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## **Determination of the age of bruises using a bilirubinometer**

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### **Compliance with ethical standards**

### **Conflict of interest**

The authors declare that they have no conflicts of interest.

# Determination of the age of bruises using a bilirubinometer

## Abstract

The dating of bruises can be of crucial interest in clinical forensic medicine, but the macroscopic or photographic evaluation of a bruise has not been regarded as reliable. Several methods have thus been regularly evaluated for this purpose, with reflectance spectrometry yielding promising results. The objective of this study is to evaluate the value of a bilirubinometer as a tool for dating bruises on living victims.

A clinical follow-up was carried out on patients presenting a bruise with a known onset. Using a bilirubinometer, we obtained daily measurements of the bruise (bili-bruise) and of healthy skin (bili-skin). Potential confounding factors were collected: age, sex, body mass index, trauma mechanism and Fitzpatrick skin phototype.

We followed 20 patients for a total of 88 measurements of bruises. Bili-skin values showed significant differences according to skin phototype. Differences between the bili-bruise and bili-skin values ( $\Delta$ -bili) followed an increase phase, peaking between 3 and 5 days, and then there was a decrease phase. No significant  $\Delta$ -bili value differences were observed based on the suspected confounding factors.

Our results are in favour of a peak  $\Delta$ -bili value generally at day 4 or 5 post-trauma. Notably, decreasing values were not observed before day 3. Decreasing  $\Delta$ -bili values would then indicate a bruise resulting from an injury formed at least 3 days before the first measurement. Complementary work confirming such data would enable improvement of the performance of bruise dating in forensic medicine.

**Keywords:** forensic medicine, bruise, age estimation, bilirubinometer, reflectance spectrophotometry

## Introduction

The interpretation of bruises can be particularly important in a forensic context. Bruise dating can play a crucial role in validating or invalidating the testimony of a person accused of acts of physical violence. For this reason, forensic clinicians in France regularly examine living victims at the request of the legal authorities.

The microscopic analysis of a bruise sampled during autopsy by a forensic pathologist often enables the evaluation of bruise dating. However, this is obviously not possible for living victims. Direct visual observation of bruises, or observation from photographs, has been the basis of the evaluation of bruise dating for many years.

Forensic medicine handbooks also state the principles for dating bruises based on their colour. These estimates have rather broad intervals and the only statement advanced is that at least 18 hours are necessary to be able to observe the yellowish colouring of a bruise, although generally it tends to appear between 24 and 72 hours (1–3). Although blood is red, the presence of haemoglobin is what alters the visual appearance of the bruise, mainly according to its oxygenation and the depth of the lesion. Haemoglobin is composed of four heme molecules combined with a globin. From a physiopathological point of view, at the time of a trauma, an inflammatory reaction occurs when blood leaves the vessels towards the tissues. Polynuclear

neutrophils are the first cells to arrive, but cannot degrade haemoglobin. Then, the macrophages arrive at the injury site and carry out the first stage of haemoglobin degradation. This enzymatic degradation of haemoglobin requires oxygen and energy. It leads to the formation of biliverdin and carbon monoxide release as well as the presence of iron atoms in heme. Biliverdin is greenish and is quickly converted into bilirubin by the biliverdin reductase enzyme. Hemosiderin is formed with part of the released iron from heme in the macrophages, and is then bound with the dermic tissue proteins. The bilirubin (yellowish pigmentation) and the hemosiderin (brownish pigmentation) are then eliminated gradually, leading to the disappearance of the bruise (4,5).

These phenomena explain the chronological evolution of the colours of such a lesion and a bruise is classically described as adopting an initially and gradually reddish colour, becoming bluish and/or purplish, then greenish, yellowish and finally brownish. The initial colours can vary from case to case and the bruise disappears in a few days to a few weeks by resorption beginning from the periphery (1). There is no known predictive factor for determining if the disappearance of the bruise will be fast or slow.

Multiple scientific publications have concluded that direct or photographic visual evaluation of bruises with the aim of dating them is unreliable. The main arguments are the important inter-individual variances between the observers, as well as the influence of the conditions of examination, in particular, lighting (6–10). There are also differences between the threshold for the perception of the yellow colour within populations, with sensitivity decreasing as the age of the practitioner increased (11). Some authors report the same problems in the context of the examination of children, for whom a lack of bibliographical data is also underlined (12).

