Correspondence of hemoglobin values obtained by a noninvasive, cutaneous-contact method with values obtained by conventional methods from whole blood samples in a Guatemalan field setting

Caitlin Crowley, Gabriela Montenegro-Bethancourt, Claudia Arriaga, Noel W. Solomons, and Klaus Schümann

Abstract

Background. Anemia is a widespread public health issue, conventionally diagnosed by analyzing the hemoglobin concentration in whole blood samples. Aspects of safety, comfort, and cultural acceptability would be obviated if reliable, noninvasive anemia screening were available.

Objective. To determine day-to-day variations within subjects in hemoglobin measurements and the correspondence of hemoglobin values obtained by a noninvasive, photometric, cutaneous-contact method with values obtained by conventional methods from blood samples.

Methods. The hemoglobin level was determined in 40 pregnant women from the Guatemalan coastal plain (low values) and 40 men from the highlands (high values). Hemoglobin concentrations (g/dl) were measured in an automated cell counter and, in parallel, estimated with the use of the Rad-87™ Rainbow pulse CO-Oximeter placed over the nail bed of the ring finger.

Results. The mean value for invasively determined hemoglobin was 13.5 g/dl, as compared with 12.2 and 12.1 g/dl for the noninvasive nail-bed estimate at 10 and 5 minutes, respectively. Measurements using the noninvasive technology were highly stable within days and from day to day. The noninvasive screening method showed satisfactory sensitivity and specificity at hemoglobin concentrations of < 12.0 g/dl (cutoff value for nonpregnant women) and < 13.0 g/dl (cutoff value for adult men). Diagnostic discrimination was poorer for the lower cutoff criteria; the anemia cutoff values were < 11.5 g/dl for school-age children and < 11.0 g/dl for pregnant women and children under 5 years of age.

Caitlin Crowley, Gabriela Montenegro-Bethancourt, Claudia Arriaga, and Noel W. Solomons are affiliated with the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), Guatemala City, Guatemala; Klaus Schümann is affiliated with the Research Center for Nutrition and Food Sciences, Technische Universität München, Freising, Germany.

Please direct queries to the corresponding author: Noel W. Solomons, CeSSIAM in Guatemala, P.O. Box 02-5339, Section 3163/Guatemala, Miami, FL 33102-5339, USA; e-mail: cessiam@guate.net.gt.

Conclusions. Noninvasive hemoglobin screening shows considerable promise, although improvement of sensitivity and specificity in the anemic range and determination periods of less than 10 minutes are desirable.

Key words: Anemia, calibration, diagnostic screening, Guatemala, hemoglobin, sensitivity, specificity

Introduction

Public health and clinical medicine have diverse needs for diagnostic assessment. For both, it is essential that diagnostic tests be reliable, innocuous, and as safe as possible, but in the public health setting they must also be culturally acceptable, technically manageable, and cost-effective. In the latter respects, requiring blood samples has recognized limitations. Blood extraction from capillaries or veins is painful and often raises objections from mothers and children alike. Moreover, in less acculturated populations, suspicions about the true motivation of doctors or investigators and concerns about negative effects on male sexual performance are often barriers to acceptance of the procedure [1]. Since the identification of blood-borne, person-to-person transmission of hepatitis [2, 3] and, more recently, of the human immunodeficiency virus in the 1980s [4, 5], safety concerns for patients, phlebotomists, and laboratory technicians in the chain of blood handling have intensified [6, 7]. The analytical procedures for diagnostic tests require highly skilled personnel and sophisticated apparatus; both factors adversely affect the accessibility and affordability of tests in field studies in the developing world. Therefore, alternatives to blood extraction, even for assessing the fundamental characteristics of this body fluid, are seen as welcome innovations.

Blood-free assessment of red blood cell status was explored by Gross et al. as early as 1996 [8]. These studies were triggered by a perceived need to target iron supplementation only to anemic individuals,

rather than to distribute iron uniformly to an entire target group. They reported proof-of-principle results for the promise of an Erlanger photometer (EMPHO), based on deep-penetrating white light [8]. Nadeau and Groner [9] carried the promise further with *in vivo* microscopic imaging of superficial, mucosal microcirculation using polarized light. This technique provided an estimation of hemoglobin that corresponded well with the values analyzed in blood samples. Rabe et al. [10], in the setting of healthy baby nurseries and neonatal intensive care populations in England, found similarly solid reliability of a transcutaneous technique using white light to estimate hemoglobin concentrations.

The aforementioned devices were used in institutional settings. More noninvasive optical, electrical, and acoustic methods of hemoglobin determination in the clinical setting were recently reviewed by McMurdy et al. [11]; yet outreach to field studies and surveys is the aspiration for a bloodless anemia screening under a public health perspective. Therefore, this study addresses how reliable, robust, and field-friendly the noninvasive, blood-free hemoglobin assessment technology is in the community scenario. World Health Organization (WHO) standard cutoff values for anemia classification at sea level range from 11.0 g/dl for pregnant women and children under 5 years of age, to 11.5 g/dl for children ages 5 to 12 years, to 12.0 g/dl for nonpregnant women, to 13.0 g/dl for adult men [12]. However, for a validation study, the wider range of hemoglobin values is more favorable as the distribution for assessing intermethod diagnostic accuracy and numerical correspondence [10, 13, 14]. Human habitation across a diversity of altitudes from the coastal plain to the mountainous highlands in Guatemala provides an appropriate setting to further evaluate the utility of a noninvasive probe across a wide spectrum of hemoglobin values. Hence, pregnant women in the lowland region are expected to trend toward having anemic values (< 11.0 g/dl), whereas adult men residing at high altitude would tend toward hemoglobin levels at the opposite end of the spectrum (> 13.0 g/dl).

