin analyzer Neo-Bil Plus from DAS. Collect10 µl of blood in a heparinized capillary tube for analysis in the Neo-Bil Plus equipment.

Appendix V: Literature review

1. Correlations

1.1 The correlation between bilirubin estimates from a smartphone application and bilirubin measurements in *serum* in newborns with varying degrees of jaundice

One of the first attempts to implement an alternative diagnostic approach was made by a group of researchers from Thailand who designed a non-invasive method for measuring bilirubin levels in 61 newborns. Images of the chest were obtained using a digital camera and manually inspected and adjusted in Photoshop. They found a significant ($p<0.05$) Pearson correlation of 0.86 between serum bilirubin and bilirubin estimated values obtained from the images. [25]

Using a digital camera to obtain images of the sclera in 110 newborns, *Leung et al*. showed a Pearson correlation of 0.75 between the sclera color and total serum bilirubin (TSB) level, concluding that although such correlation is not high enough to be used to predict the absolute TSB level, their technique may be helpful as a screening method to identify newborns with TSB above 205 μmol/L. [26]

In 2014, *De Greef et al.* reported the first results of *BiliCam*, a low-cost smartphone-based medical device that uses an embedded camera and a paper color calibration card. They conducted a study on 100 newborns to correlate *BiliCam* bilirubin estimates with TSB levels and Transcutaneous bilirubinometer (TcB) results. They concluded that *BiliCam* could not substitute TSB testing. Still, they showed statistically equivalent performance as the TcB, so it can be used as an effective screening tool to decide whether TSB testing is needed. Later on, the same group reported the results of a sample of 530 newborns with different ethnicities, including white, African American, Hispanic and Asian American, with an overall correlation of 0.91 - and 0.91, 0.90, and 0.88 for each group, respectively. Using two decision rules, sensitivities in identifying newborns with high TSB were 84.6% and 100%, and specificities were 75.1% and 76.4%, concluding that *BiliCam* provides precise estimates of TSB levels and can be used to effectively screen newborns for jaundice. [27]

There have been suggested different algorithms for the decision support systems of the applications. A study from Turkey reported the results of an advanced image processing

technology along with a color calibration card to analyze smartphone images of 40 healthy and 40 jaundiced newborns. It showed that the obtained bilirubin estimates were consistent with TSB results with a compliance rate of 85%. A more recent Turkish study from 2021 shows that using an algorithm based on the deep-learning classification of infants can help improve the results for server-based applications. The study classified the newborns into two groups according to their bilirubin levels, created a training model for the algorithm, and tested this on 40 subjects. The two-group classification accuracy of the developed algorithm was 92.5% for this 40-subject test group. Thus, they concluded that server-based estimations of neonatal jaundice could be estimated by a deep-learning-based classification followed by multiple linear regression. [28]

In Norway, a group of researchers developed a smartphone application based on professor Randeberg's research on bio-optics. Using a novel color calibration card that reflects differences in newborn skin color with varying degrees of melanin and bilirubin, a clinical trial on 134 Caucasian newborns showed a good correlation with TSB of 0.84. [20]

In 2020, the Chinese smartphone app *BiliScan* was tested on 369 Chinese Asian newborns. This study showed a moderate agreement with a correlation of 0.5 and concluded that the app required additional improvements for accurate measurements across wider ranges of TSB. A validation study of the app in 2021 showed a moderate agreement with a correlation of 0.6, and it was concluded that *BiliScan* tends to overestimate TSB and cannot replace it. However, it is an available method that can help to recognize jaundice in an early phase and minimize the number of invasive pricks. [29]

Although a huge step has been taken to achieve the need for an affordable and available method for neonatal jaundice, there are still gaps to have the ideal one, which may have the same accuracy in all types of skin and can be used on any smartphone.

1.2 The correlation between bilirubin estimates from a smartphone application and bilirubin estimates from a *standard transcutaneous device* in newborns with varying degrees of jaundice

In 2016 *Rong et al.* used an automated smartphone image-based bilirubin system (AIB) and a color calibration card for predicting jaundice and compared the results with TcB and TSB in 215 preterm and term neonates. There was no significant difference between AIB, and a correlation of 0.503 for the whole population and 0.628 for term neonates was found. [30]

