Skin colour measurements of jaundiced neonates

Master thesis by A.F. Wagter

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Preface

This thesis is written as the final part of my MSc Biomedical Engineering at the Delft University of Technology. The aim of this study was to search for a new non-invasive method for monitoring neonatal hyperbilirubinemia, which could be used in support of the current practice, to reduce the need for blood sampling.

The document consists of two main parts: a general introduction and a scientific paper. The general introduction provides background information on neonatal hyperbilirubinemia and the context for the medical research. The paper presents the results of the observational study on skin colour measurements of jaundiced neonates.

Hereby I would like to show my gratitude to all of the following people for their support and contributions.

First of all I would like to thank my supervisors; Tom Goos for all the help on navigating in the medical field and in particular the neonatology department of the Erasmus Medical Centre, and Professor Dr. Jenny Dankelman for her guidance and constructive criticism.
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Finally I would like to thank my family and my friends for all they have done that has led me to this point; the completion of my studies at the TU Delft.

Ava Wagter
Delft, March 2017

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List of abbreviations

B	*Blue*
CMYK	*Cyan-Magenta-Yellow-Key*
G	*Green*
GA	*Gestational age*
IQR	*Interquartile range*
MDC	*Minimum detectable change*
PCA	*Principal Component Analysis*
RGB	*Red-Green-Blue*
R	*Red*
SD	*Standard deviation*
SEM	*Standard error of the mean*
TcB	*Transcutaneous bilirubinometry*
TSB	*Total Serum Bilirubin*

1. General introduction

Neonatal hyperbilirubinemia is a condition affecting newborns. As is indicated in the name, it means there is a surplus of bilirubin in the body. Bilirubin is a breakdown product of haemoglobin [ref]. Normally enzymes are responsible for removing this by-product. The level of bilirubin in the blood becomes elevated in neonates specifically due to an increased breakdown of red blood cells, combined with a low activity of the enzyme responsible for removing the bilirubin[1,2]. Both of these phenomena are related to naturally occurring processes in the first days after birth, therefore hyperbilirubinemia is a commonly occurring condition. Indications for an increased risk of having elevated bilirubin values are prematurity, malnutrition, blood group antagonism and other conditions affecting blood cell breakdown or liver function[1,3].

Since bilirubin is a yellow pigment, when a build-up of bilirubin in blood and the subcutaneous tissues occurs it becomes visible through the capillary veins. It will show as yellow discolouration of the skin, the white of the eyes, and mucous membranes[2,3]. This symptom is called icterus, or jaundice in layman's terms.

Having elevated levels of bilirubin is not intrinsically harmful. However, when the level of bilirubin in the blood surpasses a patient specific threshold, the risk of bilirubin crossing the blood-brain barrier becomes hazardous. Since bilirubin is neurotoxic, the possible consequence of this situation is a certain extent of encephalopathy; brain dysfunction, presenting itself in symptoms such as cerebral palsy, hearing loss and other physical or intellectual disabilities [1,4,5].

In order to prevent children from experiencing severe hyperbilirubinemia, treatment by phototherapy is used. The infant's skin is exposed to intense blue light in the range of 430-490 nm, which causes a chemical reaction facilitating bilirubin excretion [6][Stokowski 2006]. If phototherapy is not effective (enough), the final option is to perform a blood exchange transfusion [7–9]]. The potentially dangerous levels of bilirubin vary for each individual depending on many factors; i.e. birthweight, postnatal age in hours, and the risk indicators. The guidelines for treatments levels, taking into account these factors, have been visually represented in multiple hyperbilirubinemia nomograms [10,11], indicating the patient specific intervention threshold (Figure 1). The nomograms for all gestational ages can be viewed in Appendix A. Nomograms - patient specific intervention threshold.

The incentive for this graduation project came from a start-up company, which aims to develop a phototherapy solution for the home situation. Currently, treatment by phototherapy is done solely in the hospital. The newborn needs to stay in the incubator a number of days, with eye shades to protect the eyes from the light. A major disadvantage of this treatment is that the newborn needs to be naked in the incubator separated from the mother and family in the first days of its life, which is undesirable for bonding and feeding of the infant. Treatment usually takes place in a high care department and not in the normal maternity department of the hospital, driving up the cost of nursing and care. This motivated to start developing a new portable phototherapy device, suitable to be used at home. A new challenge that arises when translating the current treatment to a new solution for the home situation is in monitoring the condition of the neonate, which is why this is the focus of this research.

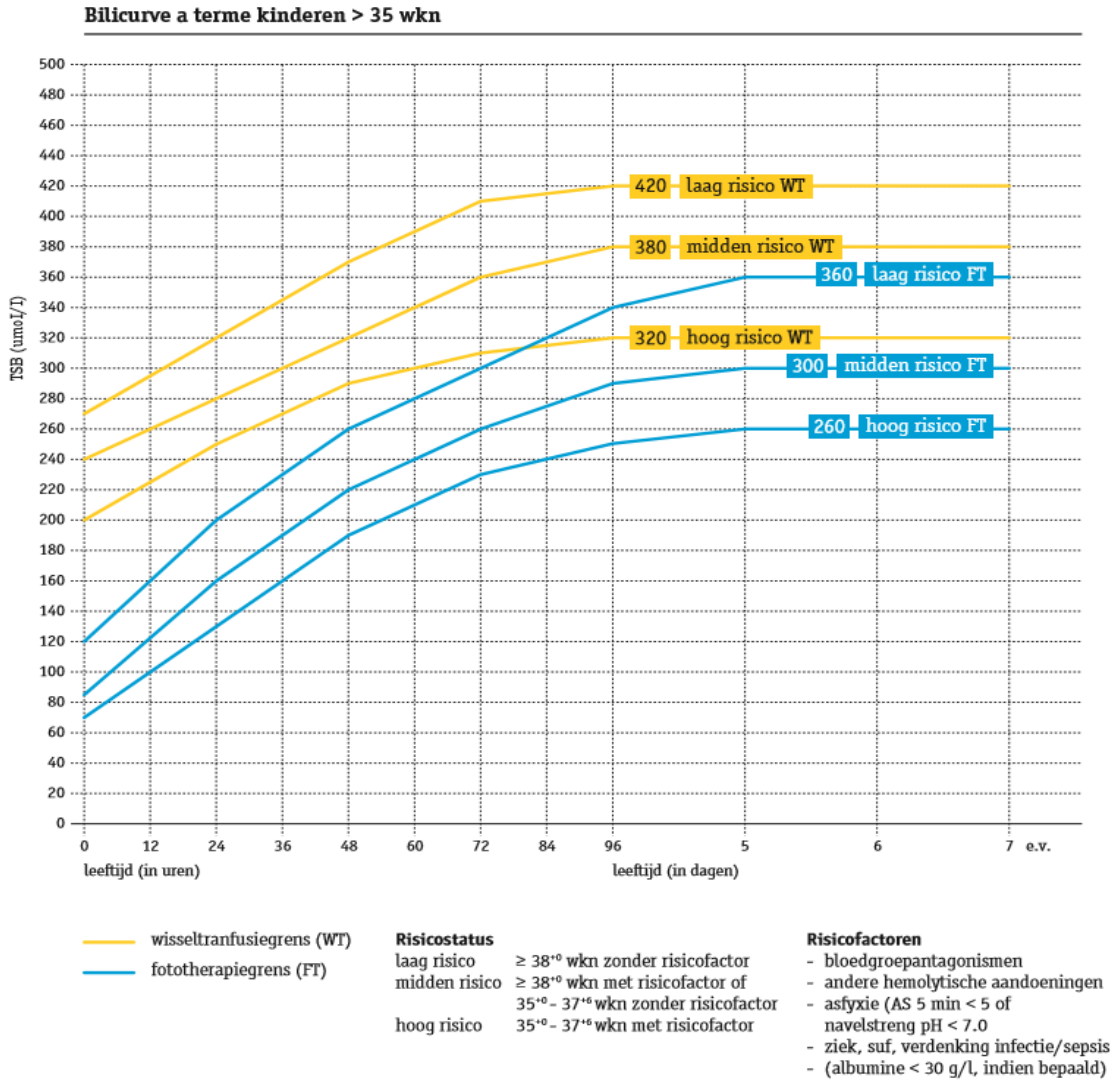

Figure 1: Example of a hyperbilirubinemia nomogram. Specified for patients over 35 weeks of gestation

Monitoring for hyperbilirubinemia consists of firstly identifying the neonates whom have, or are at the risk of developing, significant hyperbilirubinemia and secondly to keep supervising these children and to intervene with treatments if needed. The assessment of hyperbilirubinemia takes place with the following steps; the suspicion of hyperbilirubinemia usually occurs through (subjective) visual assessment of jaundice, optionally the jaundice can be measured through transcutaneous bilirubinometry to estimate the total serum bilirubin, and finally the condition must be quantified by evaluating the level of bilirubin in the blood [12].

The golden standard in diagnosing neonatal hyperbilirubinemia is determining the total serum bilirubin (TSB) in the blood [10,11]. The interval in which this is determined tends to be 24 hours, but the frequency can increase up to approximately every 6 hours depending on the severity of the hyperbilirubinemia and hospital policy. Drawing blood by heel puncture for each TSB determination is invasive and the procedure is stressful for infant and parents.

To reduce the invasiveness and stress it would be desirable to reduce the amount of blood draws from the patient, without compromising the monitoring of the hyperbilirubinemia. A manner in which the extent of hyperbilirubinemia can be observed is through detection of jaundice. With a decrease of hyperbilirubinemia, the extent of jaundice will decrease as well. However, visual detection of jaundice is subjective and deemed to be unreliable [13,14]. The visible extent of jaundice in an infant is very patient specific. A similar level of bilirubin in the blood of two neonates might lead to a different degree of yellowing of the skin. Thus it is found that healthcare professionals and parents are able of recognising jaundice but they are not very capable at assessing the clinical severity [15–17]. Another complicating factor is skin pigmentation; some studies suggest jaundice is harder to detect in infants with a dark complexion[18].

A method to quantify the degree of yellowness of the skin is through colour measurements of light reflection of the skin. A well-known example of this method is transcutaneous bilirubinometry (TcB). The measurement of the colour of the skin is converted through an algorithm to generate an estimation of the TSB value[19]. TcB has been found to be reliable as a screening method. However, when the infant is undergoing phototherapy, the infant's skin is bleached by the light and it is found that the TcB device can't give an accurate measurement [20,21].

In order to be able to monitor the neonates during phototherapy at home, a new non-invasive method of monitoring is required. The desire is to ultimately incorporate this method into the new portable phototherapy device, which would mean the device has to be able to be on the infant at all times. The aim of the following study was to test a new hypothesis regarding a non-invasive method of monitoring.

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2. *Skin colour measurements of jaundiced neonates over 30 weeks as an indication of development of hyperbilirubinemia*

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Abstract

Background: Monitoring neonatal hyperbilirubinemia during phototherapy is currently done solely by determining the total serum bilirubin (TSB) in the blood. A non-invasive method of monitoring would be desirable but the current transcutaneous bilirubinometry measurements are not deemed reliable during phototherapy.
Objectives: To evaluate whether the change in TSB can be monitored through assessing the change in non-invasive measurements of skin colour over time.
Methods: In an observational multicentre study, multiple transcutaneous skin colour measurements where collected over time. Within each subject, two subsequent skin colour measurements are compared for the change in skin colour and the change in TSB.
Results: For the preliminary findings of the study, on 15 neonates (GA 33+4 (30+3-37+3) weeks, birthweight 1875 (1078-3465)) skin colour measurements were performed during blood sampling for TSB determination. The change of skin colour expressed in Yellow, and in a linear regression of Green and Blue components, show to correctly predict the change of TSB up to 86,4% of the cases, with a sensitivity of 100% and a specificity of 80%.
Conclusion: The change in colour values has predictive capacity for the change in TSB over time. Skin colour appears to change with a delay compared to TSB change. The greatest potential lies in measuring on a proximal location of the body. Monitoring the change in skin colour should be further explored as an option to support and partially replace TSB determination.

Key words: neonatal hyperbilirubinemia, jaundice, skin colour, transcutaneous measurements

INTRODUCTION

Clinical relevance of neonatal hyper-bilirubinemia is high as it is estimated to occur in 60% of full term and 80% of preterm neonates[1,2]. Fortunately in the majority of these neonates the condition will not become severe; merely 4% of all neonates[3] will need treatment in order to prevent high levels of bilirubin in the blood which can lead to kernicterus, neurological damage resulting in physical or intellectual disabilities[4,5]. Despite the relatively low need for treatment, due to the high occurrence and the potentially extreme consequences of hyperbilirubinemia, it is of great importance to adequately monitor neonates in the first days of life when they may experience hyperbilirubinemia.

Current methods in monitoring neonatal hyperbilirubinemia are essential but have their own limitations. Determining the total serum bilirubin (TSB) is invasive, which creates a barrier for the initial checking of levels of bilirubin. Furthermore, the frequency with which the TSB will be determined once a child is diagnosed with significant hyper-bilirubinemia will be limited. Transcutaneous bilirubinometry offers a non-invasive method[6]. Although this has proven to be effective to some extent[7,8], due to inaccuracies with high TSB and ineffectiveness during phototherapy[9], transcutaneous bilirubinometers are solely deemed suitable as a screening device, and still they are not used in all care centres.

These limitations drove the desire to find a different method of monitoring hyper-bilirubinemia, which is non-invasive and effective during phototherapy, thus reducing the number of blood draws needed, allowing for more frequent measuring, and perhaps generating the possibility for monitoring jaundice in the home situation in the future.

A new method is evaluated; by taking multiple skin colour measurements of a single neonate over time, the overall trend of the jaundice can be tracked. For each measurement, a comparison to the previous measurement will show either a decline, an incline or a continuation in the extent of yellow colour in the skin. The trend of the jaundice for a single child is presumed to correlate with the increase or decrease in neonatal hyper-bilirubinemia over time. If this correlation exist, it would be possible to determine whether an infant is recovering from the hyperbilirubinemia or if the condition is progressing and (additional) intervention is required.

This method would not replace the determination of the TSB, but could reduce the frequency with which it has to be determined, and thus reduce the number of samples drawn. Furthermore it could give an early indication on whether the current applied therapy works adequately or not.

By using the trend of colour measurements, only the relative change in the degree of yellow is relevant. Therefore it is not necessary to determine how a single measurement of colour relates to the level of TSB. Using the relative change instead of the absolute value is convenient since the natural skin tone of each neonate will have a different extent of yellow, and it will differ how jaundiced a neonate will appear for a specific level of TSB[10].

The aim of this study was to determine whether non-invasive measurements of skin colour over time can be used as a method to monitor the trend of hyperbilirubinemia in neonates.

METHODS

A prospective observational multicentre study was performed at the departments of Neonatology and Obstetrics of the Sophia Children's Hospital - Erasmus Medical centre in Rotterdam, the Amphia Hospital in Breda, and the Maasstad Hospital in Rotterdam, all located in The Netherlands.

The medical ethics committee of the Erasmus Medical Centre study found the study not to lie within the scope of the Medical Research Involving Human Subjects Act (WMO). Due to the non-invasive and non-encumbering nature of the study, and the time constraint posed by

the fleeting nature of hyperbilirubinemia, obtaining deferred parental consent was permitted. The research protocol for the medical ethical application can be found in Appendix B.

Included parameters

The main study parameter was the relative change of skin colour over time. In order to determine whether this change was an useful indicator for the progression of hyperbilirubinemia, it was compared to the change in bilirubin level in the blood.

Parameters which could influence the main study parameter and have been accounted for in the research are: the skin type of each infant, treatment with phototherapy and the effect of cephalocaudal progression on the jaundice.

A dark skin type is known to increase difficulty for detecting jaundice[11]. The trend of skin colour measurements is also expected to be different for light skinned babies and more dark skinned babies. In order to examine the effect of skin tone on the correlation of the trend of colour measurements and the progression of the hyperbilirubinemia, both Caucasian and non-Caucasian babies are included in the study so these groups can be analysed separately and can be compared.

Treatment with phototherapy has been described to bleach the skin that is exposed to the phototherapy compared to skin that is not exposed to phototherapy[9,12]. Whether exposed skin holds a better or poorer correlation to the actual severity of the jaundice is unknown. Many participants included in the study were expected to undergo phototherapy during the participation in the study, since the conditions during phototherapy tends to make them more eligible for inclusion. However it is common for a neonate to wear a diaper while undergoing phototherapy, which means the area is largely shielded from the light. In order to examine the effect of exposure to light, both the skin underneath the diaper and exposed skin areas has been measured.

Hyperbilirubinemia follows a cephalocaudal progression, as described by Kramer[13]. The jaundice will first be visible in the cranial area and as the condition becomes more severe, the jaundice will progress to more distal regions of the body. As a result, the extent of jaundice will differ for different body parts. This is accounted for in the study by measuring colour on several locations, ranging from more cranial to more caudal.

Other parameters that were collected were the neonate's temperature, gestational age, birth weight, birth date and time, whether the neonate was wearing a diaper, whether the neonate was receiving phototherapy and the intensity of the therapy if applied.

Measurements

The research consisted of several repeated sets of cutaneous measurements with a colour sensor done on each neonate. A set of measurements was performed at the time of a blood draw for the determination of TSB. All blood samples had been requested in accordance to clinical practice, no additional samples were taken for this study. Since the main outcome is the trend of the skin colour in relation to the TBS, a minimum of two sets of measurements per neonate was required in order to be able to analyse the data. The total number of sets of measurements was determined by the number of blood draws which had been requested by the physician.

Many subjects received phototherapy as treatment for hyperbilirubinemia during the study. The phototherapy was not interrupted while the measurements were performed.

A set of measurements consisted of five cutaneous measurements on different locations, namely on the forehead, the sternum, laterally on a hip, between the umbilicus and the os pubis, and on the sole of a foot. The measurements on the forehead, the sternum, the hip and the sole of the foot were compared in order to investigate the effect of the cephalocaudal progression on jaundice. The measurements between the umbilicus and the os pubis of neonates which

were wearing a diaper were compared with the measurements of the thigh, in order to investigate the effect of bleaching of the skin by phototherapy.

Next to the set of measurements, two additional measurements were performed; a colour measurement of the forehead half an hour before a blood draw, and a colour measurement of the forehead half an hour after a blood draw. These measurements provide information on a possible delay in change. Doing more measurements would have been desirable, however this comprise was made in order to not disturb the neonates to frequently. The forehead was chosen since this is an easily accessible location, which is likely to be uncovered, thus resulting in minimal disturbance for the neonate. See Appendix C for the full research procedure.

Device

Since the intention was to research whether the method of using change in skin colour is useful in monitoring, and not whether a specific device is useful, a simple set up was developed for the measurements of the skin colour. (Appendix D.) The set up consisted of an handheld, battery powered, colour sensor device and a display where the colour measurement was presented. The device contained a colour sensor having a 3-channel (RGB) photodiode sensitive to the red (λp=650 nm), green (λp=550 nm) and blue (λp=450 nm) regions of the spectrum. The RGB colour space is one of the simplest and most commonly used models to describe colours and RGB sensors are subsequently a much used colour sensor type. A light source was built into the device, a white LED light source of 380-780 nm, with a luminous intensity of 370 mcd.

During a measurement, the device was placed onto the neonate's skin. White light from the LED was directed towards the skin, which then reflected back to the RGB sensor. The collected light represented the colour of the skin. The colour data was then displayed in the red, green and blue components of the specific colour.

Subjects

All infants born at a gestational age equal or greater than 30 weeks whom were hospitalized in one of the participating hospitals, and of whom more than one TSB value has been determined on suspicion of neonatal hyperbilirubinemia, were eligible for inclusion. Infants experiencing sepsis during the study period were excluded from the study, since large temperature fluctuations are expected to influence the measured skin colour.

Statistical analysis

Sample size

Since this is a pilot study and no comparable study data exists, a limited number of participants is included. As suggested by Julious [14], at least 12 participants should be included in each group in a pilot study in order to optimize for feasibility and for precision about the mean and the variance. Based on these findings and on the feasibility of executing the research due to incidence of hyperbilirubinemia, 36 participants will be included.

Colour data

The colour measurements are expressed in red, green and blue values, which change over time. In order to correlate relative change of skin colour over time with change of bilirubin in the blood over time, two approaches of handling the colour data were explored. The first approach is to use a single colour, or Yellow, a combined value of the RGB values. The change in this colour value is evaluated on whether it has predictive abilities for change in TSB. The second approach was to use the RGB colour values in a linear regression to find factors for RGB with which they best estimate the TSB values. The change in the predicted values is compared to the change in TSB.

Yellow is a component of the Cyan, Yellow, Magenta and black Key (CMYK) colour space. In RGB colour space, the colour yellow is described as a combination of red and green. For a completely yellow the respective RGB

values would be 255,255,0. In CMYK colour space, the same colour would be described by the CMYK values 0,0,1,0.

RGB to Y conversion is established as following:

$$R' = \frac{R}{255} \qquad G' = \frac{G}{255} \qquad B' = \frac{B}{255}$$

$$K = 1 - \max(R', G', B')$$

$$Y = \frac{1 - B' - K}{1 - K}$$

To compare the relative change of skin colour over time with the change of TSB over time, a logistic mixed effect regression analysis will be used. The relative change of skin colour and the change in TSB are represented by dichotomous variables indicating an increase or decrease from one time point to the next. Race is indicated dichotomously by either Caucasian or non-Caucasian. The logistic regression analysis will indicate the probability that the skin colour will increase if the TSB increases. (IBM SPSS Statistics 24, Armonk, New York, USA).

The effect of cephalo-caudal progression is a categorical variable, which will be assessed by analysing the measurements of the forehead, the sternum, thigh and sole of the foot. The logistic mixed effect regression model will be applied for each measurement location. In this way the different locations can't be compared to find the best site for measurements, but the outcomes will show whether each site is suitable for this method.

The effect of phototherapy will be assessed through a different logistic mixed effect regression model, where change in colour is the dependent variable, and we have change in TSB and the presence of phototherapy as independent covariates.

RESULTS

The results show the preliminary findings of the study, after the inclusion of 15 patients who were enrolled from August 2016 to December 2016 with obtained informed consent from the legal guardians. The patient data is represented in table 1.

Table 1: Patient characteristics.

