Innovative Non- or Minimally-Invasive Technologies for Monitoring Health and Nutritional Status in Mothers and Young Children

The Role of a New Noninvasive Imaging Technology in the Diagnosis of Anemia

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ABSTRACT Traditionally, numerical data regarding the status of a patient are a combination of measurements made at the point of care (POC) and those made in the laboratory on specimens withdrawn from the patient. We report here on our experiences with a new method for a noninvasive determination of anemia, as defined by blood hemoglobin (Hb) concentration. This method is based on a novel technology, orthogonal polarization spectral imaging, which provides high quality digitized images of the microcirculation using reflected light. Measurements of Hb, based on the analysis of these images at the POC, were found to compare favorably with results obtained with traditional laboratory methods. Additional advantages of these new POC technologies are that they will make possible completely new measurements that may have no direct analog with existing methods. For example, orthogonal polarization spectral imaging can give feedback regarding microvascular density, which also may be reduced in anemic subjects. This information may give earlier and different insights regarding the patient status in nutritional deficiency anemia than an Hb concentration only. However, additional research will be required to confirm the accuracy and utility of this measurement, especially in adult and pediatric populations, where anemia is more commonly encountered. The ultimate success of POC testing will require collaboration between the attending health care professional, the laboratory and institutional management to rapidly assimilate improved methodologies and new information to provide benefits to the patient. J. Nutr. 131: 1610S–1614S, 2001.

KEY WORDS: hemoglobin, hematocrit, polarization, microcirculation, anemia

Accurate numerical data regarding the nutritional status of the patient are essential in effective health care. Traditionally, these data are obtained by combining the results of noninvasive observations and measurements (blood pressure, temperature, pulse, pallor, etc.) made at the point of care (POC) with the results from remote laboratory measurements [hemoglobin (Hb), ferritin, vitamin B-12, etc.] made on specimens withdrawn from the patient. One obvious drawback to this scheme is that all of the data required for the clinician to proceed are generally not available until hours (or days) after the patient encounter. This dramatically slows the treatment process, especially if the results of initial laboratory tests indicate that additional testing may be required. Additional drawbacks involved in extracting the specimen by venipuncture include the risk of exposure to blood-borne pathogens as well as the added costs of handling and disposing of the laboratory waste.

These concerns have resulted in increasing research emphasis aimed at developing effective methods for performing additional testing at the POC (Price and Hicks 1999). Thus, in general, there is a gradual shifting of testing from the laboratory to the patient’s bedside driven by the combined desire to improve the effectiveness of health care while reducing costs.

The challenge for medical device manufacturers is to find means by which the data necessary for assessment or monitoring of the patient’s nutritional status are available at POC. The first and most obvious approach to dealing with this challenge has been to make traditional laboratory testing methods portable and bring them closer to the POC. This approach is still invasive using the same blood specimen and the same measurement technology. However, the results may be obtained sooner because it is not necessary to transport the specimen to the laboratory and process it along with hundreds of other specimens.

An alternative and a more desirable approach would be to
develop methods that extend the noninvasive measurements typically made at POC to include critical tests that are currently performed in the laboratory. In pursuing this approach, the technology is generally new and different. However the additional benefits are obvious: 1) results are available in real-time, allowing direct interaction between the patient and the health care professional in managing patient care; 2) testing can be performed at point-of-care, allowing monitoring of the parameter; and 3) the complications and hazards involved in specimen handling are completely eliminated.

One significant concern regarding this approach is the transferability of results obtained using new and different methods of measurement. For instance, when a noninvasive method is compared with an invasive method, the fact that the measurements are made on different samples can introduce a variance, not generally considered, coming from the nature and variability of specimen collection.

In this article, we report on our experiences in developing and evaluating a new method for a noninvasive determination of anemia, as defined by the Hb concentration of the blood. The new method is based on a novel technology, orthogonal polarization spectral (OPS) imaging, which provides high quality digitized images of the microcirculation in reflected polarization spectral (OPS) imaging, which provides high quality digitized images of the microcirculation in reflected light (Groner et al. 1999). Measurements based on the analysis of these images were compared with results obtained in vitro with traditional laboratory methods.

MATERIALS AND METHODS

Noninvasive measurement method

The OPS imaging instrument used in these studies was a prototype of a commercial device, the HEMOSCAN, developed by Cytometrics (Philadelphia, PA). The optics are contained in a hand-held probe. The optical portion that is inserted into the subject’s mouth is 6.5 cm long and 8 mm in diameter. In OPS imaging, polarized light is first directed back through the optics toward the camera. However, a second polarizer or analyzer oriented precisely orthogonal to that of the first polarizer blocks the portion of the light that has retained its polarization. Therefore, an image is formed using only the depolarized light returning from deep within the tissue.

