Quantitative and localized spectroscopy for non-invasive bilirubinometry in neonates

Bosschaart, N.

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CHAPTER 2

Limitations and opportunities of transcutaneous bilirubin measurements

Although transcutaneous bilirubinometers exist for over 30 years, the clinical utility of the technique is limited to a screening method for hyperbilirubinemia, rather than a replacement for invasive blood sampling. This study investigates the reason for this limited clinical value and addresses possibilities for improvement. To obtain better insight into the physiology of bilirubin measurements, we developed and evaluated an optical transcutaneous bilirubinometer that determines not only the cutaneous bilirubin concentration (TcB), but also the blood volume fraction (BVF) in the investigated skin volume. For 49 neonates (gestational age 30±3.1 weeks, postnatal age 6(4-10) days) at our neonatal intensive care unit, we performed 124 TcB and 55 BVF measurements. The TcB correlated well with the total serum bilirubin concentration (TSB) (r=0.88) with an uncertainty of 55 µmol/L. The BVF in the measured skin volume ranged between 0.1–0.75%. The performance of our bilirubinometer is comparable to existing transcutaneous devices. The limited clinical value of current bilirubinometers can be explained by the low BVF in the skin volume that is probed by these devices. Since the TcB depends for over 99% on the contribution of extravascular bilirubin, it is a physiologically different parameter from the TSB. Hence, the standard method of evaluation that compares the TcB to the TSB is insufficient to fully investigate the clinical value of transcutaneous bilirubinometers, i.e. their predictive value for kernicterus. We suggest that the clinical value may be improved considerably by changing either the method of evaluation, or the technological design of transcutaneous bilirubinometers.

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2.1 Introduction

Jaundice is a common and often harmless clinical condition in neonates. However, severe jaundice (hyperbilirubinemia) may result in kernicterus (bilirubin encephalopathy), causing irreversible brain damage to the patient. It is therefore vital to monitor the neonate's bilirubin levels to prevent hyperbilirubinemia by providing adequate treatment in case the bilirubin levels exceed the acceptable limits [1].

The current gold standard to measure bilirubin levels is invasive blood sampling, commonly performed by a heel stick, followed by laboratory analysis of the blood sample. This method provides the total serum bilirubin concentration (TSB), which can be related to the need for treatment in look-up tables that take into account the gestational age and birth weight of the neonate. Although over the years this method has been proven to be successful in preventing kernicterus [1], it is has its drawbacks. Invasive blood sampling is painful and stressful for the neonate, resulting in blood loss and an increased risk of developing osteomyelitis and infections at the site of sampling [2,3]. In addition, the method is laborious and time consuming, lacking the possibility for immediate diagnosis or bed-side monitoring of bilirubin levels.

A possible alternative for invasive blood sampling is transcutaneous bilirubinometry, a non-invasive and painless method that provides an instantaneous read-out of the cutaneous bilirubin concentration (TcB). Transcutaneous bilirubinometry is based on optical spectroscopy, which relates the amount of light absorption by bilirubin (i.e. the yellow color of the skin) to the concentration of bilirubin in the skin. The first transcutaneous bilirubinometer was introduced in 1980 [4]. Since then, several other devices have been developed, and important adjustments, such as the correction for the presence of other skin chromophores (i.e. melanin and hemoglobin), were made to improve their accuracy [5-9]. These 'second generation' bilirubinometers are suitable for screening of hyperbilirubinemia, leading to a considerable decrease of the number of hospital readmissions [10] and a decrease of the amount of required blood samples from patients [11]. Hence, the use of transcutaneous bilirubinometers is recommended in clinical practice guidelines on the management of hyperbilirubinemia [12]. However, after more than 30 years of development, still no transcutaneous bilirubinometer has proven itself to be a worthy replacement for invasive blood sampling. Reasons for this limited clinical value may be diverse – e.g. the technological design of the bilirubinometers, the method of clinical evaluation and variations between patients – but have not been described or investigated thoroughly in literature. A better understanding of these reasons is important for both the interpretation of the measured TcB value from a patient, and for a possible improvement of the clinical value of transcutaneous bilirubinometers. Therefore, we developed and evaluated a new transcutaneous bilirubinometer that can aid to this understanding.