A colorimetric approach for dating bruises has been described in the literature. This method is based on the characterization of a colour starting from the three primary colours (red, green and blue), using a colorimeter. Although this technique allows precise description of colours, the colour of the underlying skin is too much of a confounding factor to enable the estimation of the age of a bruise. The integration of other basic colours could nevertheless enable improvements for this approach (13,14).

Magnetic resonance imaging has been tested as a predictive tool for dating bruises in an animal model (15) and for artificially created subcutaneous hematomas in humans (16), with prominent initial results in this area of research.

The use of reflectance spectrometers for dating bruises has also been explored and has shown promising results. The peaks of absorption of the breakdown products of haemoglobin, and in particular bilirubin, could be used for that purpose. Indeed, these breakdown products first increase (degradation of haemoglobin) then decrease (elimination of bilirubin). However, additional data are necessary in order for conclusions usable for practice to be drawn. Moreover, the studies published with such monitoring used expensive equipment or equipment not available on the medical market (4,17,18). Bilirubinometers, or jaundice meters, are portable devices designed for tracking neonatal jaundice. They are easy-to-use and non-invasive devices for estimating bilirubin levels in new-borns. It can be used via simple application on the skin on the sternum and/or the face, and a luminous flash is delivered. The basic

technology used in these devices is reflectance spectrophotometry. This study aims to bring practical insight into the use of a bilirubinometer for dating bruises on living victims in the exercise of forensic medicine. It consists of a daily follow-up, with a bilirubinometer, of patients presenting bruises with a known date of occurrence.

## **Material and methods**

The study was carried out prospectively for 4 months in the orthopaedic and traumatologic surgery unit of the Lille University Hospital Center in the north of France. The data collected for this study were anonymized and notified to the CNIL (National Commission for Data Protection and Liberties) according to French regulations.

The patients included were provided verbal and written information on the rationale and methods of the study. They were given an information leaflet and their written agreements were collected. It was also specified to them that their assent was constantly revocable.

### **Inclusion and exclusion criteria**

All patients presenting a post-surgical bruise after total knee prosthesis implantation were included. Patients hospitalized after a traumatic event and presenting one or more bruises were also included as long as they could precisely describe the date and mechanism of the trauma at the origin of the bruise. The patients were also selected outside of any active judiciary procedure to avoid other bias.

For the duration of the study, the exclusion criteria were the presence of a disorder of coagulation or an abnormal bilirubinemia value ( $>1.3$  mg/dL) in the preoperative assessment.

### **Measurement technique**

Bilirubinometers measure the distribution of a complex radiation according to its frequency or wavelength. Reflectance corresponds to the report of the intensity of the wave reflected compared to the incidental wave. After the sending of a luminous flash by a probe, the bilirubinometer's sensor thus analyses the signal reflected by the different layers of the skin. Yellower or more icteric skin absorbs more zone wavelengths corresponding to the blue part of white light (450/500 nm). These wavelength values are in accordance with the peak absorbance of bilirubin, which is found towards 460 nm. Other factors affect the reflectance of skin, in particular the maturity of the dermis, and melanin and haemoglobin.

Based on these principles, the bilirubinometers are calibrated to obtain an estimated index of transcutaneous bilirubin, which is correlated in tables of equivalence in terms of bilirubinemia. To obtain this value index, the bilirubinometer probe (coupled with the sensor) is applied to the skin with minimum pressure to send a flash of white light. The light is transmitted via an optical fibre beam arranged in a circle on the periphery of the sensor. This light is partially absorbed and partially reflected by the cutaneous and subcutaneous layers. The part of the light recovered by the bilirubinometer sensor is re-transcribed with a quantified value (19–22).

The bilirubinometer used in this study was a Minolta JM-102. Two measurement sites were initially selected for each patient and monitored daily: one in the centre of the bruise, labelled 'bili-bruise', the other at 10 centimeters away from the bruise, in a cutaneous zone without any lesion, labelled 'bili-skin'. The measurements were repeated daily by the same examiner and at the same time of the day (between 7:00 and 8:00 A.M), from day 1 when a bruise was observed after bandage removal, until the patient was discharged. The bilirubinometer probe was applied to the skin so that the entire sensor was in contact with the skin, then 3 successive measurements were taken of the bili-bruise and bili-skin areas each. The average value of the 3 indexes collected at each measurement site was retained and rounded with a margin of 0.5. These steps were taken because of the scientific recommendations concerning the Minolta JM-102 bilirubinometer, which recommend 2 measurements per site and which specify that values close to 0.1 produce 'a false feeling of precision' (19).