One year later, another Chinese group assessed the accuracy of the same AIB system using the smartphone app *Biliscan for Newborn Jaundice* and compared results with TSB and TcB of 296 datasets from 194 neonates. The accuracy of AIB was not inferior to TcB, and the TSB value was $\leq$ 20 mg/dL, but it decreased with higher bilirubin values. The correlation between AIB and TSB was 0.824, so it was concluded that *Biliscan for Newborn Jaundice* is useful for dynamic monitoring moderate jaundice of neonates and early infants at home. [31] Nowadays, this application is available online for free. Its accuracy and reliability have been tested in an Indian population of 35 neonates, taking images of the sternum and abdomen. They found a correlation of 0.6 for the sternum and 0.55 for the abdomen. [32]

1.3 The correlation between bilirubin estimates from a smartphone application and bilirubin estimates from a *visual inspection* in newborns with varying degrees of jaundice

To our knowledge, there are no studies that present a comparison between bilirubin estimates made by a smartphone application and bilirubin estimates from a visual inspection in newborns. However, some studies present results of the correlation between visual inspection and TSB. *Moyer et al.* studied the correlation among 244 observations made by 2 groups of health workers and bilirubin levels in infants with gestational age > 36 weeks. Bilirubin ranged from 0.4 to 16.7 mg/dL. They found a Pearson coefficient of 0.43 and 0.54 for the 2 groups of observers when comparing the visual assessment and TSB. However, the agreement between observers regarding the presence of jaundice was low.

Another finding was that the presence of any visible jaundice beyond the lower chest (between nipples and umbilicus) had the best combination of sensitivity and specificity for a 12 mg/dL

bilirubin value, suggesting that infants without jaundice below the nipple line were not likely to have a 12 mg/dL or higher bilirubin value. However, 81% of infants with bilirubin values less than 12 mg/dL also presented jaundice below the nipple line, concluding that this observation was only useful to exclude high bilirubin levels and that visual assessment is neither accurate nor reliable for neonatal jaundice diagnosis. [33]

In another study, five neonatologists and 17 nurses made 3532 visual clinical assessments with the principle of cephalocaudal progression, named *BiliEye*, in 1129 term and late term infants (> 35 weeks) before discharge from the hospital on days 2 to 5 of life. The level of TSB was measured at the same time. Although a good correlation (0.752) between *BiliEye* and TSB was found, it was concluded that visual assessment is not a reliable screening tool for significant neonatal hyperbilirubinemia before discharge. Neonates with TSB levels in high-risk zones may be misdiagnosed as low-risk with inadequate follow-up. [9]

However, in 2012 *Acosta-Torres et al.* compared Kramer's scale values with TSB in 50 newborns with NNJ of 3 different ethnic groups (Caucasian, indigenous, and African-American). They found a correlation index of 0.93 ($p<0.005$) and no differences among ethnicities, concluding that Kramer's scale is a safe, non-invasive, and costless method that helps prevent kernicterus and should be implemented in health facilities lacking bilirubinometers. [34]

Some other considerations have been made regarding skin color. *Knudsen and Brodersen* concluded that measuring yellow color in the skin is not a reliable indicator of high bilirubin levels because it depends upon other factors such as plasma pH, albumin concentration, and basic skin color bilirubin concentration. [35]

2. Epidemiology

2.1 The global and regional incidences of neonatal hyperbilirubinemia

The global incidence of severe neonatal hyperbilirubinemia has been estimated in two studies of large datasets. *Bhutani et al*.[2], was the first to first to do this and was published in 2013. They used a mathematical model for its estimations and concluded with a global incidence of 359 per 100 000 births. They included incidences of extreme neonatal hyperbilirubinemia, defined as *"TSB ≥ 428 µmol/L or those treated with exchange blood transfusion"*

Another study, done by *Slusher et al*.[36], was published in 2017 and relied on population-based studies in numerous countries worldwide for its estimation. They concluded with a global incidence of 99 per 100 000 births and included incidences of neonates with severe neonatal jaundice, defined as *"jaundice associated with acute bilirubin encephalopathy (ABE)/kernicterus and/or exchange transfusions (ET) and/or jaundice-related death"*.

Regionally, the incidences of neonatal hyperbilirubinemia have been tried to be estimated in numerous studies worldwide. However, the definitions of neonatal hyperbilirubinemia vary for many studies and are therefore difficult to compare. Generally, it can be seen that low-income countries use definitions of neonatal hyperbilirubinemia with lower TSB values (TSB ≥ 170) compared to high-income countries (TSB ≥ 425-510). Additionally, the studies take different approaches on data collection. Some studies are conducted on a single population in the respective country, while other studies retract information from previously written articles, databases provided at the hospital, or reports they receive from clinicians. Generally, low-income countries tend to rely on information from medical forms with limited laboratory investigations, while high-income countries use databases.