For example (as shown in the top portion of Fig. 1), when an ink jet-printed cross is observed in reflected light, some light is reflected back from the surface and a significant amount of light is scattered within the paper medium. The result is limited contrast and some apparent voids in the ink. This problem is solved with OPS imaging by taking advantage of the fact that photons change their direction more easily than they change their polarization. As shown in the lower portion of Figure 1, when a second polarizer (analyzer) oriented orthogonal to the first is added in front of the CCD camera, light reflected from the surface and single scattering light returned from slightly below the surface are blocked. Therefore, the light detected has penetrated the medium by several scattering path lengths and has been depolarized. Thus, an embedded contrast object closer to the surface (the ink jet cross) seems to be illuminated from the back.

The analysis for the Hb concentration of blood with OPS imaging starts with an image of the sublingual microcirculation (see Fig. 2 for examples). A wavelength region, centered at a peak in the Hb absorption spectrum (548 nm), was chosen for optimal imaging of the microcirculation and for the Hb measurement. The detailed method for the calculation of systemic Hb has been described previously (Nadeau and Groner 2000). Briefly, each image is divided into vessel and background regions whose relative intensities are measured. From these measurements, the optical densities of a number of vessel segments are calculated and then used to determine Hb concentration by Beer’s law. Averaging these results allows an estimate of the subject’s systemic Hb concentration. In addition, the instrument keeps track of the average number of measurable vessel segments per area and calculates an average result (vessel segment density) for this parameter as well.

In vitro Hb method

The in vitro method for Hb analysis was performed on a fresh blood specimen (typically 3 ml) obtained by venipuncture. To perform the analysis, the sample of whole blood was diluted (~200 times) in a reagent that hemolyzed the red blood cells. The released Hb was then converted to cyanomet Hb. The resulting liquid was transferred to a measurement cell, where it was then measured spectrophotometrically at a wavelength of 540 nm. Calculation of Hb concentration is based on the application of Beer’s law. The in vitro
Comparison of multiple linear regression lines was made using GraphPad Prism, Version 3.00 for Windows (GraphPad Software, San Diego, CA; www.graphpad.com).

RESULTS

Comparison of Hb methods

Hemodilution studies in pigs were performed to demonstrate the linear response of in vivo Hb predictions compared with the traditional in vitro measurement. As shown in Figure 3, when the pigs were hemodiluted from ~100 g/L to 30 g/L in graded steps, there was a linear correlation between the in vivo and in vitro method. Comparison the individual regression lines for the three experiments using analysis of covariance demonstrated that there were no significant differences between the slopes or intercepts. The pooled slope was equal to 1.0 and the intercept to −0.002.

Figure 4 shows the comparison of the results obtained by the two methods when performed upon a series of 71 human subjects. A correlation coefficient of 0.93 is noted with good general agreement regarding the classification of individual subjects into compromised (anemia) and normal categories (medical decision point of 120 g/L). However, quantification of these results with regard to relative error is more challenging.

The comparison of the noninvasive method with the in vitro method made by doing a simple regression analysis has as a fundamental assumption that the reference method (x-axis) is without error. It is now found (by the specimen variation study) that this is not true. Consequently, the data were analyzed with a more general approach to establishing equivalence adapted from the method of Blackwelder (1982). When this was done, it was found that a 97% confidence level was achieved for equivalency between the two methods at the level of specimen variability (5 g/L) for the venipuncture.

Measurement of vessel segment density

The normal range of vessel segment density was calculated for a series of 50 ambulatory and putatively normal subjects.
(Hb between 125 g/L and 160 g/L). It was found to have a mean of 37 segments/mm² with 95% of the results between 25 segments/mm² to 50 segments/mm². Of the 10 anemic subjects (Hb < 120 g/L), 3 had vessel densities substantially lower than this normal range. These included the two most severely anemic subjects, suggesting that a reduction of vessel density may sometimes accompany a severe or long-standing anemic condition.

**Variability**

The results of the specimen variation study are summarized in Table 1 after the variation was subjected to a nested analysis of variance procedure and broken down into three components. It was found that, on the average, there was nearly a 2-g/L difference in the mean Hb from one arm to the other, a 1.4-g/L difference between the first tube and the second tube, and an ~1-g/L difference in replication of the measurement. Thus, the total variability in the comparison method (95% confidence) was roughly 5 g/L, with most of the variation due to specimen collection. This was true despite the great care observed in the specimen collection. In typical circumstances, the situation may be even worse. Recently, van Assendelft and Simmons (1995) addressed the sources of variability due to specimen and even tourniquet error. As an example, if a tourniquet was left on too long, an increase in Hb concentration of up to 30 g/L could be observed. Exercising before a blood draw or even walking as opposed to resting can create a difference of up to a 15 g/L. For these reasons, it is not surprising that many physicians have learned not to respond by varying treatment to changes of <10 g/L in the reported Hb concentration.