Patients characteristics								
Patient no	Sex	GA	Weight	No.	Yellow		Bili	
1	F	34 + 6	2085	4	0,235	(0,183 – 0,287)	148	(104 – 161)
2	F	34 + 6	1078	4	0,211	(0,178 – 0,270)	86	(80 – 95)
3	M	31 +2	1180	4	0,151	(0,120 – 0,258)	103	(38 – 131)
4	F	30 + 3	1275	16	0,256	(0,216 – 0,344)	169	(121 – 261)
5	F	33 + 6	1540	7	0,261	(0,125 – 0,305)	186	(132 – 205)
6	F	37 + 3	3075	2	0,319	(0,312 – 0,327)	233	(228 – 238)
7	F	37 + 3	3465	2	0,360	(0,355 – 0,364)	255	(250 – 259)
8	M	31 + 2	1700	5	0,337	(0,329 – 0,341)	228	(184 – 276)
9	M	36 + 0	2700	3	0,352	(0,284 – 0,367)	239	(214 – 274)
10	M	32 + 6	1615	4	0,297	(0,262 – 0,321)	179	(147 – 199)
11	M	33 + 6	1280	2	0,273	(0,236 – 0,310)	139	(114 – 163)
12	M	33 + 6	2165	2	0,311	(0,299 – 0,322)	140	(138 – 142)
13	F	33 + 0	1420	2	0,236	(0,217 – 0,255)	151	(147 – 155)
14	F	30 + 3	1150	3	0,286	(0,217 – 0,241)	153	(125 – 156)
15	M	32 + 4	2390	3	0,358	(0,252 – 0,426)	187	(178 – 263)
Mean		33 + 4	1875	4	0,273	(0,120 – 0,426)	172	(38 – 276)

Data presented as median (range). Median Yellow was determined from measurements of the forehead

Within the enrolment time frame, 29 patients were approached for parental consent. Out of this group 15 patients were included in the study, the remaining 14 patients were excluded either due to a lack of parental consent or since it was not possible to obtain two sets of measurements. Since this is a pilot study, thus including only a small sample size, the study population is not expected to be representative of the total population.

Of the study population 12 subjects were patients in the Sophia Children's Hospital, Patient 15 was a patient in the Amphia Hospital, and Patient 8 and Patient 10 were hospitalized subsequently in the Sophia Children's Hospital and in the Amphia Hospital.

Factor determination

One approach in handling the colour data was to use the RGB values in a regression to find the best estimate of the TSB values. For this approach multiple models were used to compare their efficacy.

- Linear regression; one analysis was done for the R and B values (RB), and another for the G and B values (GB). Due to the high collinearity of R and G, these colour values cannot be used in linear regression simultaneously.
- Optimization through brute force regression; which was performed both without (BF1) and with (BF2) the use of a constant value in the formula.

From these models the weighing factors of the input variables can be determined. For each model, the formula to find the predicted value of the TSB based on the colour values can be seen in table 2.

Table 2: Models for predicting TSB

	Predicted TSB values
RB	*TSB = 125 + 3R - 3,7B*
GB	*TSB = 245 + 2,7G - 3,7B*
BF 1	*TSB = 4R + 1G - 5B*
BF 2	*TSB = 115 + 3R + 0.3G - 3,9B*

Colour values over time

For all patients, the measured RGB values on each location on the body are plotted over time. An example can be seen in Figure 2, which represents the measurements of the forehead over time for patient 4. The RGB values show high multicollinearity, changing similarly over time. In Figure 3 the RGB values are expressed in Yellow, and in the regression models, these values are plotted with the TSB values over time. For RGB plots over time on multiple patients and multiple locations, refer to Appendix E.

For further analysis the actual colour values will not be taken in regard but instead the relative change over time. The change of skin colour is assessed by the difference in two consecutive colour measurements, which will give a delta value. The delta will be expressed dichotomously, where each delta is either an increase or decrease over time. The change in TSB is computed in the same fashion, so that the behaviour of the skin colour and TSB over a specific time period can be compared.

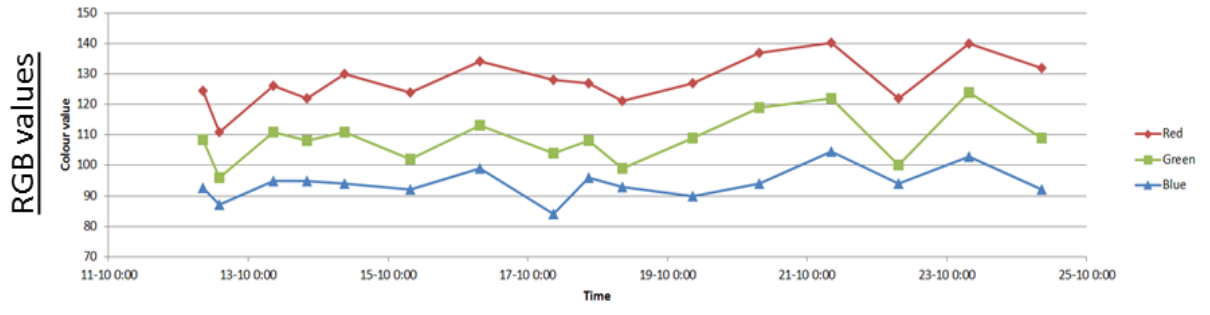

Figure 2: Example of RGB colour values over time – Patient 4, forehead

Expressed colour values over time – Patient 4, forehead

Figure 3: Values of Yellow, linear regression RB and GB, and of BF1 and BF2 over time of the forehead for patient 4.

Prediction of change in TSB through change in colour

With the logistic regression analysis, the single colour values of R, G, B, and the combined Yellow colour values are compared for consecutive values, the change is expressed dichotomously. With the change, the models are assessed on the probability that the TSB will increase if the skin colour increases. The results, in Table 3, shows the R and the G as a non-significant result, thus not being a suitable model for predicting the change in TSB. B has an inverse relation, showing a decrease in B signifies an increase in TSB. The Yellow colour value however does provide a significant result, with an odds ratio of 4,17. The odds of the model correctly predicting the direction of change is 4,17 times greater than the odds of a wrong prediction.

Table 3: Odds ratio for R, G, B, and Yellow

	Estimate	*Odds ratio*
R	*0.0236*	*0.98*
G	*-0.0002*	*0,99*
B	*1.0085*	*0,36***
Yellow	*1.4266*	*4,17****

*** = p < 0.001, ** = p < 0.01

The change in the predicted values, using the factors determined for the linear regressions and the brute force optimizations, is also evaluated for the probability that the skin colour will increase if the TSB increases, as shown in Table 4. The linear regression of GB shows to have the highest odds of predicting the change correctly.

Table 4: Odds ratio for regression models

	Estimate	Odds ratio
RB	1.145	3,14**
GB	2.0675	7,91***
BF 1	1.4906	4,44***
BF 2	1.5092	4,52***

*** = p < 0.001, ** = p < 0.01

Due to the simplicity of the method of Yellow, the relatively good results is interesting. The linear regression of GB appears to give the best predictive ability for change. Therefore the results of Yellow and the linear regression of GB will compared in the following analyses.

Measurement fault

The measurement fault of the RGB device was evaluated prior to the study. In consistent measurements, which consisted of repeated measurements within a short time frame on three locations on the body of a single adult, the standard deviation was found to be 0,00538 in Yellow and 1,942 in GB.

Additional uncertainty was introduced in the study due to researcher variability, neonate variability and environment conditions. The total measurement fault was assessed by performing repeated measurements on the forehead of each patient during the study, which showed a standard deviation of 0,00952 in Yellow and 2,648 in GB.

When assessing the change of colour, the measurement fault has to be taken into account. The minimum detectable change (MDC) is used to find the range where measurements might overlap, thus introducing uncertainty in the results.

$$Range\ MDC = \pm 1{,}96 * \sqrt{2} * SEM$$

For Yellow and GB this results in the ranges of [-0,0128, 0,0128] and [-3,571, 3,571].

Relative change of skin colour

When assessing the measurements locations individually, it can be seen whether the change in Yellow, or the change in GB, and the change in TSB show the same behaviour over time. In Figure 4 and 5, the delta values of the measurements of the forehead are plotted. The range of the MDC is plotted as dotted lines to show where the uncertainty of the measurement lies. For all plot of the delta values, refer to appendix F.

When looking at the graph, a distinction can be made on where on the graph the data point lie and what this means for the analysis.

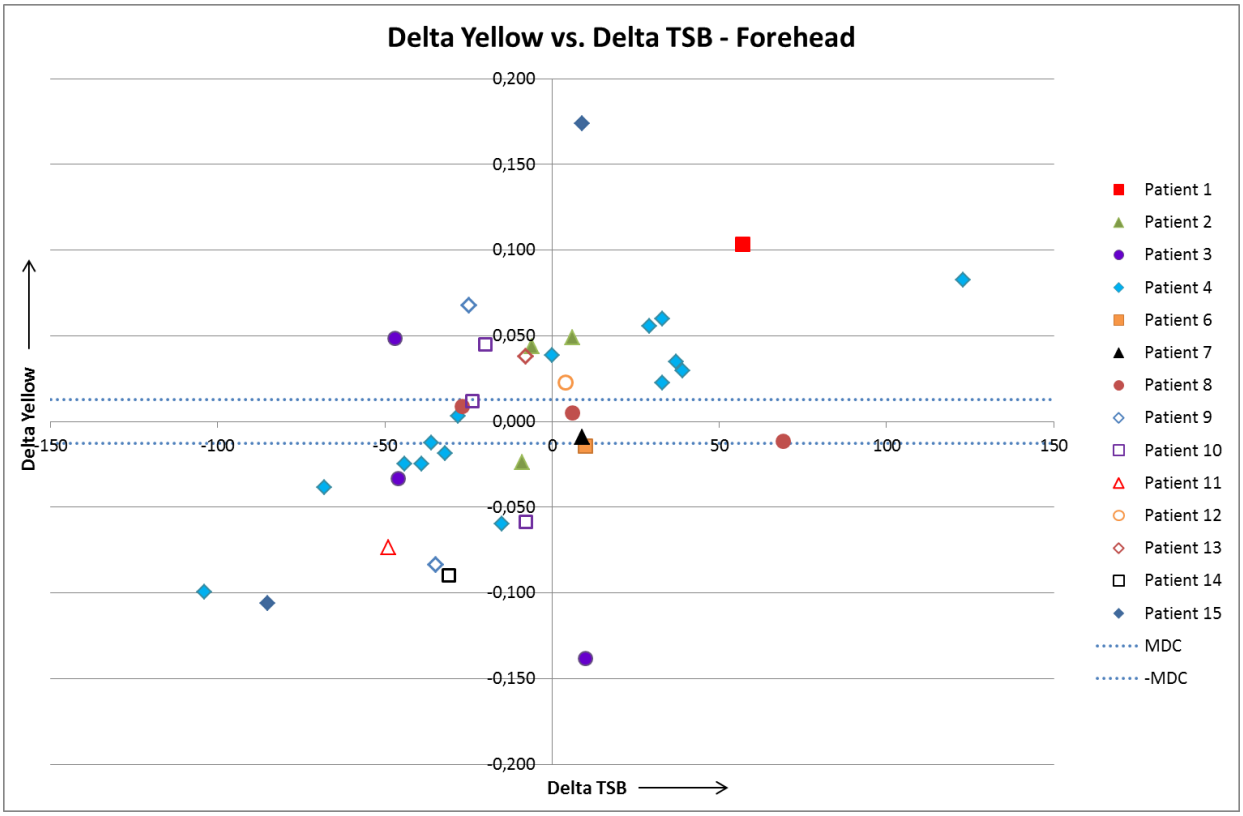

Figure 4: Plot of Delta Yellow vs. Delta TSB for all patient data of the forehead

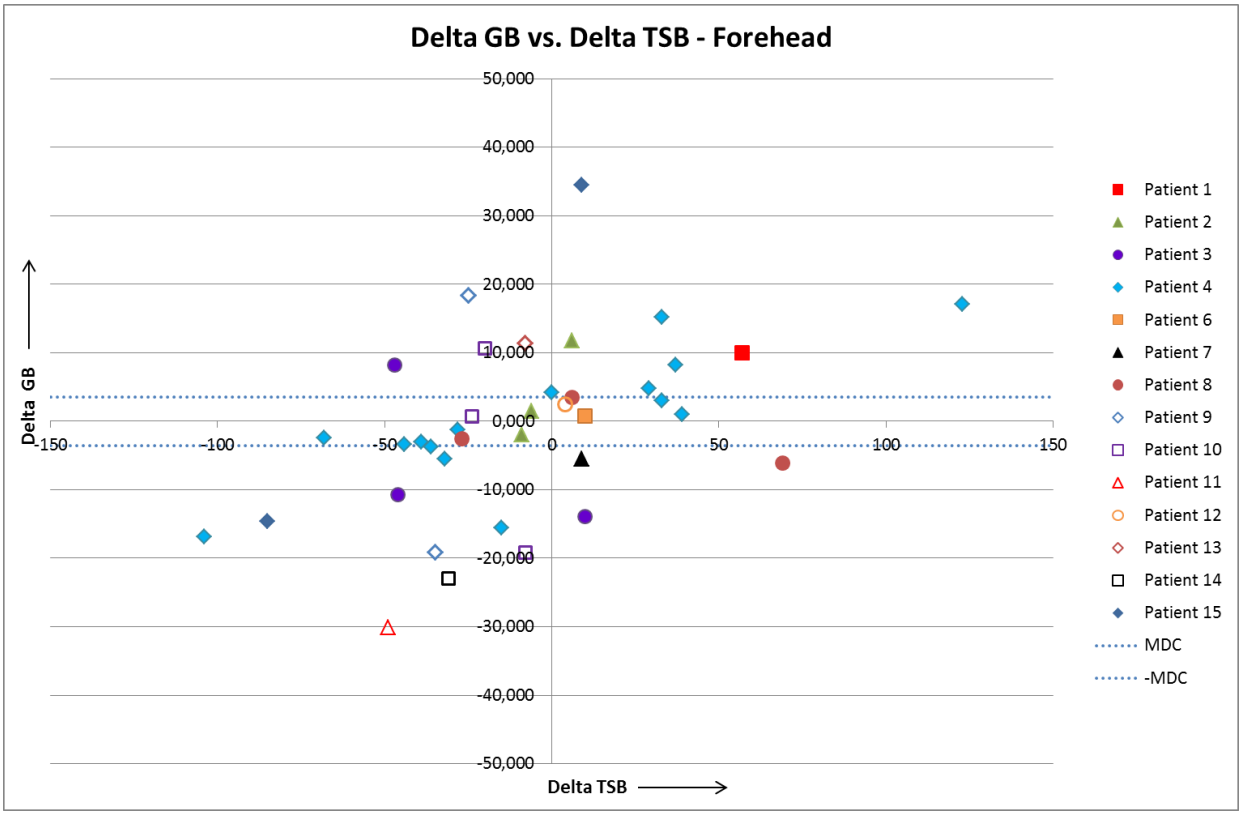

Figure 5: Plot of Delta GB vs. Delta TSB for all patient data of the forehead

The data points in the areas 'positive Delta TSB-positive Delta colour' and negative TSB-negative Delta colour' are indicated as correct, since these would be predicted correctly if only looking at the skin colour. The area 'negative Delta TSB-positive Delta colour' is indicated as false positive, and the area 'positive Delta TSB-negative Delta colour' as false negative. In Table 5 and 6, the number of data points in each area are summarized.

Table 5: Odds ratio and distribution of data points for Yellow, of each measurement location

Yellow	Odds ratio	% Correct	% False negative
Forehead	4,28*	65,8%	10,5%
Sternum	8,39*	71,4%	16,7%
Diaper	1,86	56,3%	21,9%
Hip	5,71**	71,1%	13,3%
Sole of foot	1,78	62,8%	20,9%

*** = p < 0.001, ** = p < 0.01

Table 6: Odds ratio and distribution of data points for GB, of each measurement location

GB	Odds ratio	% Correct	% False negative
Forehead	9.14**	73,7%	7,9%
Sternum	9.88**	76,2%	11,9%
Diaper	4.00.	68,8%	18,8%
Hip	11.44***	77,8%	11,1%
Sole of foot	3.21.	65,1%	18,6%

** = p < 0.01, * = p < 0.05

Table 7: Standard deviations for all data per location

Location	SD – Yellow	SD - GB
Forehead	0,062	49,03
Sternum	0,060	44,34
Diaper	0,056	41,53
Hip	0,055	41,99
Sole of foot	0,048	41,88

The logistic regression analysis shows that the forehead, the sternum and the hip are all locations on the body which have a high probability of an increase in TSB when the colour increases, which are also the locations with the highest percentage of correct data points. Beneath the diaper and on the sole of the foot show to have non-significant results.

When gathering the data of all patients, for each of the locations on the body the distribution of the data is defined, to assess the validity of the measurements for that specific measurement location. The standard deviations in Yellow and GB for each of the locations are shown in Table 7.

Effect of phototherapy

The area between the umbilicus and the os pubis, which is located beneath the diaper and therefore, is blocked from (an extent of) phototherapy, gives an indication of the effect of phototherapy on the change of colour. From the logistic regression analysis, the area beneath the diaper shows as not a suitable location for predicting the change in TSB, whereas the hip is.

Delay in change of skin colour

When comparing Delta TSB vs Delta Yellow for the measurements on the forehead, before, during, and after the TSB determination, the after measurements show less false positive and false negative data points, and a smaller deviation from the trend as can be seen in Figure 6 and 7. (Appendix G.) These findings are summarized in Table 8 and 9. The logistic regression analysis confirms the findings, showing the probability of an increase in skin colour when the TSB increases is by far the greatest for the measurements 30 minutes after the TSB determination. This could indicate there is lag between the change of TSB and change of skin colour.

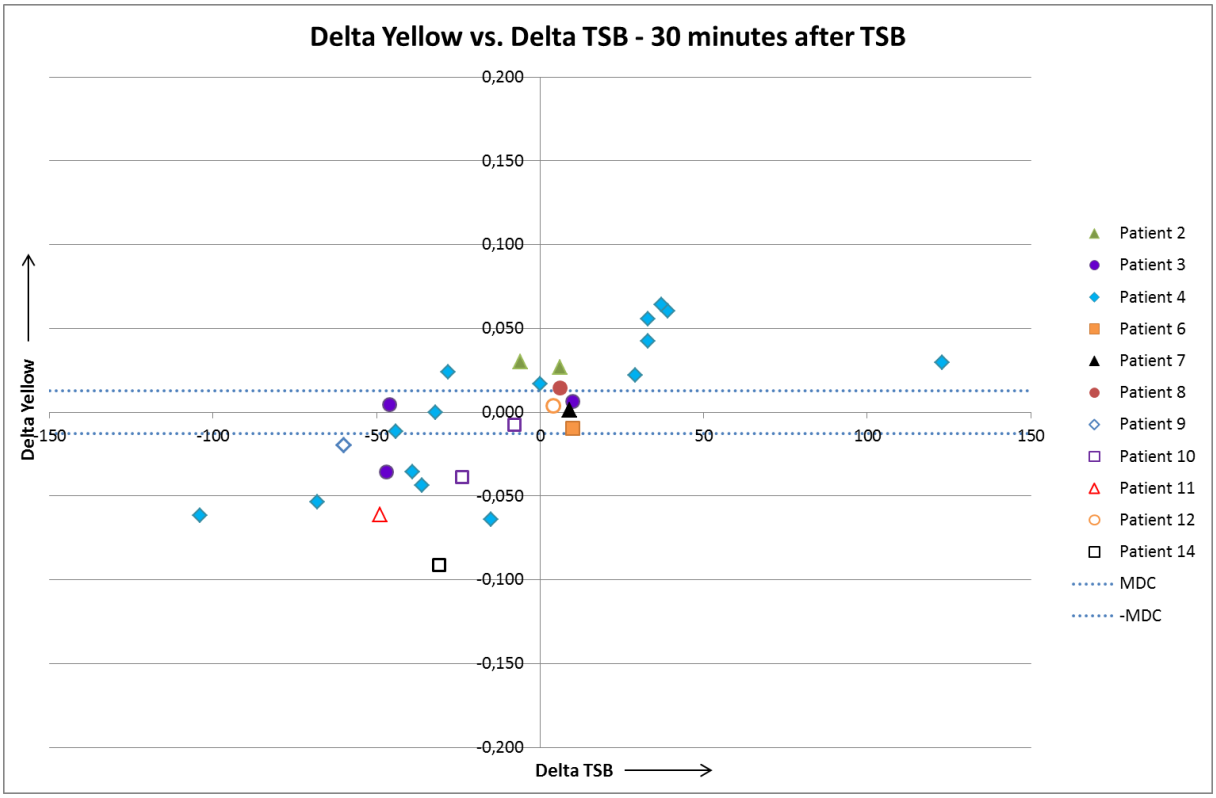

Figure 6: Plot of Delta Yellow vs. Delta TSB for the measurements of 30 minutes after TSB determination

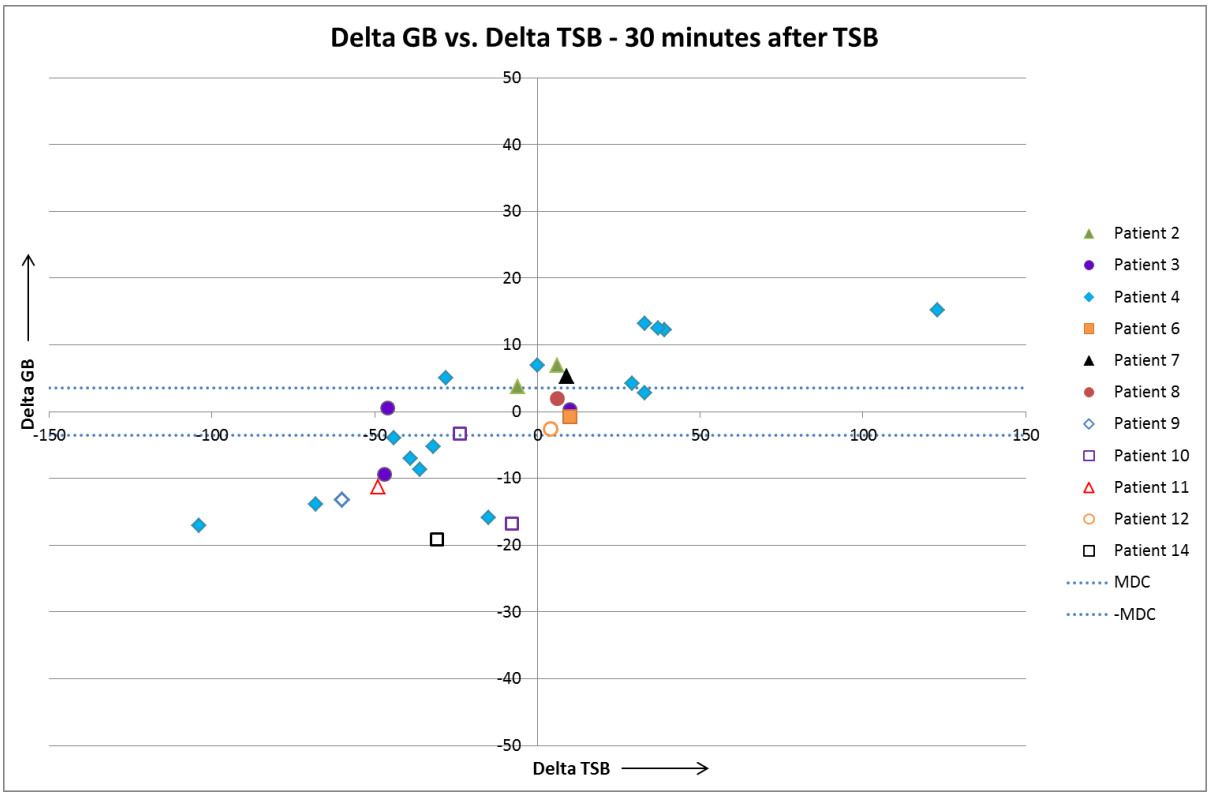

Figure 7: Plot of Delta GB vs. Delta TSB for the measurements of 30 minutes after TSB determination

Table 8: Odds ratio and distribution of data points of Yellow, 30 minutes before, during and 30 minutes after TSB determination, on the forehead

Yellow	Odds ratio	% Correct	% False negative
Before	1.99	58,3%	16,7%
During	4,28*	65,8%	10,5%
After	35.77**	82,8%	3,4%

** = p < 0.01, * = p < 0.05

Table 9: Odds ratio and distribution of data points of Yellow, 30 minutes before, during and 30 minutes after TSB determination, on the forehead

GB	Odds ratio	% Correct	% False negative
Before	2,40	60,7%	17,9%
During	9.14**	73,7%	7,9%
After	16,25**	79,3%	6,9%

** = p < 0.01

DISCUSSION

The aim of this study was to evaluate whether through non-invasive measurements of skin colour over time the trend of hyperbilirubinemia in neonates can be monitored. Although from this study no conclusion can be made on the viability of using this method, it can be seen that there is potential in monitoring the skin colour.

