

## Accuracy of transcutaneous bilirubin measurement in full-term newborns at 3400 meters above sea level

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### Abstract

**Background:** Neonatal jaundice is a frequent benign condition in newborns. However, a rapid diagnosis must be established for its most appropriate treatment. The objective of this study was to measure the correlation between total serum bilirubin (TSB) and transcutaneous bilirubin (in forehead and sternum) in full-term newborns at 3400 m above sea level. **Methods:** We conducted a prospective and cross-sectional study in full-term newborns with clinical jaundice from the Hospital Regional in Cusco-Peru. General characteristics and measurement of TSB, transcutaneous forehead bilirubin (TcBF), and transcutaneous bilirubin in the sternum (TcBS) were explored. Correlation, sensitivity, and specificity were calculated. Receiver operating characteristic (ROC) curves were constructed using the SPSS statistical package, version 22.0. **Results:** A total of 123 newborns were evaluated. The mean bilirubin values were  $13.7 \pm 3.5$  for TcBF,  $14.1 \pm 3.1$  for TcBS, and  $13.8 \pm 3.9$  for TSB. In addition, Pearson correlation coefficients between TSB/TcBF and TSB/TcBS were 0.90 and 0.91, respectively ( $p < 0.001$ ). For the percentile 95 cut-off point, a sensitivity of 93% and 100% and a specificity of 89% and 80% were obtained for TcBF and TcBS, respectively, with an area under the curve of 0.813 for TcBF and 0.815 for TcBS ( $p < 0.001$ ). **Conclusions:** Measurement of transcutaneous bilirubin is a fast and painless method that can be considered a reliable tool for screening and monitoring neonatal jaundice, but not for a definitive diagnosis to decide the use of phototherapy in full-term newborns at 3400 m above sea level.

**Key words:** Neonatal jaundice. Less developed countries. Newborn.

### Precisión de la bilirrubinometría transcutánea en recién nacidos a término a 3400 metros sobre el nivel del mar

#### Resumen

**Introducción:** La ictericia neonatal es una condición benigna y frecuente en los recién nacidos, en quienes es preciso hacer un diagnóstico rápido para el tratamiento más adecuado. El objetivo de este estudio fue determinar la correlación entre la bilirrubina sérica total (BST) y la bilirrubina transcutánea (frente y esternón) en recién nacidos a término a 3400 metros sobre el nivel del mar. **Método:** Estudio prospectivo y transversal en recién nacidos con ictericia clínica en el Hospital Regional de la Ciudad de Cusco, Perú. Se exploraron las características generales y se midieron la BST, la bilirrubina transcutánea en la frente (BTcF) y la bilirrubina transcutánea en el esternón (BTcE). Se calcularon la correlación, la sensi-

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bilidad y la especificidad, y se elaboraron las curvas de características operativas del receptor (ROC) con el paquete estadístico SPSS 22.0. **Resultados:** Se evaluaron 123 recién nacidos. El promedio de la BST fue de  $13.8 \pm 3.9$ , el de la BTcF fue de  $13.7 \pm 3.5$  y el de la BTcE fue de  $14.1 \pm 3.1$ . La correlación entre BST/BTcF y BST/BTcE fue de 0.90 y 0.91, respectivamente ( $p < 0.001$ ). Para el punto de corte del percentil 95 según el nomograma Bhutani se obtuvo una sensibilidad del 93% y el 100%, y una especificidad del 89% y el 80%, para la BTcF y la BTcE, respectivamente, con un área bajo la curva ROC de 0.813 para la BTcF y de 0.815 para la BTcE ( $p < 0.001$ ). **Conclusiones:** La medición de la bilirubina transcutánea es un método rápido e indoloro, y podría ser considerado confiable para el despistaje y el seguimiento de la ictericia neonatal, mas no para un diagnóstico definitivo con el fin de decidir el uso de fototerapia en recién nacidos a término a 3400 metros sobre el nivel del mar.

**Palabras clave:** Ictericia neonatal. Países en desarrollo. Recién nacido.

## Introduction

Neonatal hyperbilirubinemia or jaundice (yellow color of the mucous membranes) is defined as total serum bilirubin (TSB) concentration  $> 3\text{-}5$  mg/dl<sup>1</sup>. This pathology is a benign and frequent condition in newborns<sup>2,3</sup> that manifests itself due to the liver's enzymatic immaturity<sup>4</sup> and is more frequently observed in premature newborns<sup>5</sup>. The importance of early diagnosis rests on avoiding complications such as acute encephalopathy and kernicterus, the latter being a chronic complication associated with cerebral palsy, deafness, dental dysplasia, ophthalmoplegia, and personality disorders<sup>6</sup>.

