

ANNEX 1

Indicators of the iron status of populations: red blood cell parameters

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1. Introduction

Iron is an essential nutrient that plays a central role in many metabolic processes. Aerobic metabolism is critically dependent on maintaining normal concentrations of several iron-containing proteins that mediate oxygen transport, storage and utilization, particularly when the tissue demand for oxygen is increased by physical activity. Pioneering research over the last 50 years, much of it stemming from concepts developed and validated experimentally by Dr Clement Finch and his coworkers, led to the recognition that a negative iron balance resulting from an iron intake insufficient to match losses from the body despite compensatory changes in the rate of absorption and, to a more limited extent, excretion could be divided into three stages based on the severity of the potential effect on physiological functions. The evaluation of functional impairment was related entirely to erythropoiesis for two reasons: the effects of changes in iron status on blood elements are readily evaluated, while the effect on the enzymes in other tissues necessitates obtaining biopsy samples. The red blood cell pool is the largest functional iron compartment in the body. Its requirements therefore have a dominant influence on studies of iron transport and storage. The first stage (iron deficiency) is characterized by the absence of measurable iron stores; the second (iron deficient erythropoiesis) by evidence of a restricted iron supply in the absence of anaemia; and the third (iron deficiency anaemia) by a haemoglobin concentration that falls below the normal threshold for age and sex. The iron indicators that can be used to identify the three stages of iron deficiency are discussed in the other literature reviews in this annex.

WHO recognized the public health importance of nutritional anaemia over 50 years ago (1) and haemoglobin threshold values to classify anaemia were first published in the report of a 1958 WHO Study Group (2). The thresholds were chosen arbitrarily. Revised thresholds were published in 1968 (3) based on a review of five earlier reports. The following text dealing with the recommendations is taken from the 1968 report: "The report (2) of the 1958 WHO Study Group recommended haemoglobin values below which anaemia could be considered to exist. These figures were chosen arbitrarily and it is still not possible to define normality precisely (4). However, more recent data (5–8) indicate that the values given previously should be modified. It is recommended that, in future studies, anaemia should be considered to exist in those whose haemoglobin levels are lower than the figures given below (the values are given in g/100 ml of venous blood of persons residing at sea level):

children aged 6 months to 6 years:	11
children aged 6–14 years:	12
adult males:	13
adult females, non-pregnant:	12
adult females, pregnant:	11 "

Five references were provided by WHO for the more recent data. Four referred to published papers and one to unpublished observations. None of the published references dealt specifically with the development of normal ranges. The first paper (5) described a series of observations in 312 healthy Norwegian men, aged 15–21 years. Capillary blood samples were used. A haemoglobin concentration <130 g/l was observed in 3.5% of the sample. The second paper (6) was an evaluation of venous blood samples from 149 pregnant women and did not provide any specific recommendations. The third paper (7) dealt with a series of experimental observations of venous

blood samples from 82 pregnant women. There were four groups of volunteers: one group served as a control, one received 1000 mg iron intra-muscularly and the remaining two were given a dose of 39 mg oral iron either once or twice a day. The authors suggested that a threshold of 104 g/l should be used to classify anaemia in the last trimester of pregnancy. The fourth paper (8) was a report of 600 men aged 35–64 years and 200 women aged 55–64 years in Wales. Venous blood samples were used. The study contained individuals who responded to iron therapy. No specific recommendations for thresholds for anaemia were given.

The WHO thresholds cited above were used by DeMaeyer and Adiels-Tegman (9) in their landmark paper which is still often quoted as the basis for estimates of the global prevalence of both all anaemia and iron deficiency anaemia. DeMaeyer and Adiels-Tegman recognized the importance of distinguishing between iron deficiency and other causes of anaemia, and proposed deriving the prevalence of iron deficiency anaemia by subtracting the prevalence of anaemia in men (assuming that the prevalence of nutritional iron deficiency in this group would be negligible in most countries) from the prevalence in other groups, thereby deriving the prevalence of iron deficiency anaemia in these groups. By this means they calculated that generally a little less than 50% of the anaemia could be attributed to iron deficiency. They had no way of estimating the prevalence of iron deficiency without anaemia.

