Targeted Provision of Oral Iron: The Evolution of a Practical Screening Option[1–3]

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ABSTRACT

Universal oral iron supplementation, undertaken according to 1998 WHO guidelines, produced adverse consequences among some children in malaria-endemic areas. Prompted by the Pemba trial, which revealed excessive hospitalizations and deaths, WHO advised that iron supplementation in such regions be accompanied by previous screening for iron deficiency. This agenda, however, poses issues of cost, benefit, acceptability, technical feasibility, and reliability of such screening. The cost of equipment and personnel is balanced against savings from iron supplements spared and treatment for morbidity averted. Costs aside, the most efficacious acceptable screening approach for avoiding hospitalization and deaths must be fielded. Screening before supplementation can be used to assess hematological, iron, and possible inflammatory status to differentiate the source of decreased hemoglobin concentration. Iron deficiency has often been inferred from hematological status markers. The need for extraction of blood, albeit capillary in origin, and high assay costs limit the use of validated methods in screening. Noninvasive methods, i.e., not requiring the extraction of blood, provide the most acceptable and potentially least expensive approach for determining hematological or iron status. Although a noninvasive technique for iron and inflammatory status would be the ideal, it is unattained. Field-friendly, skin-probe hemoglobin devices, derived from instruments for clinical settings, are being developed and tested for eventual rollout in malarial areas. Given a firm grounding for the theoretical requirements needed to advance the screening agenda, evaluation and monitoring of the performance of screening devices can proceed hand in hand. *Adv. Nutr. 3: 560–569, 2012.*

Introduction

Public health measures are generally taken to ensure the health of the public as a whole. A classic example of this universal approach is childhood immunizations, now with vaccinations against a large number of communicable diseases (1). Fluoridation of municipal water supplies is another common example of a universal public health measure. Targeting of specific subpopulations, however, is also seen in public policy. In fact, the action of the father of epidemiology, Dr. John Snow, to eliminate the propagation of cholera in the 1854 London epidemic, did not stop the consumption of all drinking water in London, but rather that water consumed in the confluence of the Broad Street pump serving the Soho district. Selective application of health measures is not atypical. Obviously, screening with Papanicolau cervical exfoliative cytology examination is reserved for women and prostatic-specific antigen for men. In fact, even in the domain of immunization, there was a degree of individual screening; persons with eczema skin rashes were excluded from receiving smallpox vaccinations because of the danger of contracting disseminated vaccinia as a consequence of the immunization (2).

Origins of the imperative to screen for oral iron supplementation in malaria areas

In 1998, at the request of the International Nutritional Anemia Consultative Group (INACG)[6] and the WHO, Stoltzfus and Dreyfuss (3) reviewed the extant literature and proposed age- and sex-specific guidelines for prophylactic oral iron and

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[6] CeSSIAM, Center for Studies of Sensory Impairment, Aging, and Metabolism; Hb, hemoglobin; ZPP, zinc protoporphyrin.

folic acid supplements for situations in which anemia constituted a public health problem. For young male and female children of normal birth weight, daily supplementation with 12.5 mg of elemental iron and 50 μg of folic acid was recommended from 6 to 12 mo if the age-specific prevalence of anemia is <40% and from 6 to 24 mo if the age-specific prevalence is >40%. One of the authors of the guidelines, in conjunction with institutional colleagues and international collaborators, later joined an initiative to undertake a field assessment of the validity, efficacy, and safety of the public health recommendation. One study was conducted in the lowland plains of Nepal (4), and the other on the island of Pemba, part of the Zanzibar archipelago of Tanzania (5). The latter site is holoendemic for *Falciparum* malaria, which had not yet been addressed by effective programs of bed nets and chemoprophylaxis. The trials had a parallel objective of evaluating oral zinc supplementation, and the potential for iron-zinc interactions to affect the efficacy of one or the other interventions. Among the 4 treatment arms were iron and folic acid only; zinc only; iron, folic acid, and zinc; and no treatment (placebo).

The aftermath of Pemba

As discussed extensively elsewhere in the symposium proceedings in this issue of *Advances in Nutrition*, periodic interim evaluation in the data-monitoring and safety process uncovered a tendency toward excessive adverse outcomes in the 2 arms of the Pemba trial that included the administration of iron and folic acid (5). Although neither the excessive mortality nor the excessive rate of hospitalization had reached statistical significance, the strength of the trend lines was sufficient to motivate the suspension of the iron treatment arms out of an abundance of caution. Later, with formal analysis of the data for publication, a combined excessive risk of mortality or acute hospitalization of 11% proved to be significant at the 0.03 probability level (5).