The forehead, the sternum and the hip show to be promising areas for conducting the measurements. It is interesting to find that the sole of the foot is not a suitable location for measurements. Although these locations where chosen since it was assumed the cephalo-caudal progression would have an effect on the measurements, it is not possible to conclude whether the unsuitability of the sole of the foot is due to the cephalo-caudal progression. Since the sole of the foot is a peripheral location, local temperature could influence the perceived colour. Another influencing factor could be the exposure to phototherapy. Even though the feet where not blocked from the phototherapy, in practice they are hardly exposed to the light. Since the area beneath the diaper also received little to no light from phototherapy and showed to be an unsuitable location, this might point to a poorer correlation of the skin colour and TSB for skin receiving no phototherapy, which is an unexpected result.

When looking into a possible delay in change of skin colour, there is a significant difference in the outcomes for the different measurements, suggesting the skin colour does indeed change with a delay compared to the change of the TSB.

The range of RGB values could influence the outcome if the ranges are smaller or larger for the different measurement moments, since a smaller range could indicate better measurements or less outliers. However the range of the measurements is quite similar for the different measurements, as can be seen in Table 8.

Table 10: Median and IQR for the measurements 30 minutes before, during and 30 minutes after TSB determination

	Median Yellow	IQR
Before	0,2747	0,2245 - 0,3165
During	0,2725	0,2399 - 0,3210
After	0,2672	0,2329 - 0,3127

Due to the very small sample size of non-Caucasian patients in this subset of the study population, the effect of race on the correlation could not be determined. In the analysis of the total study population, race will be taken into account.

A limitation in this study was the measurement fault of the device and of the measurement conditions. As can be seen in Figure 4 and 5, multiple data points lie within the range of MDC. If the data points within the MDC range are removed from the analysis, this influences the obtained results, as can be seen in Table 11 and 12. For most locations, the outcome is improved after the removal of the uncertain data points.

The viability of using the models is dependent on the type of errors which are made. False negative data points are much more dangerous than false positive data points, especially when the delta TSB is large, since this would mean children with a rising TSB would not be identified.

Table 11: Distribution of data points of Yellow per location without data points in MDC range

Yellow	% Correct	% False negative
Forehead	74,2%	6,5%
Sternum	80,6%	11,1%
Diaper	61,5%	19,2%
Hip	65,8%	15,8%
Foot sole	60,6%	24,2%
After (forehead)	85,7%	0%

Table 12: Distribution of data points of GB per location without data points in MDC range

GB	% Correct	% False negative
Forehead	68,0%	12,0%
Sternum	84,4%	6,25%
Diaper	68,2%	18,2%
Hip	84,4%	6,25%
Foot sole	74,1%	18,5%
After (forehead)	86,4%	0%

Since the measurements 30 minutes after the TSB determination has the highest percentage of correct predictions while not having a false negative (after eliminating the MDC range), this is deemed interesting as a basis for further research. The most promising combination of variables is to take the measurement on the forehead, sternum, or hip combined with a time delay. The distributions of data points shows both model have a high sensitivity. The specificity is lower, however if this method is used as an addition in monitoring hyperbilirubinemia, this is less relevant. If an increase in skin colour takes place, the TSB would still be determined to know the absolute value of bilirubin. However, when a decrease in skin colour is observed, this method could replace a blood sample which would otherwise be needed to monitor the neonate.

If the results after the analysis of the total study population show to be similar to the obtained results on this subpopulation, then recommendations for further research are as following.

1. Improve the colour sensor device to reduce measurement fault.
2. Increase the frequency of the measurements.
3. The possibility to leave the sensor on a single location of the body would also reduce measurement fault.
4. Use a proximal location on the body.
5. Conduct more research into lag, using the increased frequency of measurements
6. Increase minimum number for sets of measurements

CONCLUSION

In conclusion, measuring the skin colour over time could be an asset to monitoring neonates for hyperbilirubinemia. The method might be used as an addition to TSB determination. When measuring the skin colour of the forehead with a small time delay, little to no TSB increases are missed. As such, it could replace (some of) the blood sampling needed after the initial determination of TSB.

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Appendix

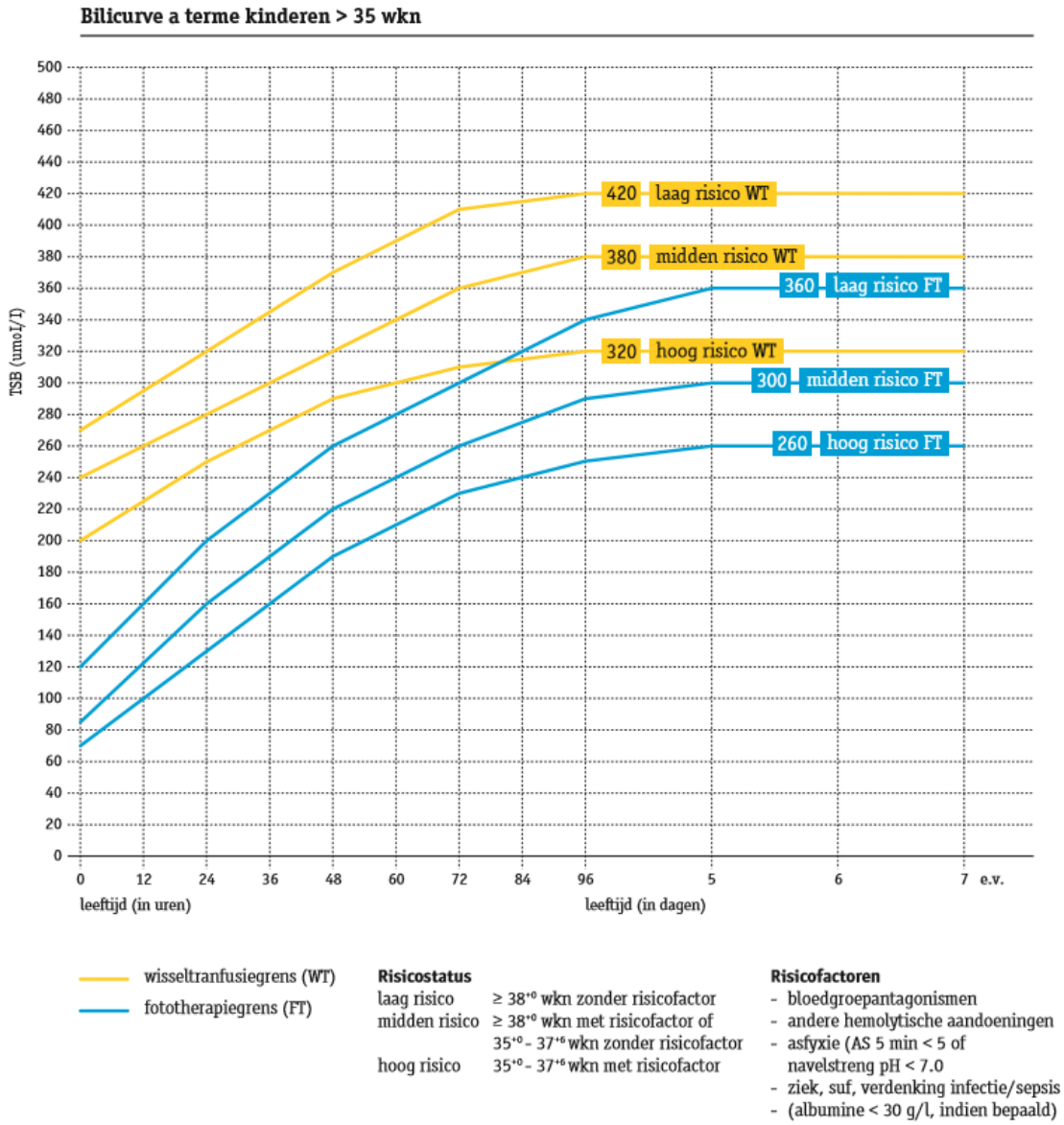
Table of content of the appendix

A. Nomograms - patient specific intervention threshold

A very important aspect of the AAP guideline is the nomograms. In order to provide some insight into the patient-specific risk for developing severe neonatal hyperbilirubinemia, Bhutani created an hour-specific bilirubin nomogram.38 In the graph, see Figure 6, one can plot the TSB value against the age of the infant defined in hours and assess the resulting risk.

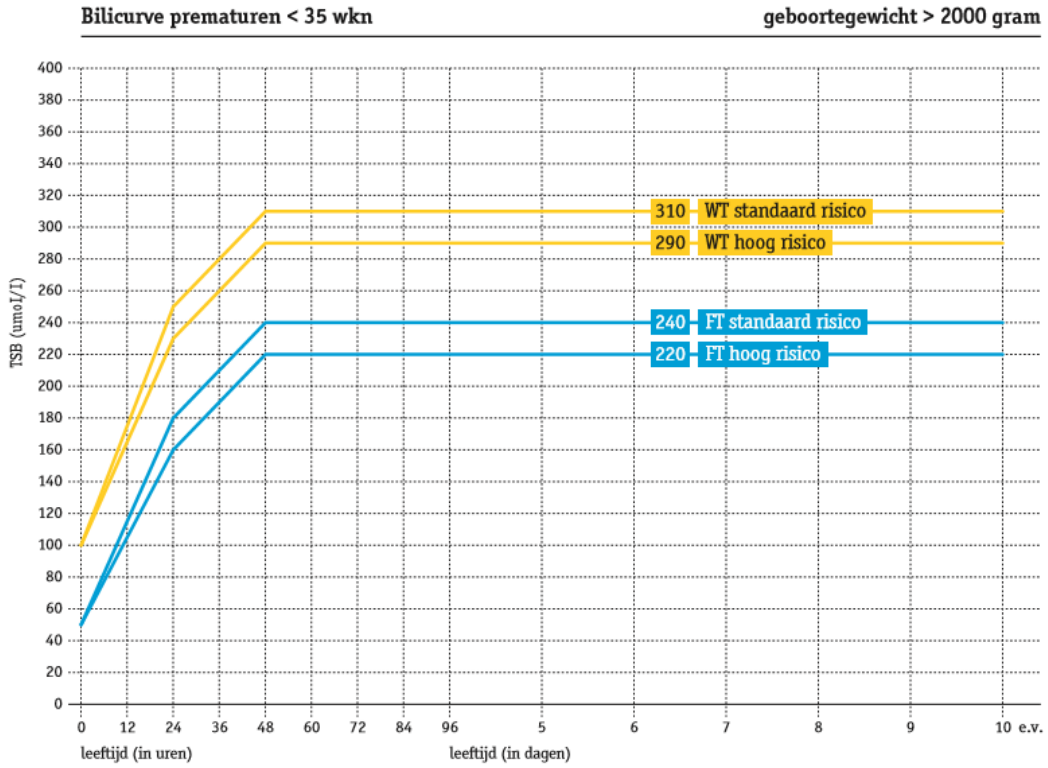
Depending on the risks factors the specific neonate complies with, which have been defined before, the moment of intervention will differ. For high, medium and low risk cases, the TSB level at which phototherapy or exchange transfusion should be initiated has also been defined in the nomogram

For patients with a gestational age over 35 weeks



Nomograms - patient specific intervention threshold

For patients with a gestational age under 35 weeks, and with a birthweight of over 2000 grams



wisseltranfusie WT

fototherapie FT

Richtlijn voor het gebruik van fototherapie en wisseltransfusies gebaseerd op TSB (µmol/l) <35wkn

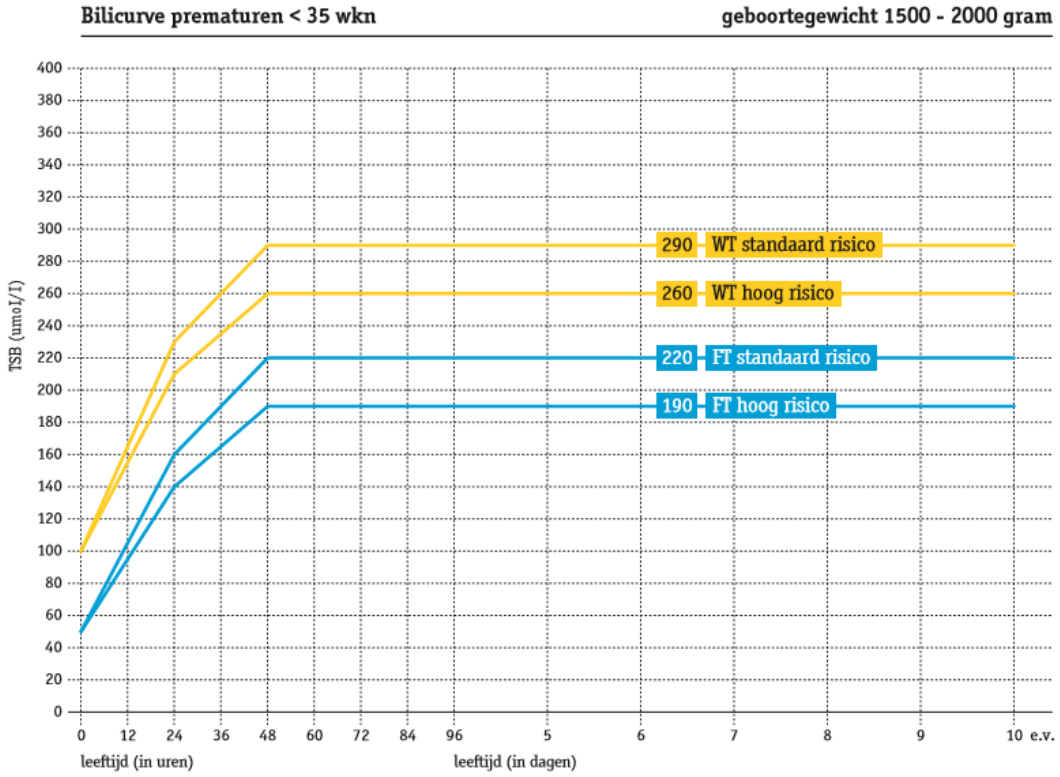
1. bepaal risicostatus (standaard of hoog risico)
2. bepaal leeftijd in uren op de X-as
3. zet TSB in de curve
4. start fototherapie als FT-grens overschreden is. Fototherapie kan gestopt worden als TSB onder de FT-grens is.
5. overweeg wisseltransfusie als WT-grens overschreden is, ondanks intensieve fototherapie.

Hoog risico factoren (dagelijks te bepalen)

- asfyxie: apgar score < 3 na 5 min.
- hypoxemie: PaO^2 < 5.3 kPa > 2 uur (recente 24 u)
- acidosis: pH < 7.15 > 1 uur (recente 24 u)
- hemolysis met positieve coombs
- klinische of neurologische verslechtering zoals sepsis waarvoor vasopressoren, meningitis, intracraniele bloeding > gr 2.

Nomograms - patient specific intervention threshold

For patients with a gestational age under 35 weeks, and with a birthweight of 1500 - 2000 grams



wisseltranfusie WT

fototherapie FT

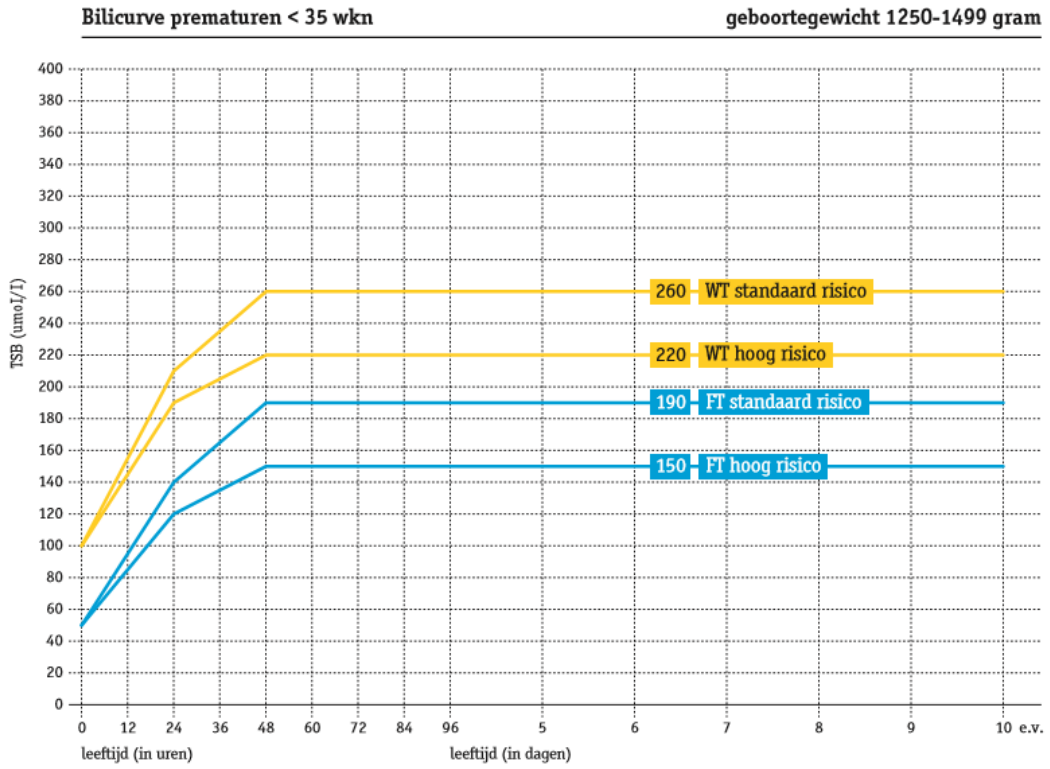
Richtlijn voor het gebruik van fototherapie en wisseltransfusies gebaseerd op TSB (µmol/l) <35wkn

1. bepaal risicostatus (standaard of hoog risico)
2. bepaal leeftijd in uren op de X-as
3. zet TSB in de curve
4. start fototherapie als FT-grens overschreden is. Fototherapie kan gestopt worden als TSB onder de FT-grens is.
5. overweeg wisseltransfusie als WT-grens overschreden is, ondanks intensieve fototherapie.

Hoog risico factoren (dagelijks te bepalen)
- asfyxie: apgar score < 3 na 5 min.
- hypoxemie: PaO^2 < 5.3 kPa > 2 uur (recente 24 u)
- acidosis: pH < 7.15 > 1 uur (recente 24 u)
- hemolysis met positieve coombs
- klinische of neurologische verslechtering zoals sepsis waarvoor vasopressoren, meningitis, intracraniele bloeding > gr 2.

Nomograms - patient specific intervention threshold

For patients with a gestational age under 35 weeks, and with a birthweight of 1250 - 1500 grams



wisseltranfusie WT

fototherapie FT

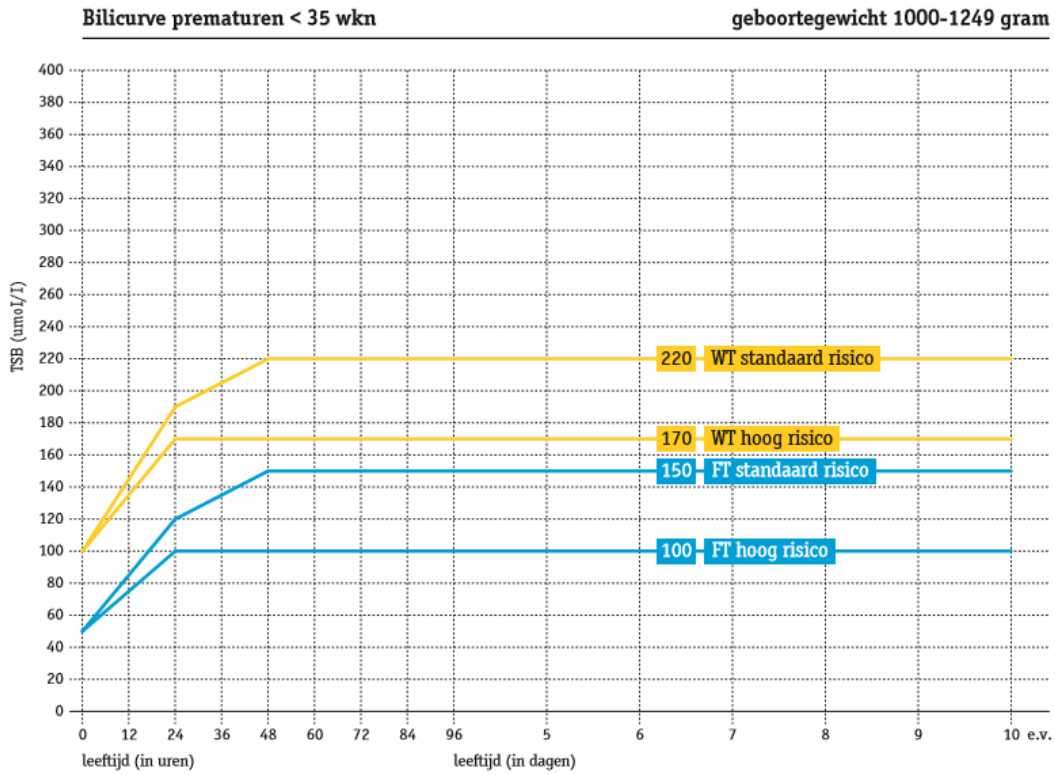
Richtlijn voor het gebruik van fototherapie en wisseltransfusies gebaseerd op TSB (µmol/l) <35wkn

1. bepaal risicostatus (standaard of hoog risico)
2. bepaal leeftijd in uren op de X-as
3. zet TSB in de curve
4. start fototherapie als FT-grens overschreden is. Fototherapie kan gestopt worden als TSB onder de FT-grens is.
5. overweeg wisseltransfusie als WT-grens overschreden is, ondanks intensieve fototherapie.

