Estimating the prevalence of iron deficiency in the first two years of life: technical and measurement issues

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National-level data on iron deficiency is not available for most countries and many rely on the prevalence of anemia as a proxy estimate, assuming that approximately 50% of anemia cases are caused by iron deficiency. Anemia, however, has multiple causal factors and the risk attributable to any one cause will depend on its relative importance in a population in relation to other causes. The present review summarizes current estimates on the prevalence of iron deficiency and anemia in children younger than 2 years and evaluates the strengths and weaknesses of currently available indicators of iron deficiency in children. Anemia prevalence is insufficient to estimate the prevalence of iron deficiency in children younger than 2 years. The methods widely used to assess iron deficiency at the population level rely on venous blood samples and are complicated and costly to implement.

INTRODUCTION

Anemia is a major public health concern around the world. It is estimated that up to two billion people are anemic, with women and young children at highest risk of adverse health consequences from this condition.[1] It has been estimated that approximately 50% of anemia is due to iron deficiency (ID).[2] Infants and young children are at particular risk for developing ID due to their rapid growth in the first 2 years of life and to the use of complementary foods with low iron content and/or poor bioavailability.

The iron pool of infants is dynamic and difficult to characterize.[3,4] In this early phase of life, growth and cognitive development are progressing rapidly and the danger of prolonged ID is greatest. A growing body of literature shows the negative and possibly irreversible effect that ID in infancy has on mental, behavioral, and motor development.[5–8] The magnitude and consequences of ID warrant interventions in populations at high risk. This highlights the need for accurate, efficient, and economical tools for the identification of iron status in infant populations.

The objective of this review is to summarize current estimates on the prevalence of ID and anemia in children younger than 2 years and to review the strengths and weaknesses of currently available indicators of ID in children.

IDENTIFICATION OF ANEMIA AND IRON DEFICIENCY IN INFANTS

Anemia, diagnosed as a hemoglobin concentration below a given cutoff point (110 g/L for infants), reflects insufficiency in the mass of circulating red blood cells.[1] In individuals, the cause of anemia is identified based on a combination of family history, patient history (i.e., length of gestation, sources of blood loss, or parasitic infection), dietary intake and sources and inhibitors of iron absorption, and biochemical measures of iron status, among other hematological tests.[3,9] If hemoglobin is low and ID is suspected (as a result of biochemical tests and/or patient history), dietary counseling about iron sources and a 2-month course of iron supplementation is recommended. Some recommend that the diagnosis of iron-deficiency anemia (IDA) be confirmed only after

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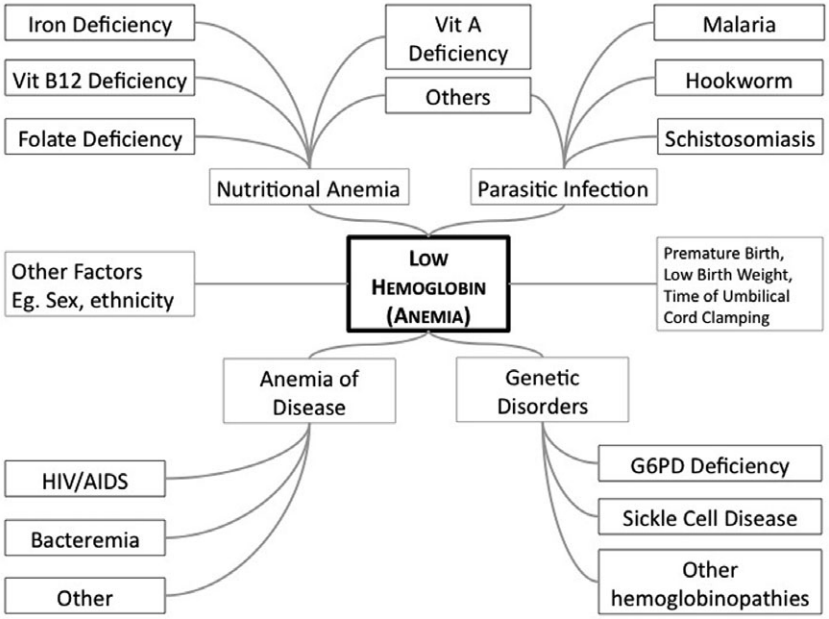

Figure 1 **Determinants of hemoglobin concentration and the risk of anemia in populations.**

treatment for ID is completed and be based on whether the hemoglobin concentration responded to treatment.[9,10] Typically, due to the difficulty associated with interpreting normal values in infants, measures of hemoglobin concentration are not obtained until the age of 9–12 months, or 6 months for at-risk infants (i.e., low-birth-weight and premature infants).[10]