As advised (19), the bilirubinometer was calibrated before the daily series of measurements among patients by obtaining a zero value via application on a white plate acting as the control.

### **Data collection**

Several data were collected for each patient, including the date and hour of the traumatic event and/or the surgery, age, sex, body mass index (BMI), brachial perimeter (mid upper arm circumference), skin phototype [1–6 according to the classification of Fitzpatrick (23)].

To adhere to the exclusion criteria, several elements in the patients' files were searched but not recorded in the database: medical history, treatment, bilirubinemia and coagulation factors.

When the bilirubinometer measurements were taken, the date, hour and sites of measurement, as well as the size and the colour of the bruise were recorded.

Each patient could not be followed identically and thus different numbers of measurements were collected for each subject. Indeed, a patient could be hospitalized a certain number of days after the traumatic event from which the bruise originated, and lost to further follow-up after discharge, where the date varied on a case-by-case basis.

### **Statistical analysis**

Data are presented as means ( $\pm$  standard deviation) or number (percentage). First-available bili-skin values between skin phototypes 2 and 3 were compared using the Mann-Whitney U test; no comparison with skin phototype 4 was done given the small number of subjects with skin phototype 4 ( $n = 3$ ). We assessed the evolution of bili-bruise values during the 7-day follow-up using a linear mixed model by including subjects as random effects to take into account the correlation between repeated measures within the same subject. Normality of model residuals was checked with normal probability plots. Post-hoc pairwise comparisons with day 1 values were performed using linear contrast after Bonferroni correction for multiple comparisons. We also used a linear mixed model to compare the bili-bruise and bili-skin values at

each time point by including in the model the site of measurement, time and measurement  $\times$  time interaction. The evolution curve of intra-subject differences between the bili-bruise and bili-skin values was fitted using penalized B-spline regression. Finally, we assessed the impact of the subjects' characteristics (i.e. age, sex and BMI) on the intra-subject differences between bili-bruise and bili-skin values using a linear mixed model that included the subjects' characteristics, time and subjects' characteristics  $\times$  time interaction. Statistical testing was done at a two-tailed  $\alpha$  level of 0.05. Data were analysed using the SAS software package, release 9.4 (SAS Institute, Cary, NC).

## Results

We included 20 consecutive patients (mean age,  $58.5 \pm 14.2$  years; 5 men; mean BMI,  $31.1 \pm 6.6$  kg/m<sup>2</sup>) with post-surgical cutaneous bruise in the present study, with daily bilirubin measurements of the cutaneous layer with and without bruise within 7 days following surgery. A total of 88 bilirubin measurements were made (mean number of measurements per patient, 4.4, range, 2–7).

The traumatic event from which the bruise originated was total knee prosthesis implantation in 13 cases (65%), venipuncture in 4 cases (20%) and blunt trauma in 3 cases (15%).

### Bili-skin values

The bili-skin values ranged between 8 and 14 (mean, 10.5). No change in bili-skin values was observed during the 7-day follow-up in any subject. We found a significant difference in bili-skin values between skin phototypes based on the first-available bili-skin values (Figure 1). As shown in Figure 1, the bili-skin values of skin phototype 2 were lower compared to skin phototypes 3 and 4, with a difference reaching significance for comparison between skin phototypes 2 and 3 ( $p = 0.024$ ).

### Bili-bruise values

The bili-bruise values ranged between 7.5 and 23 (mean, 13.6). The individual patient's changes are reported in Figure 2. In the linear mixed model, we found a significant time effect for the bili-bruise values ( $p < 0.0001$ ). In post-hoc comparison with day 1 values, day 3 to day 7 values were significantly greater than day 1 values ( $p < 0.05$  adjusted for multiple comparisons), with a greater difference observed with the day 5 values (mean difference, 4.5; 95% confidence interval [95%CI], 3.1–5.9).