Our bilirubinometer differs from existing devices in light detection geometry and method of analysis, which enabled us to determine both the bilirubin concentration and the blood content inside the investigated skin volume. In general, the skin volume that is probed by transcutaneous bilirubinometers consists of both intravascular tissue space (within e.g. capillaries, arterioles) and extravascular tissue space, as illustrated in Figure 1.2a. Since the bilirubin concentration in the extravascular space is likely to be lower, the added information of blood content (i.e. quantification of intravascular space)

provides us with better insight into the physiology of TcB measurements. Moreover, whereas existing bilirubinometers use built-in calibration factors to correct for the concentration differences between the intravascular and extravascular space [5-9], the design of our bilirubinometer allowed us to measure the 'true' bilirubin concentration in the skin. Following this more thorough approach, we evaluate the performance of our device in a patient group of 49 neonates at our neonatal intensive care unit. Finally, we will use our findings in an effort to answer the question asked by many neonatologists and pediatricians: can transcutaneous bilirubinometry *replace* invasive blood sampling?

2.2 Methods

2.2.1 Patients

In a patient group of 49 neonates who were admitted to the neonatal intensive care unit of the Emma Children's hospital of the Academic Medical Center in Amsterdam, 124 blood samples were taken for TSB. Each blood sample was preceded by a TcB measurement with our bilirubinometer (within a time window of 30 minutes). Approval of the local medical ethical committee and informed consent from the patient's legally authorized representative was obtained. Blood samples were only taken for clinical reasons and were obtained by capillary heel stick or from arterial lines. The patient group varied in gestational age from 25 to 40 weeks (mean ± standard deviation (SD): 30.0 ± 3.1 weeks), postnatal age from 2 to 84 days (<7 days: 65 measurements, ≥7 days: 59 measurements, median and 25^{th} – 75^{th} percentile: 6 (4-10) days), birth weight from 620 to 4140 grams (mean ± SD: 1480 ± 660 grams) and ethnicity (Caucasian: 33, Mediterranean: 11, Negroid: 5). The majority of the patients were subjected to more than one measurement during their admission at the hospital, with a time lapse of one day to 3 weeks between measurements and a median of 2 measurements per patient.

2.2.2 Measurement device

The technical details of our transcutaneous bilirubinometer will be described in detail in Chapter 3 [13]. The device differs from other bilirubinometers in probe design, number of wavelengths, and the underlying physical model to extract the bilirubin concentration. Compared to existing bilirubinometers, this offers the unique advantage of quantifying other skin chromophore concentrations than the TcB, such as hemoglobin. In short, the device measures the skin absorption coefficient – a universal measure for the amount of light absorption in the skin (Section 1.3) – at every wavelength between 450 and 600 nm. The absorption coefficient contains the contributions of all skin chromophores (light absorbing molecules), which are bilirubin, oxygenized hemoglobin, deoxygenized hemoglobin and melanin in neonatal skin. For each individual chromophore, literature values of the chromophore-specific absorption coefficients are known for a standard concentration [14,15]. The measured skin absorption coefficient is the sum of all individual concentration dependent chromophore contributions. Therefore, by fitting the known chromophore-specific absorption coefficients to the skin absorption coefficient using a nonlinear least-square

Levenberg-Marquardt algorithm, we obtain the concentrations of bilirubin (TcB), oxygenized hemoglobin ($TcHbO_2$), deoxygenized hemoglobin (TcHb) and melanin.

The TcB was measured on the glabella of the forehead of the patients and was compared to their TSB, which was obtained from invasive capillary blood sampling at the heel maximally 30 minutes after the TcB measurement and analyzed by a Bilimeter 3 (Pfaff Medical, Germany). The uncertainty in the TSB determination by this device was ±2.8% of the measured value (95% confidence limit), which accounts for 1-11 µmol/L in the investigated range of 40-400 µmol/L.