The Rad-87™ (Masimo Corporation, Irvine, CA, USA) was used to collect digital readings that were then compared with values provided from a conventional venous blood test. The expectation was that bloodless screening might provide a more accurate assessment, that is, closer correspondence with the whole blood standard values in the mid-range values than at the extreme ends of the hemoglobin spectrum. Since minimal measurement time is desirable for field studies, we compared readings at 10 minutes, as recommended by the manufacturer, with readings at 5 minutes. We present here the results of a validation study in adult volunteers simulating the procedures and environment for the application of noninvasive hemoglobin screening in service to public health surveying.

Methods

Study design

The study was a cross-sectional survey of adult subjects for the calibration of a screening method to determine human hemoglobin concentrations with the corresponding values from laboratory measurements in whole blood. The goal of sampling was designed to include an abundance of extreme values at both the anemic and the plethoric ends of the hemoglobin concentration spectrum for sensitivity and specificity assessment. Enrollment involved intentional oversampling in a population expected to have a high prevalence of low values (pregnant lowland women) and of high values (highland men). The diagnostic discrimination analyses involved modeling across the WHO discrete age- and sex-specific cutoff criteria for classifying anemic individuals at sea level [12].

Subjects

Eighty adults were recruited from two departments located in the western region of Guatemala. Forty men 18 years of age or older came from two communities of the highland region of Totonicapán, which lies approximately 2,600 m above sea level and has average temperatures ranging from 6° to 22°C. The men were primarily recruited at the health center of San Francisco El Alto. The remaining male subjects were residents of Tacajalbe, a neighboring village within the department of Totonicapán, who agreed to participate at the local health post.

Forty pregnant women attending one of two health centers in the coastal region of Retalhuleu were also invited to participate. The majority of the subjects were patients already attending the health center located in Retalhuleu proper, while the remaining participants were pregnant women presenting at the health center of the nearby community of El Asintal. At approximately 240 m above sea level, both locations experience year-round tropical climate, with average temperatures between 21° and 34°C [15].

Interested residents of the community or individuals attending the health centers were eligible to participate. Those with chronic diseases or a history of adverse reactions to venous blood extraction and pregnant women who had had blood work done within the previous 4 weeks were excluded from the study. No children were included in the sample. This study was approved by the Human Studies Committee of the Center for Studies of Sensory Impairment, Aging, and Metabolism. A written informed consent form outlining potential risks and benefits, confidentiality, and voluntary participation was given to and authorized by all subjects. Printouts of complete hematologic results, including a brief explanation of hemoglobin

ranges and guidelines for follow-up, were provided to all participants.

Measurements of whole blood hemoglobin concentrations

The subjects had 3 ml of blood drawn from the cubital vein of their nondominant arm by a local laboratory technician. The samples were stored in a cooler at 4°C until they were delivered to the laboratory for analysis on the same day. Complete hematologic analysis was done for all samples on the day of blood sampling at the laboratory in Quetzaltenango, using the automated HumaCount–Human device (Corporación Analíticos, Guatemala) after adequate calibration and quality control. Most subjects received both invasive and noninvasive tests within approximately 1 hour, and at a maximum of 2 hours, of each other.

Digital estimations of hemoglobin concentration with the noninvasive probe

In parallel with blood sampling, hemoglobin concentrations were estimated noninvasively with the use of the Rad-87™ with Rainbow Set technology (Masimo Corporation). This device uses 7+ wavelengths, ranging from 500 to 1,400 nm. In male subjects, an adult-size finger clip was placed on the nail bed of the ring finger of the nondominant hand. The entire hand was covered with a dark-colored sock to reduce interference from external light. A child-size finger clip was placed on the nail bed of the ring finger of the nondominant hand in female subjects. To prevent the hands of participants in the hot coastal regions from overheating, while still reducing external light, a small, black plastic sleeve was used to cover the subject's ring finger. Any excess debris was removed from the nail bed prior to applying the clip, if necessary. All participants were asked to rest their hand on a flat surface for the duration of the test. Timing began when a value for Hb concentration first appeared on the display. Hemoglobin values were recorded in grams per deciliter at baseline and after 5 and 10 minutes.

Reproducibility exercises

Ten control subjects (eight women and two men) in Guatemala City were tested repeatedly with the Rad-87™ Rainbow probe over a period of 10 days to determine day-to-day variability within the same subject. In addition, eight subjects (seven women and one man) were tested at four different times over the course of one day to evaluate within-day variability.

In this field study, we also examined the correspondence of the standard 10-minute digital reading with the value obtained after only 5 minutes of finger-clip application. These were compared as explained below.

Data handling and statistical analysis

All data were recorded manually at the time of measurement. For each subject, hemoglobin measurements were recorded when the value first appeared and after both 5 and 10 minutes. Each subject was identified with a single patient identifier. Values were later keyed into Microsoft Excel 2002, version 10 for Windows. Blood results were labeled using the same identifier and entered into a separate database along with complete patient information.