Although thorough, this process is impractical for determining iron status in populations. In many countries and in global databases,[11] anemia prevalence has been used as a proxy measure of ID. In infants between the ages of 6 and 24 months, anemia is determined as a hemoglobin concentration <110 g/L. Anemia is an important indicator of health status (in individuals and populations) but the extent to which it is an appropriate indicator of iron status has been strongly criticized.[12,13] Use of hemoglobin concentration is highly practical and can be assessed immediately in field studies using a single drop of capillary blood, avoiding the need for processing, transportation, and storage of samples. However, many factors other than iron status influence hemoglobin concentration, including, possibly, the source of the blood sample (capillary or venous blood)[14] and the altitude above sea level at which the individual resides; cutoff points also vary by age, sex, and physiological status. Recent estimates suggest that between 22% and 33% of infants and young children in low-income countries have IDA,[15] whereas estimates of the prevalence of anemia range from 5% to over 90% in many of these same countries.[13,16] Many other nutritional and non-nutritional causes of anemia have been identified, including deficiency of other micronutrients (vitamin A, folic acid, vitamin B_{12}), parasitic infection (e.g., malaria, helminth), genetic disorders, and chronic infection and disease, among others (Figure 1).

It has been estimated that 50% of anemia cases are caused by ID.[17] However, the exact origin of this calculation and a clear definition of the assumptions used to generate it are not clear. Furthermore, in populations, the risk attributable to any individual cause of anemia will depend on the prevalence of that cause in relation to others in the population.[18] Given the vast variability in the distribution of malaria, hemoglobinopathies, and other causes among countries, it is likely that this figure is not accurate in all contexts.

For nutritional surveillance data to be useful to countries, reliable estimates of the prevalence of health problems and their underlying direct and indirect causes are required.[19] The prevalence of anemia permits assessment of the burden of disease in populations but it is not an adequate estimate of the extent to which this is due to ID and/or other causes. Therefore, it is also not sufficient to provide the necessary information for the design or evaluation of programs to alleviate the problem. There is a clear need for consensus on which biomarkers are appropriate for measuring the iron status of infants and young children in populations.

BIOMARKERS OF IRON STATUS IN INDIVIDUALS AND POPULATIONS

A wide variety of biomarkers exist for the assessment of iron status as used in a variety of clinical and population-level settings (Table 1). The sensitivity, specificity, and fea-

Table 1 **Biomarkers for the assessment of iron status in individuals and populations**.

Biomarkers not discussed in the present review	Biomarkers discussed in the present review
Bone marrow iron	Hemoglobin
Erythrocyte protoporphyrin	Zinc protoporphyrin
Hematocrit	Total iron-binding capacity
Hepcidin	Transferrin saturation
Mean cell volume	sTfR
Mean cell hemoglobin	Serum ferritin
Non-transferrin-bound iron	Serum or plasma iron
Red cell distribution width	
Reticulocyte hemoglobin concentration	
sTfR/serum ferritin ratio	
Body iron stores	

Abbreviation: sTfR, soluble transferrin receptor.

sibility of each for identifying ID and iron excess vary greatly. This review focuses on the subset of biomarkers used widely in population settings, specifically hemoglobin, serum ferritin (SF), soluble transferrin receptor (sTfR), zinc protoporphyrin (ZPP), and measures used in the calculation of transferrin saturation (TSat) (serum iron and total iron-binding capacity). The application and methodology for the others are reviewed fully in the publications of Gibson[20] and Bowman and Russell.[21]

The recommended biomarkers and their cutoff points for the diagnosis of ID and anemia issued by the World Health Organization (WHO) over the past 50 years are presented in Table 2. With each report, the specific diagnostic criteria recommended for ID and IDA have varied over time. In the 1950s, hemoglobin was identified as a measure of anemia, and the reference values for identifying anemia in infants were similar to those existing today (hemoglobin < 108 g/L).[22] Other early measures were red blood cell count, packed cell volume, and mean corpuscular hemoglobin. In a 2001 publication,[17] serum ferritin and erythrocyte protoporphyrin were identified as indicators for assessing iron status in populations, although the suggested cutoff levels for determining ID using these indicators were modified in 2007.[1] With the advent of immunoassay technology, increasingly complex measures have become more common, including sTfR. Zinc protoporphyrin (ZPP), plasma iron, and transferrin saturation also received attention in this publication.[23] An ongoing review is currently being conducted by the WHO of a number of iron status indicators, with SF, sTfR, and ZPP preliminarily identified for particular attention.[23] The selection of indicators for the assessment of iron status of populations is subject to a number of constraints, including cost, technical considerations (i.e., storage of samples), need for venipuncture, and cultural practices.