A call for a targeted approach to oral iron supplementation in malarial areas

The outcomes of this trial prompted a more detailed look at and evaluation of guidelines for iron supplementation and for the application of the 1998 recommendation in regions of intense *Falciparum* endemicity (3). As a result of the experience in Tanzania, the WHO called a consultancy in Lyon, France, in 2006 to react to and address those findings (6). It had several recommendations related to improving the safety of the administration of iron to children in malarial areas, which included providing standard treatment and measures such as insecticide-treated bed nets, appropriate antimalarial and antibiotic administration, and prophylactic iron, either from fortified foods or in an oral form taken with food. Recommendations made in Lyon specifically state that "universal iron supplementation…should not be implemented without the screening of individuals for iron deficiency…This mode of iron administration may cause severe adverse events in iron-sufficient children" (6).

The recommendation concerning screening by the Lyon consultancy, like many "official" mandates, ran into the problem that available technology for conducting the screening procedures in the field did not yet exist. The Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) in Guatemala, and the Hildegard Grunow Foundation in Germany took up the challenge to advance the technology to allow for compliance with screening. Thus, this review focuses on techniques to assess hemoglobin (Hb) values noninvasively and on the involved trade-offs. It comments on theoretical and practical aspects of noninvasive integumental scans for Hb concentration and on the available experiences gathered from literature review, consultation with colleagues, and field trials with such methods.

Theoretical and conceptual issues in screening

Universal application and universal coverage are such strong imperatives in public health theory and practice that selective application after individual screening raises considerable attention and consideration. This requires accepting the premise of steering iron-replete children in malarial areas away from exposure to oral iron. Thereafter, the consequences of that policy decision in terms of costs, benefits, and intrinsic limitations must be recognized in terms of the complex trade-offs implied.

Considerations for screening

To begin, we recognize 2 basic options for providing iron supplementation: universal supplementation and targeted distribution following an initial screening to determine who is iron deficient. In comparing these 2 options, the consequences of not receiving iron when needed are weighed against receiving excess iron when not needed. Because both of these can have serious adverse effects, an effective program to save both lives and costs will include screening to ensure that the appropriate individuals and populations are targeted for supplementation.

The trade-off between risks and benefits

Initiating conscious action to intervene for either a clinical (treatment) or public health (prophylactic) purpose inevitably seeks a benefit for an individual or individuals across a population. An efficacious intervention sometimes, however, implies a concomitant harmful consequence for the treated party or adverse outcomes induced in some members of the larger population (7,8). In fact, in the universal public health prophylaxis scenario, some people may not benefit at all, and others may only be harmed, also without receiving any benefit.

Stoltzfus et al. (9,10) commented on more generalized implications of the particular instance of the Zanzibar setting at the time of the Pemba trial. This commentary includes modeling a risk-benefit analysis and proposes scenarios in which screening of candidates for oral iron supplementation would be useful (10). The modeling performed by Stoltzfus et al. was based only on the zinc protoporphyrin (ZPP) marker (of iron deficiency), which was measured in a subsample of the population, ignoring Hb as a marker of

anemia. Without considering the potential of any adverse iron-malaria interaction, it would fit the threshold-for-action criteria of Stoltzfus et al., that an anemia prevalence of >45%, as detected in Pemba, calls for intervention with iron and folic acid (10). Universal supplementation with the iron and folic acid regimen as recommended in 1998 would be predicted to reduce the risk of adverse events of hospitalization and death overall in the population compared with no intervention; this occurs because iron deficiency itself was a risk factor for the adverse outcomes. In contrast, when iron deficiency is less common, as in populations with a prevalence <45%, universal supplementation with iron and folic acid would be expected to cause more harm than good through the exposure of oral iron to an even higher number of iron-replete children. To produce the beneficial effects of iron in iron-deficient children who need the nutrient, one must separate them from iron replete children with a screening component of any iron supplementation program (3).

There is a caveat in the scenario of a prevalence of iron deficiency >45%, however, because the authors state that "it increases risk in a subpopulation of children," referring to the iron-replete individuals within the catchment area of the malarial zone. They state that, referring to the Pemba parameters and rationale, "universal supplementation would be a rational choice at the population level, although some children would be harmed by it" (10). Mortality is one of the categories of harm in play. Thus, from an ethical standpoint, this conclusion for the same risk-benefit analysis runs contrary to a Hippocratic judgment standpoint. In a high iron deficiency prevalence situation, adverse events among iron-deficient individuals occur as the result of inaction by the authorities, i.e., not supplementing with iron. When universal supplementation is applied, the minority of iron-replete individuals suffer the adverse consequences, but exclusively as a result of the action taken by the authorities in exposing everyone to iron in a nonselective manner. In adherence to the *primum non nocere* (first do no harm) Hippocratic principles, the benefit for some does not compensate for the harm done to others, as the latter is a predictable effect of the intervention. The pathway toward effective screening has been pursued from the perspective that the attributable harm from an intervention must be maximally mitigated by screening in all scenarios.