Descriptive statistics were calculated for the pooled sample and separately for both 40-subject subpopulations using Microsoft Excel 2002. The arithmetic mean, standard deviation, median, 25th and 75th percentiles (interquartile range), and maximum and minimum values were determined. Various regression correlation coefficients were applied, including the Pearson product-moment and the Spearman rank-order correlation coefficient. Specifically for the comparison of readings at 10 and 5 minutes, the Lin concordance coefficient, a measure of intertest identity, was applied [16]. Correlations between variables were calculated using Microsoft Excel 2002, SPSS version 17, and the NIWA Statistical Calculator [17]. The differences among the distributions of hemoglobin estimation by whole blood and digital readings were assessed by repeated-measures analysis of variance (MANOVA), with a post hoc test of LSD, using SPSS version 17. Finally, conventional diagnostic discrimination analysis for screening (digital readings) versus reference standard (whole blood) generated the sensitivity, specificity, and positive and negative predictive values. WHO thresholds for diagnosing anemia were used to determine these values. The standards for pregnant women and children under 5 years of age (11.0 g/dl), school-age children (11.5 g/dl), nonpregnant women (12.0 g/dl), and adult men (13.0 g/dl) were utilized [12].

Results

Within-subject variation of digital readings of hemoglobin concentration with the noninvasive probe

Repeated measures were taken in subjects to assess internal consistency of the noninvasive probe device. **Table 1** summarizes day-to-day variation of hemoglobin values on 10 once-daily repetitions within a span of 15 elapsed days, as recorded by the noninvasive apparatus in eight subjects, across two observers (C.C. and C.A.). The pooled coefficient of variation was 7.2 ± 2.9% if calculated as the mean of an individual's variance, or 5.3% as the coefficient of variation of the global mean. A similar test-retest exercise for variation within a single day was conducted by the two observers

TABLE 1. Day-to-day variation of digital readings of hemoglobin concentration with the noninvasive probe

| Subject | Hemoglobin concentration (g/dl): sequential repetitions within subjects on different days (10-min reading) | | | | | | | | | | Descriptive statistics | | |
	1	2	3	4	5	6	7	8	9	10	Mean	SD	CV%
A	13.4	13.3	13.2	12.9	11.7	13.3	14.6	14.8	16.5	14.7	13.8	1.3	9.6
B	13.8	9.8	12.7	13.2	12.6	14.2	12.7	10.5	11.4	12.4	12.3	1.4	11.3
C	11.2	14.7	15.1	13.7	13.8	13.2	13.4	13.1	13.2	13.8	13.5	1.0	7.7
D	10.8	11.9	12.4	13.4	12.5	12.3	14.0	12.2	13.4	12.6	12.6	0.9	7.1
E	12.8	14.0	13.9	10.4	14.1	11.9	12.0	12.7	11.8	14.1	12.8	1.3	9.9
F	11.8	12.6	12.6	11.6	11.8	12.1	11.6	12.8	13.0	12.9	12.3	0.6	4.5
G	13.2	14.2	14.7	12.9	13.2	13.9	14.1	13.0	13.4	14.0	13.7	0.6	4.4
H	13.3	14.1	13.7	12.9	13.9	13.6	14.3	13.6	14.3	13.5	13.7	0.4	3.2
Mean											13.1	0.9	7.2
SD											0.7	0.4	2.9

across a total of eight subjects. Four measurements were collected over four 2-hour intervals on a single calendar day. The pooled coefficient of variation was 4.4 ± 2.1% (tabular data not shown).

Characteristics of the distribution of the whole blood hemoglobin values of the sample

The histogram distribution of the whole blood hemoglobin values, as associated with the origins in the low-altitude, female and high-altitude, male subgroups, is illustrated in **figure 1**. Hemoglobin measurements as determined by the whole blood sample covered a broad array of values, ranging from anemic to plethoric, with a range from 9.1 to 19.5 g/dl, and a median value of 13.3 g/dl (interquartile range, 11.0 to 15.6 g/dl) (**table 2**). The mean value of hemoglobin concentrations determined by standard procedures across all 80 subjects was 13.5 ± 2.6 g/dl.
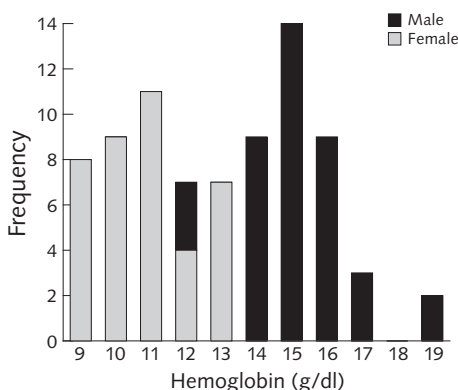

FIG. 1. Histogram distribution of whole blood hemoglobin values of the 80 individuals in the selected Guatemalan sample recruited to extend the spectrum and oversample at both extremes of potential human red cell concentration. Gray bars represent the pregnant women sample of the coastal lowlands, and solid black bars represent the highland-dwelling men sample

By subgroups, among the 40 pregnant women residing along the coastal plain, the mean value was 11.3 ± 1.3 g/dl, with a range from 9.1 to 13.8 g/dl. The median value hovered around the WHO reference limit for anemia among pregnant women, at 11.0 g/dl (interquartile range, 10.3 to 12.0 g/dl). Overall, highland men had higher hemoglobin concentrations, with a mean value of 15.7 ± 1.4 g/dl. The median value was also higher than that of lowland women (15.6 g/dl; interquartile range, 14.7 to 16.5 g/dl), and the difference between the minimum and maximum values (12.4 and 19.5 g/dl, respectively) was higher and considerably larger.