For 55 measurements, also the total blood hemoglobin concentration ([tHb]) was determined from the capillary blood sample by an XE-5000 analyzer (n = 34, Sysmex, Germany), or from an arterial line sample by a Rapidlab 1265 bloodgas analyzer (n = 21, Bayer Health Care, Germany). From these measurements, we determined the blood volume fraction (BVF) inside the measurement volume of the transcutaneous bilirubinometer, using BVF = (TcHb + $TcHbO_2$)/[tHb] x 100%. The measurement volume of this transcutaneous bilirubinometer comprises approximately 1 x 2.4 mm (depth x width) in neonatal skin [13].

2.3 Results

Figure 2.1 shows the measured TcB versus the TSB for all 49 patients and 124 measurements. Since most evaluation studies of transcutaneous bilirubinometers only regard patients in the first week after birth, difference is made between measurements on patients with a postnatal age less than 7 days (triangles) and 7 days or more (open circles). As expected, the TcB value in the investigated skin volume is approximately a factor 4 lower than the corresponding TSB value (linear regression on all 124 measurements yields TcB = 0.26*TSB + 0.90). Regardless of this difference, the TcB correlates well with the TSB (Spearman $r = 0.88$, $p < 0.01$, all 124 measurements), which is comparable to the clinical performance of commercial transcutaneous bilirubinometers [5-9,16]. The uncertainty in the measured TcB values is visualized in Figure 2.1 by their 95% prediction limits (PL). If we want to use these TcB measurements in clinical practice, we need to correct the measured TcB for the previously mentioned factor of ~4, resulting in an uncertainty of 55 µmol/L (2*SD of the difference between the corrected TcB and the TSB). This uncertainty is also comparable to the clinical performance of commercial transcutaneous bilirubinometers [5-9,16]. The correlation between the TcB and the TSB does not alter when we regard only the measurements on patients with a postnatal age less than 7 days (Spearman $r = 0.88$, $p < 0.01$, $n = 65$), but it is lower when we regard only the measurements on patients with a postnatal age of 7 days or more (Spearman $r = 0.81$, $p < 0.01$, $n = 59$). This may be due to age related skin changes (thickening) and other, not investigated parameters. The influence of other patient characteristics (ethnicity, gestational age, birth weight) on the correlation between the TcB and the TSB was not assessed.

We found that the BVF in the measurement volume ranged between 0.1% and 0.75% (mean±SD: 0.35±0.12%), which is slightly lower than the average BVF of 2-5% in well perfused adult human skin [15], but comparable to the vessel density (vessel length*diameter per tissue area) of 0.20-0.23% observed in orthogonal polarization

spectral images of the microcirculation of neonatal skin at the inner upper arm [17]. Since the BVF accounts for the amount of intravascular space present in the measurement volume of the transcutaneous bilirubin measurement, we can conclude that over 99% of the bilirubin contributing to the measured TcB, is extravascular bilirubin. Furthermore, no correlation exists between the measured BVF and TcB (Pearson $r = 0.03$, $p > 0.05$), which implies that high TcB values cannot be associated with a higher vessel density in the measurement volume.

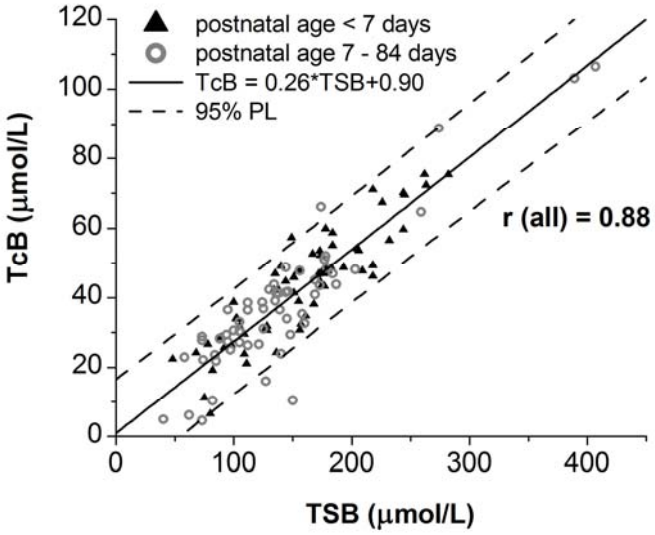

Figure 2.1 *Comparison of 124 patient measurements (postnatal age < 7 days and ≥ 7 days) of the TcB to the TSB, with 95% PL of the linear regression TcB = 0.26*TSB + 0.90 (Spearman r = 0.88, p < 0.01). Conversion of units: 1 µmol/L = 0.0585 mg/dL bilirubin. Note the difference in horizontal and vertical scaling.*