### The bili-bruise and bili-skin value differential ( $\Delta$ -bili)

When the bili-bruise values and bili-skin values were compared, there was no significant difference at day 1 but a significant difference in favour of greater bili-bruise values at day 2 to day 7 ( $p < 0.05$  adjusted for multiple comparisons). The highest estimated difference between the bili-bruise and bili skin values was found at day 5 (mean difference, 5.3; 95%CI, 4.9–6.3), confirming the previously observed peak bili-bruise values. The estimated difference was 4.7 (95%CI, 3.8–5.6) at day 4 and 3.9 (95%CI, 2.6–5.2) at day 6.

We therefore calculated the intra-patient difference between the bili-bruise and bili-skin values (labelled  $\Delta$ -bili) to compare the samples (Table 1 and Figure 3).

### **Influence of patient demographics on $\Delta$ -bili**

In the linear mixed model, we found no difference in the changes of  $\Delta$ -bili with age (interaction with time,  $p = 0.96$ ), sex (interaction with time,  $p = 0.48$ ) and BMI (interaction with time,  $p = 0.99$ ). In addition, when time interaction was removed from the linear mixed model, age, sex and BMI were not associated with  $\Delta$ -bili levels ( $p > 0.15$ ).

## **Discussion**

In this study, a significant difference was found between the values measured by the bilirubinometer based on whether the measurement was taken of a bruise or a healthy cutaneous zone. The chronological evolution of the bili-bruise values followed an increase then decrease phase (Figure 2). The peak separating the phases ranged between 4 and 5 days for 18 cases (90%) and was at 3 days for 2 cases (10%). The findings were the same for the  $\Delta$ -bili values. The results thus indicate peak bili-bruise or  $\Delta$ -bili values generally at day 4 or 5, but not before day 3 after the traumatic event from which the bruise originates.

These observations are in accordance with the results of a study of bruises by reflectance spectrometry by Kim et al (24), where a maximum subcutaneous concentration of bilirubin was found 80 hours after the traumatic event. Only one bruise (bruise n° 2) presented an evolution with 2 peaks: one at day 3 and one at day 5. This particular chronology could be due to new deep bleeding within the bruise (causing a new local accumulation of bilirubin). Indeed, it concerned a post-surgical bruise of total knee replacement, and drain ablation could explain this phenomenon. The  $\Delta$ -bili and bili-bruise values of bruise n° 5 plateaued between days 4 and 5, and then the patient was lost to follow-up because he had left the hospital. This plateau enabled the estimation that the precise location of the peak is between these 2 days. A measurement at day 6 would have probably enabled better interpretation of the case. The values for bruises n° 2 and n° 5 do not, however, contradict the observation of peak bili-bruise/ $\Delta$ -bili values not before day 3 after the formation of a bruise.

A bruise very often originates from a trauma, but can sometimes occur because of a disorder of coagulation or an unknown cause. The bruise results in a coloured spot, more or less extended and that does not disappear upon the skin being pressed (1,2,25). For a bruise to appear, three criteria must be met (13):

- The skin must undergo sufficient trauma so that cutaneous or subcutaneous vessels break, but without causing cutaneous effraction. This is generally blunt trauma.
- The blood pressure must be sufficient so that the blood cells leave the vessels towards the tissues.
- Blood coming from the injured vessels must be sufficiently close to the cutaneous surface to be visible. This factor is influenced in particular by the anatomical properties of the cutaneous localization concerned.

In the evaluation of dating bruises, the following factors are described in the literature as possibly confounding: age, sex, BMI, skin phototype and type of trauma (4). In our study, these factors were collected and the evolution of the  $\Delta$ -bili values during the clinical follow-up did not reveal statistically significant differences.



A bilirubinometer was used in this study because it is portable, easy to use, available on the medical devices market, non-invasive and fairly affordable. We chose to carry out 3 measurements per site to be able to identify the 'aberrant' values more easily. Indeed, abnormally low values can be obtained in the event of poor application of the probe on the skin. Abnormally high values can also be observed in the presence of naevi, freckles or hairs in front of the probe. A bilirubinometer would also be easily usable in daily forensic medicine practice to provide assistance for dating bruises. For example, in the case of a victim of alleged physical abuse, the practitioner could take a first measurement using a bilirubinometer on an ecchymotic cutaneous zone and on a healthy zone. By examining the victim again the following day and by taking the same measurements, it would be possible to determine if the bruise corresponds to the increase phase or decrease phase of the bilirubinometer's values. In the event of obtaining equal bilirubinometer values on two consecutive days, a third day of observation would be necessary to determine if the values are increasing or decreasing. Based on our results, when a bruise has decreasing bilirubinometer values (bili-bruise) from one day to another, it can be concluded that the trauma goes back to at least 3 days before the first measurement. This assumption, which is subject to the limits of our study and confounding factors to be defined, should be the objective of additional studies for possible validation.