The trade-off between costs and benefits

Another more frequently enunciated important trade-off is that of costs and benefits, often expressed as a cost-benefit ratio. The concept is much deeper and complex than the apparently straightforward nature of the term. If one looks at the diversity of public health costs that could be brought into the consideration of targeting, there are multiple layers to estimate and balance in trade-off scenarios. If the global bottom line were to reduce adverse events such as hospitalization and death by the targeted distribution of iron, one could begin with the alternative costs of initiating and maintaining the measures to minimize malarial transmission such as provision of impregnated bed nets and antimalarial drugs. In the absence of these interventions, one could calculate the cost of iron and its distribution along with the expenses involved in the screening procedure for eligibility (hematological or iron and inflammatory status) at the start of iron supplementation. The latter could be priced with only the start-up (equipment costs) and recurrent supplies or reagents, and, more precisely, should also include equipment maintenance and depreciation, training and monitoring of operating personnel, and so forth.

Benefits could also be seen as cost savings or as the costs for averting a specific negative outcome. Cost savings then could be calculated as the decrease in payment for the iron supplements not given to iron-replete children. If half of the population were iron replete and screened out of a planned universal trial, one would theoretically have saved half of the allotted cost for iron supplements across the catchment area. All of these estimations would have to be set into a specific size of a child population because there are economies of scale to be considered. As to additional benefit outcomes, the number of cases of iron deficiency corrected would not be useful or discriminative insofar as both universal and deficient-only targeting would ideally have reached and assisted the same children.

In the context of the acute hospitalization and deaths that are consequent to iron exposure in the iron-replete children, the benefit of adverse events averted, compared with the cost of averting an adverse event, would become a reasonable choice for cost-benefit analysis. In fact, this was the approach in the modeling conducted by Stoltzfus et al. (10) using the context of the Pemba trial experience (5). Because everything from the catchment population size to the monetized values of personnel, services, and iron supplements for Zanzibar would not generalize to other malarial zones of the world, the dollar amounts in the calculations are of little use to cite or discuss.

The need for oral iron supplementation in malarial areas is critical, but cost concerns cannot be ignored. On the saving side, one may also add the estimated costs of treating the sequelae of more severe malaria, as caused by giving iron to iron-replete children. As a corollary to Hippocratic perspective asserted previously, however, not screening and not targeting oral supplementation programs are, from an ethical point of view, not an option within the policy guidance of the Lyon consultancy (6).

Trade-offs between hematological status and iron status assessment

Another potential trade-off results from the selection of whether to use hematological or iron status as the basis for diagnosis. As discussed previously, Stoltzfus et al. (10) proposed ZPP screening, which looks at iron status independent of anemia. The verbatim language of the Lyon consultancy also specifies iron deficiency as the target condition. As discussed later, current technological promise for truly noninvasive, compared with minimally invasive, screening lies in Hb screening, which looks at anemia independent of iron status.

In fact, a comprehensive examination of risk and benefit from oral iron supplementation in the Pemba study within the subsample of 1609 individuals was based on a combination of both diagnostic dimensions (**Table 1**) (5). Overall, 75.6% of children in the substudy were iron deficient and 63.3% were anemic. We have adapted the pertinent, more complex relationships used by Sazawal et al. (5) in Table 1. As shown in the Table 1, the risk-benefit assessment was based on 4 combination categories: iron replete/anemic, iron replete/nonanemic, iron deficient/anemic, and iron deficient/nonanemic. Given this 4-quadrant analysis, only the combined category of iron deficient and anemic, representing 55.4% of the sampled population, would have benefitted (in terms of a sizable 49% reduction in harm) by targeting for both diagnostic parameters. Notably, iron-deficient children who were not anemic would have neither reduced nor increased risk. Indeed, iron-replete children would be harmed with a 50% to 100% increased risk of adverse outcomes. Therefore, screening should most urgently be aimed at keeping this group out of the harm's way by avoiding exposure to extra iron. If circumstances only allowed for establishing a child as nonanemic and designating him or her for exclusion from oral iron supplementation, a two-thirds reduction of severe risk of adverse events would be achieved. The remaining one third of those at high risk of iron-induced harm would not have been detected nor protected from intervention.

The trade-off between sensitivity and specificity

In general, there is an inherent trade-off between sensitivity and specificity in diagnostic discrimination, except when there is an incredible constellation of perfect correspondence between standard and screening methods, providing a sensitivity and specificity both of 100%, as seen in Nadeau and Groner (11). This is especially critical in issues of oncology, for example, in which one has an obligation to maximize sensitivity in screening to catch all potential tumors and to optimize specificity before embarking on radical ablation procedures (12). Illustrative examples of understanding the sensitivity-specificity trade-off and the operational consequences for less grave situations such as fecal screening for *Helicobacter pylori* (13) or HbA_{1C} for diabetes mellitus (14) are available. In the specific case of anemia, it is particularly important that anemia is able to be detected throughout the entire range of the cutoff criteria of interest, which would be at least <11.0 g/dL for a sea-level population of pregnant women and children younger than 5 years of age (15). With regard to whether the priority in screening is to find anemic children so that they can be treated or to find nonanemic children so that they can be excluded from treatment, the guidance that we get from the Lyon consultancy (6) suggests that specificity should be prioritized to best accomplish the latter in a malaria endemic area.