**DISCUSSION**

POC testing is becoming a significant alternative to standard laboratory testing in today’s health care system. Recent technological advances (especially in the area of biophotonics) have enabled an alternative concept for POC testing, where results of critical tests are obtained in real-time by noninvasive methods. Thus, the attending physician (or health care worker) in the near future may, using an instrument no more complicated than otoscope, obtain numerical data regarding a patient’s bilirubin, Hb, glucose, or white blood cell count.

Although the benefits of such advanced technology are obvious, there are some challenges that are not immediately apparent. Because by their very nature these new methods will involve determinations made on different types of tissue samples, of particular concern are issues regarding the nature and the variability of the specimen and the effect on results. Thus, when comparing the results from these new noninvasive POC testing instruments to existing in vitro methodology, consid-

![FIGURE 4](https://academic.oup.com/jn/article-abstract/131/5/1610S/4686805)

**TABLE 1**

_Venipuncture specimen variability in humans_

<table>
<thead>
<tr>
<th>Component</th>
<th>Standard deviation</th>
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<tbody>
<tr>
<td>Arm to arm</td>
<td>1.8</td>
</tr>
<tr>
<td>Tube to tube</td>
<td>1.4</td>
</tr>
<tr>
<td>Replicates</td>
<td>1.1</td>
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</tbody>
</table>

Specimen variability was measured, as described, using 40 subjects with hemoglobin (mean ± standard deviation) = 132.8 ± 2.25 g/L. All specimens assayed using Beckman Coulter MDII analyzer.

**TABLE 2**

_Analytic variability in vitro versus in vivo in humans_

<table>
<thead>
<tr>
<th>Source</th>
<th>In vitro</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard deviation</td>
<td>%CV</td>
</tr>
<tr>
<td>Instrument</td>
<td>1.10</td>
<td>0.82</td>
</tr>
<tr>
<td>Specimen</td>
<td>2.28</td>
<td>1.71</td>
</tr>
<tr>
<td>Total</td>
<td>2.53</td>
<td>1.90</td>
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</tbody>
</table>

CV indicates coefficient of variation.

In vitro measurements were made using a standard clinical analyzer and in vivo measurements were made using an OPS imaging device. In vitro variability was determined in 40 subjects as described in the text. In separate studies, total in vivo variability was determined from repeated samplings of 42 subjects. Instrument repeatability was obtained as described. In vivo specimen variability was calculated from the total and instrument variability assuming that they were independent contributions.
eration must be given to both sources of variability: the instrument and the specimen. In the process of developing a noninvasive method for Hb, we encountered difficulty in obtaining the type of agreement traditionally associated with this classic test. Further study demonstrated that the problem was not in the imprecision of the instrument or the accuracy of the method but in the hidden and often ignored variability of the specimen. When a more general approach to analysis was used, equivalency between the two methods (at the level of specimen variability) was found for a limited data set. Additional studies are currently being performed to see whether this result can be extended to a wide class of both normal and abnormal subjects.

Other challenges will also arise as these new methods become available regarding comparisons between testing at POC and results from traditional laboratory methods. In some cases (as in the Hb concentration example), the comparisons will be relatively simple because only the methods are new. Anemia, as defined by a low Hb value, is conceptually the same whether the measurement is made in vivo or in vitro. However, use of new technologies such as OFS imaging will also engender completely new measurements with no direct analog in existing laboratory methods. As an example, the direct measurements of vessel segment density may provide more complete insights regarding the patient status in nutritional deficiency anemia. Figure 2 shows a comparison of the sublingual microcirculation of two female subjects. One has a normal Hb of 140 g/L and the other is an iron-deficient anemic with an Hb of 98 g/L. A striking difference in the images is the relative paucity of vessels in the anemic subject. Not only are the vessels lighter (due to reduced Hb concentration), but also there is a reduction in vessel segment density (22 segments/mm² in this example vs. the average for normal subjects of 37 segments/mm²). This difference was not apparent in all subjects with similar Hb concentrations. Thus, decreased vessel segment density may offer an additional and useful measurement in assessing nutritional iron deficiency anemia. However, additional research will be required to confirm the accuracy and utility of this measurement, especially in adult and pediatric populations, where anemia is more commonly encountered.

The ultimate success of POC testing will require collaboration between the attending health care professional, the laboratory and institutional management to rapidly assimilate improved methodologies and provide the benefits to the patient.

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LITERATURE CITED