Neonatal jaundice is a significant morbidity and mortality issue<sup>7</sup>, accounting for 1309.3 deaths per 100,000 live births worldwide in 2016, the seventh leading cause of neonatal death<sup>8</sup>. In 2012, the prevalence of neonatal jaundice was 29%, representing the leading cause of medical consultation and neonates' hospitalization in Peru<sup>9</sup>.

The measurement of TSB is traditionally the most widely used diagnostic method worldwide, but it involves an invasive, costly, and time-consuming procedure. Another option is to measure transcutaneous bilirubin (TcB), a painless, cheap, fast, and non-invasive method that minimizes blood collection in newborns<sup>10,11</sup>. The American Academy of Pediatrics and the Canadian Academy of Pediatrics recommend TcB measurements on all newborns before discharge<sup>12,13</sup>. Moreover, it is used as a diagnostic method in some countries<sup>14</sup>. Due to these recommendations, studies have been conducted in different population groups to confirm the correlation of values obtained by transcutaneous and serum bilirubinometry and determine if the transcutaneous diagnostic method shows adequate sensitivity and specificity for diagnosing jaundice in newborns<sup>15</sup>.

The city of Cusco is located in the highlands of Peru at an altitude  $> 3400$  m above sea level (masl). In this

geographical area, it is necessary to verify if the transcutaneous method is as effective as the serum method since the physiological adaptation in healthy populations at high altitudes causes sustained hypoxia, increased cardiac output, and hemoglobin increase<sup>16,17</sup> of 1.05 g/dl for every 1000 m of altitude<sup>18</sup>. This consideration is essential since bilirubin is the final product of the catabolism of hemoglobin<sup>1</sup>.

## Methods

A prospective and cross-sectional study was conducted between January and March 2017 in the Neonatology Service of the Hospital Regional of the city of Cusco, located in the mountains of Peru, at an altitude of more than 3400 masl.

The study included full-term newborns with clinical jaundice, Apgar score  $> 7$  (minute 1), and a normal physical exam. Newborns with the following characteristics were excluded: those born at home, needing resuscitation maneuvers, with congenital malformations, who received previous therapy, and those born to mothers with an obstetric pathology such as preeclampsia, diabetes mellitus, placental insufficiency, toxoplasmosis, rubella, cytomegalovirus, herpes simplex, or HIV.

Using convenience sampling, we collected data from newborns with clinical jaundice who met the selection criteria (the calculated sample size using the formula for diagnostic tests was 123 newborns) through a revised card evaluated by experts in the field. General data, such as gestational age by the Capurro method, life span in hours, sex, weight, height, birth route, Kramer's scale, and measurement of total and transcutaneous serum bilirubin, were included in the study.

The Ethics Committee of the Hospital Regional of Cusco approved the research project. Data were collected under the authorization of the hospital director and the head of the Neonatology service, ensuring participants' anonymity and confidentiality, and with the

voluntary informed consent of the mothers of each newborn.

As soon as the selection criteria were met, venous blood samples were collected to measure TSB; after 30 min, transcutaneous bilirubin (forehead and sternum) was determined. We used the Konica Minolta/JM-103 transcutaneous bilirubinometer (Drager Medical Inc, Telford, Pennsylvania), which determines the intensity of yellow in the subcutaneous cellular tissue. The transcutaneous bilirubinometer use was standardized according to the manufacturer's guidelines by placing it vertically to the skin and disinfecting it with alcohol (70%) before and after its use on each newborn.

The frequencies and percentages of the categorical variables were included in the descriptive analysis, as well as the best measure of central tendency and dispersion of the quantitative variables. Pearson's correlation was used to compare the measurement of transcutaneous bilirubin (in the forehead and sternum) with TSB values after the Kolmogorov–Smirnov normality test. A linear scatter plot was also calculated to determine the correlation curve trend. Bland-Altman graphs were used to determine if the difference between the methods was stable in all measured bilirubin ranges. TSB  $\geq$  percentile 95 (high-risk hyperbilirubinemia) was used as the gold standard. According to the Buthani nomogram<sup>19</sup>, two cut-off points were used to determine transcutaneous bilirubin (in the forehead and sternum): percentile 95 and percentile 75 for postnatal age in hours, to compare the results in case the cut-off point was lowered. Sensitivity and specificity were calculated, and the receiver operating characteristic (ROC) curves were constructed to evaluate the predictive capacity of TcB (in the forehead and sternum). The results were considered statistically significant with  $p < 0.05$ . The SPSS software version 22.0 was used.