2.4 Discussion

The evaluation of our transcutaneous bilirubinometer showed that its performance is comparable to existing devices and therefore, it does not lead directly to an improved clinical value for transcutaneous bilirubinometry. However, with our device we were able to estimate also the blood volume fraction in the probed skin volume, which aids to better understanding of the differences between transcutaneous and blood sampling bilirubin measurements. From our results we can learn two important lessons: 1) the contribution of intravascular bilirubin to the measured TcB is less than 1%, and 2) the extravascular bilirubin concentration is approximately a factor 4 lower than the TSB. Hence, we can draw the conclusion that the TcB is a physiologically different parameter from the TSB, since the first contains mainly the contribution of extravascular bilirubin and the latter equals the bilirubin concentration in the intravascular space. Awareness of this difference is important when interpreting TcB measurements on patients, because it is not straight forward to relate a TcB value to a TSB value [18]. The relation between the TcB and the TSB depends on numerous factors regulating the extravasation and clearance of bilirubin in the extravascular space. Many of these factors are difficult to model, such as the photo break-down of bilirubin by phototherapy or day light influences [19], or are still not well understood, such as the

cephalocaudal progression of jaundice [20]. This raises a new, but relevant question: is it justified to evaluate the clinical value of the TcB by comparing it to the TSB?

Any new medical diagnostic technique needs to prove that its performance is equal to, or better than that of the gold standard, in order to replace it [21]. Therefore, to test the performance of transcutaneous bilirubinometers, their outcome is commonly compared to the outcome of the total serum bilirubin measurements from invasive blood sampling. However, because of the complex nature of the processes that regulate the extravasation and clearance of bilirubin [18-20], the uncertainty in the TcB measurement is considerable when comparing it to the TSB (Figure 2.1). Since the accuracy of our bilirubinometer in measuring bilirubin absorption is approximately 15% [13], we can assume that the majority of the uncertainty in our TcB evaluation is caused by the physiological difference between the two parameters [22,23]. Because of this physiological difference, transcutaneous bilirubinometry will never equal the performances of the gold standard, invasive blood sampling [22]. This is the major reason why transcutaneous bilirubinometry has not yet replaced invasive blood sampling, and that it is primarily used as a screening method for hyperbilirubinemia. Fortunately, this does not imply that we have reached an end station in the clinical utility of transcutaneous bilirubinometry. We stress that the clinical value of transcutaneous bilirubinometry can be increased considerably, by choosing either a medical, or a technological approach to solving the problem. Both approaches will be explained in more detail below.

2.4.1 Medical approach to enhanced clinical value

In patients with hyperbilirubinemia, the clinically relevant parameter is the risk for developing kernicterus. The TSB has proven to be a good predictor for kernicterus, and extensive research and risk analyses have led to the TSB limits that we use today for treatment decision making. However, these limits are not conclusive, especially in extreme premature infants, since some patients developed kernicterus even though the TSB was well below the safety limits [24]. This explains that the debate on the exact values of these limits is still ongoing [24,25]. Thus, although the TSB is a good predictor, it is not the perfect predictor for kernicterus. An increasing amount of studies suggest that the free bilirubin (unbound to albumin) concentration is a considerably better predictor for kernicterus than the TSB [25,26]. However, since no widely available method exists to determine the free bilirubin concentration, the TSB remains the gold standard for determining the risk for developing kernicterus [12,26].