Serum ferritin

SF is an iron storage protein and its concentration in serum is a reflection of body iron stores. Since its use began in the 1970s, SF has become one of the most widely used biomarkers of iron status in populations, being used extensively to evaluate the efficacy of interventions to reduce ID.[1,24,25] Even in the first stages of ID, SF reflects

Table 2 **Biomarkers and historically recommended cutoff points for determining the iron status of infants.**

Biomarker	Recommendations from WHO (1959)[22]	Recommendations from UNICEF/UNU/WHO (2001)[17]	Recommendations from WHO/CDC (2007)[1]
Hemoglobin (for identification of anemia)	<108 g/L	<110 g/L	<110 g/L
RBC	4.1 M/μL		
PCV/Hct	<32%	<6.83 mmol/L	
MCH	79 fm		
Serum ferritin		<12 μg/L 30 μg/L*	<5 μg/L to <20 μg/L depending on age
Erythrocyte protoporphyrin		>70 μg/dL RBC >2.6 μg/g Hb >61 mmol/mol heme	>80 μg/dL RBCs >5–10 μg/g Hb
Plasma iron			<50–60 μg/dL
Soluble transferrin receptor			>20 mg/L
Transferrin saturation			<10–15%
Zinc protoporphyrin			>70–80 μg/dL RBCs

* Indicates depleted iron stores in the presence of infection, as measured by CRP or AGP.
Iron deficiency anemia in infants is typically identified as hemoglobin < 110 g/L (anemia) plus iron deficiency identified by abnormal values for two of three used biomarkers.
Abbreviations: Hb, hemoglobin; RBC, red blood cell; PCV/Hct, packed cell volume/hematocrit; MCH, mean cell hemoglobin.

body stores and can predict iron status with high specificity and moderate sensitivity.[20,26,27] SF is elevated in infection and inflammation and values must therefore be interpreted together with markers of acute-phase proteins.[28] In preschool-aged children (6–59 months), the cutoff for a diagnosis of ID is an SF value of <12 μg/L; however, in times of infection, SF values of <30 μg/L can be indicative of ID.[1] C-reactive protein and alpha1-acid glycoprotein are the most common biomarkers of inflammation.[28] However, as Bamberg[4] illustrates, SF is dynamic with a wide range of normal values and unknown laboratory values for infants under 6 months of age with ID (see Bamberg[4] for descriptive table). Thus, even taking into consideration the possibility of altered values due to infection, there is some potential for diagnostic error. The major disadvantage of using SF for population-level measures is the need for a specialized laboratory for processing and the high cost of reagents, estimated in 2007 as approximately 5–10 US dollars per sample; this is in addition to the cost required to then measure acute phase proteins.[1] Furthermore, few laboratories are capable of processing SF on very small serum volumes and venous sampling is usually required.

Soluble transferrin receptor

Transferrin receptor is a membrane-bound protein that is essential for importing circulating transferrin-bound iron into the cell. The serum or soluble transferrin receptor (sTfR) is a truncated version of the cellular transferrin receptor, existing in the blood and detectable in serum using an immunoassay (e.g., ELISA) or immunoturbidometry.[29] sTfR levels increase as iron stores decrease and are less affected by inflammation than SF, reflecting the intensity of erythropoiesis and iron demand.[27] Thus, it has been suggested as an appropriate indicator of iron status in populations with a high risk of infection or inflammation, particularly in conjunction with SF. The disadvantages of using sTfR to determine the iron status of infants are that it indicates ID only when iron stores have been exhausted, and it varies considerably in certain population groups (e.g., it is increased in hemolytic anemia and thalassemia).[30] Additionally, the thresholds for determining ID are unclear for different age ranges within the first 24 months of life.[4] However, Olivares et al.[31] have demonstrated that sTfR may be a more useful indicator than SF for the identification of ID in infants. Furthermore, each method or kit used has individual cutoffs for detection, resulting in complex methods for large population surveys and the potential for lack of standardization. Like SF, sTfR analysis is costly both in terms of laboratory equipment requirements and testing, with each sTfR sample analysis estimated to cost between 10 and 15 US dollars.[1] At this time, sTfR is not recommended for use as the sole biomarker of iron status, thus resulting in additional serum volume and increased costs due to the need for multiple tests.