Table 1. Prediction of hospitalization and death in the Pemba substudy by hematological and iron status indicators[1]

	Children	RR (95% CI)	*P* value
Iron replete/anemic	127	2.00 (0.46–8.75)	0.36
Iron replete/nonanemic	264	1.51 (0.57–3.98)	0.41
Iron deficient/anemic	891	0.51 (0.31–0.83)	0.006
Iron deficient/nonanemic	327	0.91 (0.42–1.98)	0.82

[1] Adapted from Reference 5 with permission. Effects of supplementation with iron and folic acid with or without zinc on adverse events overall and by iron status and anemia (substudy).

Technical and instrumental issues in screening

As an urgent need to roll out an acceptable screening approach for malarial areas developed, the issues of acceptability, cost, and ability to perform in the field (field-friendliness, portability, robustness under tropical conditions) were obvious parameters to consider. Manufacturers of medical devices or academic laboratories had ongoing research or products that theoretically lent themselves to the field screening agenda. Being minimally invasive, that is, requiring only capillary blood from a finger, heel, or earlobe prick, or totally noninvasive, as with a direct contact probe to the surface of the skin or fingernail, rose to the fore as considerations related to the cost, safety, and community acceptance of the screening procedures.

Screening approaches for hematological status

Screening of hematological status would seek to produce a sensitive and specific diagnosis of anemia, although not specific to iron-deficiency anemia. To be valid for field application, the method should come close to duplicating the results obtained from conventional Hb measurement using the manual cyanmethemoglobin method or an automated cell-counter hematogram analyzer.

Historical and practical aspects of capillary-blood Hb concentration

A practical system capable of usefully reliable measurement of Hb concentration based on capillary blood collected in a special cuvette (HemoCue, HemoCue AB, Ängelholm, Sweden) was developed by Swedish designers and used extensively in anemia survey research. As with any other device requiring blood extraction, the obvious drawbacks are pain, discomfort, fear, and the need for proper handling and disposal. Within the past 30 y, blood extraction has been of increasing concern with the risk of blood-borne viral infections such as HIV and hepatitis. The HemoCue was evaluated in 2 widely circulated articles, one by Neufeld et al. (16), in children in the central highlands of Mexico at Cuernavaca and another by Morris et al. (17), in children in both Honduras and Bangladesh. The best reported sensitivity and specificity among the findings of Neufeld et al. (16) were 84% and 93%, respectively, with a general inclination toward better specificity than sensitivity. If, as with the question of malaria-zone screening, the premium is on exclusion of those not anemic, then specificity—accurately detecting those not affected by iron deficiency anemia—is more relevant than sensitivity for detection. With respect to the study by Morris et al. (17), interpretation of the correspondence and diagnostic discrimination is somewhat

vitiated in that the same (venous) blood sample was used to generate the whole-blood Hb and the HemoCue value. This was used as a proxy for a capillary sample. In the modeling approach of Morris et al. (17), the best-case scenario sensitivity and specificity were 80% and 95%, respectively. More recently, the HemoCue has been widely tested with different outcomes. Some studies found it not to be sufficiently accurate to base therapeutic decisions on (18,19), whereas others found a good correlation with laboratory data (20). The difference between venous and capillary blood is likely to contribute to such differences. The agreement of both methods provides an adequate estimate of population anemia prevalence, but less so on an individual level (21).

Historical aspects of noninvasive integumentary scanning for Hb concentration

In the way of non-invasive methods for determining hematological status, the earliest devices were developed within the past 10–15 y. Key features of the early devices and their characteristics are shown in **Table 2**. Gross et al. (21), working with German engineering collaborators (Siemens) in Jakarta, Indonesia, were the pioneers in the arena of noninvasive measurement of circulating Hb and the detection of anemia. A decade before the revelations of the Pemba trial, Gross et al. (21) were concerned by the possible harmful consequences of indiscriminant distribution of oral iron and sought a practical and acceptable approach for selecting candidates for the intervention. They developed the Erlangen microlight-guide photometer, which evaluated Hb concentration in tissue and capillary beds by using the reflection of backscattered white light. The use of this device was restricted to laboratory conditions.

Subsequently, Nadeau and Groner (11) published another approach, in the *Journal of Nutrition*, which involved orthogonal polarized spectral imaging using a sublingual probe to discriminate between anemic and nonanemic individuals. Findings suggest that the device provided perfect sensitivity and specificity, correctly identifying all those anemic as anemic and those nonanemic as not anemic. At this point, use of transdermal monitoring of hematological status took a decidedly clinical and hospital-focused turn, in which the potential for continuous assessment of Hb concentration in neonatal intensive care units and surgical suites surged forward. This potential motivated medical instrument companies to invest in hospital-directed devices. Another approach using white light projected through the skin was investigated by Rabe et al. (22) in 2005. In this perinatal study, a probe was applied to the forearm of preterm and term infants. Findings suggested that the relationship between noninvasive and invasive or the gold standard method was very stable and unbiased and demonstrated a correlation of 0.98.