Table 1 shows incidences estimated in five studies from low-income countries and eight studies from high-income countries. Their results demonstrate that high-income countries generally have lower incidences of severe neonatal hyperbilirubinemia ranging from 7.1-42 per 100 000 births compared to low-income countries, with incidences of neonatal hyperbilirubinemia ranging from 5.5-34%. However, there are numerous limitations to this conclusion such as the limited quality of the studies from low-income countries, the lacking definitions of hyperbilirubinemia in some of the studies, and variation in population included in the studies.

Table 1: Incidences of neonatal hyperbilirubinemia.

Study	Country	Definition	Incidence	CI
Olusanya et al.	Nigeria	TSB ≥ 170 µmol/L	5.5%	4.9-6.2
Worke et al.	Ethiopia	-	42.4% (of all complications)	-
Simiyua et al.	Kenya	"jaundice"	34.4%	-
Mwaniki et al.	Kenya	"jaundice"	9.2%	-
Nyangabyaki-Twesigye et al.	Uganda	TSB ≥ 170 µmol/L	7.75%	-
Donneborg et al.	Denmark	TSB ≥ 450 µmol/L	42 / 100 000	28-69 / 100 000
Qattea et al.	US	P59.9 Diagnoses Neonatal hyperbilirubinemia	19.6%	-
Sgro et al.	Canada	TSB ≥ 425 µmol/L	40 / 100 000 (1) 12 / 100 000 (2)	-
Manning et al.	UK	TSB ≥ 510 µmol/L	7.1 / 100 000	5.8-8.6 / 100 000
McGillivray et al.	Australia	TSB ≥ 450 µmol/L	9.4 / 100 000	-
Bhutani et al.	California	TSB ≥ 428 µmol/L	19.2 / 100 000	-
Zoubir et al.	Switzerland	TSB ≥ 425 µmol/L	17 / 100 000	-

2.2 The global and regional morbidity and mortality of neonatal hyperbilirubinemia

The global incidence of kernicterus has been estimated in the same study by *Bhutani et al.*[2] from 2013 to be 56 per 100 000 births, with a higher incidence in low-income countries (73 per 100 000) than in high-income countries (10 per 100 000). In another four regional studies from high-income countries, the incidence ranged from 0.6-13.6 per 100 000 births.

The global mortality rate of deaths caused by neonatal hyperbilirubinemia was estimated in the study of *Bhutani et al*[2] to be 114 000 deaths (out of 134 million). They also found this to be higher in low-income countries (119 per 100 000 births) compared to high-income countries (1 per 100 000 births).

Another study, by *Olusanya et al*.[37] looked at the data from the Global Burden of Disease study from 2016, a large dataset provided by researchers who review multiple studies worldwide and integrate these into one large dataset. From this dataset, *Olusanya et al.* concluded a mortality rate of 1496 per 100 000 births owed to neonatal hyperbilirubinemia.

Regional mortality rates are continually being issued in order to provide clinicians with tools to prevent avoidable deaths. In four studies from low-income countries, it was found a mortality rate ranging from 1.3-13.1% compared to a mortality rate from 0-0.03% in two studies from high-income countries. Several studies from high-income countries did not provide numbers for mortality, probably because there were none. For example, a large study done in Denmark, by *Donneborg et al.*[38] found that none of the 12 infants who acquired kernicterus spectrum disorder (KSD) died. All of these findings on morbidity and mortality are summarized in Table 3.

Table 3: Mortality rates and kernicterus/bilirubin encephalopathy cases. The first three studies base their mortality rates on all births, while the.

Study	Country	Mortality rate	Kernicterus
Bhutani et al.[2]	Global	**114 000 / 134 million** 119 / 100 000 (1) 1 / 100 000 (2)	**56 / 100 000** 73 / 100 000 (1) 10 / 100 000 (2)
Olusanya et al.[37]	Global	**1496 / 100 000** 1309 / 100 000 (3) 187 / 100 000 (4)	113 401 DALYs lost (5)
Olusanya et al.[39]	Global	7th cause of death (3) 9th cause of death (4)	-
Olusanya et al.[40]	Nigeria	4-13.1 %	-
Simiyu et a.l[41]	Kenya	7.8 %	-
Mwaniki et al.[42]	Kenya	1.2 %	-
Nyangabyaki-Twesigye et al.[43]	Uganda	1.9 %	-
Donneborg et al.[38,44]	Denmark	0	1.2 / 100 000
Qattea et al.[45]	US	0.03 %	13.6 / 100 000
Manning et al.[46]	UK	0.2 / 100 000	0.9 / 100 000
McGillivray et al.[47]	Australia	0	0.6 / 100 000