Correspondence of 10-minute and 5-minute digital readings of hemoglobin concentration with the noninvasive probe

The manufacturer-recommended scanning duration is 10 minutes. To determine for future reference whether a shortened procedure would provide equally valid results, we recorded the 5-minute digital hemoglobin value from the noninvasive device as well. The correspondence of the paired values at respective time points for each subject is shown in the scatterplot in **figure 2**. A strong relationship between readings was reflected by the Pearson coefficient ($r = 0.95$) and Spearman rank-order coefficient ($r = 0.95$). Furthermore, the Lin concordance coefficient between hemoglobin values at 10 and 5 minutes indicates a robust relationship of the readings taken at different times ($r = 0.95$). As shown in **table 2**, the overall median hemoglobin values generated by the digital readout for both 10- and 5-minute time points for the entire sample were identical at 11.9 g/dl.

Correlation of whole blood hemoglobin values with digital readings of hemoglobin concentration obtained with the noninvasive device

Figure 3 displays the correlation between digital readings provided by the noninvasive device and values

TABLE 2. Comparison of means and distributions of hemoglobin values from alternative measurement methods across the entire 80 subjects in the sample

Measurement method	Hemoglobin concentration (g/dl)						
	Mean	SD	Min	25%	50%	75%	Max
Whole blood	13.5	2.6	9.1	11.0	13.3	15.6	19.5
Digital reading, 10 min	12.2	1.7	8.9	10.8	11.9	13.5	15.7
Digital reading, 5 min	12.1	1.6	8.9	10.8	11.9	13.5	15.9

determined by whole blood analysis. Although measurements taken at 10 minutes have a slightly stronger Pearson correlation ($r = 0.81$) than those taken at 5 minutes ($r = 0.75$), both readings demonstrate a direct, positive correlation with the hemoglobin values determined by the invasive method. However, the true regression line at both time points had a slope below 1.0 (45° angle), which would represent quantitative numerical correspondence between methods.

The average digital readings obtained with the use of the noninvasive probe were slightly lower than the conventionally determined whole blood mean, as shown comparatively in **table 2**. When analyzed by MANOVA and followed by LSD post hoc analysis, the whole blood values were significantly different from both digital readings ($p < .001$), but no difference was found between digital values at 10- and 5-minute intervals ($p = .966$). The median at both 10- and 5-minute readings with the noninvasive device was 11.9 g/dl, which was also only slightly less than the value obtained with the invasive test. Moreover, the Rad-87™ produced a similarly wide range of values on the lower end of the spectrum. The noninvasive probe was able to capture a low value of 8.9 g/dl at both reading intervals. However, major limitations were seen toward the upper limit, as maximum outputs of only 15.7 and 15.9 g/dl at 10

and 5 minutes, respectively, were recorded digitally, as compared with 19.5 g/dl with the standard method (**table 2**).

Sensitivity, specificity, and predictive accuracy of digital readings of hemoglobin concentration at different diagnostic cutoff criteria for anemia classification

The noninvasive device proved to be only moderately sensitive at higher cutoff values for classifying anemia.

FIG. 3. Scatterplots of paired values for hemoglobin in the 80 selected participants. The horizontal axis represents the whole blood hemoglobin values. The vertical axis represents the digital reading from the noninvasive device. The broken diagonal line represents the 45° line of identity, and the solid line represents the least-squares regression line. Panel A displays the 10-min digital reading. The Pearson correlation coefficient is $r = 0.81$, $p < .001$. Panel B displays the 5-min digital reading. The Pearson correlation coefficient is $r = 0.75$, $p < .001$
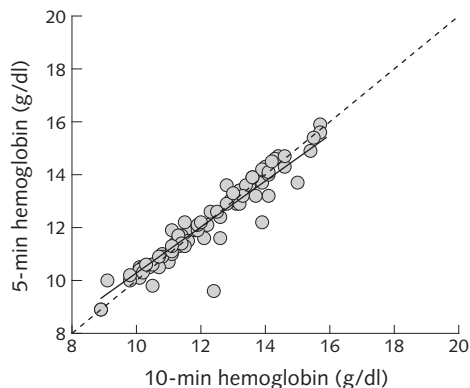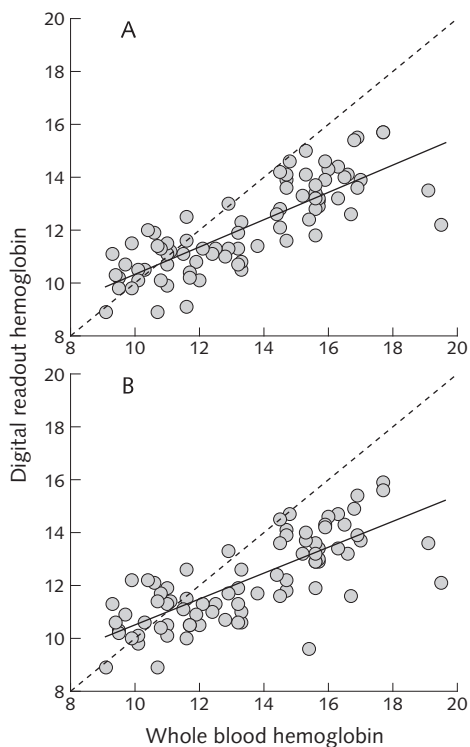