Table 2. Historical exploration of noninvasive, integumentary, light-probe technology to assess hemoglobin concentration

Device: Erlanger microlight-guided photometer Light source: backscattered white light from tissue capillary beds Probe site: earlobe, palm, finger Intended application: noninvasive public health surveying and screening Author/year: Gross et al., 1996 (21)
Device: HEMOSCAN Light source: orthogonal polarized spectral imaging Probe site: sublingual Intended application: immediate point-of-care anemia diagnosis, for clinical or public health applications Author/year: Nadeau and Groner, 2001 (11)
Device: Mediscan 2000 Light source: white light spectroscopy Probe site: forearm Intended application: hospital-based neonatal intensive care surveillance Author/year: Rabe et al., 2005 (22)

Practical aspects of noninvasive integumentary scanning for Hb concentration

The final requirements for a practical and useful noninvasive, skin-probe device— whether for Hb or tissue ZPP— would relate to a series of performance characteristics ensuring adequate sensitivity and specificity under many conditions of priority application, namely, for screening in children in malarial areas. These factors would relate to, among others things, variation in skin pigmentation, the full range of Hb concentrations, light conditions, the heat and humidity of the tropics, electricity supply demands, stability of performance with frequent transport and use, and small body size (and finger size where finger-clip sensors are required). The current studies in Guatemala are generic and focused on instruments themselves, using adult volunteers; most of the issues, however, except those of the small size of young children, are emerging as part of the current performance trials.

Systematic evaluation of the validity and performance under field conditions of each of four portable devices that are depicted in **Figure 1** was conducted in Guatemala. The Masimo Rad-87^{TM} has been evaluated twice, once alone (23) and again in a comparative study with the first generation of the Haemospect® (MBR Optical Systems GmbH) (24). In the former study, it proved to be highly stable within the day and over 14 d, but diagnostic discrimination was poor, especially in the lower concentration range that signifies anemia. An almost identical set of findings was confirmed in the second evaluation (24). Meanwhile, Masimo came out with a compact, handheld model, the Pronto-7^{TM}, which was subjected to the validity and field evaluation protocol. Among the issues encountered with this device was a 3-g/dL deviation from the actual whole-blood Hb concentration (C. Crowley and C. Arriaga, unpublished results, 2010). Masimo recalled the Pronto-7^{TM} devices and, meanwhile, relaunched an improved version.

The best experience in terms of accuracy, correspondence, sensitivity, and specificity at the time of testing was with the first-generation Haemospect®, which used direct application of the penlike probe to the palm or forearm (24). The correlation coefficient of 0.94 demonstrates an

Masimo Corporation, Rad-87™ 	Probe site: finger (nail bed) Digital Read time: 10 minutes Size: Bench-mounted, 908 g Power source: Rechargeable battery and AC power Photosensitivity: Mild sensitivity to external light Temperature sensitivity: Decreased performance in low temperature Field Durability: Long read time and short battery life not suitable for repeated field trials
Masimo Corporation, Pronto-7™ 	Probe site: finger (pad) Digital Read time: 1 minute Size: Compact handheld, 296 g Power source: Rechargeable battery Photosensitivity: Mild sensitivity to external light Temperature sensitivity: Performance compromised by high skin temperature Field Durability: Moisture accumulation in clip prevented repeated trials
MBR Optical Systems, Haemospect® (1st generation) 	Probe site: Palm or forearm Digital Read time: 1 minute Size: Handheld, 544 g Power source: Rechargeable battery Photosensitivity: Moderate sensitivity to external light Temperature sensitivity: Mild Field Durability: Adequate *Digital output required external reprocessing of spectra before final reading was given
MBR Optical Systems, Haemospect® (2nd generation) 	Probe site: Finger (pad) or forearm Digital Read time: ~30 seconds* Size: Handheld, 544 g Power source: Rechargeable battery Photosensitivity: High sensitivity to external light Temperature sensitivity: Mild Field Durability: Delicate hardware, failed with repeated use in field setting *Digital output required external reprocessing of spectra before final reading was given

Figure 1 Photographic representation and systematic summary of descriptive and performance characteristics for 4 noninvasive light-probe devices tested in Guatemala.

extraordinarily high strength of association between digital readings from the Haemospect® and the value for whole-blood Hb (Fig. 2). The data are relatively tightly distributed and are not biased with respect to measurement across the range of readings by Bland-Altman (data not shown). Furthermore, nearly identical central tendencies of the digital and invasive methods (medians of 13.4 g/dL and 13.8 g/dL, respectively) suggest that the device would be suitable for measuring anemia at the population level.