From the results in Section 2.3 we know that transcutaneous bilirubinometry measures the extravascular, rather than the intravascular bilirubin concentration. Whereas bilirubin extravasation in the skin depends on both the concentration of unconjugated bilirubin that is free, and bound to albumin, in the brain it depends only on the concentration of free, unconjugated bilirubin that extravasates through the intact blood brain barrier and enters the subcortical cells [1]. The latter is caused by the absence of extravascular albumin space in the central nervous system [18]. However, the TcB may be more closely related to the risk for developing kernicterus than the TSB, since the TcB is a measure for the extravasated (unconjugated) bilirubin concentration and the TSB is a measure for the intravascular (added conjugated and unconjugated) bilirubin concentration. The added value of the TcB in the prediction of central nervous

system symptoms has been demonstrated before [27]. The TcB may therefore even be an equally good, or better predictor for kernicterus than the TSB, as has been recognized by others as well [7,18,22]. Hence, the true clinical value of current transcutaneous bilirubinometers should not be investigated by comparing the TcB to the TSB, but rather by clinical assessment of the TcB as a predictor for kernicterus. Such a study requires an extensive risk analysis in a large variety of patients over multiple hospitals, because the incidence of kernicterus is low at present.

Since the TcB reading may be influenced by the brand of the device [16], and the accuracy of the reading is influenced by the age of the patient [28] and the measurement site on the body [7], the influence of these factors needs to be included in the proposed risk analysis. We also need to keep in mind that the reproducibility of TcB measurements (coefficient of variation ±4% [4,6]) is lower than the reproducibility of TSB measurements (coefficient of variation <2% [16]), although the latter does not take into account errors that may occur in the preanalytical phase of TSB measurements, e.g. during blood sampling and sample handling [29]. The difference in reproducibility between TcB and TSB measurements may result in larger safety margins for treatment decision making based on TcB measurements, but these safety margins will primarily depend on the predictive value of the TcB on kernicterus.

2.4.2 Technological approach to enhanced clinical value

Another possibility lies within a new design for transcutaneous bilirubinometers. Until now, all transcutaneous bilirubinometers measure the TcB in a tissue volume consisting mainly of skin cells and hence, the extravascular, rather than the intravascular bilirubin concentration is measured. In order to achieve a one to one comparison with the TSB, the measurement volume of transcutaneous bilirubinometers needs to be confined to the intravascular space, i.e. a volume consisting of blood only as illustrated in Figure 1.2b. This requires a spectroscopic technique that is capable of measuring the absorption coefficient in a confined volume of choice, such as a blood vessel. As described in Chapter 1, low-coherence interferometry (LCI) based techniques have proven that precise control of photon path lengths – and therefore measurement volume, in the case of single scattering events – is possible [30-33]. The possibilities for localized spectroscopy by LCI have only been marginally investigated in optical coherence tomography studies [32,33], which aimed on image contrast enhancement with relatively low spectral resolution, rather than accurate quantification of chromophore concentrations. However, we propose that the development of an LCI based technique that is optimized for spectroscopic purposes can be realized (LCS, Chapter 1). Hence, the feasibility of such a technique for transcutaneous bilirubinometry should be investigated.

2.5 Conclusion: can transcutaneous bilirubinometry *replace* invasive blood sampling?

In this Chapter, we tried to obtain better understanding of the reasons for the limited clinical value of transcutaneous bilirubinometers by evaluating a new design for a transcutaneous bilirubinometer. Although this device did not perform better than

existing devices, it did give us better insight into the physiology related to transcutaneous bilirubin measurements. The cutaneous bilirubin concentration (TcB) measured by transcutaneous bilirubinometry is a physiologically different parameter from the total serum bilirubin concentration in blood (TSB), since the TcB consists for over 99% of the contribution of extravascular bilirubin. Due to the complex nature of the processes that regulate the supply and clearance of bilirubin in the extravascular space, a one to one comparison of the TcB to the TSB is currently impossible. Therefore, transcutaneous bilirubinometry will not replace invasive blood sampling, if the criterion for this replacement remains that the TcB should equal the TSB. However, we suggest that two approaches can result in a better clinical value for transcutaneous bilirubinometry: 1) a medical approach, requiring an extensive risk analysis for the predictive value of the TcB for the occurrence of kernicterus and 2) a technological approach, where the measurement volume of the transcutaneous bilirubinometer is confined to the intravascular space, enabling a one to one comparison with the TSB. Both approaches are worth investigating, since a non-invasive alternative to invasive blood sampling in bilirubinometry will reduce pain, complications and diagnostic time for patients with hyperbilirubinemia.

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