## Results

A total of 195 patients were evaluated, of which 123 full-term newborns with clinical jaundice met the selection criteria. Seventy newborns (56.9%) were female, with a mean weight of  $3251.79 \pm 545$  g, mean length of  $49.29 \pm 2.5$  cm, mean age of  $53 \pm 16.6$  h of life, and mean gestational age according to Capurro method of  $38.8 \pm 1.4$  weeks. Of the total number of newborns, 115 (93%) showed an adequate birth weight. The most common delivery route was cesarean section in 72 (57.7%) births. Fifty-two (42.2%) were grade IV on the Kramer scale. The mean transcutaneous bilirubin in the forehead (TcBF) was  $13.7 \pm 3.5$ , the mean transcutaneous

**Table 1.** General characteristics of newborns

Characteristic	Mean $\pm$ SD (n = 123)
Gestational age (Capurro method)	38.8 $\pm$ 1.4
Weight (g)	3251.7 $\pm$ 545
Adequate birth weight	115 (93%)
Low birth weight (LBW)	8 (7%)
Length (cm)	49.29 $\pm$ 2.5
Hours of life	53.9 $\pm$ 16.6
Sex	
Male	53 (43.1%)
Female	70 (56.9%)
Birth route	
Cesarean section	72 (57.7%)
Vaginal	51 (42.4%)
Kramer's scale	
II	13 (9.7%)
III	42 (34.9%)
IV	52 (42.2%)
V	16 (13.1%)
TcBF	13.7 $\pm$ 3.5
TcBS	14.1 $\pm$ 3.1
TSB	13.8 $\pm$ 3.9

SD: standard deviation; TcBF: transcutaneous bilirubin in the forehead; TcBS: transcutaneous bilirubin in the sternum; TSB: total serum bilirubin.

bilirubin in the sternum (TcBS) was  $14.1 \pm 3.1$ , and the mean TSB was  $13.8 \pm 3.9$  (Table 1).

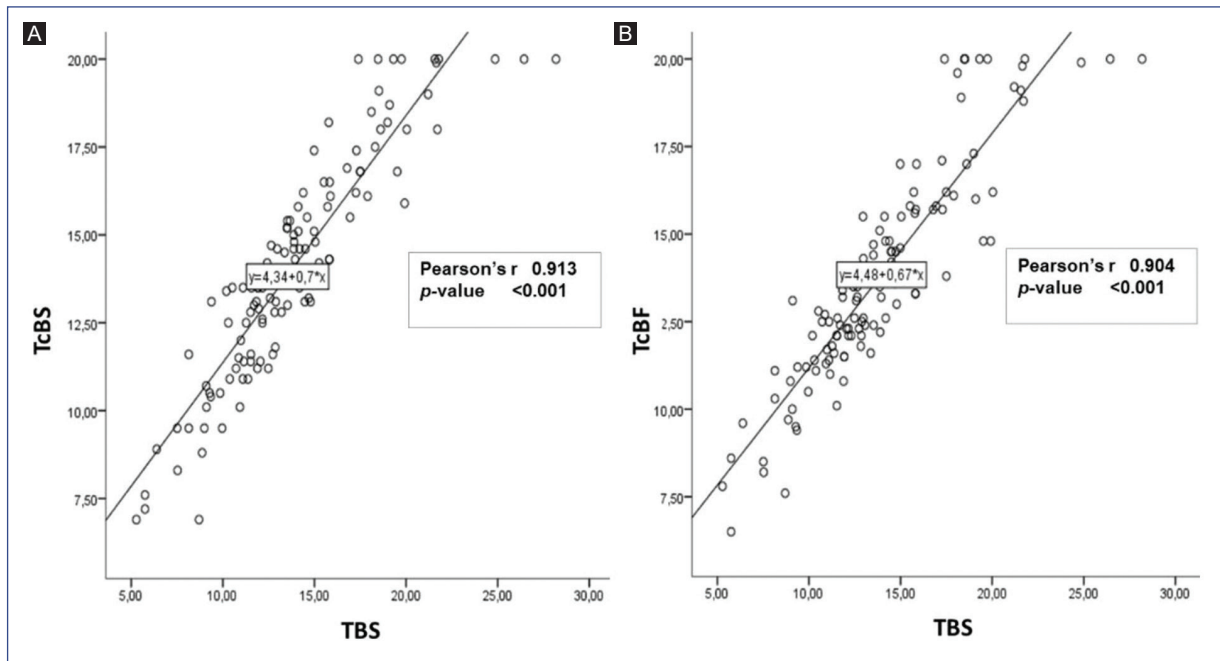
Pearson's correlation between TSB/TcBF and TSB/TcBS was 0.90 and 0.91, respectively ( $p < 0.05$ ), as shown in the linear scatter plots (Fig. 1).