Hoog risico factoren (dagelijks te bepalen)
- asfyxie: apgar score < 3 na 5 min.
- hypoxemie: PaO^2 < 5.3 kPa > 2 uur (recente 24 u)
- acidosis: pH < 7.15 > 1 uur (recente 24 u)
- hemolysis met positieve coombs
- klinische of neurologische verslechtering zoals sepsis waarvoor vasopressoren, meningitis, intracraniele bloeding > gr 2.

Nomograms - patient specific intervention threshold

For patients with a gestational age under 35 weeks, and with a birthweight of 1000 - 1250grams



wisseltranfusie WT
fototherapie FT

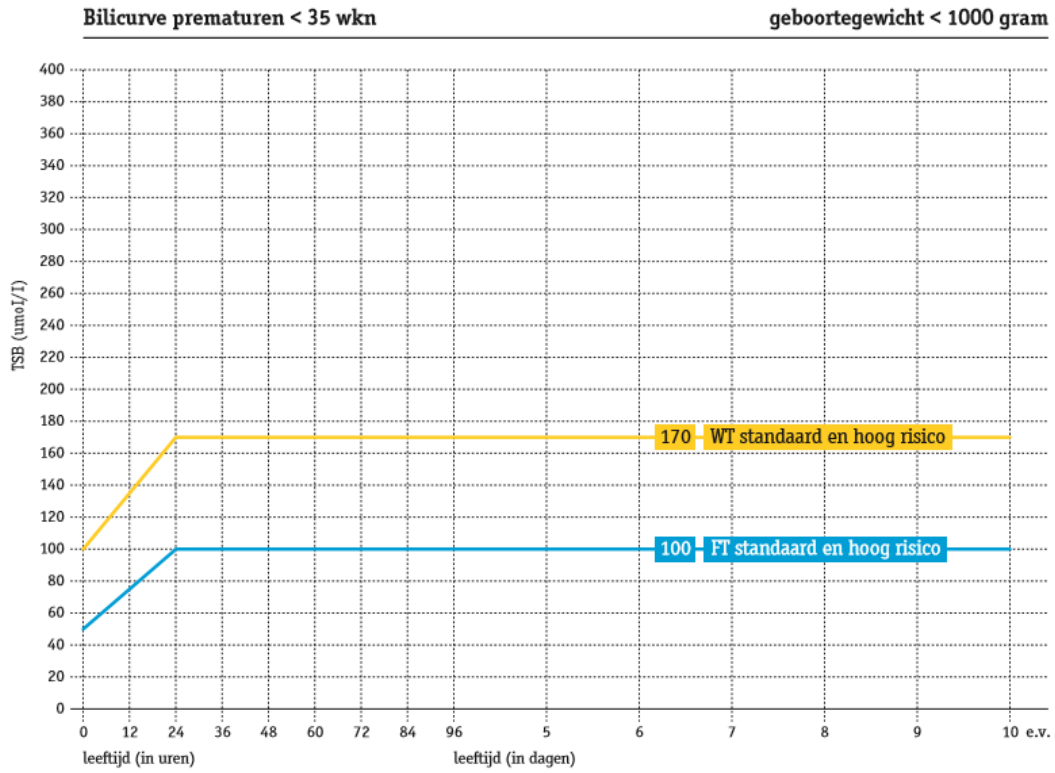
Richtlijn voor het gebruik van fototherapie en wisseltransfusies gebaseerd op TSB (µmol/l) <35wkn

1. bepaal risicostatus (standaard of hoog risico)
2. bepaal leeftijd in uren op de X-as
3. zet TSB in de curve
4. start fototherapie als FT-grens overschreden is. Fototherapie kan gestopt worden als TSB onder de FT-grens is.
5. overweeg wisseltransfusie als WT-grens overschreden is, ondanks intensieve fototherapie.

Hoog risico factoren (dagelijks te bepalen)
- asfyxie: apgar score < 3 na 5 min.
- hypoxemie: PaO^2 < 5.3 kPa > 2 uur (recente 24 u)
- acidosis: pH < 7.15 > 1 uur (recente 24 u)
- hemolysis met positieve coombs
- klinische of neurologische verslechtering zoals sepsis waarvoor vasopressoren, meningitis, intracraniele bloeding > gr 2.

Nomograms - patient specific intervention threshold

For patients with a gestational age under 35 weeks, and with a birthweight below 1000 grams



wisseltranfusie WT
fototherapie FT

Richtlijn voor het gebruik van fototherapie en wisseltransfusies gebaseerd op TSB (µmol/l) <35wkn

1. bepaal risicostatus (standaard of hoog risico)
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4. start fototherapie als FT-grens overschreden is. Fototherapie kan gestopt worden als TSB onder de FT-grens is.
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- klinische of neurologische verslechtering zoals sepsis waarvoor vasopressoren, meningitis, intracraniele bloeding > gr 2.

Skin colour measurements of jaundiced neonates over 30 weeks as an indication of development of hyperbilirubinemia

(October 2015)

- May 2015: adaptation section 11.5: text in accordance to old and new Measure regarding Compulsory Insurance for Clinical Research in Humans
- Sept 2015: adaptation section 9.1, 9.2 and 12.5: text in accordance to WMO amendment on reporting SAE and temporary halt (section 10 of WMO)
- Oct 2015: adaptation section 4.4 – comment [CCMO15], 8.2 and 10.1 with respect to methodology/statistics

PROTOCOL TITLE 'Skin colour measurements of jaundiced neonates over 30 weeks as an indication of development of hyperbilirubinemia'

Protocol ID	**SCIN – Skin Colour In Neonates with jaundice**
Short title	Skin colour measurements of jaundiced neonates
EudraCT number	Not applicable
Version	1
Date	July 19th, 2016
Coordinating investigator/project leader	Prof. *dr*. I.K.M. Reiss Erasmus MC - Sophia Children's Hospital Head of Neonatology Department of Paediatrics Room Sp-3432 Wytemaweg 80 Postbus 2060 3000 CB Rotterdam
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Subsidising party	Erasmus MC - Sophia's Children's Hospital Department of Paediatrics Division of Neonatology Wytemaweg 80 Postbus 2060 3000 CB Rotterdam
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Laboratory sites <*if applicable*>	Not applicable
Pharmacy <*if applicable*>	Not applicable

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor or legal representative: **Head of Department:** ***Prof. Dr Edmond H.H.M. Rings*** ***Head of Paediatrics***		
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TcB	**Transcutaneous Bilirubinometry**
TSB	**Total Serum Bilirubin**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Infants affected by neonatal hyperbilirubinemia are routinely monitored by repeated measurement of the total serum bilirubin (TSB) Drawing blood is invasive and the procedure is stressful for infant and parents.

A non-invasive way to estimate the extent of hyperbilirubinemia is through assessing the skin colour of the child with transcutaneous bilirubinometry (TcB). However, this method is not reliable when a child is undergoing phototherapy. We hypothesise that the serum bilirubin levels can be monitored by taking multiple skin colour measurements of a single child over time. For each measurement, a comparison to the previous measurement will show either a decline, an incline or a continuation in the extent of yellow colour in the skin. The trend of the jaundice for a single child is presumed to correlate with the increase or decrease in neonatal hyperbilirubinemia over time.

This method would not replace the determination of the TSB in blood, but could reduce the frequency with which it has to be determined, and thus reduce the number of samples drawn.

Objective: Primary objective: To determine whether a correlation exist, for Caucasian and non-Caucasian babies, between the trend of hyperbilirubinemia over time and the gradient of skin colour, measured on the skin surface. Secondary objective: To determine the effect of bleaching of the skin by phototherapy or cephalocaudal progression of jaundice on the correlation.

Study design: This is a prospective observational study. Skin colour will be measured around the time of the blood draw for a Total Serum Bilirubin determination. The measurement will be done in five locations on the body with a colour sensor that is rested on the skin. Along with this measurement, one location of the body will be measured with the colour sensor half an hour before and half an hour after the blood draw.

Study population: All infants born at gestational age of 30 to 42 weeks whom are admitted in the Sophia Children's Hospital, Erasmus Medical Center in Rotterdam, the Maasstad Hospital in Rotterdam or in the Amphia Hospital in Breda, and of whom more than one TSB value needs to be determined for monitoring of neonatal hyperbilirubinemia, are eligible for inclusion.

Intervention (if applicable): Not applicable

Main study parameters/endpoints: The main study parameter is the relative change of skin colour compared to the change in bilirubin level in the blood.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Next to standard care, the burden of the research will be minimal. Approximately every 6 hours, a sensor will be placed on the skin shortly at five sites. When the infant is no longer monitored for hyperbilirubinemia, the participation to the study will be stopped.

Neonatal hyperbilirubinemia is a specific pathology for infants of this age. The efficacy of skin colour measurements cannot be guaranteed for neonates when the method is validated on adults, since the characteristics of the skin of both groups are different.

2. *INTRODUCTION AND RATIONALE*

Infants severely affected by neonatal hyperbilirubinemia have to be monitored and, if necessary, treated for this condition. The golden standard in establishing the development of neonatal hyperbilirubinemia, is through determining the total serum bilirubin (TSB) in the blood.1 The interval in which this is determined tends to be 6, 12 or 24 hours, depending on the severity of the hyperbilirubinemia and hospital policy. Drawing blood by heel puncture for each TSB determination is invasive and the procedure is stressful for infant and parents.

For this reason it would be desirable to reduce the amount of blood draws of the patient, without compromising the monitoring of the hyperbilirubinemia. A manner in which the extent of hyperbilirubinemia can be observed is through detection of jaundice. With a decrease of hyperbilirubinemia, the extent of jaundice will decrease as well. However, visual detection of jaundice is unreliable.2–5 A method to quantify the degree of yellowness of the skin is through colour measurements of light reflection of the skin. A well-known example of this method is transcutaneous bilirubinometry (TcB). The measurement of the colour of the skin is converted through an algorithm to generate an estimation of the TSB value. Transcutaneous bilirubinometry has been found to be reliable as a screening method.6 However, when the infant is undergoing phototherapy, the TcB device can't give an accurate measurement since the infant's skin is bleached by the light.7

A solution to the problem is hypothesized; by taking multiple skin colour measurements of a single child over time, the overall trend of the jaundice can be tracked. For each measurement, a comparison to the previous measurement will show either a decline, an incline or a continuation in the extent of yellow colour in the skin. The trend of the jaundice for a single child is presumed to correlate with the increase or decrease in neonatal hyperbilirubinemia over time. If this correlation exist, it would be possible to determine whether an infant is recovering from the hyperbilirubinemia or if the condition is progressing and additional intervention is required.

This method would not replace the determination of the TSB in blood, but could reduce the frequency with which it has to be determined, and thus reduce the number of samples drawn.

By using the trend of colour measurements, only the relative change in the degree of yellow is relevant. Therefore it is not necessary to determine how a single measurement of colour relates to the level of TSB. This is convenient since the natural skin tone of each child will have a different extent of yellow, and since it will differ how jaundiced a child will be for a specific level of TSB.8

Several factors are expected to be of influence on the trend of colour measurements, and insight into their influence is desired since this is yet unknown. For this reason, these factors will be taken into account in the study design. Below, the factors will be explained.

1. A dark colour of the skin is known to increase difficulty for detecting jaundice.9 The trend of skin colour measurements is also expected to be different for light coloured babies and more dark coloured babies. In order to examine the effect of skin tone on the correlation of the trend of colour measurements and the progression of the hyperbilirubinemia, both Caucasian and non-Caucasian babies will be included in the study.
2. Hyperbilirubinemia follows a cephalo-caudal progression, meaning the jaundice will first be visible in the cranial area. As the condition becomes more severe, the jaundice will progress to lower regions of the body.10,11 As a result, the extent of jaundice will differ for different body parts. This will be accounted for in the study by measuring colour on several locations, ranging from more proximal to more distal.
3. Skin which is exposed to phototherapy will be bleached compared to skin which is not exposed to phototherapy.7 Whether exposed skin holds a better or poorer correlation to the actual severity of the jaundice is unknown. Many participants which will be included in the study are expected to undergo phototherapy during the participation in the study, since the conditions during phototherapy will tend to make them more eligible for inclusion. However the participants are also expected to wear a diaper while undergoing phototherapy, which means the area will be largely shielded from the light. In order to examine the effect of exposure to light, both the skin underneath the diaper and exposed skin areas will be measured.
4. When the level of bilirubin in the blood changes, the skin colour is expected to change as well. The rate of change however is unknown. If there is a large delay then this will affect the measured correlation. In order to find whether an delay occurs, multiple colour measurements will be done around the time of a TSB determination.

3. *OBJECTIVES*

Primary Objective:

To determine whether a correlation exist, for Caucasian and non-Caucasian babies, between the trend of hyperbilirubinemia over time and the gradient of yellow colour of the skin over time, measured on the skin surface.

Secondary Objectives: :

To determine the effect of bleaching of the skin by phototherapy on the correlation.

To determine the effect of cephalocaudal progression of jaundice on the correlation.

To determine whether an delay between the change in TSB value and the change in skin colour occurs.

4. *STUDY DESIGN*

For this prospective observational study repeated sets of cutaneous measurements with a colour sensor will be done on each child. A set of measurements will be done at the time of each requested blood draw for the determination of TSB which will be done in accordance to clinical practice; every 6-12 hours depending on the severity of the hyperbilirubinemia. Since we are interested in a trend, a minimum of two sets of measurements is required. The total number of sets of measurements will be determined by the number of blood draws which will be requested by the physician.

A set of cutaneous measurements consists of five measurements on different locations, namely on the forehead, the sternum, laterally on a hip (either left of right), between the umbilicus and the ospubis, and on the sole of a foot. One set of measurements will take less than 5 minutes. The measurements on the forehead, the sternum, the hip and the sole of the foot can be compared in order to investigate the effect of the cephalo-caudal progression on jaundice. The measurement between the umbilicus and the os pubis (underneath the diaper) can be compared with the measurement of the thigh, in order to investigate the effect of bleaching of the skin by phototherapy.

In addition to the set of measurements, two more measurements will be done; a colour measurement of the hip half an hour before and half an hour after each blood draw. This will provide information on a possible delay in change. The hip is chosen since this is an easily accessible location, which is likely to be uncovered, thus resulting in minimal disturbance for the child.

The skin colour measurements will be performed by shining white light onto the infant's skin, where the reflected light will be collected by a RGB sensor.

Most subjects are expected to receive phototherapy as treatment for hyperbilirubinemia during the study. The treatment will not be interrupted while the measurements are done. The sensor's head will cover an area of the skin, which will impede the phototherapy light of shining onto the sensor.

5. *STUDY POPULATION*

5.1 Population (base)

All infants born at gestational age of 30 to 42 weeks which are hospitalized in the Sophia Children's Hospital, Erasmus Medical Center, the Maasstad Hospital and in the Amphia Hospital, and of whom (it is expected that) more than one TSB value needs to be determined on suspicion of neonatal hyperbilirubinemia, are eligible for inclusion.

5.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Born at a gestational age equal to or greater than 30 weeks
- Hospitalized at the Erasmus Medical Centre, Maasstad hospital or the Amphia hospital
- Blood will be tested for TSB on suspicion of jaundice

5.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Born at a gestational age lower than 30 weeks
- Neonates of whom the parents have not given informed consent
- If hospitalization is terminated before at least two TSB samples have been obtained
- Neonates with sepsis

5.4 Sample size calculation

Since this is a pilot study and no comparable study data exists, a required sample size cannot be calculated. For pilot studies, the range of participants per group can fluctuate largely. As suggested by Julious[12], at least 12 participants should be included in each group in a pilot study in order to optimize for feasibility and for precision about the mean and the variance. Isaac & Michael[13] recommend a sample size of 10-30 subjects.

Based on these findings and on the feasibility due to incidence of hyperbilirubinemia, a sample size of 18 participants per group is deemed appropriate.

Therefore a total of 36 participants will be included, consisting of 18 Caucasian and 18 non-Caucasian participants. When the total number of participants is reached, the data will be analysed to verify whether statistical significance can be achieved. If this is not the case, an expansion of the sample size will be requested with a power calculation based on the results of the pilot group.

5.5 Investigational product/treatment

Not applicable

5.6 Use of co-intervention

Not applicable

5.7 Escape medication

Not applicable

6. *INVESTIGATIONAL PRODUCT*

6.1 Name and description of investigational product(s)

Not applicable

6.2 Summary of findings from non-clinical studies

Not applicable

6.3 Summary of findings from clinical studies

Not applicable

6.4 Summary of known and potential risks and benefits

Not applicable

6.5 Description and justification of route of administration and dosage

Not applicable

6.6 Dosages, dosage modifications and method of administration

Not applicable

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable

6.8 Drug accountability

Not applicable

7. *NON-INVESTIGATIONAL PRODUCT*

7.1 Name and description of non-investigational product(s)

Not applicable

7.2 Summary of findings from non-clinical studies

Not applicable

7.3 Summary of findings from clinical studies

Not applicable

7.4 Summary of known and potential risks and benefits

Not applicable

7.5 Description and justification of route of administration and dosage

Not applicable

7.6 Dosages, dosage modifications and method of administration

Not applicable

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable

7.8 Drug accountability

Not applicable

8. *METHODS*

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The main study parameter is the relative change of skin colour over time. In order to determine whether this change is an useful indicator for the progression of hyperbilirubinemia, it will be compared to the change in bilirubin level in the blood.

8.1.2 Secondary study parameters/endpoints

Parameters which could influence the main study parameter and therefore need to be accounted for are the skin type of each infant (Caucasian/non-Caucasian), whether an infant is being treated with phototherapy, and the effect of cephalo-caudal progression on the jaundice.

8.1.3 Other study parameters

The additional parameters which will be monitored/described are;

- The infants' temperature
- The gestational age
- Birth weight
- Age in hours at first measurement
- Possible medication the infant receives before or during measurements
- Whether the infant is lying in an incubator or an open bed
- Which devices are being used for phototherapy

8.2 Randomisation, blinding and treatment allocation

Not applicable

8.3 Study procedures

When a physician/nurse suspects an infant having neonatal hyperbilirubinemia, , blood will be drawn by performing a heel prick in order to determine the TSB level in the blood (according to standard care). Half an hour before, around and half an hour after this heel prick colour measurements will be done, by placing a sensor on the skin on five locations on the body. After the TSB level is established the physician will determine whether treatment or further monitoring is required. At each following heel prick for the determination of TSB

requested by the physician, colour measurements will be conducted before, during and after the prick.

8.4 Withdrawal of individual subjects

Subjects can withdraw from the study at any time for any reason without any consequences. The treating physician can decide to withdraw a subject from the study for any medical reasons.

8.4.1 Specific criteria for withdrawal

Not applicable

8.5 Replacement of individual subjects after withdrawal

Since the study is not patient specific, but looking into the feasibility of a method, a subject can easily be replaced by a new person after withdrawal. The only requirements for a replacing subject is that they fall into the same group (Caucasian/non-Caucasian) as the withdrawn subject, and they fit into the study population.

8.6 Follow-up of subjects withdrawn from treatment

Not applicable

8.7 Premature termination of the study

Not applicable

9. *SAFETY REPORTING*

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events, except for the following SAEs:

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

Not applicable

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

10. *STATISTICAL ANALYSIS*

10.1 Primary study parameter(s)

The dependent variable, the relative change of skin colour over time, is a continuous variable, which will be quantified by calculating a binary variable indicating an increase/decrease from one time point to the next. The independent variable, change of bilirubin in the blood over time, is also a quantitative, continuous variable.

A logistic mixed effect regression analysis will be used, whereby change in colour is the dependent variable, and we have change in TSB, time and race as independent covariates.

10.2 Secondary study parameter(s)

Skin type is a categorical variable, for which the effect on the dependent variable will be analyzed in the logistic mixed effect regression model, as an independent covariate.

The effect of cephalo-caudal progression is a categorical variable, which will be assessed by analyzing the measurements of the forehead, the sternum, thigh and sole of the foot. The logistic mixed effect regression model will be applied for each measurement location. In this way the different locations can't be compared to find the best site for measurements, but the outcomes will show whether each site is suitable for this method.

The effect of phototherapy will be assessed through a different logistic mixed effect regression model, where change in colour is the dependent variable, and we have change in TSB, time and the presence of phototherapy as independent covariates

10.3 Other study parameters

10.4 Interim analysis

Not applicable

11. *ETHICAL CONSIDERATIONS*

11.1 Regulation statement

This study will be conducted in accordance to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

The request for a TSB determination will be executed instantly, meaning the time between the moment of potential inclusion in the study and the need for the colour measurement will be half an hour. If parents or caregivers are present they will receive the information needed to make an informed decision from a member of the research team. Unfortunately they will have to make the decision to participate in the study rather quickly. If they need more time to decide, the researcher will come back at an agreed upon time, at least an hour later. The first sample will not be included in the study.

Often the parents or care givers will not be present at the moment it is determined that phototherapy is needed, so they will not be available to fully inform them about the study. To prevent having to approach, disturb, worry and inform all parents and caregivers of all the babies that might need a TSB determination, deferred consent is requested. If parents or care givers are not present, the first measurements will be performed without their consent, and information is provided to them as soon as they are available. At that time parents/caregivers can decide to join/continue the study or withdraw. Because this is a low risk study, this is deemed acceptable. Whenever one or both parents at any moment reject to participate in the study the measurements will be halted and all data will be deleted

11.3 Objection by minors or incapacitated subjects

The Code of conduct relating to expressions of objection by minors participating in medical research is applicable. Both parents, care givers or all legal representatives must give consent for the inclusion of the neonate into this study

11.4 Benefits and risks assessment, group relatedness

There is no additional risk for the patients included in this study.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

There are no incentives and no compensations given to either the neonates or their parents when they are approached for this study or when they participate.

12. *ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION*

12.1 Handling and storage of data and documents

All the data per patient will be coded with a chronological number.

12.2 Monitoring and Quality Assurance

Not applicable

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study

report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

<Please mention the arrangements made between the sponsor and the investigator concerning the public disclosure and publication of the research data. >

13. *STRUCTURED RISK ANALYSIS*

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

d. Selectivity of the mechanism to target tissue in animals and/or human beings

e. Analysis of potential effect

f. Pharmacokinetic considerations

g. Study population

h. Interaction with other products

i. Predictability of effect

j. Can effects be managed?

13.2 Synthesis

14. *REFERENCES*

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C. Research procedure (Dutch)

Onderzoeksprocedure Huidskleurmetingen bij geelzucht

In dit document staat de procedure beschreven voor het uitvoeren van de studie ''Huidskleurmetingen bij neonaten met geelzucht, als een indicatie van het verloop van hyperbilirubinemie.''

Inhoudsopgave

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Beschrijving onderzoek

Naam onderzoek: Huidskleurmetingen bij neonaten van meer dan 30 weken zwangerschap met geelzucht, als een indicatie van het verloop van hyperbilirubinemie.