When considering the diagnostic discrimination capabilities of the first generation of Haemospect® devices, several relevant cutoff criteria determined by the WHO (15) were used. At the 11.0-g/dL cutoff, which is applied to children younger than 5 y of age and pregnant women, the Haemospect® had strong diagnostic ability for identifying those who are not anemic (specificity of 98%), yet did not do as well when identifying those who are anemic (sensitivity of 67%). Likewise, at the 11.5-g/dL cutoff, which applied to children ages 5 to 12 y, specificity was high, yet sensitivity was only fair. At the cutoff used for teenagers and nonpregnant women (12.0 g/dL), sensitivity improved to 88% and specificity remained high. At the highest cutoff, which applied to adult men, the digital device demonstrated very good diagnostic discrimination (97% sensitivity, 100% specificity) (15).

Subsequent to this trial, in a second generation of the Haemospect®, the sensor application mode was changed from a direct skin probe to a finger clip. When run through

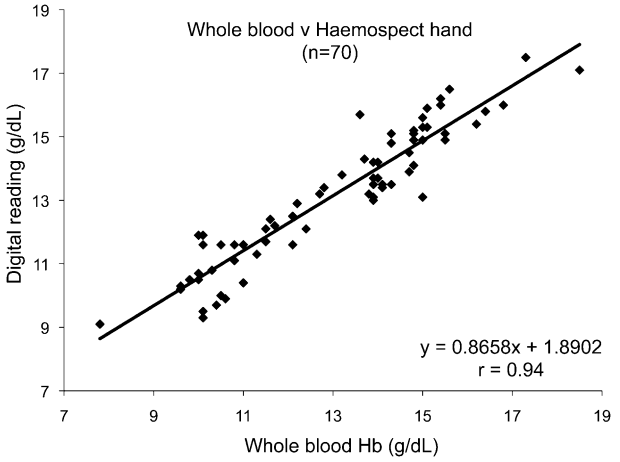

Figure 2 Scattergram of the values for whole blood hemoglobin (Hb) concentration in g/dL on the *x*-axis versus the corresponding values for the light-probe from the Haemospect placed on the forearm on the *y*-axis. Reproduced from Reference 24 with permission.

the field evaluation trial, the newer variant failed to confirm the promise of the earlier model. The strength of the association of the Hb output of the noninvasive device was $r = 0.43$, that is, only half of that of its predecessor (C. Crowley C and C. Arriaga, unpublished results, 2010). This device is currently being redesigned.

The 4 devices, both the first- and second-generation Haemospect® systems and the Rad-87™ and Pronto-7™ were portable and able to be field tested. All were easily transportable, although the Rad-87™ was the heaviest and most cumbersome and required a table or flat surface for placement when carrying out a reading. Each of the other 3 was handheld, and the compact Pronto-7™ allowed the easiest 1-hand use. Read time varied considerably between the Rad-87™, which required nearly 10 min, and the other devices, which produced a reading in anywhere from 30 to 60 s. The relatively short measurement times allowed for a large number of subjects to be evaluated in 1 workday and on 1 battery charge. Due to the long read-out time and short battery life of the Rad-87™, few subjects could be tested without the use of an AC power source. Although this device can be run off external power during testing, battery life proves to be a more valuable attribute in remote field settings where electricity is not always readily available. The Pronto-7™, although allowing for more rapid continuous readings, consumed more power than both Haemospect® systems, which could remain in use for several hours before battery life decreased. The Haemospect® software, however, was not finalized and, due to too limited calculation capacity in the device, required reprocessing of the spectra before a reading could be considered. This means that although digital output time was quite fast, final results were not yet available until collected spectra were reanalyzed at the manufacturer's headquarters; lack of immediately usable output was a drawback for field use of this device, which is currently being redesigned.

The sensor probes were applied to either the hand or finger (Fig. 1). Most used a finger-clip sensor that was placed on the tip of the finger, with the exception of the first-generation Haemospect®. Sensors of both the Pronto-7™ and the second-generation Haemospect® made contact with the pad of the finger, whereas the Rad-87™ reflected through the nail bed. The first-generation Haemospect® used a pen-like probe that could be placed on either the palm or the forearm. The second-generation Haemospect® used a finger clip for adult subjects, but the sensor could be removed from the clip and used as a flat probe on the forearms. Although this sensor provided versatility for measurement sites, the newer hardware was very delicate, and the light-transmitting sensor cords had to be replaced soon after field testing began. Both Masimo devices were designed for use with either an adult- or child-sized finger clip, although a Pronto-7™ child-size clip was under development. Clip size did prove to have an effect on ease or feasibility of reading, particularly among young children or other small subjects.

The most prominent issue affecting feasibility of field use resulted from the temperature and light sensitivity of the devices. Because all of the noninvasive devices relied on reflected white light and demonstrated some degree of photosensitivity, they generally required some sort of covering to prevent interference from external light. In particular, the second-generation Haemospect® showed extreme sensitivity to ambient light. Extreme skin temperatures also adversely affected read-outs. The Rad-87™ demonstrated poorer performance in colder climates, where subjects' hands were cold and circulation potentially compromised. This same effect was seen with the Pronto-7™, in particular with elderly subjects with poorer perfusion. The performance of Pronto-7™ was also affected when the clip was continuously exposed to warm hands, creating a temperature gradient between the probe and the subject. Condensation accumulated in the clip sensor from repeated readings and resulted in incomplete tests.