Zinc protoporphyrin

During normal erythropoiesis, iron is inserted into the porphyrin ring complex of hemoglobin in the final step of synthesis. However, in ID, a zinc ion is alternately incorporated, thereby increasing the concentration of ZPP. The presence of ZPP can be detected using flourimetry techniques and it can be quantified as a risk factor for ID.[1] The use of ZPP detection in field surveys of infants may be beneficial due to its small sample requirements (i.e., a drop) and portable detection tools. However, the poor specificity of ZPP for identifying ID is a significant limitation of this technique. ZPP is elevated in response to lead poisoning, chronic infections, and hemoglobinopathies.[30] Additionally, quantification techniques vary, leading to differing cutoff values indicative of ID and limiting the potential to make comparisons across surveys. Consensus regarding ZPP threshold cutoffs, specifically for infants younger than 2 years, are needed before this biomarker can be used reliably for this group.

Serum iron, total iron-binding capacity, and transferrin saturation

Serum iron and total iron-binding capacity rely on the detection of transferrin, an iron-delivery protein found in plasma. Detection of serum iron measures the amount of ferric iron bound to serum transferrin and is thus decreased in individuals with ID or chronic inflammatory disorders.[1] Serum iron does not detect iron that is contained in hemoglobin and it fluctuates significantly throughout the day and after meals, making it an inaccurate method unless in reference to other measures of iron status.[30] When combined with total iron-binding capacity, a method that utilizes reagent iron to saturate a sample of transferrin, these two indicators can be used to calculate transferrin saturation (TSat). TSat estimates a percentage of occupied iron-binding sites on transferrin. A lower percentage, thus lower TSat value, is indicative of diminished iron status. Like SF and sTfR, most laboratory methods to measure serum iron and total iron-binding capacity require an adequate aliquot of serum, which is usually obtained only from venous samples. This presents important limitations, particularly with respect to infant subjects under 2 years of age.

Total body iron

Total body iron (TBI) is a measure that has been used to estimate the quantity of iron in the body stores of neo-

Table 3 **Estimates of the worldwide prevalence of anemia in children 0–4.99 years of age by WHO region.**

WHO region	Anemia prevalence (1993–2005)	
	Total affected population (in 000's)	Prevalence (%)
Africa	93,200	64.6
Asia	170,000	47.7
Europe	6,100	16.7
LAC	22,300	39.5
North America	800	3.4
Oceania	700	28.0
Overall	293,100	47.4

Data from the World Health Organization[16]
Abbreviations: LAC, Latin America and the Caribbean.

nates at birth. TBI is estimated using hemoglobin concentration and an estimate of body iron stores (usually SF),[32] which provides a more complete picture of iron status than either of the measures individually. Although its application in population-level surveys could be limited, it may be a useful indicator for assessing the impact of intervention programs on iron status. It has been suggested that TBI underestimates the total amount of body iron in infants because the estimated cutoff values have been extrapolated from adult males and limited data is available on standard levels for infants.[30]

TECHNICAL AND CULTURAL CONSIDERATIONS IN SELECTING BIOMARKERS FOR ASSESSING IRON STATUS IN POPULATIONS

When selecting biomarkers for surveys of iron status or the evaluation of interventions in lower-income countries, a number of technical and cultural factors must be taken into consideration. The need for venous blood samples, as required by a number of iron-status indicators, requires cold-chain, adequate storage facilities and standardized and adequately equipped laboratories. Samples should not be collected in locations where minimum quality control criteria for the sampling and the handling and processing of samples cannot be guaranteed. Although obtaining venous blood samples from infants younger than 24 months of age has been deemed a low-risk procedure, it has also been suggested that taking venous blood samples from severely iron-deficient infants and small children may exacerbate the condition.[33,34] In this case, strategies should be in place within a survey to ensure that iron stores can be replenished using appropriate supplements.

Attention to cultural acceptance of public health research techniques is integral to the success of surveillance and evaluation strategies. For example, it has been reported that in many parts of Africa, taking blood samples is a sensitive issue, with some participants reporting skepticism of research motivations and reluctance to participate due to cultural beliefs.[35] In a sample of 23 Malawian mothers, 47% reported that the collection of a 15-mL blood sample from infants was too large of a sample.[36] Although these barriers may be overcome by adequate education and information, local beliefs in relation to blood sampling should be taken into consideration when training field staff to adequately respond to concerns, particularly in situations in which sampling from infants and small children is vital.