The present study population is not representative of the French population in terms of age (older) and sex ratio (more women) (26). This is mainly due to recruitment bias, as only patients admitted to a hospital service were included. Moreover, total knee replacement implies a significant proportion of relatively older subjects. However, all measurements are adequate with peak values not occurring before 3 days, which encourages the study of broader populations over a longer duration. Only people with skin phototype 2, 3 or 4 were involved in our study. This is partly due to the weather in the north of France, which is not very sunny. Our results thus cannot be extrapolated to people with skin phototype 1, 5 and 6. For skin phototype 6 (black skin), a test measurement was performed on a patient not presenting a bruise. The values were around 24 with the bilirubinometer, which is higher than the peaks observed for the bruises in our study. The extension of this study to other skin phototypes will thus be crucial in view of practical use. Some authors have described experimental protocols of follow-up of healthy volunteers with bruises induced by cutaneous pinching or a suction pump (6,18). This type of follow-up would make it possible to carry out better data collection. We had not considered such a protocol initially because of the difficulty in gaining ethics committee approval, as well as finding healthy volunteers. Nevertheless, our encouraging results would allow us to argue for the introduction of such a protocol in the face of competent authorities as regards to biomedical research.

## **Conclusion**

The use of a bilirubinometer for dating bruises in forensic medicine is an innovative approach, which to date has not been described in the literature to our knowledge. We observed increasing then decreasing bilirubinometer values of bruises followed daily, with a peak not before 3 days, and generally observed around 4–5 days. Our results are encouraging and could be used as a basis for subsequent studies in a broader

population. The confirmation of such data would make it possible to improve the estimation of the age of bruises in forensic medicine.

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## Tables

Table 1. Mean  $\pm$  SEM of delta-bili values at each time point

### **Figures**

Figure 1. Distribution of the first-available bili-skin values according to the subject's skin phototype. Mean values are indicated.

Figure 2. Evolution of bili-bruise values in patients

Figure 3. Average evolution of delta-bili values from day 1 to day 7. The smoothing curves are shown using penalized B-spline regressions; 95% prediction limits are reported.

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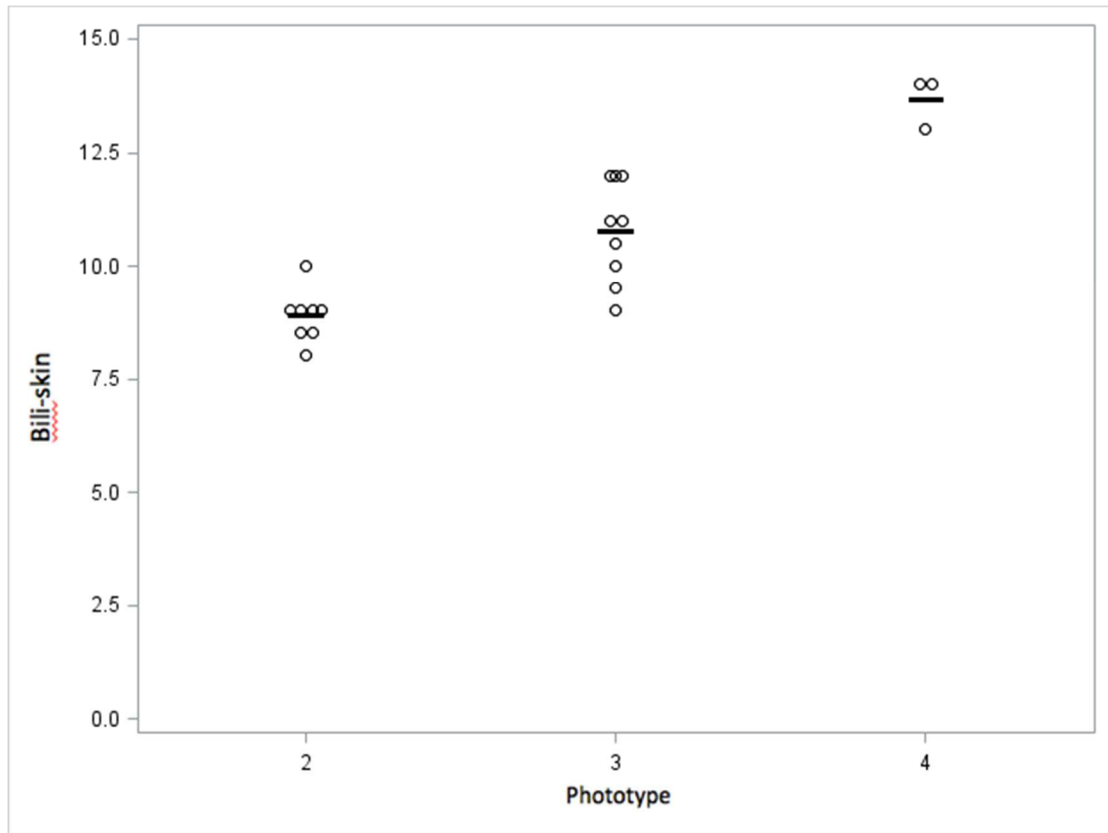
The authors would like to thank the members of the surgical and orthopaedic and traumatology department of Lille Regional Teaching University Hospital who participated in the project.