The thresholds proposed by WHO in 1968 are, with minor modifications, still regarded as the international standards for evaluating nutritional iron deficiency anaemia. Moreover their validity has been confirmed by analyses of the US Second National Health and Nutrition Examination Survey after excluding subjects with abnormal tests of iron status. The following thresholds are given in the most recent WHO, UNICEF, UNU guide for programme managers (10):

Children 6 months to 59 months	110 g/l
Children 5–11 years	115 g/l
Children 12–14 years	120 g/l
Non-pregnant women (above 15 years of age)	120 g/l
Pregnant women	110 g/l
Men (above 15 years of age)	130 g/l

Most epidemiological surveys in developing countries have focused on women and children. The prevalence of anaemia in men has only been used to evaluate nutritional iron deficiency in a few studies. Iron deficiency anaemia is therefore still generally considered to account for about 50% of the anaemia in surveys that do not include specific measurements of iron status.

2. Relationship between anaemia and iron deficiency

2.1 Physiological control of haemoglobin levels

Haemoglobin concentrations reflect the composite effects of mechanisms that control the sizes of both the red cell mass and the plasma volume. The red cell mass in healthy human beings is maintained by the stimulation of red cell production by the humoral factor erythropoietin. A sensing mechanism within the kidney that is responsive to tissue oxygen concentration controls the release of erythropoietin from renal peritubular cells.

Red cells circulate in the blood for about 90–120 days, necessitating the replacement of about 1% of the red cell mass each day. In normal human beings red cell mass

is controlled by the rate of red cell production, because red cell loss due to senescence is relatively fixed. Red cell mass is increased when oxygen delivery to the blood in the lungs is reduced, such as by high altitude or smoking.

Plasma volume is determined by a complex set of hormonal and vascular factors that control salt and water homeostasis, blood pressure and vascular permeability.

The range of normal haemoglobin concentrations used to evaluate individuals for clinical diagnostic purposes is customarily determined from the distribution of haemoglobin concentrations in healthy population groups. An arbitrary proportion of the healthy population (usually 2.5%) is assumed to fall below the appropriate threshold. The variation in haemoglobin values in healthy human beings is relatively large; in women aged 18–49 years the mean haemoglobin concentration is 140 g/l while the value 2 standard deviations (SD) below the mean is 120 g/l, a difference of about 14%. Table 1 gives the normal mean values and lower limits for a Caucasian population published in a popular current textbook of haematology.

TABLE 1

The mean and lower standard deviation (–2 SD) of normal haemoglobin concentrations (g/l) in a Caucasian population

Age range	Mean	–2 SD
3–6 months	115	95
0.5–2 years	120	110
2–6 years	125	115
6–12 years	135	115
12–18 years females	140	120
12–18 years males	145	130
18–49 years females	140	120
18–49 years males	155	135

Adapted, from Hoffman (11), with permission of the publisher.

2.2 Relationship between iron deficiency and anaemia

Individuals with iron deficiency anaemia are a subset of all the anaemic individuals in a population. They can be identified as iron deficient by using measurements of laboratory indicator(s) that are more specifically influenced by iron status than the haemoglobin concentration. However the proportion of low haemoglobin values is often used alone to predict the prevalence of iron deficiency anaemia. This approach is flawed for several reasons.

There is a significant overlap in the distribution of haemoglobin values of iron sufficient individuals and those with true iron deficiency anaemia, meaning anaemia that responds to treatment with iron. Garby et al. (12) demonstrated this by showing an overlap in the distribution of haematocrit values between iron sufficient and iron deficient individuals (determined by their responses to treatment with iron) in a group of apparently healthy women of childbearing age from Uppsala in Sweden. In this population a woman with a haemoglobin concentration at the WHO threshold of 120 g/l (equivalent to a haematocrit of 36%) would have a probability of only 58.5% of being iron deficient (13). Margolis et al. (14) reported similar results for children. Two other approaches have been used to demonstrate the overlap in the distributions of haemoglobin values between iron deficient and iron sufficient samples of people. Cook et al. (15) estimated the prevalence of anaemia in pregnant and non-pregnant