FIG. 2. Scatterplot of paired digital values for hemoglobin in the 80 selected participants. The horizontal axis represents digital readouts given by the noninvasive device at 10 minutes, whereas the vertical axis represents those taken at 5 minutes. The broken diagonal line represents the 45° line of identity, and the solid line represents the least-squares regression line. The Lin concordance coefficient is $r = 0.95$

Two-by-two contingency tables for 11.0 and 12.0 g/dl cutoff values assess the ability of the noninvasive apparatus to diagnose anemia (**table 3**). In the $n = 80$ population, 6 subjects appeared to be nonanemic when they were anemic according to standard analysis (false negative), whereas 10 and 11 subjects (measured at 10 and 5 minutes, respectively) appeared to be anemic, although they were not anemic (false positive) at the 11.0 g/dl cutoff value. At the 12.0 g/dl cutoff value, the number of false negatives was slightly less (2 and 4 for measurements at 10 and 5 minutes, respectively), whereas the number of false positive values was higher (14 and 17

for measurements at 10 and 5 minutes, respectively). **Table 4** outlines the sensitivity, specificity, and positive and negative predictive values of hemoglobin measurements obtained by the noninvasive technique, across all 80 subjects and by subgroup. For values over 16.0 g/dl, the noninvasive device was evaluated for accuracy of determining plethoric values in male subjects. The female subpopulation was evaluated for cutoff values below 13.0 g/dl. The sensitivity of the digital readings was highest at both 10 and 5 minutes, at a cutoff value of 13.0 g/dl (97% within the total population, 100% among the female subpopulation). Specificity,

TABLE 3. Examples of two-by-two contingency table diagnostic discrimination analyses for selected cutoff criteria for anemic classification using the noninvasive probe in the selected Guatemalan sample

$n = 80$, all subjects				$n = 40$, women, lower hemoglobin range			
< 11.0 g/dl							
		Whole blood				*Whole blood*	
		Anemic	Nonanemic			Anemic	Nonanemic
Digital	Anemic	11	10	*Digital*	Anemic	11	10
(10 min)	Nonanemic	6	53	*(10 min)*	Nonanemic	6	13
		Whole blood				*Whole blood*	
		Anemic	Nonanemic			Anemic	Nonanemic
Digital	Anemic	11	11	*Digital*	Anemic	11	10
(5 min)	Nonanemic	6	52	*(5 min)*	Nonanemic	6	13
< 12.0 g/dl							
		Whole blood				*Whole blood*	
		Anemic	Nonanemic			Anemic	Nonanemic
Digital	Anemic	27	14	*Digital*	Anemic	27	10
(10 min)	Nonanemic	2	37	*(10 min)*	Nonanemic	2	1
		Whole blood				*Whole blood*	
		Anemic	Nonanemic			Anemic	Nonanemic
Digital	Anemic	25	17	*Digital*	Anemic	25	10
(5 min)	Nonanemic	4	34	*(5 min)*	Nonanemic	4	1

TABLE 4. Sensitivity, specificity, and predictive accuracy of digital readings of hemoglobin concentration at different diagnostic cutoff criteria for anemia classification[a]

	Digital (10 min)								Digital (5 min)							
	$n = 80$				$n = 40$				$n = 80$				$n = 40$			
Cutoff (g/dl)	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV
< 11.0	65	84	52	90	65	57	52	68	65	83	50	90	67	57	52	68
< 11.5	82	72	53	91	82	22	56	50	77	74	53	90	77	28	57	50
< 12.0	93	73	66	95	93	9	73	33	86	67	63	85	86	9	76	14
< 13.0	97	61	67	96	100	0	83	NC	97	59	66	96	100	0	83	NC
> 16.0	0	100	NC	84	0	100	NC	68	0	100	NC	84	0	100	NC	68
> 17.0	0	100	NC	95	0	100	NC	90	0	100	NC	NC	0	100	NC	90

NC, not calculable; NPV, negative predictive value; PPV, positive predictive value; SEN, sensitivity; SPE, specificity
a. For cutoffs < 13 g/dl, the female subpopulation ($n = 40$) is used, corresponding to the first four data rows; for cutoffs > 16 g/dl, the male subpopulation ($n = 40$) is used, corresponding to the last two data rows.

however, was greater at the lowest cutoffs for determining anemia: 84% among the total population, at a 10-minute reading. The noninvasive device was 100% specific, yet 0% sensitive for assessing plethoric values within the total population and the male subgroup.

Discussion

Examples of replacing diagnostic blood extraction with less invasive procedures are extensive. The original approach to assessing lactose maldigestion in association with lactose intolerance involved administering 50 to 100 g of lactose in water and drawing blood at intervals over 3 or more hours to measure the increase in circulating glucose [18, 19]. With the advent of technology to quantify hydrogen (H_2) in expired air, a lactose maldigestion breath test was validated [20, 21]. This shift in techniques rapidly permitted the innovation of portable gas chromatographs [22] for outreach to population studies [23, 24] and the use of lower oral doses of lactose, even in their natural food forms of milk and yogurt [25, 26]. More recently, bloodless transcutaneous sensors for blood gases and pH values [27–29] have emerged as additional examples of noninvasive technology innovations.