Doel onderzoek: Door het meer of minder geel worden van de huid van een kindje kunnen we zien of het kind zieker wordt of beter. Het onderzoek gaat om het objectief meten van de huidskleur om te zien of hiermee een betrouwbare methode kan worden ontwikkeld om de geelzucht non-invasief in de gaten te houden. Hierdoor zou het aantal bloedprikken kunnen worden verminderd en worden kind en ouders minder belast.

Uitvoer onderzoek: Een kleursensor wordt op meerdere plekken op de huid gehouden om de huidskleur te bepalen. Dit wordt op 3 momenten rondom een bloedprik voor bilirubine gedaan. Deze data van de huidskleur wordt vergeleken met de data van de bilirubine waardes.

Categorie kinderen (inclusie criteria):

- Neonaten tussen de 30 tot 42 weken zwangerschapsduur waarbij men een bilirubine wil bepalen.

Exclusie criteria (geen deelname) indien:

- Neonaten < 30 weken zwangerschapsduur
- Geen toestemming van ouders/ verzorgers
- Pathologische hyperbilirubinemie; bloedgroep antagonisme of G6PD deficiëntie
- Sepsis

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Betrokken artsen: Prof dr Irwin Reiss, Erasmus MC
Dr Anthon Hulsmann, Amphia Ziekenhuis
Dr Martin Baartman, Maasstad Ziekenhuis

Alle benodigde materialen zijn te vinden in/bij de studie map

- De Onderzoeksprocedure
- De Informatiebrieven & Toestemmingsformulieren
- De formulieren Patiëntgegevens
- De formulieren Meetgegevens
- De meetapparatuur
- De hygiënische zakjes

Stappenplan

Elke baby boven 30 weken zwangerschapsduur waarvan hyperbilirubinemie wordt verdacht en waarvoor een bloedprik wordt aangevraagd, is een potentieel proefpersoon. De onderzoeksprocedure wordt uitgevoerd volgens de onderstaande stappen.

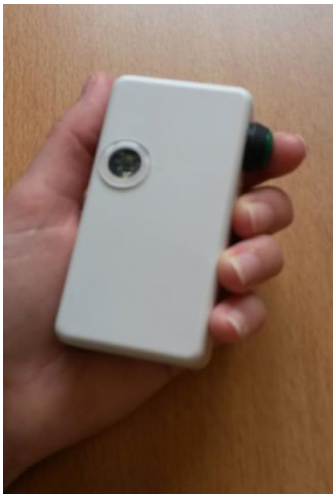
1. Er wordt een bilirubine controle door de verpleging afgesproken met het lab.
2. De arts assistent of verpleegkundig specialist geeft ouders uitleg met betrekking tot het onderzoek en vraagt toestemming aan de ouders/ verzorgers.
3. Het ondertekende toestemmingsformulier wordt bewaard in de ordner Informatie & Toestemmingsformulieren
4. Het formulier Patiëntgegevens wordt ingevuld. Op dit formulier wordt de patiënt ID ingevuld waarmee de patiënt bij het onderzoek zal worden aangeduid.
5. De tijd van de bilirubinemeting wordt bepaald en aan de arts assistent doorgebeld.
6. De 3 meetmomenten worden ingepland a.d.h.v. het tijdstip voor de bloedprik ter bilirubine controle, circa 30 minuten voor dit tijdstip, rond het tijdstip en circa 30 minuten erna.
7. 30 minuten voor de tijd van de bilirubinemeting wordt 3 keer een huidskleurmeting van het voorhoofd gedaan door de arts assistent of verpleegkundige. De gegevens worden genoteerd op het Meetgegevens formulier.
8. Wanneer de laborante binnen komt om het kind te prikken, wordt de verpleegkundig specialist of arts assistent gebeld. Rond dit moment worden huidskleurmetingen op 5 plekken gedaan. De gegevens worden genoteerd op het Meetgegevens formulier.
9. De arts assistent of verpleegkundige doet 30 minuten na het prikken nog 3 keer een huidskleurmeting van het voorhoofd. De gegevens worden genoteerd op het Meetgegevens formulier.
10. Het meetgegevens formulier wordt bewaard in de studiemap onder de tab ingevulde Meetgegevens.
11. Bij de aanvraag van elke volgende bilirubine controle worden punt 4 t/m 9 uitgevoerd.
12. Na de laatste bilirubine controle worden de ouders bedankt voor hun medewerking.

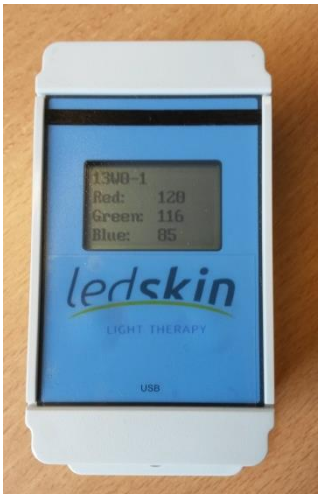
Meet apparatuur

De meetapparatuur bestaat uit:

1. Sensor (afbeelding links), waarmee de huidskleur wordt gemeten

2. Display (afbeelding rechts), waarop de meetgegevens worden weergegeven.





- De doorzichtige lens op de sensor is de plek waar de kleur wordt gemeten, deze plek moet dan ook op de aangewezen plekken op de patiënt worden geplaatst.

- Wanneer de sensor op de aangegeven positie is geplaatst, moet de knop op de sensor ongeveer 5 seconden worden ingehouden, totdat de gegevens op het display komen te staan.

- De meting geeft drie waardes, de R-waarde, de G-waarde en de B-waarde. Deze waardes worden van het display afgelezen en moeten op het meetgegevens formulier worden ingevuld. (Let op! Op het display lijkt de 0 erg veel op de 8 doordat er een punt in de 0 staat, als volgt → ʘ)

- Na ongeveer 10 seconden schakelt de display automatisch uit.

Hygiëne

Voor gebruik moeten eerst de eigen handen worden gedesinfecteerd.

Er wordt een afsluitbaar zakje gepakt en de sensor wordt hierin gedaan. Het zakje afgesloten met de druksluiting.

Nadat de sensor is gebruikt voor een meetmoment moet het hygiënische zakje worden weggegooid.



Huidskleurmetingen

Rond elke bloedprik voor bilirubine zijn er 3 meetmomenten. Bij deze momenten worden de metingen gedaan op de hieronder aangegeven plekken.

Moedervlekken of andere pigment vlekken moeten hierbij worden ontweken. Indien dit niet mogelijk is moet dit duidelijk worden aangegeven op het formulier bij Opmerkingen.

1e meting:

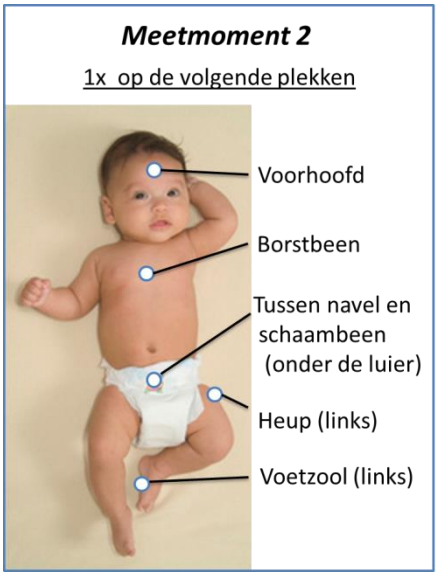
30 minuten voor de bloedprik

De eerste huidskleurmeting wordt 3 keer uitgevoerd, alleen op het borstbeen.

2e meting:

Rondom de bloedprik

Bij het tweede meetmoment worden de volgende plekken elke 1 keer gemeten, beginnend bij het voorhoofd en eindigend bij de voetzool.



3e meting:

30 minuten na de bloedprik

De laatste huidskleurmeting wordt 3 keer uitgevoerd, alleen op het borstbeen.

Hoe om te gaan met..?

Kinderen die op hun buik liggen

Wanneer een kind op zijn buik ligt wordt het kind zo gedraaid bij de metingen rondom het bloedprik . Bij de metingen 30 min voor en 30 min na het prikmoment wordt verwacht dat het voorhoofd alsnog goed bereikbaar is en dit de metingen niet in de weg zit.

Locatie huidskleurmetingen

De metingen van de heup en de voetzool moeten op de linkerzijde van het kind worden uitgevoerd tenzij dit wordt verhinderd. Bij het uitvoeren van de metingen op de rechterzijde moet dit worden vermeld bij Opmerkingen op het Meetgegevens formulier.

Verkleuringen van de huid

Indien een kind duidelijke verkleuringen van de huid heeft, door bijvoorbeeld moedervlekken, pigmentvlekken of blauwe plekken, moeten deze kleuringen vermeden worden tijdens de metingen. Hierbij kan ofwel voor een nabije, laterale, plek worden gekozen of, bij beslaan van grote plekken op de huid, worden gekozen om een meetplek over te slaan. Dit moet worden vermeld bij Opmerkingen op het Meetgegevens formulier.

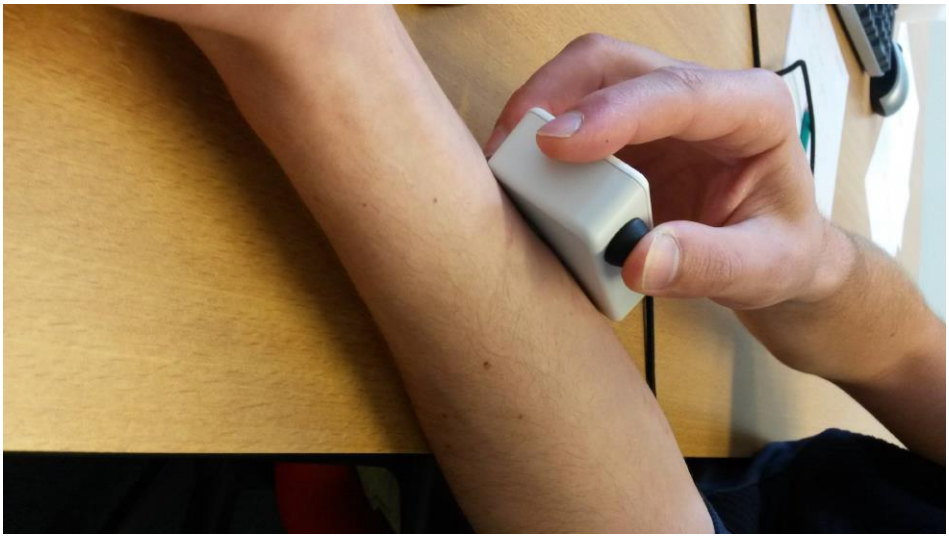
Fototherapie

Naar verwachting zullen veel proefpersonen fototherapie ondergaan tijdens deelname aan de studie. De therapie hoeft niet worden onderbroken bij het uitvoeren van de metingen.

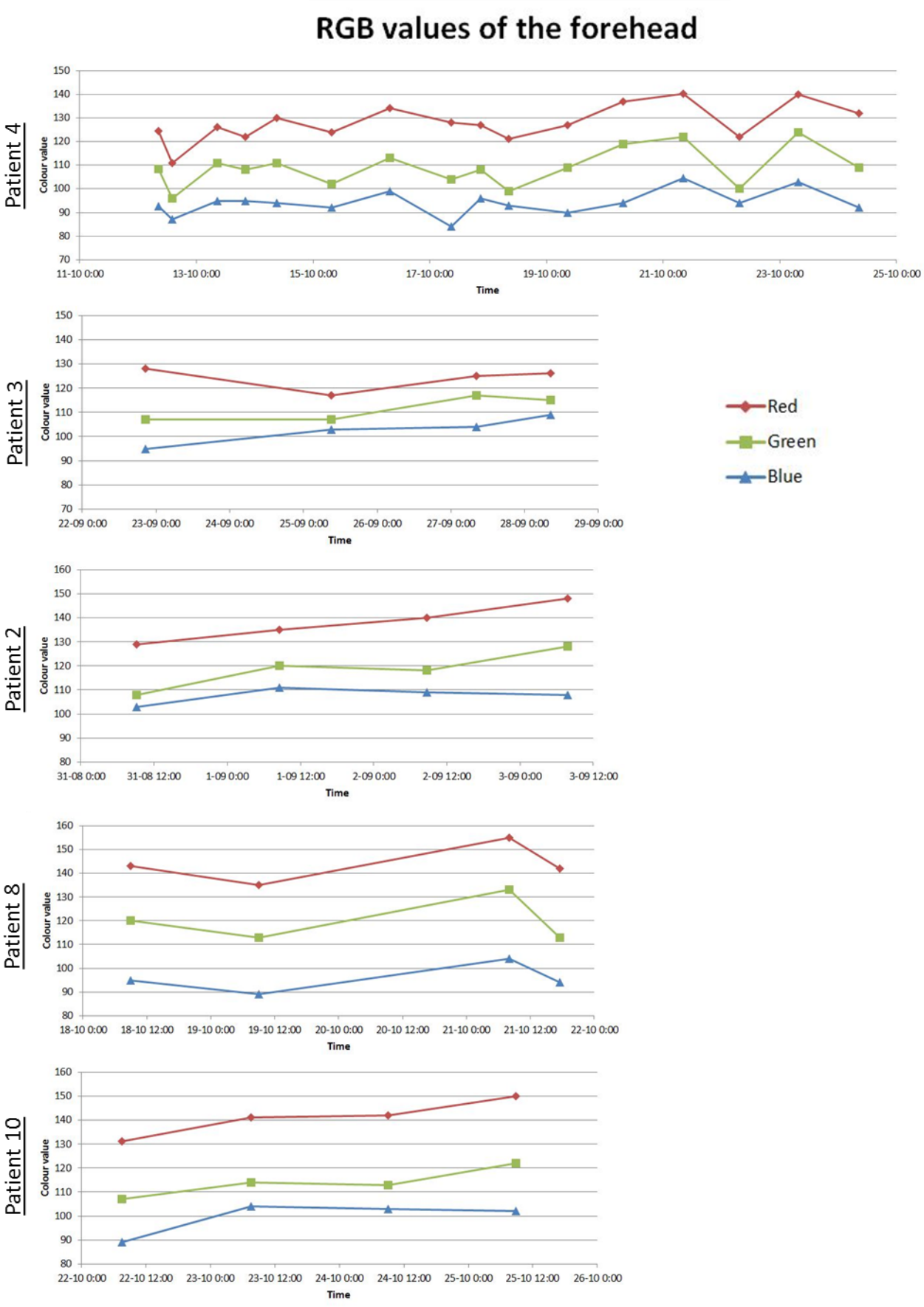
Bloedtransfusie

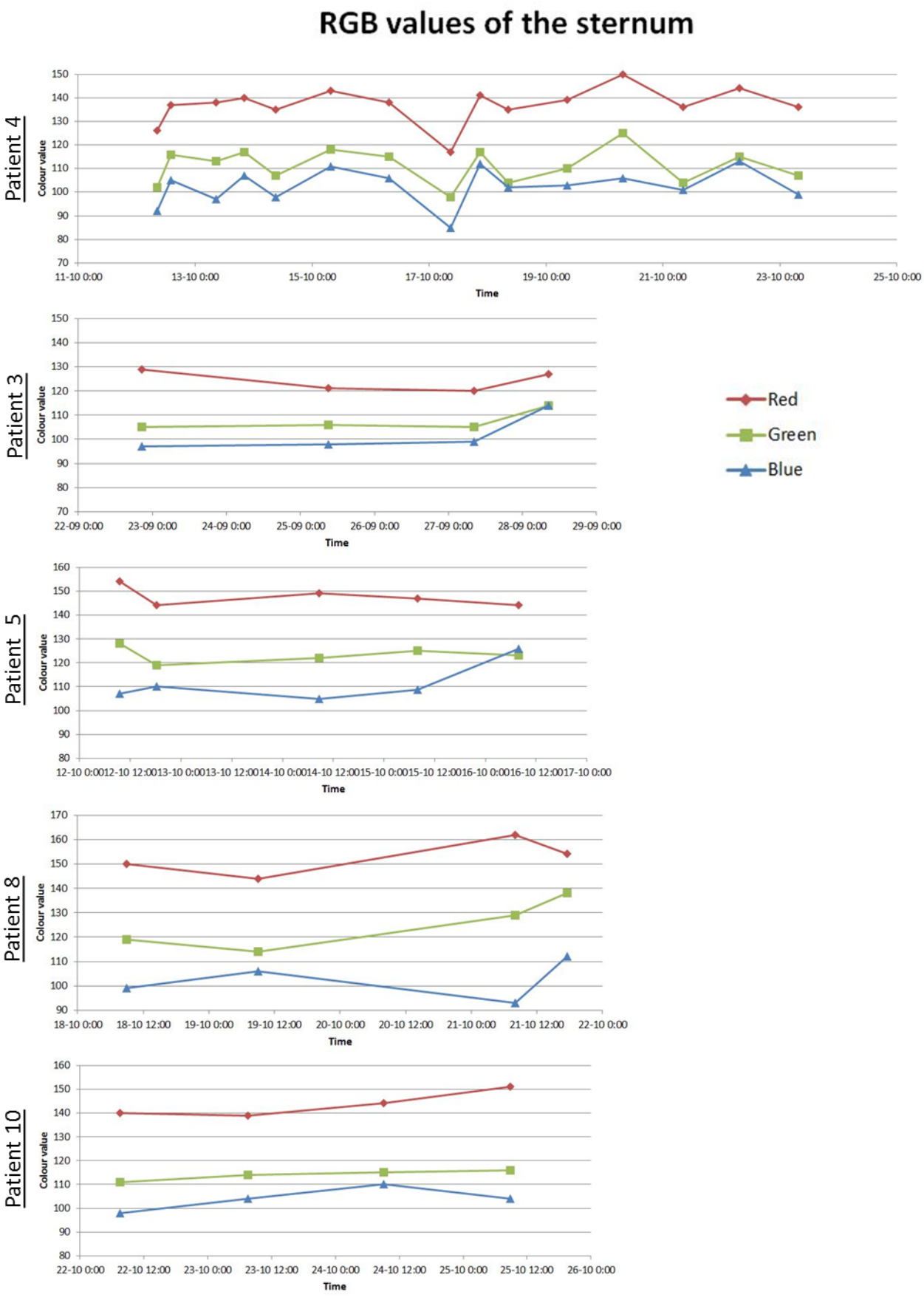
Mocht een proefpersoon een bloedtransfusie ontvangen, moet dit worden genoteerd op het formulier Meetgegevens waarbij de datum en tijdstip van de transfusie worden vermeld.