The digital screens of some devices showed messages that indicated functional problems such as low signal quality, repeat measurement, interference, and invalid spectra, which guide the operator toward correcting the issue and repeating the measurement to obtain a satisfactory reading.

Screening approaches for iron status

Acceptable and field-friendly approaches to assessing hematological status are on the horizon and move us part of the way forward in screening. A reliable companion method for iron status, however, should be part of a full solution because anemia might not be due to iron deficiency but to other causes, such as chronic disease (25).

Historical and practical aspects of capillary-blood ferritin concentration

Minimally invasive methods to assess iron status, involving capillary blood, were developed more than a decade ago. The first approach was developed in Kansas by Cook et al. (26) with dried whole-blood spots (DBS) on filter paper, with the notion of its use in large-scale population surveys for iron status. For field use, the collection of blood on a filter paper strip, analogous to the widely known prostate-specific antigen screening, is not only minimally invasive but has the advantages of conservation and storage of the specimen. It does not require freezing or refrigeration because the samples can be stored at room temperature. The disadvantage of the whole-blood approach, however, was the need to separate 2 species of ferritin. The red cells themselves contribute the majority of ferritin to whole blood; this is a variety, H-ferritin (heart ferritin) that is not useful for the assessment of iron stores. It is the L-ferritin (liver/spleen ferritin) in the serum that is the diagnostic marker for human iron reserves. The need to focus on only the latter, in the background of a 3-fold greater abundance of the former, rendered the whole-blood approach overly costly and cumbersome for the purposes of its invention. Moreover, the authors conclude that the best diagnosis of iron deficiency using the whole-blood spot assay is when transferrin receptor is also measured, and the receptor/L-ferritin ratio is the indicator of record, in particular, to discriminate anemia of

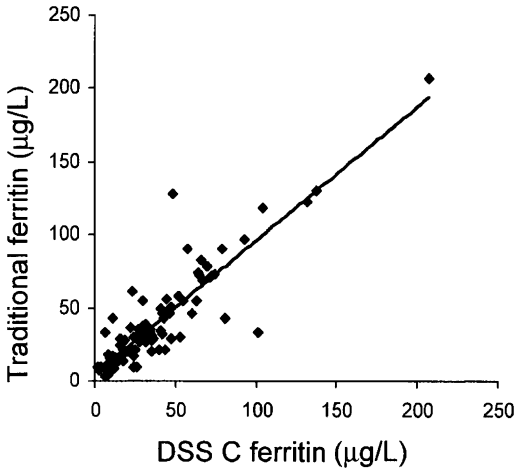

Figure 3 Relationship of the difference in serum ferritin values determined with the use of the dried serum spot (DSS) ferritin method and the traditional ferritin method, against the average values obtained with the 2 methods, for samples with serum ferritin concentrations between 0 and 50 μg/L. The dotted and dashed lines represent the mean and mean ± SD, respectively, for the difference in ferritin concentrations with the DSS C method, in which the DSS samples were prepared using self-sealing capillary tubes with a dispenser system that pushed serum onto filter paper. Reproduced from Reference 29 with permission.

inflammation from iron-deficiency anemia. This additional assay adds time and cost to the analysis procedure.

Later in the same year, Ahluwalia et al. (27), at the University of California, Davis, California, developed the analytical capacity to spot and dry aliquots of serum, separated from the red cell clot by a sophisticated gel filtration method in collecting tubes designed to receive capillary blood from a conventional finger prick. Field trials were conducted in Sri Lanka (28) and in collaboration with CeSSIAM in Guatemala (29). These trials sought to refine the approaches to extract the serum from the dried serum spot for analysis and to assess the influence of active inflammatory states in the subjects as a distortion to the interpretation of the ferritin concentration. In both sites, the studies found acceptable correspondence of the ferritin concentrations from the dried serum samples with those from the conventional venous blood sampling. The results obtained in Guatemala are illustrated in **Figure 3**. A complementary aspect of the 2 field studies, moreover, was to determine to what degree inflammation and infection in the subjects would be distorting the interpretation of ferritin as exclusively reflecting iron stores.

Erhardt et al. (30) developed a sandwich ELISA kit designed for application to 2 public health–relevant problems of low-income countries: vitamin A and iron deficiency. It simultaneously provides assays for retinol binding protein, ferritin, soluble transferring receptor, C-reactive protein, and alpha-1 acid glycoprotein, all in as small a serum or plasma volume as 30 L. Both ferritin and soluble transferring receptor are indicators of iron status. With specially designed blood collection and separation vials with capillary action, whole-blood samples from a finger, heel, or earlobe stick can be obtained. As mentioned, the method includes C-reactive protein, an inflammatory biomarker, which favors an appropriate interpretation of the ferritin values. Unfortunately, the cost of each analysis is relatively high, probably making the sandwich ELISA prohibitive for routine pre-iron screening in malarial areas.