### **Compliance with ethical standards**

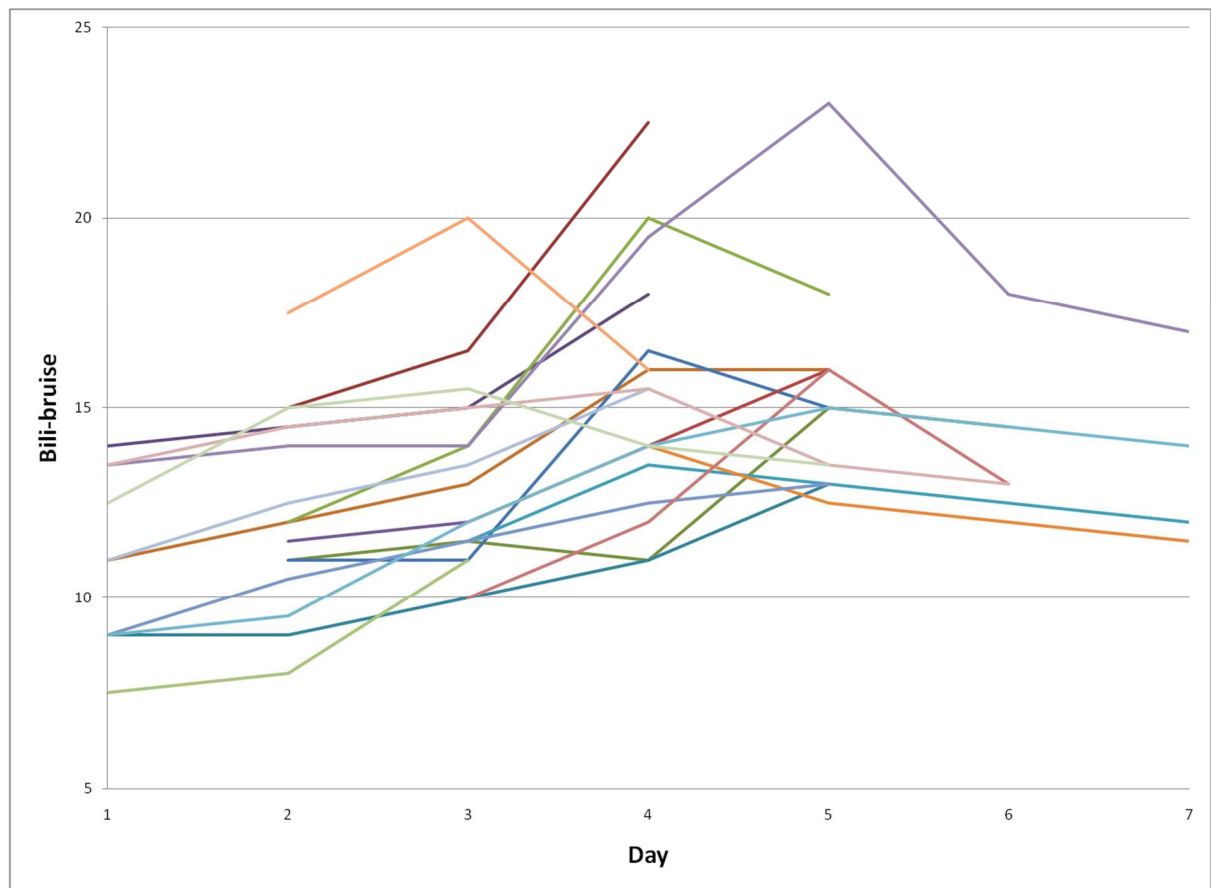
### **Conflict of interest**

The authors declare that they have no conflicts of interest.