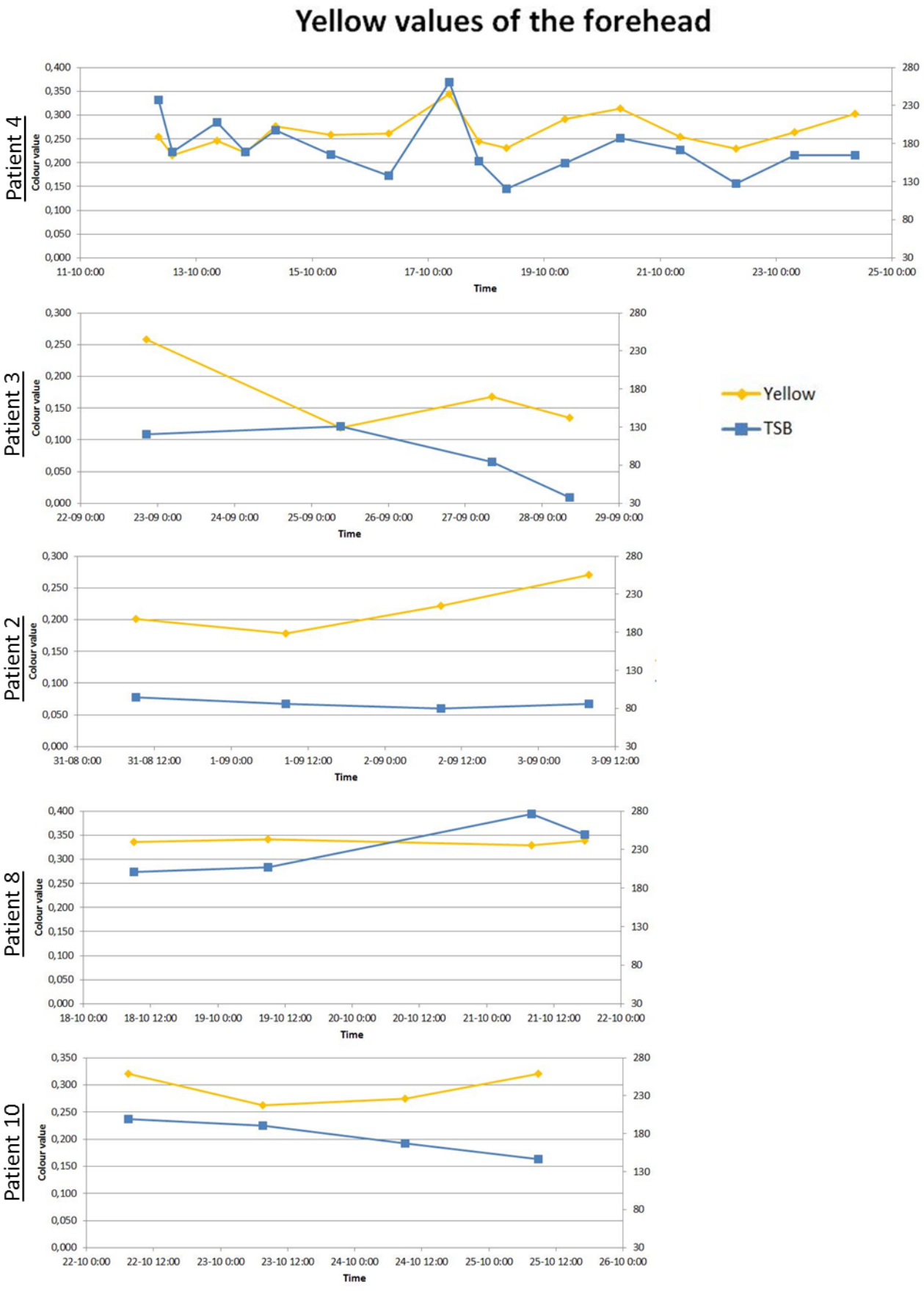
D. Device

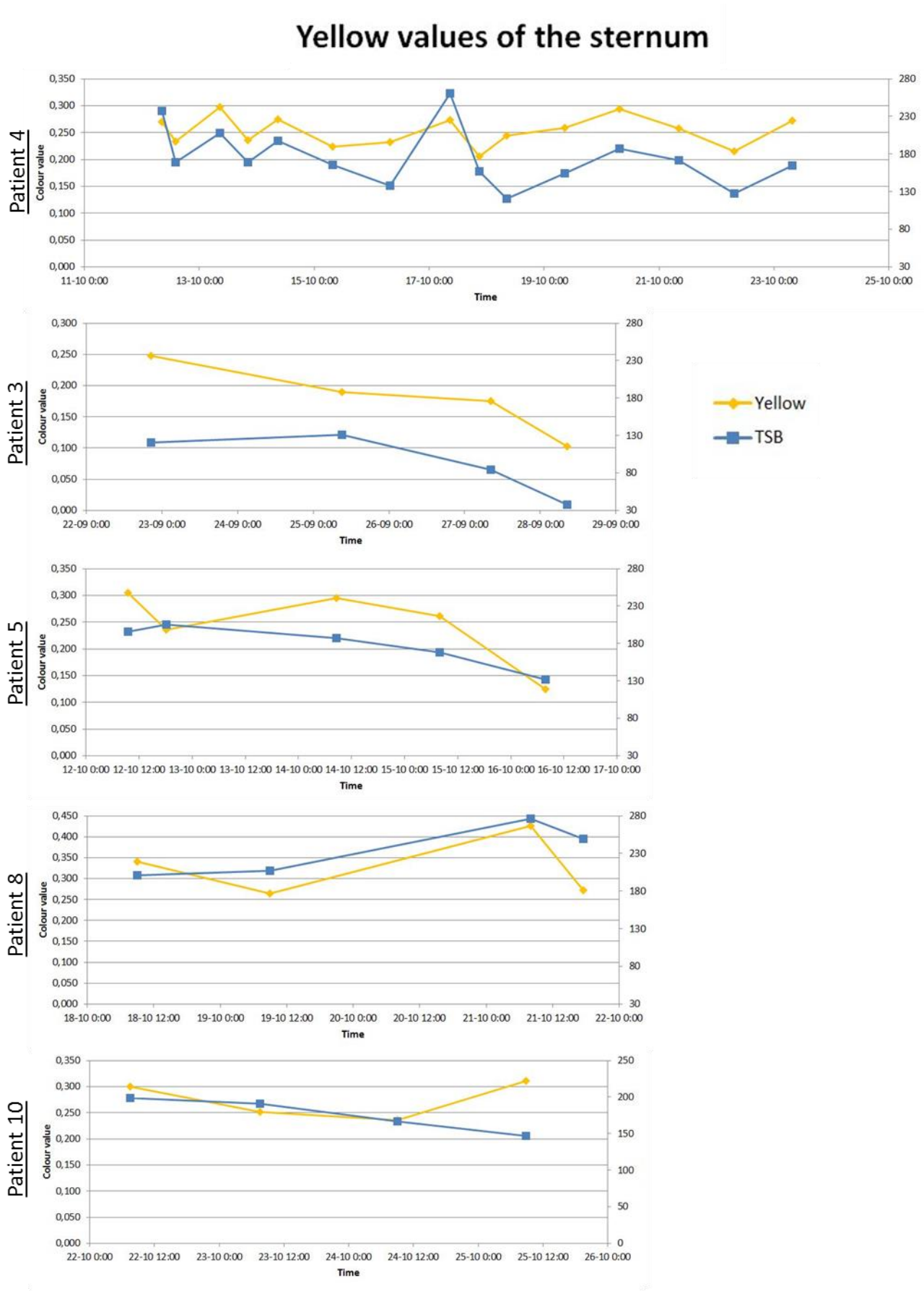


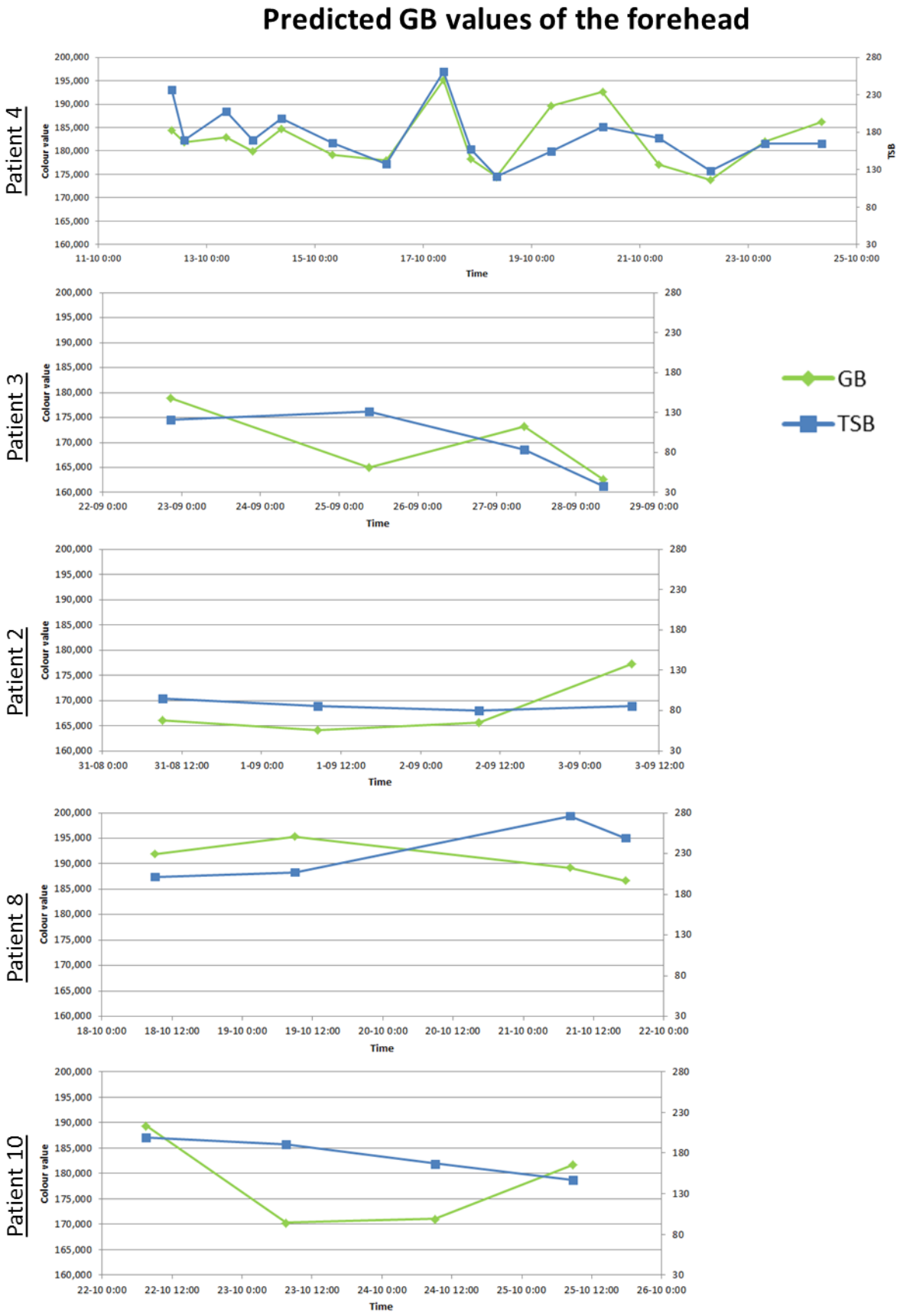
E. Colour values over time – Examples


RGB values of the forehead
Patient 4
Patient 3
Patient 2
Patient 8
Patient 10
Colour value
Time
Red
Green
Blue


RGB values of the sternum
Patient 4
Patient 3
Patient 5
Patient 8
Patient 10
Colour value
Time
Red
Green
Blue

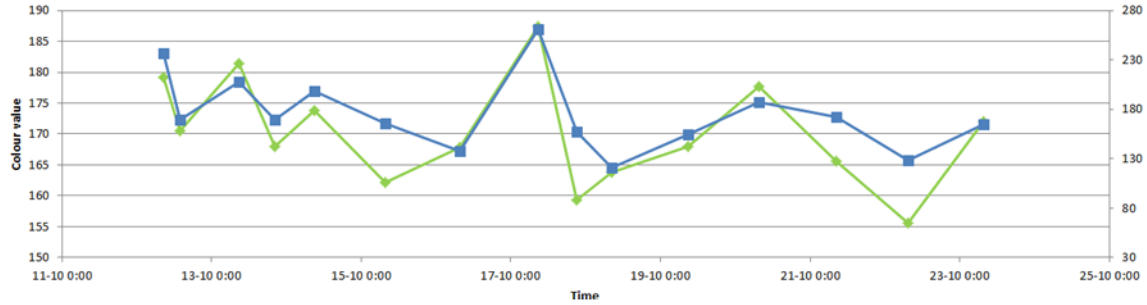

Yellow values of the forehead
Patient 4
Patient 3
Patient 2
Patient 8
Patient 10
Colour value
Time
Yellow
TSB


Yellow values of the sternum
Patient 4
Patient 3
Patient 5
Patient 8
Patient 10
Colour value
Time
Yellow
TSB

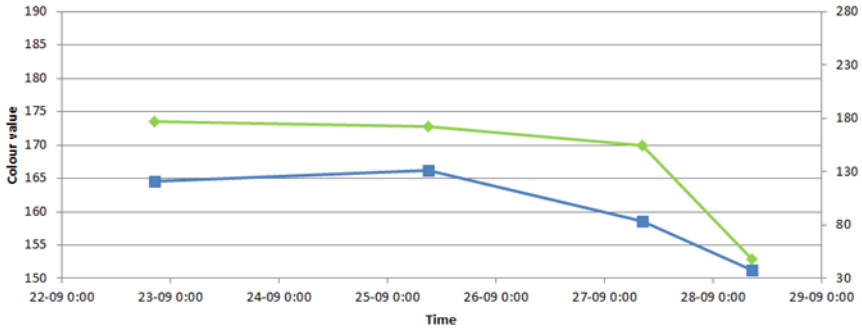

Predicted GB values of the forehead
Patient 4
Patient 3
Patient 2
Patient 8
Patient 10
Colour value
TSB
Time
GB
TSB