REGIONAL WORLDWIDE PREVALENCE OF ANEMIA AND IRON-DEFICIENCY ANEMIA

The WHO collects international data on micronutrient status in the Vitamin and Mineral Nutrition Information System.[11] Currently, data in this system are limited to vitamin A, iodine, and anemia prevalence and detailed nationally representative information on iron status is lacking. In addition, the data are available only at the national level and do not include estimates of within-country variations in prevalence (Table 3).

Examples of estimated prevalence of iron deficiency and anemia from nationally representative surveys in different populations

In recent years, a number of nationally representative micronutrient surveys have been conducted in Latin America and elsewhere. The inclusion of hemoglobin concentrations and measures of iron status in these surveys provides a more accurate estimation of the iron status of populations in these countries, but it also provides an opportunity to review some of the assumptions related to the proportion of anemia that is due to ID. Not only do the results of these surveys reveal the multifaceted nature of anemia among infants, they also serve to illustrate the varied potential impact of targeted programming and the need for relevant biomarkers to guide these decisions.

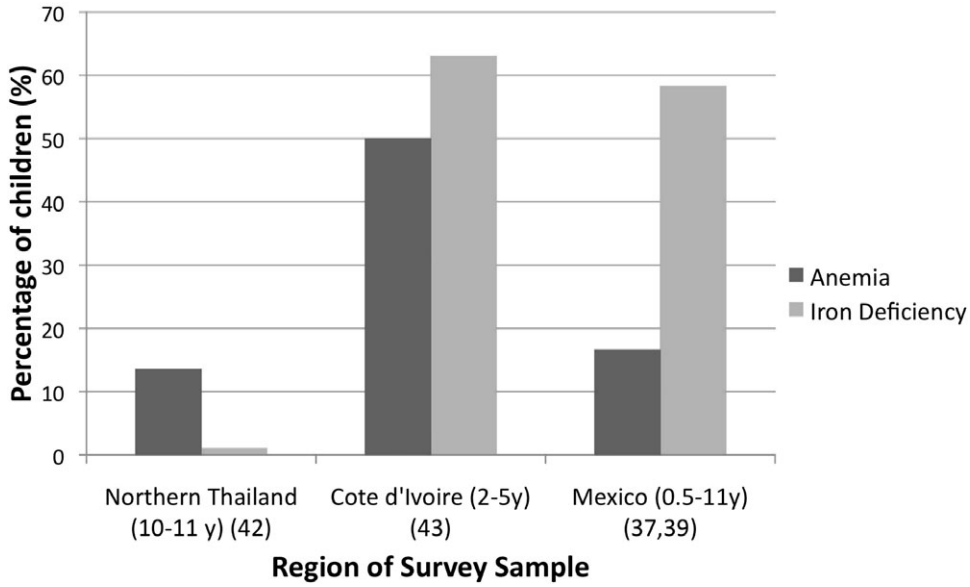

Figure 2 **Prevalence of anemia and iron deficiency in children in varying countries.**

The recent Mexican National Nutrition Surveys (completed 1999 and 2006) provide an opportunity to illustrate the importance of collecting data on both anemia and ID in infants and on the benefits of subnational as well as nationally representative surveys. In 1999, at the national level in Mexico, 13.1% of infants between the ages of 6 and 11 months and 48.9% of infants aged 12–24 months were anemic (hemoglobin < 110 g/L).[37] As has been reported elsewhere,[38] anemia prevalence was more pronounced among children from rural compared to urban areas (52.9% and 46.8%, respectively, in 12–24-month-old infants). Additionally, socioeconomic status, participation in food assistance programs, altitude, and non-indigenous ethnicity were all positively associated with hemoglobin concentration and thus with prevalence of anemia.[39,40] As expected, the prevalence of ID (TSat < 16%) was higher than that of anemia (66.6% in children 12–24 months of age). Like anemia, this prevalence of ID varied regionally (north, central, and south) and between urban and rural communities.

Analyzing data from a larger age range (0.5–11 years), it was found that approximately 62% of cases of anemia were associated with ID, and more than 20% of anemia cases in children were not associated with ID.[41] In children with IDA (low hemoglobin and TSat), 68% had abnormal serum concentrations of other nutrients that were possible contributors to anemia (30% ascorbic acid; 40.6% retinol; 11.7% folate). Additionally, the authors reported that deficiencies in vitamin A and folic acid, especially, were associated with non-ID anemia. An estimate of anemia associated with non-nutritional causes was not presented.