Table 2 presents the participants' positive and negative results for TSB according to the cut-off points for transcutaneous bilirubin. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio for TcB (in the forehead and sternum) at  $\geq$  percentile 95 and  $\geq$  percentile 75 cut-off points, according to the Bhutani nomogram, are represented.

Figure 2 shows the sensitivity and specificity ROC curves, where the area under the curve for TcBS was 0.815 and for TcBF was 0.813 ( $p < 0.001$ ). Figure 3 shows the Bland-Altman graph for the difference obtained between TSB and TcB (in the forehead and sternum) measurement methods.

## Discussion

Our study showed a high correlation between the measurement of TcB (in the forehead and sternum) and



**Figure 1.** Linear regression plots for total serum bilirubin (TSB) versus transcutaneous bilirubin (TcB) at different measurement sites. **A.** Sternum (TcBS). **B.** Forehead (TcBF).

**Table 2.** Predictive characteristics of the transcutaneous bilirubin indices in the forehead and sternum for the percentile 95 and 75 cut-off points according to total serum bilirubin

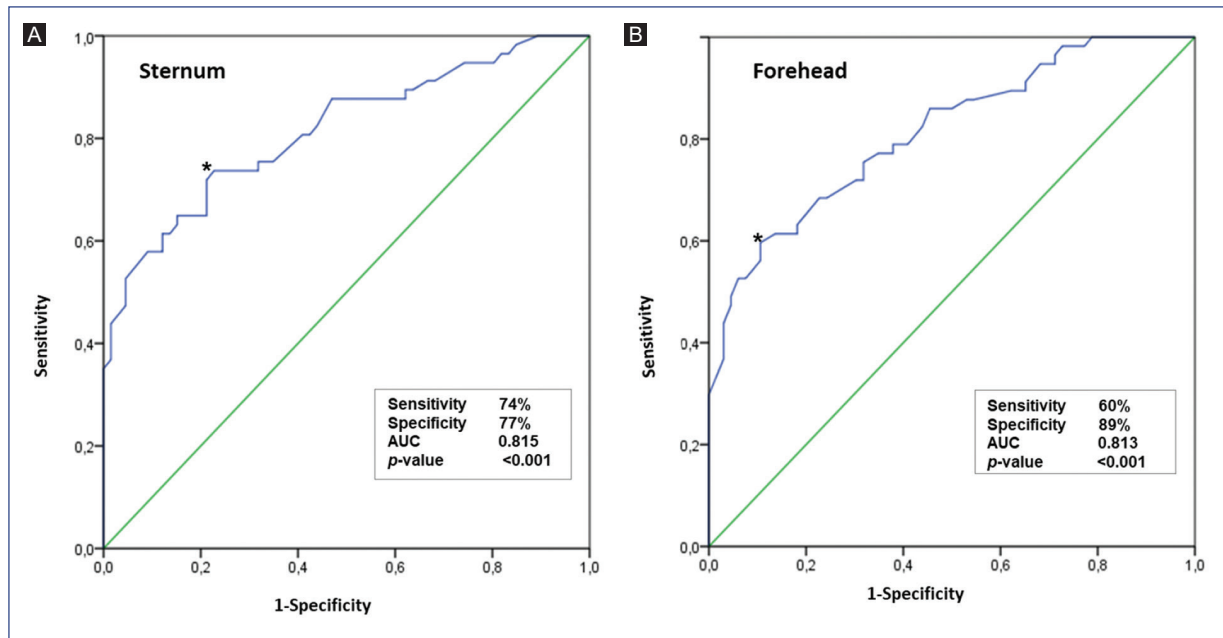
Predictive TcB	Total	TSB ≥ p95		Sen	Spe	PPV	NPV	LR+	LR-
	(n = 123)	(+)	(-)						
≥ p95 TcBS	70	57	13	100%	80%	81%	100%	5.08	0
< p95 TcBS	53	0	53						
≥ p95 TcBF	60	53	7	93%	89%	88%	94%	8.77	0.08
< p95 TcBF	63	4	59						
≥ p75 TcBS	106	57	49	100%	26%	54%	100%	1.35	0
< p75 TcBS	71	0	17						
≥ p75 TcBF	101	57	44	100%	33%	56%	100%	1.50	0
< p75 TcBF	22	0	22						