The notion of Indonesian workers in the 1990s [30] was to provide a method of selecting those individuals within a population most in need of iron intervention and thus target the administration of a nutrient with recognized adverse effects to those in need [31]. WHO developed a standard recommendation, however, for universal, across-the-board supplementation of children 6 to 24 months of age with a daily dose of 12.5 mg of elemental iron and 50 µg of folic acid [32]. The urgency of applying a system of screening and differentiated administration of iron in certain public health settings, however, emerged after the publication of a study from Pemba, Tanzania. The results suggested that iron supplementation helps children with iron-deficiency anemia to cope with the sequelae of malaria tropica, while increasing the number of hospital admissions and deaths in iron-replete children [33].

The risk factors associated with each misdiagnosis are considered to better understand the value of using the noninvasive device in an iron supplementation program. Although the apparatus correctly detected many truly anemic individuals, a number of them would have been misclassified and either inadvertently denied supplementation when they needed it or given it unnecessarily. For example, 10 of 23 nonanemic women were mistakenly classified as anemic according to the 11.0 g/dl cutoff on the basis of the digital reading at 10 minutes. If these had been children participating in an iron supplementation intervention, they would have received iron and been at greater risk for

increased hospitalization and adverse events. On the other hand, six who were truly anemic would have not been detected by the digital device and consequently not provided iron supplementation. These children would have been put at a higher risk for impaired cognitive development and increased susceptibility to infection. Thus, a device that is only 65% sensitive and 57% specific will deny supplementation to 35% of those who need it and unnecessarily and unsafely administer it to 43% who do not.

Strengths and limitations of the study

The study and its design had several strengths and a number of acknowledged caveats and limitations. The noninvasive probe device by Masimo had already shown reliability in a less mobile version in the hospital setting [10]. The experiences of the present study, in contrast, simulated the conditions of outreach in field studies within developing populations. Moreover, all of the field measurements were performed by a single observer (C.C.), who received face-to-face instruction from a technician from the Masimo Corporation in Germany.

We consider the conscious oversampling in populations expected to have higher prevalence rates of hemoglobin concentrations toward the upper and lower extremes of the human hematologic spectrum to be a strength, as it reduced hemoglobin concentration within the normal range. Thus, it allowed for more robust sensitivity and specificity testing by assuring an abundance of values at the high and low ends of the distribution pattern, which does not reflect the overall distribution of usual at-large community samples, as might be encountered in population surveys. The sensitivity and specificity values (**tables 3** and **4**) are likely to be robust across different sample distributions, but the assessment of predictive values would vary with a different hemoglobin distribution [34]. A limitation is the restriction to adult subjects, while much of the public health interest in anemia is in young children [33, 35, 36]. For pediatric applications, the requirement to keep the hand immobilized with a finger clip for 5 or 10 minutes may strain children's compliance and, in consequence, be a negative factor for acceptability to parents. However, as the ethical bar for obtaining blood samples from healthy children in the community setting is higher than that for adults [37, 38], our first proof-of-principle foray has used adult volunteers.

Some reduction in the strength of intermethod association in this study might have arisen through normal fluctuations in intravascular red cell concentration over the interval of up to 120 minutes between the sampling of whole blood and the obtaining of the digital reading.

Comparison with literature data

The experience with the EMPHO technology was largely exploratory and too fragmented in its design and presentation to permit strict comparison [8]. The Nadeau and Groner study approached the issue of noninvasive hemoglobin determination with in vivo ultramicroscopy [9]. These authors based their method on the direct visualization of the microvasculature with orthogonal polarization spectral imaging [39]. In a series of 71 subjects, the noninvasive and venipuncture methods were well correlated for classifying individuals as anemic or nonanemic ($r = 0.93$). However, the method was applied under laboratory conditions and was not further pursued, possibly because of tedious handling and evaluation or because of cost issues. Moreover, the discontinuation of the algorithm generation (personal communication, D. Johnson, 2008) poses a major barrier. Rabe et al. used an earlier, prototype model of the present Rad-87™ pulse CO-Oximeter in a neonatal care setting in southern England and found less than 5% error with the noninvasive estimation as compared with the venous hemoglobin values [10]. With the use of this model, the correlation was slightly stronger ($r = 0.98$) than with the noninvasive probe used in the Guatemalan field setting ($r = 0.81$). A comparison of Rabe's invasive and noninvasive methods using a Bland-Altman analysis suggests that variation between the two is mostly consistent, although the differences increase slightly as the average hemoglobin value increases. A similar analysis of the Guatemalan data indicates that the variability between methods remains fairly constant across the range of hemoglobin values collected, although a direct, positive relationship is shown. At the upper range of the hemoglobin concentration spectrum, blood values tended to exceed digital values, with an inversion of this relationship at the lower end. To reduce the discrepancy between invasive and noninvasive determinations, the current trend aims to improve the underlying algorithm and to increase the processing rate of the calculator in the device in order to increase the number of iteration steps in the algorithm.