Other settings have presented a similarly complex picture of anemia prevalence in children. For example, a recent report on children in northern Thailand revealed that among a sample of 190 schoolchildren aged 10–11 years, anemia prevalence was 13.5%, with only two cases of ID in the entire sample, both of whom were not anemic (Figure 2).[42] Interestingly, 61.1% of the total population and 88% of anemic children had a thalassemia or other hemoglobinopathy. This is contrasted with 0.5–11-year-old Mexican children among whom ID was apparent in 62.2% of anemic children.[41] A cross-sectional survey of children and adults in Côte d'Ivoire revealed that approximately 50% of preschool children (2–5 years of age) had anemia.[43] A staggering 80% of these study subjects were found to have ID and anemia (Figure 3). In this malaria-endemic setting, over 50% of children had malaria, although malaria incidence was not correlated with iron status. The authors noted that high rates of infection could have influenced laboratory indexes of iron status.

The estimation of anemia prevalence is vital for understanding the health of populations. However, as the above examples illustrate, the assumption that 50% of anemia is due to ID is likely not appropriate in all contexts. Until field-friendly, economical estimates of ID can be developed and tested, a better algorithm for the estimation of anemia due to ID based on the likely contribution of nutritional and other causes is urgently needed.

CONCLUSION

ID and anemia among infants are critical public health issues that warrant the implementation of targeted solu-

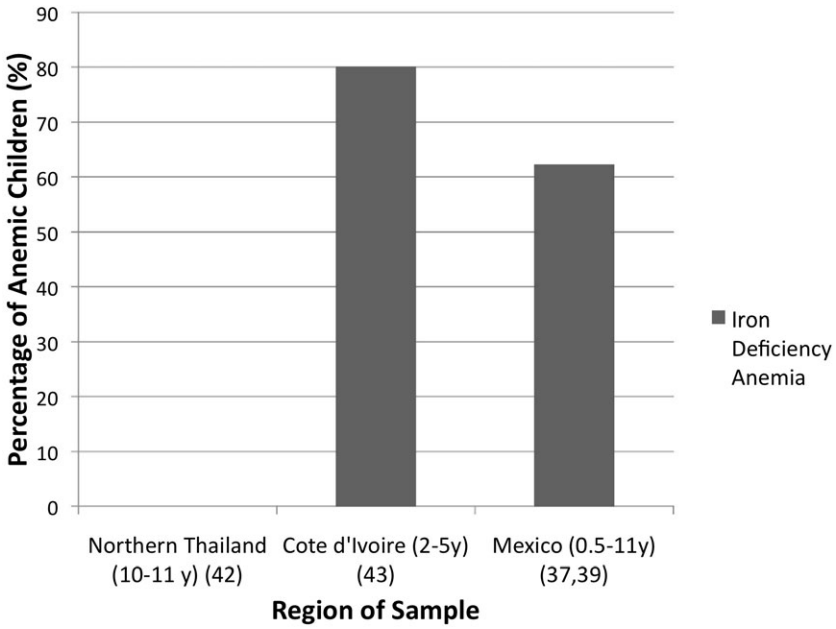

Figure 3 **Proportion of anemia attributed to iron deficiency in varying countries.** Children had anemia (hemoglobin below the appropriate cutoff) plus abnormally low iron status indicator levels.

tions. In low-income countries, approximately one of every two children suffers from anemia. Unless national data on indicators of iron status are available, at this time, estimates of the extent to which anemia is due to iron status are inadequate. ID is one major contributor to anemia around the world, but current methods for assessing the iron status of populations are insufficient. Biomarkers continue to rely on venous blood sampling and are costly and impractical for widespread use in infant populations in many countries.

There have been a number of recent efforts to review and improve the quality of indicators and ensure the appropriate utilization and interpretation of indicators of iron status. In September 2010, the WHO led a review of biochemical indicators used in the assessment of iron and vitamin A status in populations, supported by the Micronutrient Initiative, US Centers for Disease Control, and Prevention and the Government of Luxembourg.[23] The results of this review are expected in the coming months. However, it will take years before these recommendations integrate fully into survey measures and before nationally representative data are collected and reported. Also in 2010, a group of researchers met at the International Atomic Energy Agency to discuss the current knowledge and research needs related to biomarkers, including iron. This initiative, led by the National Institutes of Health with support from the Bill and Melinda Gates Foundation (http://www.iaea.org/NewsCenter/News/2010/biomarkers.html) is an attempt to gain consensus related to the appropriate uses and needs related to biomarkers and to stimulate research to improve them.