Predicted GB values of the sternum

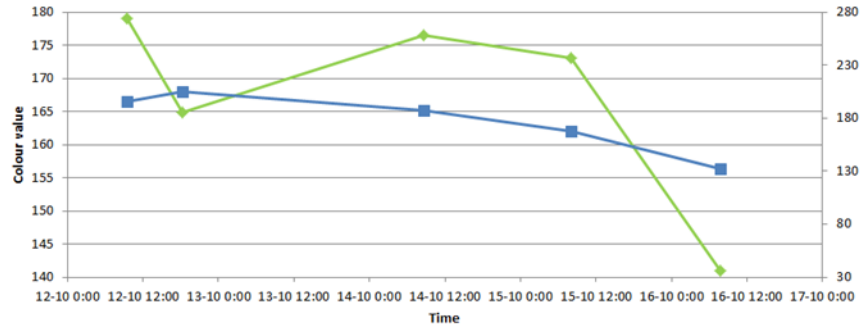
Patient 4



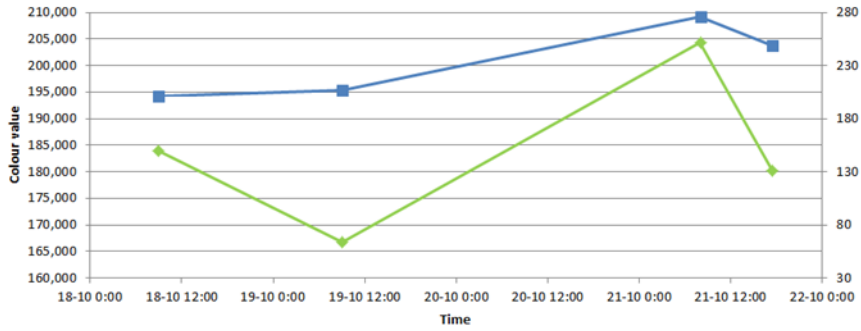
Patient 3



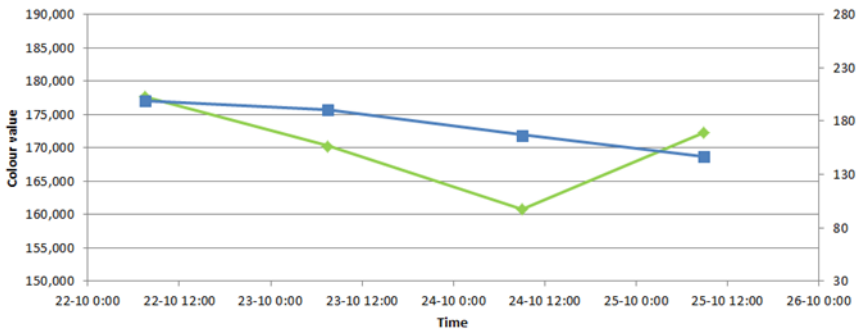
Patient 5



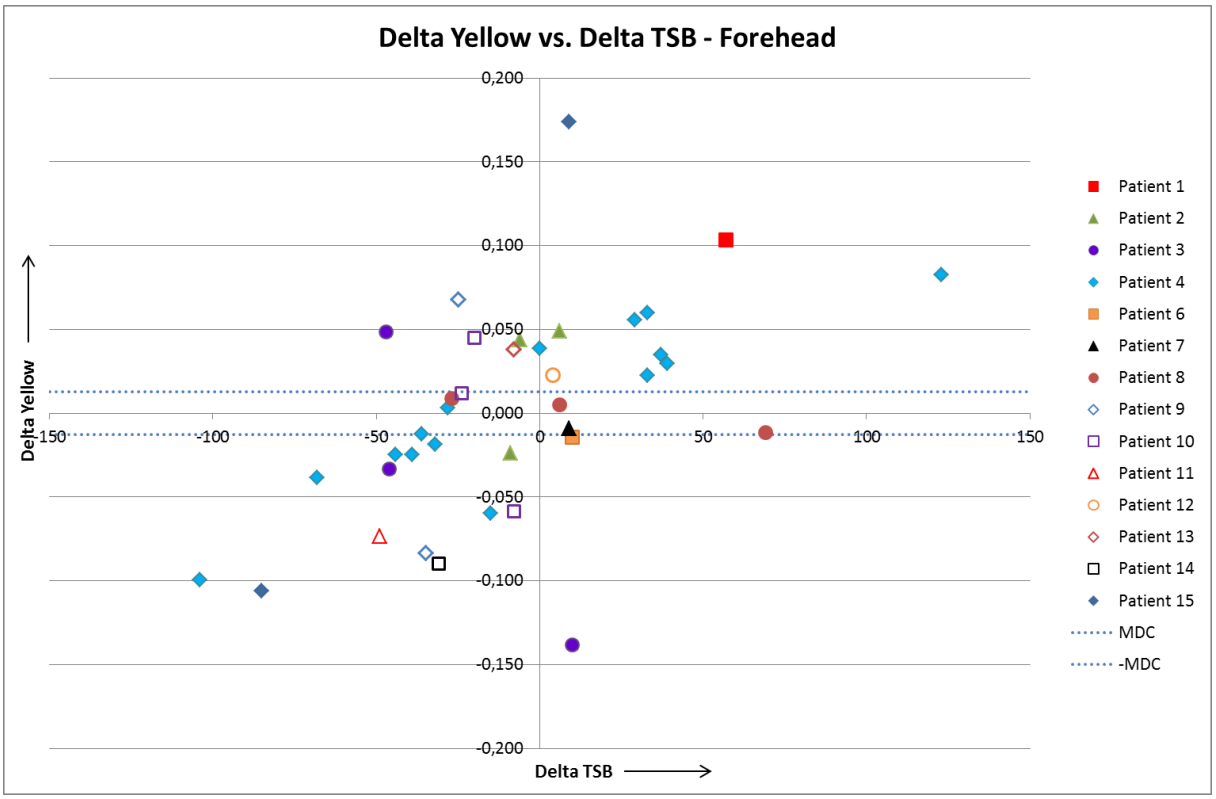
Patient 8

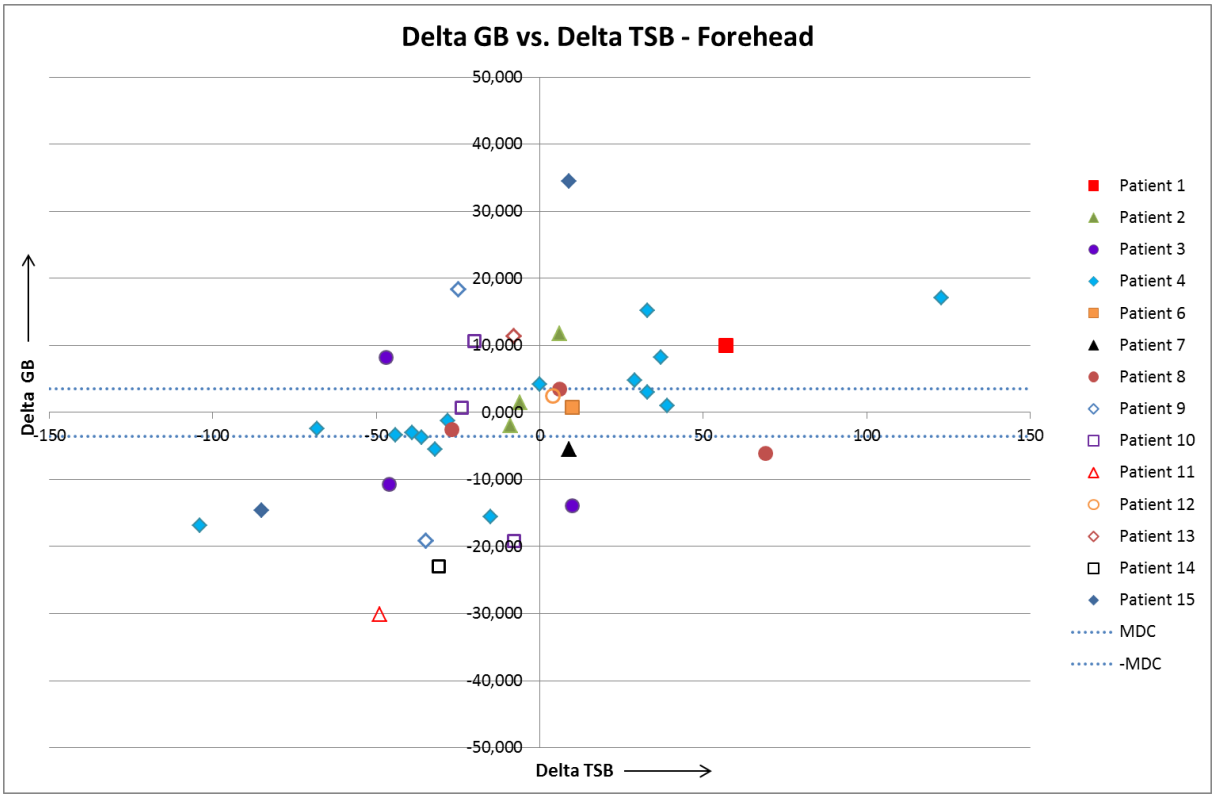


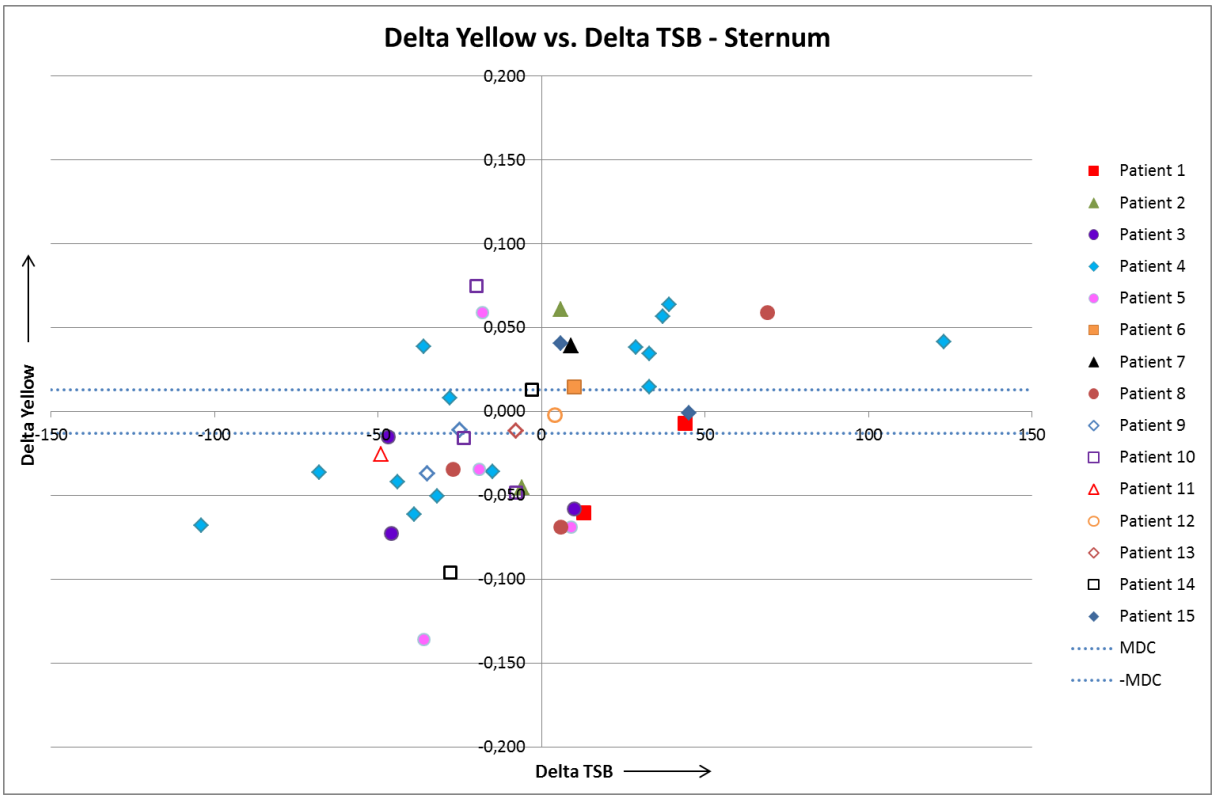
Patient 10

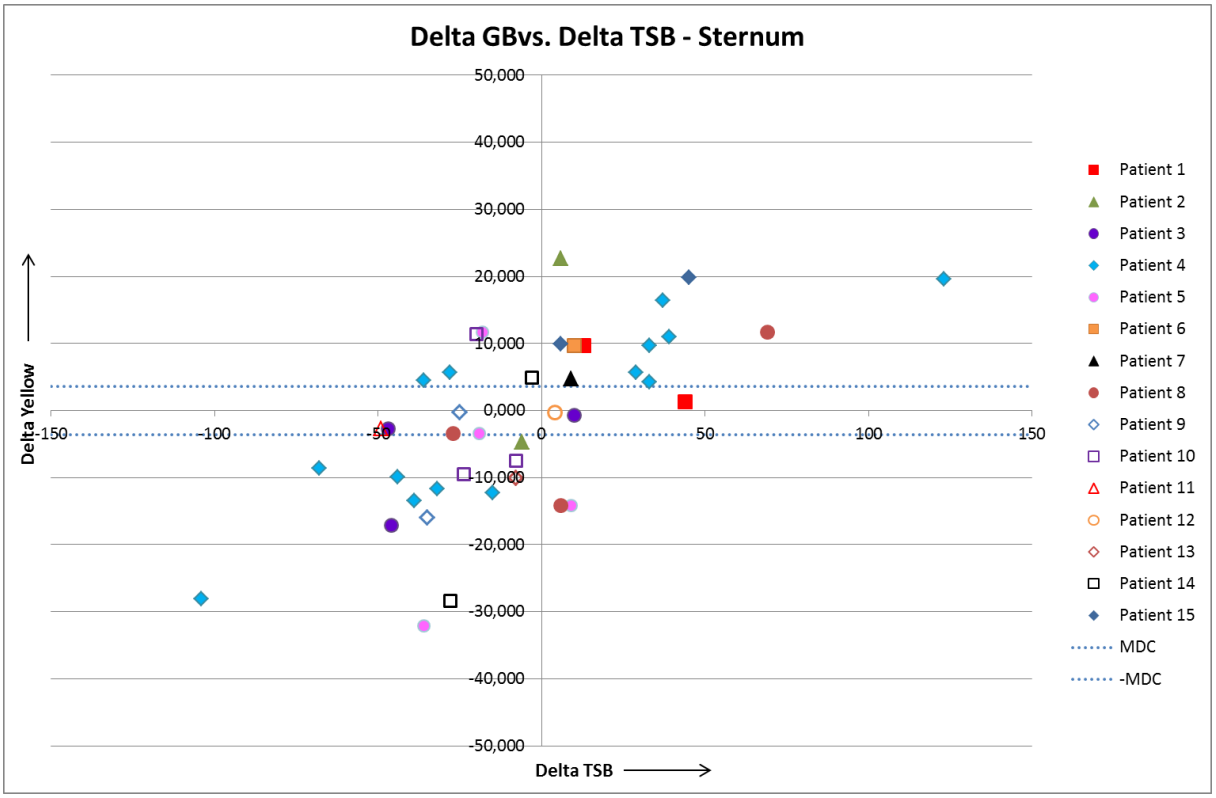


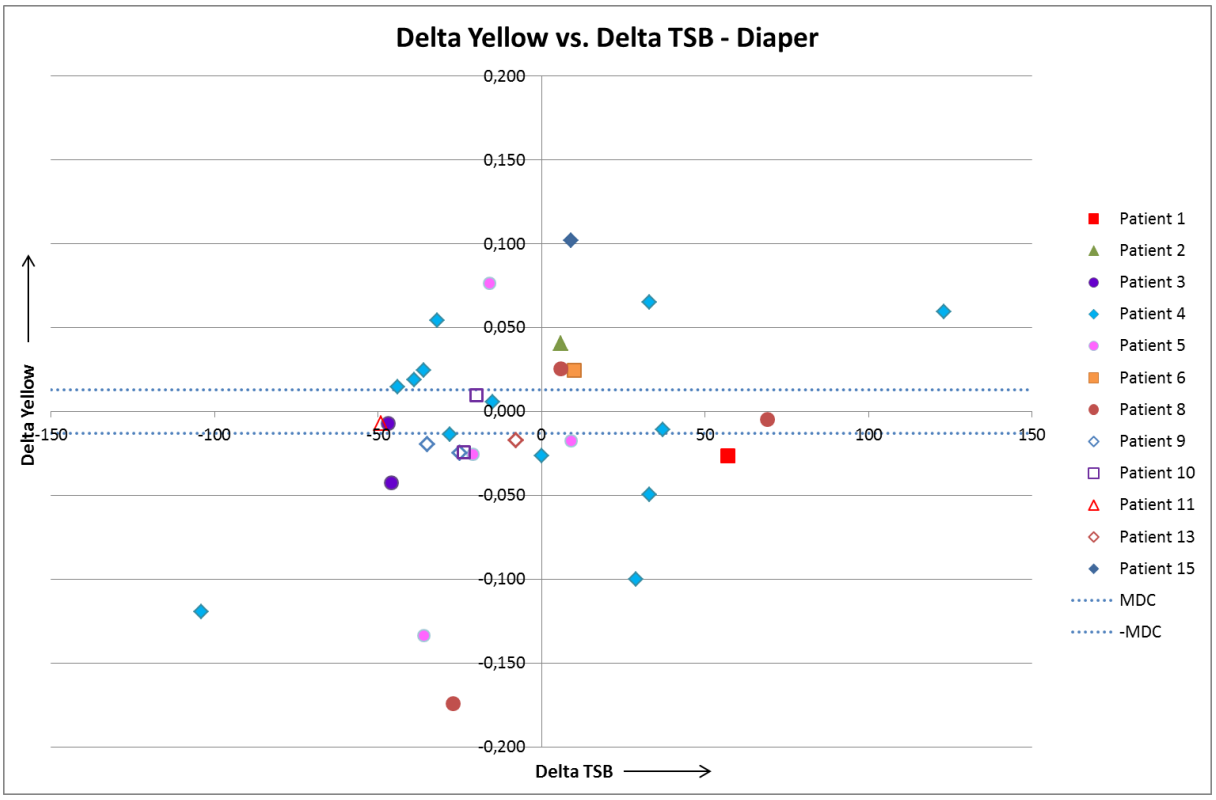
F. Plots – Delta Yellow vs. Delta TSB

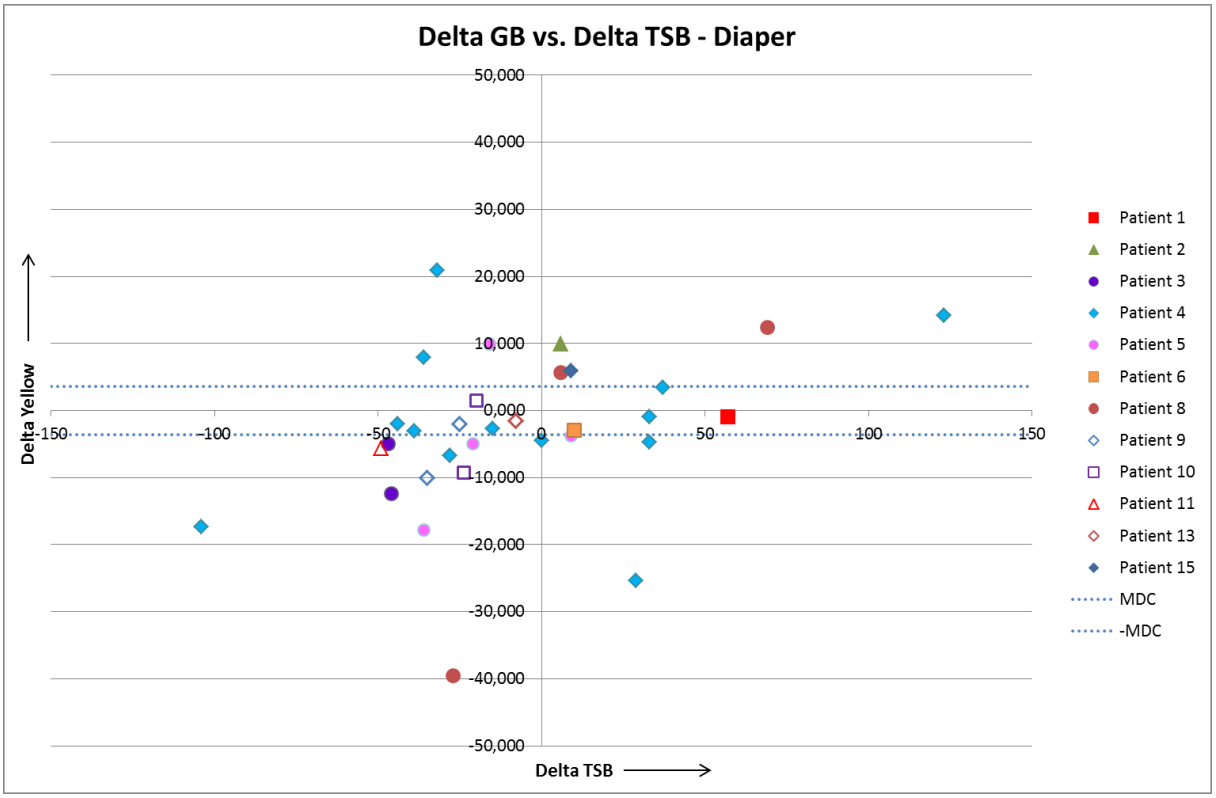


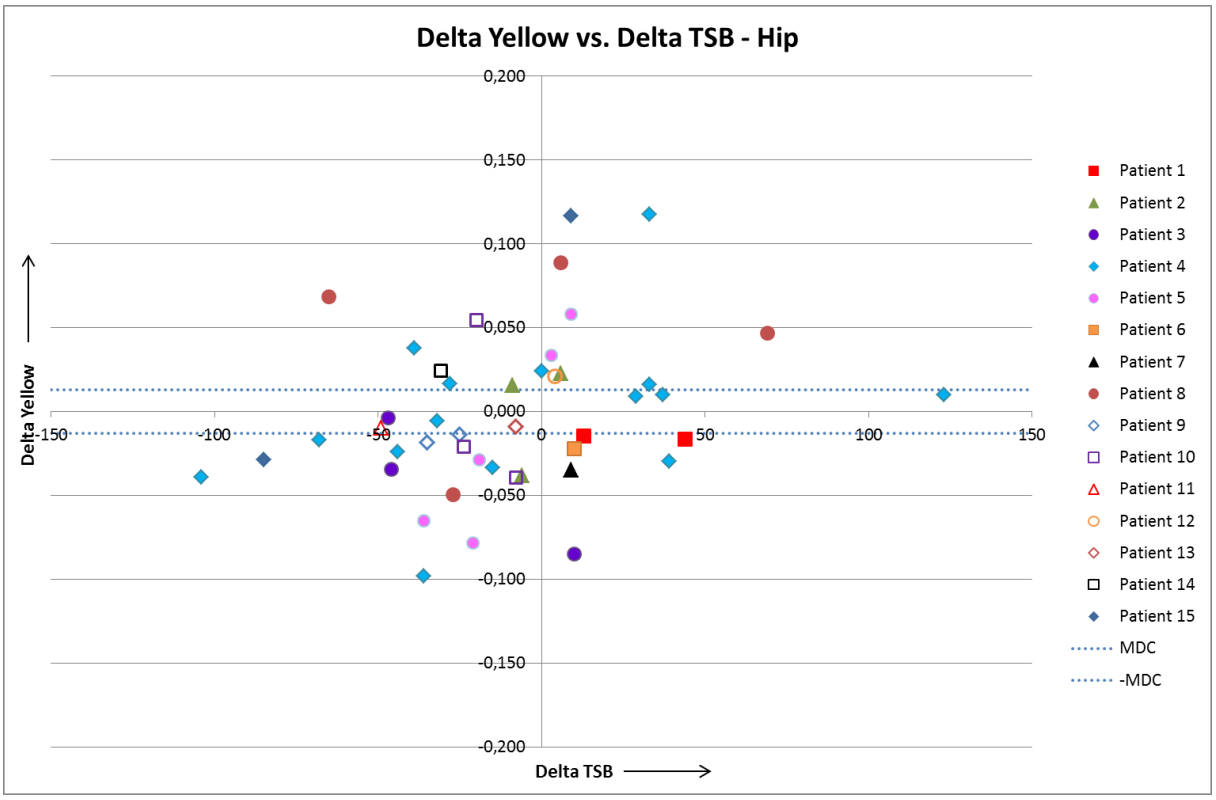


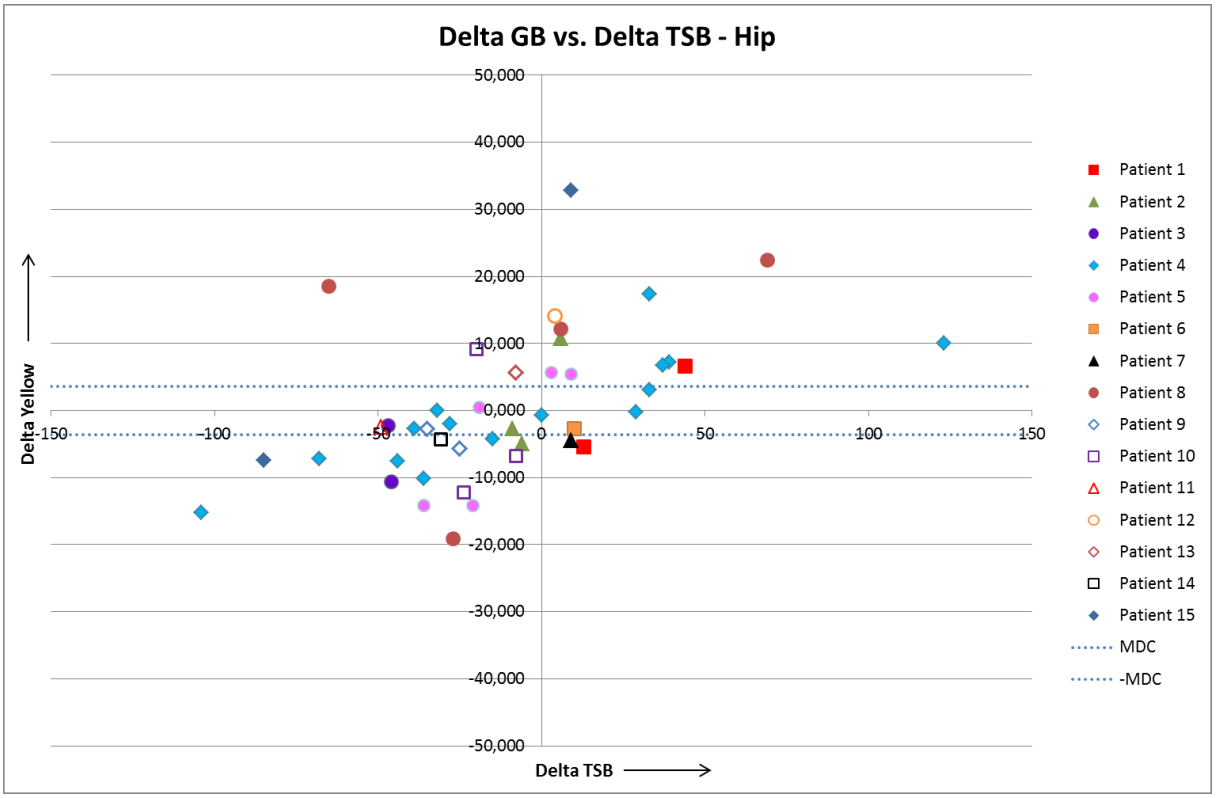

Delta Yellow vs. Delta TSB - Sternum
0,200
0,150
0,100
0,050
0,000
-0,050
-0,100
-0,150
-0,200
-150
-100
-50
0
50
100
150
Delta Yellow
Delta TSB
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
Patient 15
MDC
-MDC

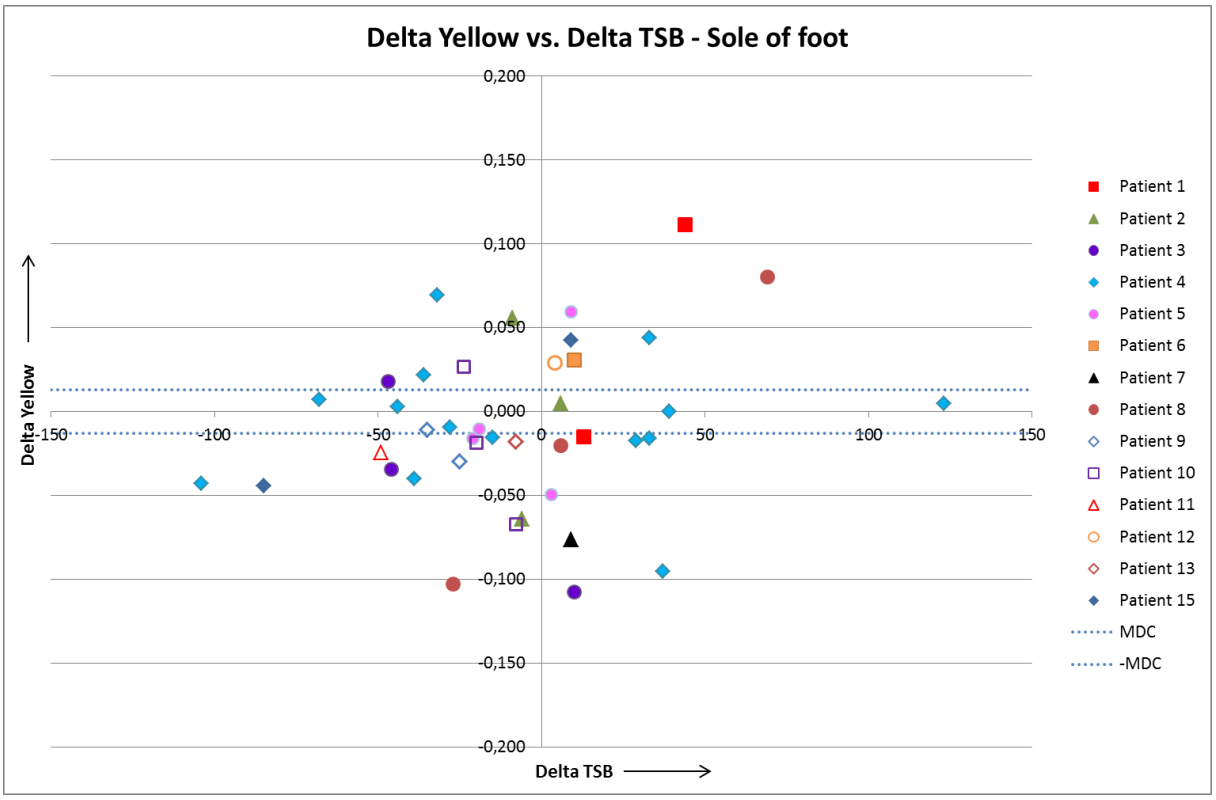

Delta GBvs. Delta TSB - Sternum
50,000
40,000
30,000
20,000
10,000
0,000
-10,000
-20,000
-30,000
-40,000
-50,000
-150
-100
-50
0
50
100
150
Delta Yellow
Delta TSB
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
Patient 15
MDC
-MDC

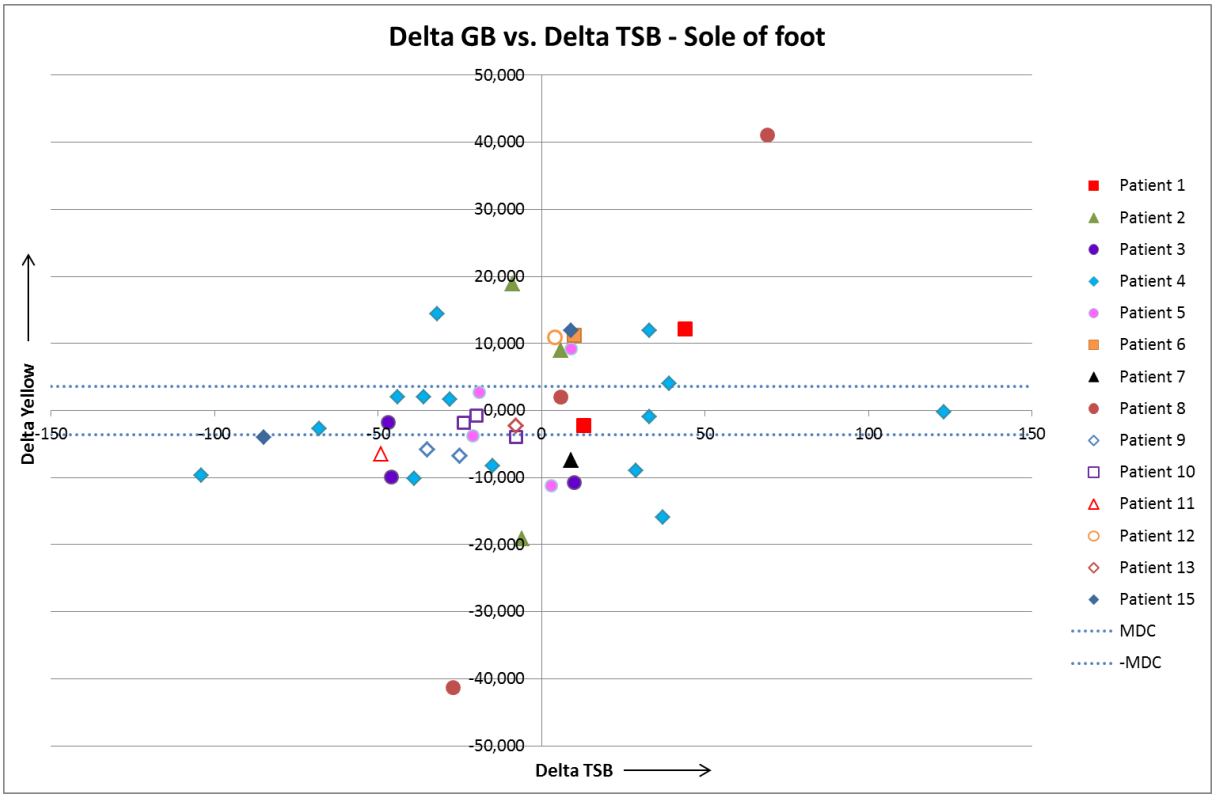

Delta Yellow vs. Delta TSB - Diaper
0,200
0,150
0,100
0,050
0,000
-0,050
-0,100
-0,150
-0,200
-150
-100
-50
0
50
100
150
Delta Yellow
Delta TSB
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 8
Patient 9
Patient 10
Patient 11
Patient 13
Patient 15
MDC
-MDC


Delta GB vs. Delta TSB - Diaper
50,000
40,000
30,000
20,000
10,000
0,000
-10,000
-20,000
-30,000
-40,000
-50,000
-150
-100
-50
0
50
100
150
Delta Yellow
Delta TSB
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 8
Patient 9
Patient 10
Patient 11
Patient 13
Patient 15
MDC
-MDC