Current promise and prospects for field application of the noninvasive probe

Noninvasive technologies, such as the Rad-87™, have potential for serving as field-friendly screening devices, although this device was originally designed to monitor hemoglobin changes over time in the clinical setting. The next generation of such noninvasive hemoglobin photometers will be designed to allow much shorter determination times, so that the question of whether to use exposure times of 5 or 10 minutes with the Rad-87™ will soon be obsolete. Although the whole blood sample has customarily been used to determine hemoglobin

levels, the noninvasive method has the potential to avoid the risks and discomfort of having blood drawn. Although the test presently takes nearly 10 minutes to display the most accurate reading, individuals are generally pleased with the immediacy of receiving their results and the relative ease of completing the test using only a finger-clip sensor. For children, the painless test is surely preferable to the invasive method, although the long testing period, which requires the patient to hold one hand still for the duration of the trial, may be a deterrent. In addition, the length of time required to take a reading limits the number of subjects who can be tested in a single day. This proved to be a logistical problem in a field setting, and would likely result in decreased participation if a large number of patients were to be screened, as some would have to wait for several hours for their turn to be evaluated with the digital test. Excessive movement, external light, and extreme temperatures all appear to affect the displayed hemoglobin values, causing readings to vary erratically or extending the length of time necessary for a final measurement. All of these factors prolong the process of obtaining the most accurate reading and contribute to the already lengthy trial period, compounding the time needed for screening a potentially large sample. With limited electricity sources for recharging in some field settings and a limited battery life, the time required to execute such trials may be problematic. Overall, the device proved to be simple to use, widely accepted among community members, and capable of accurate categorical classification of hematologic status.

In summary, the noninvasive screening method had a high sensitivity and specificity at the anemia cutoff values in a normal and low range of hemoglobin values (< 12.0 g/dl for nonpregnant women and < 13.0 g/dl for adult men). Admittedly, there are few public health or clinical situations calling for anemia screening in men. According to the results of this trial, the noninvasive probe device would find acceptable application in screening and intervention modeling in a population, such as normal women at sea level, in whom a criterion of < 12.0 g/dl is of diagnostic interest. Measurements should be taken for the indicated 10 minutes, although upon further investigation, 5 minutes may be sufficient. In either case, the noninvasive device provides an immediate result, as opposed to invasive blood drawing, which, although rapid, requires at least a 24-hour turnaround for the results. Interestingly, in the context of Guatemala or mountainous countries of the Andean or Asian regions, where the cutoff criteria for anemia increase with increasing altitude of residence [40], the present capacities of the instrument assume greater reliability for use in pregnant women and children. However, the present device would fail to represent the normal values of adults living at high altitude, since no readings above 15.9 g/dl were recorded for a sample distribution that reached 19.5 g/dl. Since not

all nutrition surveys and nutrition interventions are conducted at sea level, engineering or programming improvements to extend the responsiveness of the instrument to the high end of the spectrum would be welcome.

Finally, for the standard setting of a sea-level population, the lower cutoff criteria of interest are < 11.0 g/dl for pregnant women and children under 5 years of age and < 11.5 g/dl for school-age children. Within the cultural context of Guatemala and of many other developing societies, avoiding the extraction of blood by the use of a noninvasive, light-scanning approach would a priori be a preferred method. Beyond the discrepancy in hemoglobin values compared with the gold standard measure, the 10-minute recording time required for registering an official hemoglobin concentration reading with the Rad-87™ stands out as a major disadvantage. Although invasive, conventional blood extraction from vein or capillary beds can be accomplished in half of that time. Despite the close correspondence of the hemoglobin readings obtained at 5 minutes of scanning with the readings obtained at 10 minutes, the 5-minute reading is not yet a faithful surrogate for the measurement taken at a longer period. The poorer diagnostic discrimination around WHO cutoff values [12] would call for attention by the manufacturer to improvement

of discriminatory characteristics at the lower end of the hemoglobin spectrum into the moderately anemic range. Hence, application of the Rad-87™ in the field at the community level appears to require additional engineering modifications to achieve the consistency and accuracy reported in the fixed, institutional setting [10].

Acknowledgments

References

1. Ministry of Public Health. Encuesta nacional de micronutrientes [Guatemalan micronutrient survey]. Guatemala City: Institute of Nutrition of Central America and Panama, 1996.

2. Eyquem A, de Saint Martin J, Levaditi JC. Australia antigen and transmission of viral hepatitis to monkeys. Rev Fr Transfus 1970;13:275–84.

3. London WT, Sutnick AI, Blumberg BS. Australia antigen and acute viral hepatitis. Ann Intern Med 1969; 70:55–9.

4. Ammann AJ, Cowan MJ, Wara DW, Weintrub P, Dritz S, Goldman H, Perkins HA. Acquired immunodeficiency in an infant: possible transmission by means of blood products. Lancet 1983;1:956–8.

5. Groopman JE, Salahuddin SZ, Sarngadharan MG, Mullins JI, Sullivan JL, Mulder C, O'Hara CJ, Cheeseman SH, Haverkos H, Forgacs P, Riedel N, McLane MF, Essex M, Gallo RC. Virologic studies in a case of transfusion-associated AIDS. N Engl J Med 1984;311:1419–22.

6. Issues related to human immunodeficiency virus transmission in schools, child care, medical settings, the home, and community. American Academy of Pediatrics. Committee of Pediatric AIDS and Committee on Infectious Diseases. Pediatrics 1999;104(2 pt 1):318–24.

7. Beekmann SE, Vaughn TE, McCoy KD, Ferguson KJ, Torner JC, Woolson RF, Doebbeling BN. Hospital bloodborne pathogens programs: program characteristics and blood and body fluid exposure rates. Infect Control Hosp Epidemiol 2001;22:73–82.

8. Gross R, Glitwitzki M, Gross P, Frank K. Anaemia and haemoglobin status: a new concept and new method of assessment. Food Nutr Bull 1996;17: 27–36.

9. Nadeau RG, Groner W. The role of a new noninvasive imaging technology in the diagnosis of anemia. J Nutr 2001;131:1610S–4S.