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Declaration of interest. The authors have no relevant interests to declare.

REFERENCES

1. World Health Organization/Centers for Disease Control and Prevention. *Assessing the Iron Status of Populations*, 2nd ed. Geneva: World Health Organization; 2007.
2. World Health Organization. *The World Health Report 2002: Reducing Risks, Promoting Healthy Life*. Geneva: World Health Organization; 2002.
3. Baker RD, Greer FR, Committee on Nutrition. Clinical report: Diagnosis and prevention of iron deficiency and iron deficiency anemia in infants and young children (0–3 years of age). Pediatrics. 2010;126:1040–1050.
4. Bamberg R. Occurrence and detection of iron-deficiency anemia in infants and toddlers. Clin Lab Sci. 2008;21:225–231.
5. Lozoff B, Jimenez E, Wolf AW. Long-term developmental outcome of infants with iron deficiency. N Engl J Med. 1991;325:687–694.
6. Lozoff B, Beard J, Connor J, Felt B, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev. 2006;64(Suppl):S34–S91.
7. Beard JL. Why iron deficiency is important in infant development. J Nutr. 2008;138:2534–2536.
8. Collard KJ. Iron homeostasis in the neonate. Pediatrics. 2009;123:1208–1216.
9. Zlotkin S. Clinical nutrition 8: The role of nutrition in the prevention of iron-deficiency anemia in infants, children and adolescents. Can Med Assoc J. 2003;168:59–63.
10. Kazal LA. Prevention of iron deficiency in infants and toddlers. Am Fam Physician. 2002;66:1217–1224.