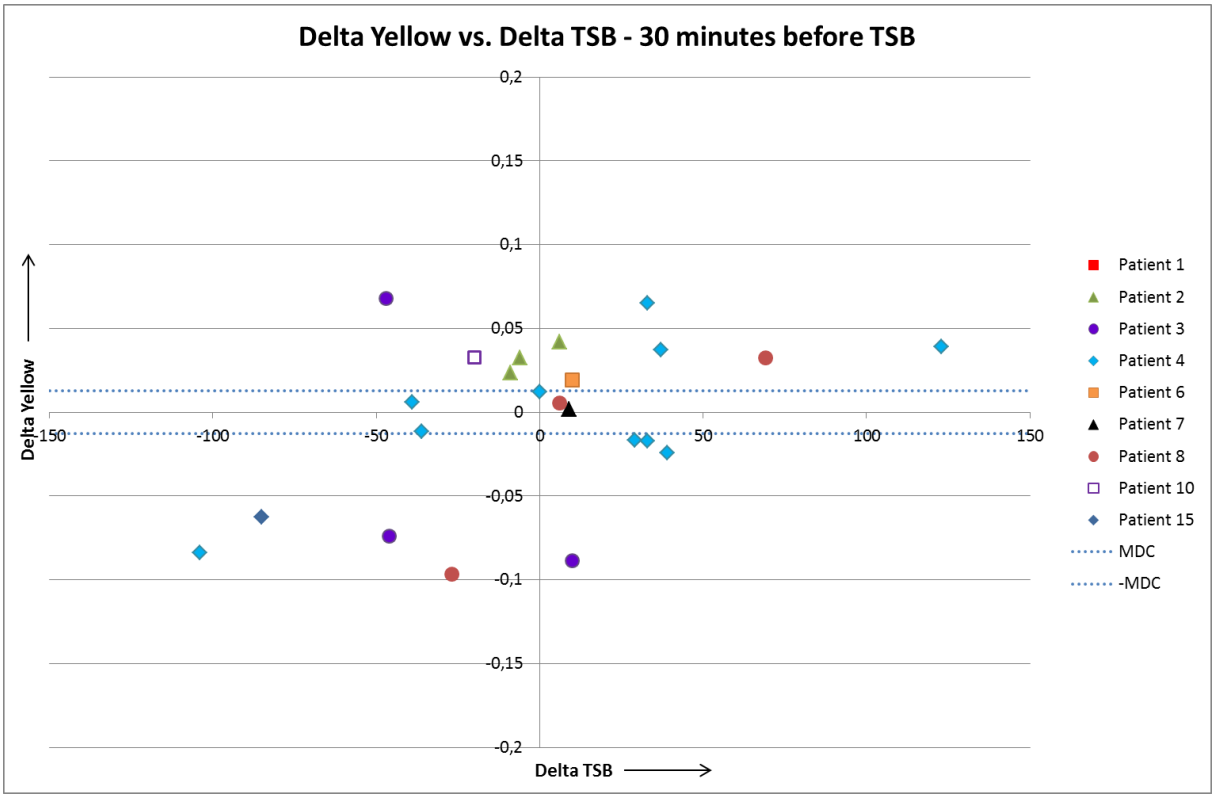

Delta Yellow vs. Delta TSB - Hip
0,200
0,150
0,100
0,050
0,000
-0,050
-0,100
-0,150
-0,200
-150
-100
-50
0
50
100
150
Delta Yellow
Delta TSB
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
Patient 15
MDC
-MDC

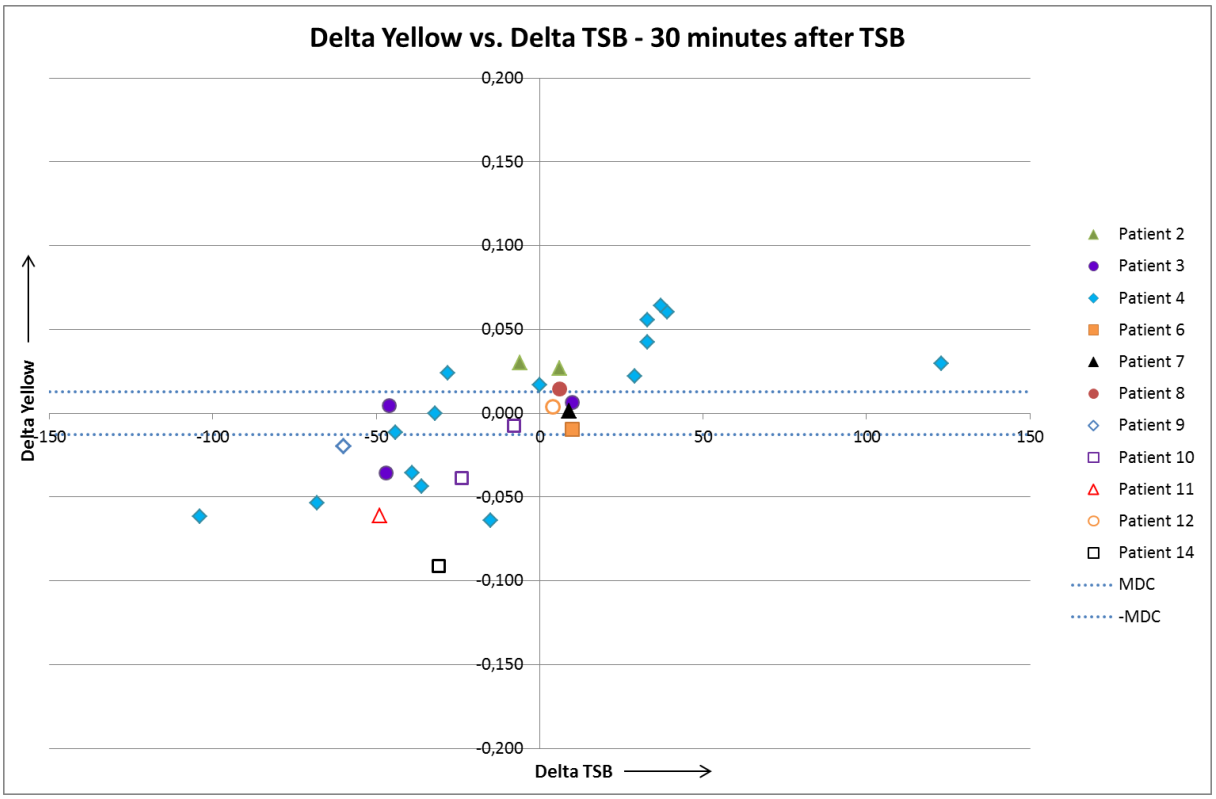

Delta GB vs. Delta TSB - Hip
50,000
40,000
30,000
20,000
10,000
0,000
-10,000
-20,000
-30,000
-40,000
-50,000
-150
-100
-50
0
50
100
150
Delta Yellow
Delta TSB
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 14
Patient 15
MDC
-MDC

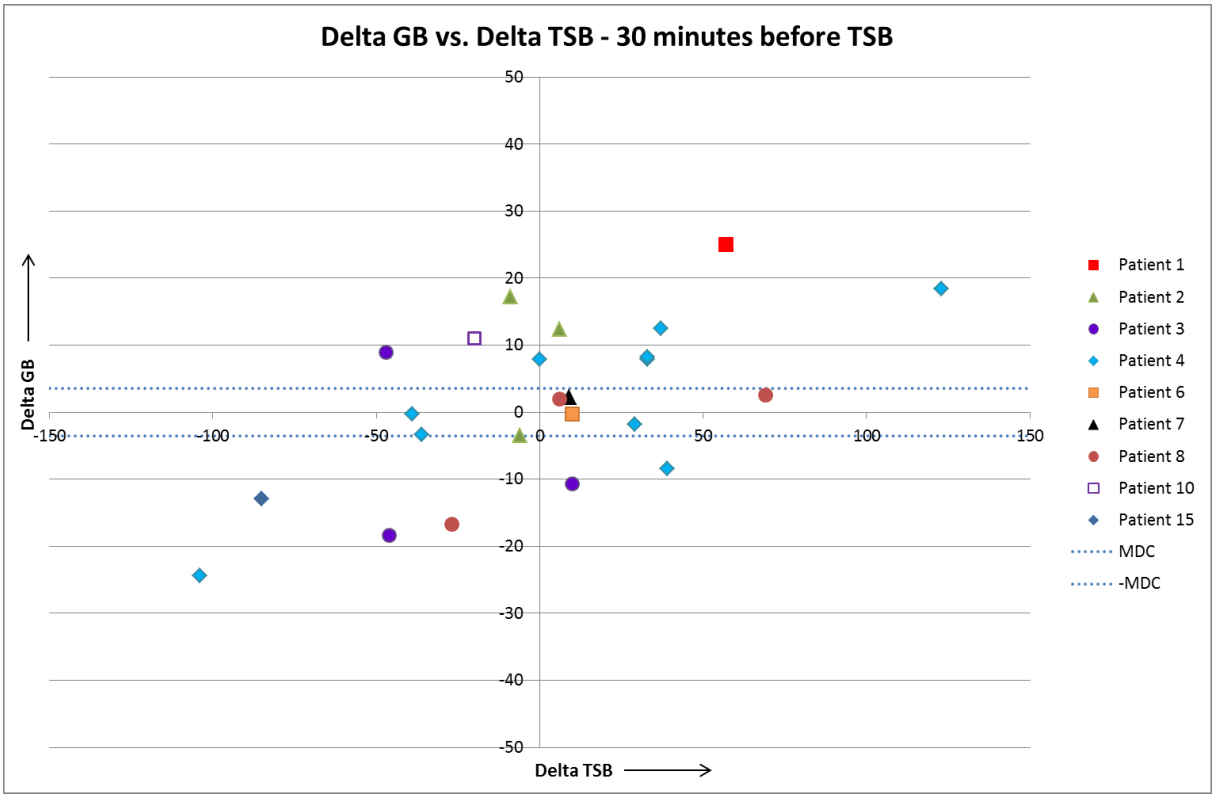

Delta Yellow vs. Delta TSB - Sole of foot
0,200
0,150
0,100
0,050
0,000
-0,050
-0,100
-0,150
-0,200
-150
-100
-50
0
50
100
150
Delta Yellow
Delta TSB
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 15
MDC
-MDC

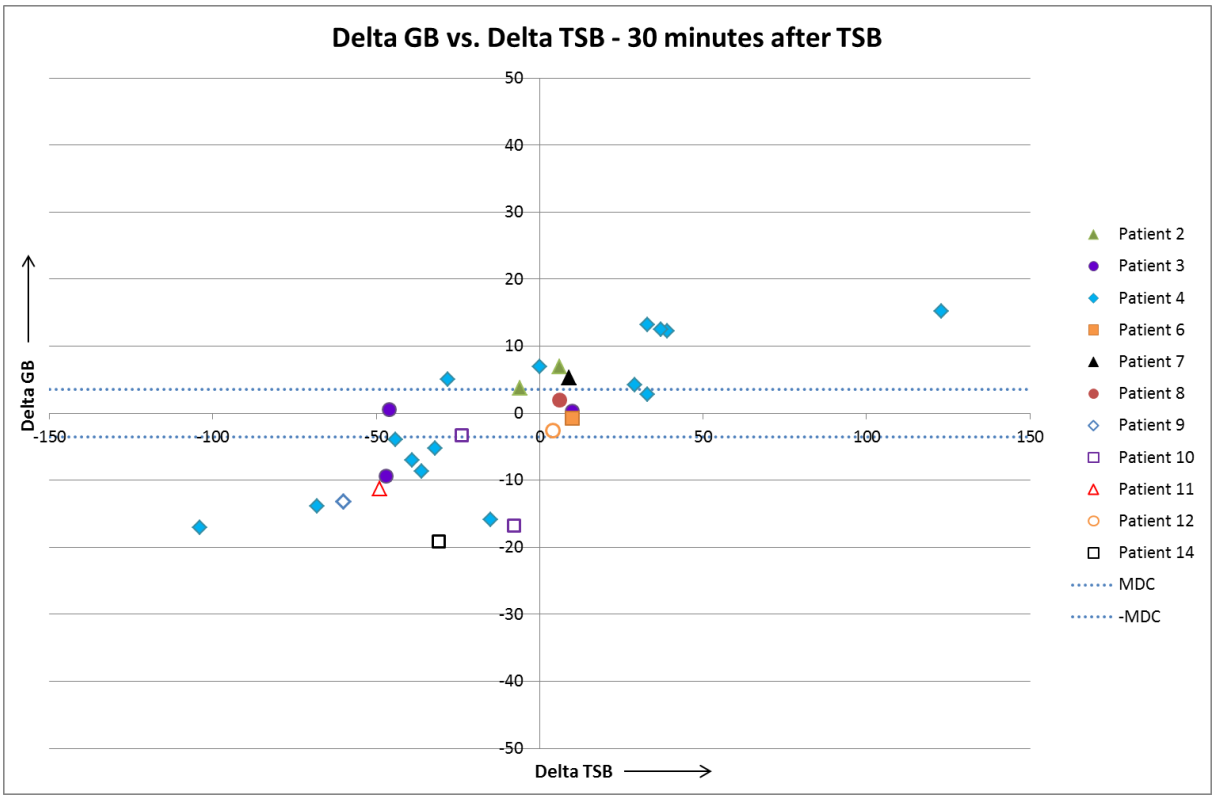

Delta GB vs. Delta TSB - Sole of foot
50,000
40,000
30,000
20,000
10,000
0,000
-10,000
-20,000
-30,000
-40,000
-50,000
-150
-100
-50
0
50
100
150
Delta Yellow
Delta TSB
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 13
Patient 15
MDC
-MDC

G. Plots on delay of change






Delta GB vs. Delta TSB - 30 minutes before TSB
Delta GB
Delta TSB
50
40
30
20
10
0
-10
-20
-30
-40
-50
-150
-100
-50
0
50
100
150
Patient 1
Patient 2
Patient 3
Patient 4
Patient 6
Patient 7
Patient 8
Patient 10
Patient 15
MDC
-MDC


Delta GB vs. Delta TSB - 30 minutes after TSB
Delta GB
Delta TSB
50
40
30
20
10
0
-10
-20
-30
-40
-50
-150
-100
-50
0
50
100
150
Patient 2
Patient 3
Patient 4
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
Patient 12
Patient 14
MDC
-MDC

H. Matlab script – Brute Force regressions

```
clear all
close all
clc

%%
Data = xlsread('RGBBilioverzicht.xls');

%% Matrix RGB per meeting en bili

[x,y] = size(Data);
%Loop for finding optimum
n=0;
steps=10;
ErrorMatrix =
zeros((20*steps)+1,(20*steps)+1,(20*steps)+1);
SomE=0;

%%
stappen=-steps:0.1:steps;

    for i=1:length(stappen);
        for j=1:length(stappen);
            for k=1:length(stappen);
                for l=1:x
                    R=Data(l,1);
                    G=Data(l,2);
                    B=Data(l,3);
                    Bili=Data(l,4);
                    a=stappen(i);
                    b=stappen(j);
                    c=stappen(k);
                    Z=a*R+b*G+c*B;
                    E=Bili-Z;
                    SomE=abs(E)+SomE;
                    n=n+1;
                end
                i1=i;
                j1=j;
                k1=k;
                ErrorMatrix(i1,j1,k1)=(SomE/n);
            n=0;
            SomE=0;
            end
        end
    end
```

```
%%
[M,I] = min(ErrorMatrix(:));
HowManyMin=sum((ErrorMatrix(:))==M);
maat=size(ErrorMatrix);
for m=1:HowManyMin
    [M,I] = min(ErrorMatrix(:));
    [I_row, I_col, I_depth] =
ind2sub(size(ErrorMatrix),I);
    a(m,1)=I_row;
    b(m,1)=I_col;
    c(m,1)=I_depth;
    ErrorMatrix(I_row,I_col,I_depth)=M+1;
end
Z=[a,b,c];
```

```
clear all
close all
clc

%%
Data = xlsread('RGBBilioverzicht.xls');

%% Matrix RGB per meeting en bili

[x,y] = size(Data);
%Loop for finding optimum
n=0;
steps=5;
ErrorMatrix =
zeros(180+1,(20*steps)+1,(20*steps)+1,(20*steps)+1);
SomE=0;

%%
stappen=-steps:0.1:steps;
for h=0:180
    for i=1:length(stappen);
        for j=1:length(stappen);
            for k=1:length(stappen);
                for l=1:x
                    R=Data(l,1);
                    G=Data(l,2);
                    B=Data(l,3);
                    Bili=Data(l,4);
```

```
                    a=stappen(i);
                    b=stappen(j);
                    c=stappen(k);
                    d=h;
                    Z=a*R+b*G+c*B+d;
                    E=Bili-Z;
                    SomE=abs(E)+SomE;
                    n=n+1;
                end
                i1=i;
                j1=j;
                k1=k;
                d1=d+1;
                ErrorMatrix(d1,i1,j1,k1)=(SomE/n);
            n=0;
            SomE=0;
            end
        end
    end
end


%%
[M,I] = min(ErrorMatrix(:));
HowManyMin=sum((ErrorMatrix(:))==M);
maat=size(ErrorMatrix);
for m=1:HowManyMin
    [M,I] = min(ErrorMatrix(:));
    [D, I_row, I_col, I_depth] =
ind2sub(size(ErrorMatrix),I);
    d(m,1)=D-1;
    a(m,1)=I_row;
    b(m,1)=I_col;
    c(m,1)=I_depth;
    ErrorMatrix(D,I_row,I_col,I_depth)=M+1;
end
Z=[a,b,c,d];
```

I. R script – Logistic regression for non-fitted data

```
# read in data
ava <- read.csv(file.choose(), header = T, sep = ";")

install.packages("lme4")
install.packages("arm")

# access packages (you may need to install this as well)
library(lme4)
library(arm)

# looking at data
dim(ava)
colnames(ava)
head(ava)


# looking at change in yellow and change in Bili:


model.yellow <- glmer(Bili.IncrDecr ~ Yellow.IncrDecr + (1|Patient),
                      data = ava,
                      family = "binomial")
summary(model.yellow)


PredictedY=fitted(model.yellow)
responseY=model.yellow@frame$Bili.IncrDecr
thresh=0.5
PredictedY2 <- cut(PredictedY, breaks=c(-Inf, thresh, Inf), labels=c("0", "1"))
# contingency table and marginal sums
YcTab <- table(responseY, PredictedY2)
addmargins(YcTab)

head(PredictedY)
head(PredictedY2)



# looking at change in r and change in Bili

model.r <- glmer(Bili.IncrDecr ~ R.IncrDecr + (1|Patient),
                 data = ava,
                 family = "binomial")
summary(model.r)

View(ava)
PredictedR=fitted(model.r)
responseR=model.r@frame$Bili.IncrDecr
thresh=0.5
PredictedR2 <- cut(PredictedR, breaks=c(-Inf, thresh, Inf), labels=c("0", "1"))
# contingency table and marginal sums
RcTab <- table(responseR, PredictedR2)
addmargins(RcTab)
```

```
# looking at change in g and change in Bili

model.g <- glmer(Bili.IncrDecr ~ G.IncrDecr + (1|Patient),
                 data = ava,
                 family = "binomial")
summary(model.g)

View(ava)
PredictedG=fitted(model.g)
responseG=model.g@frame$Bili.IncrDecr
thresh=0.5
PredictedG2 <- cut(PredictedG, breaks=c(-Inf, thresh, Inf), labels=c("0", "1"))
# contingency table and marginal sums
GcTab <- table(responseG, PredictedG2)
addmargins(GcTab)
```

```
# looking at change in b and change in Bili

model.b <- glmer(Bili.IncrDecr ~ B.IncrDecr + (1|Patient),
                 data = ava,
                 family = "binomial")
summary(model.b)

View(ava)
PredictedB=fitted(model.b)
responseB=model.b@frame$Bili.IncrDecr
thresh=0.5
PredictedB2 <- cut(PredictedB, breaks=c(-Inf, thresh, Inf), labels=c("0", "1"))
# contingency table and marginal sums
BcTab <- table(responseB, PredictedB2)
addmargins(BcTab)
```

```
## Brute force Regressions

model.bf1 <- glmer(Bili.IncrDecr ~ BF1.IncrDecr + (1|Patient),
                 data = ava,
                 family = "binomial")
summary(model.bf1)

PredictedBF1=fitted(model.bf1)
responseBF1=model.bf1@frame$Bili.IncrDecr
thresh=0.5
PredictedBF1bin <- cut(PredictedBF1, breaks=c(-Inf, thresh, Inf), labels=c("0", "1"))
# contingency table and marginal sums
BF1cTab <- table(responseBF1, PredictedBF1bin)
addmargins(BF1cTab)


model.bf2 <- glmer(Bili.IncrDecr ~ BF2.IncrDecr + (1|Patient),
                   data = ava,
                   family = "binomial")
summary(model.bf2)

PredictedBF2=fitted(model.bf2)
responseBF2=model.bf2@frame$Bili.IncrDecr
thresh=0.5
PredictedBF2bin <- cut(PredictedBF2, breaks=c(-Inf, thresh, Inf), labels=c("0", "1"))
# contingency table and marginal sums
BF2cTab <- table(responseBF2, PredictedBF2bin)
addmargins(BF2cTab)
```

J. R script – Linear and logistic regression for fitted data

```
# read in data
ava <- read.csv(file.choose(), header = T, sep = ";")

install.packages("lme4")
install.packages("arm")

# access packages (you may need to install this as well)
library(lme4)
library(arm)

# looking at data
dim(ava)
colnames(ava)
head(ava)

# creating times:
any(is.na(ava$Date.Time))
ava$date <- as.POSIXct(as.character(ava$Date.Time), format = "%d-%m-%Y %H:%M")

ava <- ava[order(ava$Patient, ava$Location, ava$date),]
ava$tdiff <- unlist(tapply(ava$date, INDEX = ava$Patient:ava$Location,
                           FUN = function(x) c(0, `units<-`(diff(x), "mins"))))
```

```
# LINEAR REGRESSION TO FIT DATA for GB

# Linear regression without tdiff

model.2 <- lmer(Bili ~ G + B + (1|Patient), data = ava)
summary(model.2)

predict.GBnoT <- fitted(model.2)
ava.2 <- data.frame(ava, predict.GBnoT)

ava.2$change_predictGBnoT <- NA

for (i in 1:269) {
  if (ava.2$predict.GBnoT[i + 1] > ava.2$predict.GBnoT[i])
  {ava.2$change_predictGBnoT[i + 1] <- 1} else
  {ava.2$change_predictGBnoT[i + 1] <- 0}
}


model.predictedGBnoT <- glmer(Bili.IncrDecr ~ change_predictGBnoT + (1|Patient),
                              data = ava.2,
                              family = "binomial")
summary(model.predictedGBnoT)

Predictedvalue1=fitted(model.predictedGBnoT)
responseGBnoT=model.predictedGBnoT@frame$Bili.IncrDecr
thresh=0.5
PredictedGBnoT <- cut(Predictedvalue1, breaks=c(-Inf, thresh, Inf), labels=c("0", "1"))
# contingency table and marginal sums
GBnoTcTab <- table(responseGBnoT, PredictedGBnoT)
addmargins(GBnoTcTab)
```

```r
# Linear regression with tdiff

model.3 <- lmer(Bili ~ G + B + tdiff +  (1|Patient), data = ava)
summary(model.3)

predict.GB3 <- fitted(model.3)
ava.2 <- data.frame(ava.2, predict.GB3)

ava.2$change_predict <- NA

for (i in 1:269) {
  if (ava.2$predict.GB3[i + 1] > ava.2$predict.GB3[i])
  {ava.2$change_predict[i + 1] <- 1} else
  {ava.2$change_predict[i + 1] <- 0}
}

model.predictedGB <- glmer(Bili.IncrDecr ~ change_predict + (1|Patient),
                           data = ava.2,
                           family = "binomial")
summary(model.predictedGB)

Predictedvalue2=fitted(model.predictedGB)
responseGB3=model.predictedGB@frame$Bili.IncrDecr
thresh=0.5
PredictedGB3 <- cut(Predictedvalue2, breaks=c(-Inf, thresh, Inf), labels=c("0", "1"))
# contingency table and marginal sums
GB3cTab <- table(responseGB3, PredictedGB3)
addmargins(GB3cTab)
```