Percentile cut-off points, according to the Bhutani nomogram. Total serum bilirubin determination is the diagnostic test of choice.

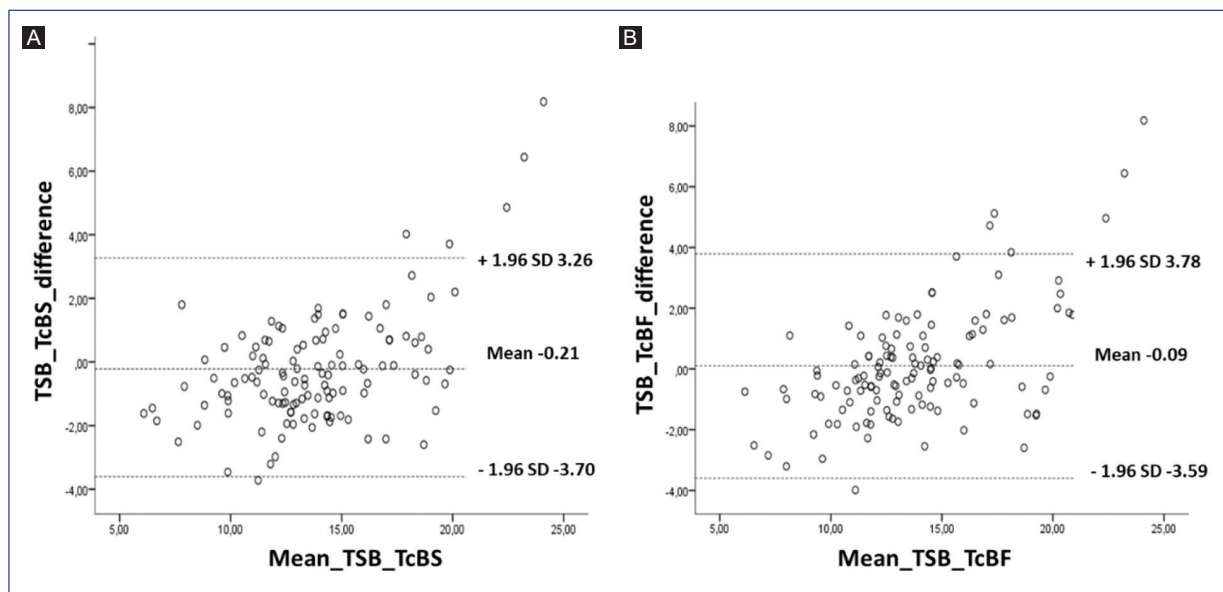
F: forehead; LR: likelihood ratio; NPV: negative predictive value; p: percentile; PPV: positive predictive value; S: sternum; Sen: sensitivity; Spe: specificity; TcB: transcutaneous bilirubin; TSB: total serum bilirubin.

serum bilirubin in newborns with clinical jaundice at more than 3400 masl. The correlations obtained were 0.91 and 0.90 for TcBS and TcBF, respectively, equal to or higher than other studies in populations at sea-level. In Mongolia, a Pearson's correlation coefficient of 0.78 was obtained<sup>20</sup>; in Bangladesh, 0.93<sup>21</sup>; in Iran, 0.82<sup>3</sup>; in the southern United States, 0.69<sup>10</sup>; and in Israel, 0.72<sup>22</sup>. The correlation was also found in studies that included low birth weight newborns, neonates

needing mechanical ventilation<sup>23</sup>, and newborns with different skin color<sup>11</sup>. Moreover, a meta-analysis showed that TcB is a reliable method in full-term and preterm infants<sup>24</sup>. Our study covered a population living at high altitude. Physiologically, higher concentrations of hemoglobin<sup>16,17</sup> and, consequently, a greater amount of bilirubin are found in this population, which could explain the more significant correlation. No differences in mean serum or transcutaneous bilirubin levels were observed.