11. World Health Organization. *Vitamin and Mineral Information System (VMNIS*). 2011. Available at: http://www.who.int/vmnis/en/. Accessed 12 October 2010.
12. Domellof M, Dewey KG, Lonnerdal B, Cohen RJ, Hernell O. The diagnostic criteria for iron deficiency in infants should be reevaluated. J Nutr. 2002;132:3680–3686.
13. Lutter CK. Iron deficiency in young children in low-income countries and new approaches for its prevention. J Nutr. 2008;138:2523–2528.
14. Neufeld LM, Garcia-Guerra A, Sánchez-Francia D, Newton-Sánchez O, Ramírez-Villalobos MD, Rivera-Dommarco J. Hemoglobin measured by Hemocue and reference method in venous and capillary blood: A validation study. Salud Publica Mex. 2002;44:219–227.
15. Lozoff B, Armony-Sivan R, Kaciroti N, Jing Y, Golub M, Jacobson SW. Eye-blinking rates are slower in infants with iron-deficiency anemia than in nonanemic iron-deficient or iron-sufficient infants. J Nutr. 2010;140:1057–1061.
16. World Health Organization. *Worldwide Prevalence of Anemia 1993–2005: WHO Global Database on Anemia*. Geneva: World Health Organization; 2008.
17. World Health Organization. *Iron Deficiency Anemia: Assessment, Prevention, and Control. A Guide for Programme Managers*. Geneva: World Health Organization; 2001:WHO/NHD/01.3.
18. Willett W. *Nutritional Epidemiology*, 2nd ed. New York, NY: Oxford University Press; 1998.
19. Neufeld LM, Tolentina L. Nutritional surveillance – developing countries. In: Caballero B, ed. *Encyclopedia of Human Nutrition*, 2nd ed. Oxford, UK: Elsevier; 2005:371–381.
20. Gibson RS. *Principals of Nutritional Assessment*, 2nd ed. New York, NY: Oxford University Press; 2005.
21. Beard JL. Iron. In: Bowman BA, Russell RM, eds. *Present Knowledge in Nutrition*, Vol I, 9th ed. Washington, DC: International Life Sciences Institute; 2006:430–444.
22. World Health Organization. Technical Report Series No. 182: Iron Deficiency Anemia; Report of a Study Group. Geneva. World Health Organization. 1959;182.
23. World Health Organization. *Nutrition: Call for Public Comments, Scientific Data and Information: Indicators for the Assessment of Vitamin A and Iron Status in Populations in preparation for the WHO Nutrition Guidance Expert Advisory Group Monitoring and Evaluation Subgroup Meeting*. Version Current September 2010. 2010. Available at: http://www.who.int/nutrition/topics/micronutrients_guideline_ME_call_for_comments/en/index.html. Accessed 1 October 2010.
24. Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. Am J Clin Nutr. 1974;27:681–687.
25. Mei Z, Cogswell ME, Parvanta I, et al. Hemoglobin and ferritin are currently the most efficient indicators of population response to iron interventions: An analysis of nine randomized controlled trials. J Nutr. 2005;135:1974–1980.
26. Jeremiah ZA, Buseri FI, Uko EK. Iron deficiency anaemia and evaluation of the utility of iron deficiency indicators among healthy Nigerian children. Hematology. 2007;12:249–253.
27. Biesalski HK, Erhardt JG. Ch. 4: Diagnosis of nutritional anemia – laboratory assessment of iron status. In: Badham J, Zimmermann MB, Kraemer K, eds. *The Guidebook: Nutritional Anemia*. Basel, Switzerland: Sight and Life Press; 2007:15–16.
28. Thurnham DI, McCabe LD, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferretin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: A meta-analysis. Am J Clin Nutr. 2010;92:546–555.
29. Ooi CL, Lepage N, Nieuwenhuys E, Sharma AP, Filler G. Pediatric reference intervals for soluble transferrin receptor and transferrin receptor-ferritin index. World J Pediatr. 2009;5:122–126.
30. Zimmermann M. Methods to assess iron and iodine status. Br J Nutr. 2008;99(Suppl):S2–S9.
31. Olivares M, Walter T, Cook JD, Hertrampf E, Pizarro H. Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy. Am J Clin Nutr. 2000;72:1191–1195.
32. Miller MF, Stoltzfus RJ, Mbuya NV, Malaba LC, Iliff PJ, Humphrey JH, ZVITAMBO Study Group. Total body iron in HIV-positive and HIV-negative Zimbabwean newborns strongly predicts anemia throughout infancy and is predicted by maternal hemoglobin concentration. J Nutr. 2003;133:3461–3468.
33. Janofsky J, Starfield B. Assessment of risk in research on children. J Pediatrics. 1981;98:842–846.
34. Rao R, Georgieff MK. Iron in fetal and neonatal nutrition. Semin Fetal Neonatal Med. 2007;12:54–63.
35. Nuffield Council on Bioethics. Ch 3: Social and cultural issues. In: *The Ethics of Research Related to Healthcare in Developing Countries*. London, UK: Nuffield Council on Bioethics; 2002: 37–43.
36. Corneli AL, Piwoz EG, Bentley ME, et al. Involving communities in the design of clinical trial protocols: The BAN Study in Lilongwe, Malawi. Contemp Clin Trials. 2007;28:59–67.
37. Villalpando S, Garcia-Guerra A, Ramirez-Silva CI, et al. Iron, zinc and iodide status in Mexican children under 12 years and women 12–49 years of age: A probabilistic national survey. Salud Publica Mex. 2003;45(Suppl):S520–S529.
38. Tuyet Mai T, Kim Hung N, Kawakami M, Kawase M, van Chuyen N. Micronutrient status of primary school girls in rural and urban areas of south Vietnam. Asia Pacific J Clin Nutr. 2003;12:178–185.
39. Villalpando S, Shamah-Levy T, Ramírez-Silva CI, Mejía-Rodríguez F, Rivera JA. Prevalence of anemia in children 1 to 12 years of age: Results from a nationwide probabilistic survey in Mexico. Salud Publica Mex. 2003; 45(Suppl):S490–S498.
40. Rivera JA, Monterrubio EA, Gonzalez-Cossio T, Garcia-Feregrino R, Garcia-Guerra A, Sepulved-Amor J. Nutritional status of indigenous children younger than five years of age in Mexico: Results of a national probabilistic survey. Salud Publica Mex. 2003;45(Suppl):S466–S476.
41. Villalpando S, Perez-Exposito AB, Shamah-Levy T, Rivera JA. Distribution of anemia associated with micronutrient deficiencies other than iron in a probabilistic sample of Mexican children. Ann Nutr Metab. 2006;50:506–511.
42. Panomai N, Sanchaisuriya K, Yamsri S, et al. Thalassemia and iron deficiency in a group of northeast Thai school children: Relationship to the occurrence of anemia. Eur J Pediatr. 2010;169:1317–1322.
43. Asobayire FS, Adou P, Davidsson L, Cook JD, Hurrell RF. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Côte d'Ivoire. Am J Clin Nutr. 2001;74:776–782.