Returning to the DBS experience, C-reactive protein elevations were widespread in the Sri Lankan children (28), whereas in Guatemala, they were relatively uncommon (29). In conclusion, capillary blood ferritin would be less than ideal for screening selecting candidates for oral iron supplementation in malarial areas for a number of reasons. Inflammation would likely be widespread in such areas, requiring some additional indicator measurement for its control. Neither of the 2 indicators would be immediately available for decision making because neither would be of a low enough cost to be sustainable for routine screening, and invasive screening bares the potential to transmit HIV and hepatitis.

Historical and practical aspects of capillary-blood ZPP concentration

ZPP increases in red blood cells as the bone marrow supply of iron reserves becomes exhausted (iron depletion), whereas the synthesis pathway for the heme ring continues. Labbé et al. (31) were among the first to recognize the potential for an approach to diagnosis based on an abnormally elevated ZPP/heme ratio and were the developers of a practical hematofluorometer, which provides a digital ratio measurement from whole blood smeared on a glass microscope slide.

ZPP was the index of iron status in the Pemba trial (**Table 1**) (5). It is the screening procedure for iron status modeled of the considerations by Stolzfus et al. (10). CeSSIAM had a positive and successful experience with ZPP/heme ratio assessment in a field study with children in Guatemala (32) because the slide mounting of the blood can be done at the remote site and later read on the hematofluorometer in a laboratory. The technique continues in widespread use in field studies and surveys, although ZPP, like ferritin, is also susceptible to distortion by inflammation and infection (33).

Theoretical aspects of noninvasive integumentary scanning for Hb concentration

Ideally, a field-friendly, noninvasive device for assessing iron status, through ZPP analysis, would be innocuous and would provide the variable that can best be related to the subanalysis of the Pemba trial (Table 1). At this time, however, there are not yet any completed designs on the market. The concept has been picked up by several investigators. Dr. Gary Brittenham of Columbia University (personal communication, 2010) has spoken of a sublingual sensor apparatus that is currently under development as a prototype in New York. CeSSIAM has taken the initiative to speak to a research team at the Otto Beckman Laser Institute at University of

California, Irvine, regarding potential collaboration on a transcutaneous noninvasive near-infrared laser method. They thought that a near-infrared spectrum laser system could potentially measure ZPP and heme to allow for a ratio expression (B. Tromberg and M. Brenner, personal communication, 2010).

An additional consideration to hone in on anemia that is truly of iron deficiency origin, in the absence of a noninvasive light-probe approach to an iron status, would be to accompany an eventually reliable, integument-directed Hb screening method with the measurement of 25-hepcidin, a putative biomarker for iron status (34), in urine samples. This retains the noninvasiveness. Such an approach would also need prospective validation in the field setting; the additional analytical cost and lack of immediacy of the diagnostic result, moreover, could be fundamental caveats to be faced because this notion is initially considered.

Moving the screening agenda forward

From an ethical and public health standpoint, the logic of the Lyon consultancy makes impeccable policy sense. In lieu of a full understanding of the risks involved in exposing children in malarial areas, focusing any oral iron supplementation on children who are most likely to benefit from it without experiencing any adverse risk is a moral and ethical obligation. At the moment, this policy would best be served by effective screening of children for exclusion or inclusion based on knowledge of their hematological, inflammatory, and iron status.

Since the awareness of the urgency of the problem in the wake of the Pemba trial (5), incremental efforts are being made in the domain of field screening. With low-cost field applicability and acceptability to the population as the priority parameters, noninvasive testing of hematological status is currently trying to assume its place in actual screening programs. Despite the limitations of hematological screening, which do not separate nutritional anemia from other causes, devices under development are being used for anemia detection in the broad sense and for research purposes to screen for iron-deficiency anemia. A range of manufacturers have lent their engineering efforts to developing prototype or commercial devices for noninvasive screening of Hb concentrations. The performance of the devices in experimental settings (11) and clinical applications (22) attests to the fundamental soundness of the engineering principles. Going to field sites for public health screening is the current instrumentation challenge.

The fullest available channeling of infants and toddlers most likely to benefit from oral iron supplementation without harm still requires a concurrent and complementary measurement of iron status. The 2 options, ferritin and ZPP, currently would require capillary blood to be minimally invasive. Moreover, organizing the flow of data from the laboratory back to the field to decide on iron supplementation or not is difficult, expensive, and prone to error. The theoretical bases for contact-probe approaches to measure ZPP/Hb ratios across the sublingual or skin surfaces have been set (G. Brittenham, personal communication, 2010; B. Tromberg and M. Brenner, personal communication, 2010), but we cannot yet describe the advances made in the laboratory, much less in the field. Despite the fact that an operative solution to the Lyon consultation mandate (6) is not yet available, an appropriate input of resources and expertise across both the hematological and iron status field screening arenas is required to advance the targeted supplementation agenda.

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