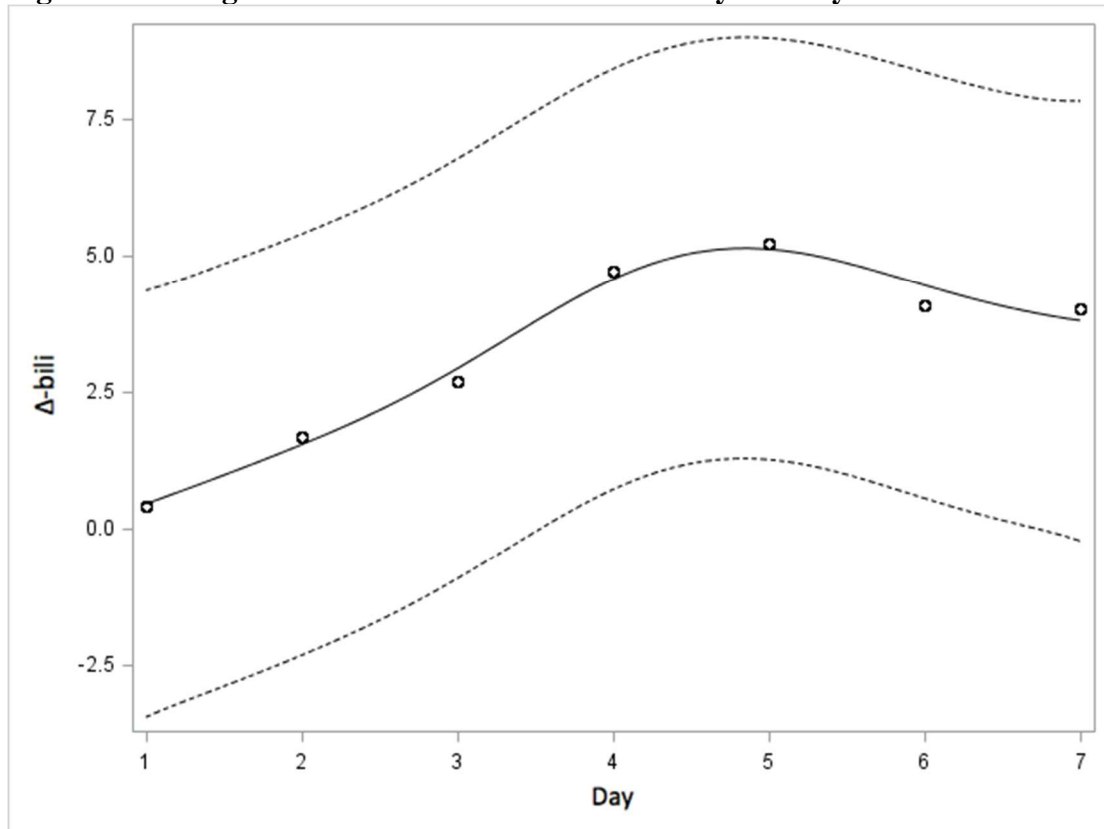
**Figure 1. Distribution of the first-available bili-skin values according to the subject's skin phototype**



Mean values are indicated.



**Figure 3. Average evolution of  $\Delta$ -bili values from day 1 to day 7**



The smoothing curves are shown using penalized B-spline regressions; 95% prediction limits are reported.

**Table 1. Mean±SEM of  $\Delta$ -bili values at each time point**

<b>Times</b>	<b><i>N</i></b>	<b><i>Mean (95% CI)</i></b>
Day 1	10	0.4 (-0.6 to 1.4)
Day 2	16	1.6 (0.8 to 2.5)
Day 3	19	2.7 (2.0 to 3.4)
Day 4	18	4.7 (3.5 to 5.9)
Day 5	14	5.2 (3.7 to 6.8)
Day 6	7	4.1 (2.3 to 5.8)
Day 7	4	4.0 (1.8 to 6.3)