**Figure 2.** Receiver operating characteristic (ROC) curves of sensitivity and specificity for testing transcutaneous bilirubin in the sternum **A:** and forehead **B:** for the cut-off point of total serum bilirubin  $\geq$  percentile 95 according to the Bhutani nomogram. AUC: area under the curve.



**Figure 3.** Bland-Altman graphs show the difference of the mean values between **A:** total serum bilirubin (TSB) versus transcutaneous sternum bilirubin (TcBS) and **B:** TSB versus transcutaneous bilirubin in the forehead (TcBF).

These results could be used for reliable clinical follow-up—as suggested by the Canadian Pediatric Society in its guidelines for detecting, managing, and preventing hyperbilirubinemia, in which correlation

coefficients in the range of 0.77-0.93 are recommended for reliable clinical use (with some exceptions)<sup>25</sup>.

According to the Bhutani nomogram, our results showed an area under the curve of 0.815 for TcBS,

slightly higher than that obtained for TcBF, which was 0.813 for the cut-off point  $\geq$  percentile 95. Therefore, both methods are useful to measure transcutaneous bilirubin indicating a high predictive value. Similarly, for the cut-off point  $\geq$  percentile 95 (high-risk zone), the sensitivity was 100% for TcBS and 93% for TcBF, with a specificity of 80% for TcBS and 89% for TcBF. Compared to other studies, the sensitivity found in this study was higher. Nahar et al.<sup>21</sup> obtained a sensitivity of 77% and a specificity of 88%. The study conducted by Hemmanti and Kiyani<sup>26</sup> showed a sensitivity and specificity of 96.6% and 99%, respectively, and Akahira-Azuma et al.<sup>20</sup> found a sensitivity of 85% and a specificity of 84%.

In contrast, these studies used a 15 mg/dl of TSB cut-off point to measure sensitivity and specificity. This measure can be biased since bilirubin levels vary with the newborn's age. However, the Bhutani nomogram covers this parameter's measurement at all ages by percentiles. The sensitivity found for TcB is better in our study and exceeds sensitivity values found in the Kramer scale<sup>27</sup>, being more effective for screening a newborn with jaundice.

Our study demonstrated higher sensitivity than specificity in the TcB for a  $\geq$  percentile 95 (high-risk zone) cut-off point. However, by decreasing the cut-off point to  $\geq$  percentile 75 (high intermediate risk zone), we observed an increase in sensitivity and a decrease in specificity. These results indicate that both cut-off points could be recommended for the measurement of TcB as screening and, above all, for post-treatment follow-up of neonatal jaundice, but not for a definitive diagnosis to determine the use of phototherapy or exchange transfusion, as recommended by other studies<sup>11</sup>.

This study presented some limitations: newborns previously exposed to phototherapy were not considered; thus, it was not possible to verify whether TcB measurements were adequate for post-phototherapy follow-up. Furthermore, considering that the measuring instruments varied in each study, it was demonstrated that bilirubinometers of different brands, including the bilirubinometer used in our study, are reliable in estimating bilirubin levels<sup>28,29</sup>.

Peru is a developing country where multiple health problems and low resources affect 21.7% of the population<sup>30</sup>. In these areas, our findings might improve the clinical confidence of TcB measurement since it has been proven to correlate well with serum bilirubin, which is the diagnostic test of choice. Therefore, hospitalization time and costs could be reduced, and blood sampling from newborns with jaundice could be

minimized. Furthermore, since this procedure can be performed with portable equipment, it would be useful in rural areas of Peru, where there is still a high prevalence of home births<sup>31</sup>.

Our study covered a high-altitude population, where a strong correlation between transcutaneous and serum bilirubin was confirmed. Therefore, measuring TcB instead of TSB could decrease hospital stay, costs, and blood sampling and could be used as a screening and follow-up tool for newborns with jaundice.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that they have followed their work center's protocols on patient data publication.

**Right to privacy and informed consent.** The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author has this document.

## Conflicts of interest

The authors declare no conflicts of interest.

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