10. Rabe H, Stupp N, Ozgun M, Harms E, Jungmann H. Measurement of transcutaneous hemoglobin concentration by noninvasive white-light spectroscopy in infants. Pediatrics 2005;116:841–3.

11. McMurdy JW, Jay GD, Suner S, Crawford G. Noninvasive optical, electrical, and acoustic methods of total hemoglobin determination. Clin Chem 2008;54:264–72.

12. World Health Organization. Worldwide prevalence of anaemia 1993–2005. Geneva: WHO, 2008.

13. Morris SS, Ruel MT, Cohen RJ, Dewey KG, de la Briere B, Hassan MN. Precision, accuracy, and reliability of hemoglobin assessment with use of capillary blood. Am J Clin Nutr 1999;69:1243–8.

14. Neufeld L, Garcia-Guerra A, Sanchez-Francia D, Newton-Sanchez O, Ramirez-Villalobos MD, Rivera-Dommarco J. Hemoglobin measured by Hemocue and a reference method in venous and capillary blood: a validation study. Salud Publica Mex 2002;44:219–27.

15. Instituo Nacional de Sismologia, Vulcanología, Meteorología, e Hidrologia. Datos Meteorologicos de los Departamentos. Available at: http://www.insivumeh.gob.gt/meteorologia/ESTADISTICAS.htm. Accessed 7 August 2010.

16. Lin LI. A concordance correlation coefficient to evaluate

reproducibility. Biometrics 1989;45:255–68.

17. National Institute of Water & Atmospheric Research. Lin's concordance. Available at: http://www.niwa.co.nz/our-services/online-services/statistical-calculators/lins-concordance. Accessed 7 August 2010.

18. Gudmand-Hoyer E, Jarnum S. The diagnosis of lactose malabsorption. Scand J Gastroenterol 1968;3:129–39.

19. Huang SS, Bayless TM. Lactose intolerance in healthy children. N Engl J Med 1967;276:1283–7.

20. Bond JH, Levitt MD. Use of breath hydrogen (H2) in the study of carbohydrate absorption. Am J Dig Dis 1977;22:379–82.

21. Newcomer AD, McGill DB, Thomas PJ, Hofmann AF. Prospective comparison of indirect methods for detecting lactase deficiency. N Engl J Med 1975;293:1232–6.

22. Solomons NW, Viteri FE, Hamilton LH. Application of a simple gas chromatographic technique for measuring breath hydrogen. J Lab Clin Med 1977;90:856–62.

23. Ellestad-Sayed JJ, Levitt MD, Bond JH. Milk intolerance in Manitoba Indian school children. Am J Clin Nutr 1980;33:2198–201.

24. Newcomer AD, McGill DB, Thomas PJ, Hofmann AF. Tolerance to lactose among lactase-deficient American Indians. Gastroenterology 1978;74:44–6.

25. Savaiano DA, AbouElAnouar A, Smith DE, Levitt MD. Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactase-deficient individuals. Am J Clin Nutr 1984;40:1219–23.

26. Solomons NW, Garcia-Ibanez R, Viteri FE. Reduced rate of breath hydrogen excretion with lactose tolerance tests in young children using whole milk. Am J Clin Nutr 1979;32:783–6.

27. Chakravarthy M, Narayan S, Govindarajan R, Rajeev S, Jawali V. Early non invasive detection of hypercarbia in two cardiac surgical patients. J Clin Monit Comput 2009;23:59–61.

28. Fife CE, Smart DR, Sheffield PJ, Hopf HW, Hawkins G, Clarke D. Transcutaneous oximetry in clinical practice: consensus statements from an expert panel based on evidence. Undersea Hyperb Med 2009;36:43–53.

29. Tobias JD. Transcutaneous carbon dioxide monitoring in infants and children. Paediatr Anaesth 2009;19:434–44.

30. Gross R, Schultink W, Juliawati. Treatment of anaemia with weekly iron supplementation. Lancet 1994;344:821.

31. Schumann K, Ettle T, Szegner B, Elsenhans B, Solomons NW. On risks and benefits of iron supplementation recommendations for iron intake revisited. J Trace Elem Med Biol 2007;21:147–68.

32. Stoltzfus RJ, Dreyfuss ML. Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia. Washington, DC: ILSI Press, 1998:1–39.

33. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK, Kabole FM. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet 2006;367:133–43.

34. Snedecor GW, Cochran WG. Statistical methods, 8th ed. Ames, Iowa, USA: Iowa State University Press, 1989.

35. Lozoff B, De Andraca I, Castillo M, Smith JB, Walter T, Pino P. Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. Pediatrics 2003;112:846–54.

36. Tielsch JM, Khatry SK, Stoltzfus RJ, Katz J, LeClerq SC, Adhikari R, Mullany LC, Shresta S, Black RE. Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. Lancet 2006;367:144–52.

37. Litton P. Non-beneficial pediatric research and the best interests standard: a legal and ethical reconciliation. Yale J Health Policy Law Ethics 2008;8:359–420.

38. Mallory GB Jr. Pitfalls in non-therapeutic research in children. Pediatr Pulmonol 2006;41:1014–6; discussion 17–20.

39. Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. Nat Med 1999;5:1209–12.

40. Dirren H, Logman MH, Barclay DV, Freire WB. Altitude correction for hemoglobin. Eur J Clin Nutr 1994;48:625–32.