women in Latin America by resolving the distribution of haemoglobin values in the sample into two Gaussian distributions. Using the WHO threshold for pregnant women of 110 g/l, 23% of the women with anaemia were wrongly classified as normal while 27% of the normal population were misclassified as suffering from anaemia. Anaemia defined by the current WHO criteria was present in 38.5% of pregnant and 17.3% of non-pregnant women. The corresponding values based on an analysis of the frequency distribution were 22% and 12% respectively. Cohen et al. (16) used a similar distribution analysis to identify iron deficiency anaemia in children aged 12–23 months in Guatemala. Dallman et al. (17) employed a “median shift” method to estimate the prevalence of iron deficiency anaemia in the United States of America. All of these examples demonstrate the significant flaw arising from the assumption that individuals with iron deficiency anaemia can be readily identified by applying simple thresholds to the distribution of haemoglobin concentrations.

The value of using haemoglobin measurements to define the prevalence of iron deficiency in healthy population groups is further diminished by the need to make adjustments to the thresholds for individuals who live at high altitudes or smoke regularly (18). There is also convincing evidence that there are small differences in the distributions of haemoglobin values between different ethnic groups. Suggested adjustments to thresholds for the ethnic groups that have been studied are given in Table 2. It is important to note that differences in the distribution of haemoglobin concentrations have only been established for a few ethnic groups.

TABLE 2

Suggested adjustments for several ethnic groups to the thresholds of haemoglobin concentration used by WHO to define anaemia

Ethnic Group	Suggested adjustment (g/l)	References
African Americans	-10.0	(18)
East Asian Americans	0	(18)
Hispanic Americans	0	(18)
Japanese Americans	0	(18)
American Indians	0	(18)
Jamaican girls (13–14 yrs)	-10.7	(19)
Indonesians from West Indonesia	0	(20)
Thais	0	(21)
Vietnamese	-10.0	(22)
Greenland men	-8.0	(23)
Greenland women	-6.0	

Several other factors limit the value of anaemia as a means to diagnose iron deficiency. Iron deficiency is not the only cause of anaemia and in some circumstances it may not be the most common cause. Vitamin A deficiency is probably the second most frequent nutritional cause of mild anaemia, while deficiencies of folic acid, vitamin B₁₂ and possibly riboflavin may also cause anaemia. Inflammatory and infectious diseases including malaria are undoubtedly as common as iron deficiency as a cause of anaemia in many developing countries. Anaemia may also result from inherited or acquired conditions that affect red cell production. People who are carriers of alpha- or beta-thalassaemia are the most likely to be included in surveys. Both carrier states may cause a mild microcytic anaemia. Finally, there may be seasonal variations in haemoglobin concentration: variation was reported in a cross-sectional

study of men in Israel (24,25). Small variations may also occur during the menstrual cycle with mean values 3 g/l lower during menses than during the luteal phase (26).

The prevalence of iron deficiency without anaemia can only be inferred if specific iron indicators are not included in epidemiological surveys. Iron deficiency may have functional consequences. An effect of iron deficiency without anaemia on the cognitive and motor development of children has not been established although there is considerable experimental evidence to support the contention that the functional consequences result from tissue iron deficiency within the brain rather than from the accompanying anaemia (27). Physical performance is known to be affected by iron deficiency in the absence of anaemia. The maximum oxygen consumption was reduced in iron deficient women without anaemia when compared with an iron sufficient control group (28). Aerobic adaptation was improved by the administration of iron supplements to trained and untrained women who were not anaemic (29,30). Although functional consequences in the absence of anaemia may not have been established for the effects of iron deficiency other than on physical performance, it is important to emphasize again the difficulty of identifying anaemia in some individuals because of the overlap in the distribution of haemoglobin concentrations between iron deficient and iron sufficient people. Furthermore individuals whose iron intake is marginal may or may not be anaemic at different times because of small changes in iron intake or bioavailability.

3. Red blood cell parameters

3.1 Haemoglobin

The measurement of haemoglobin concentration and other red blood cell parameters is well standardized. Laboratories that use particle counting and sizing equipment have access to expert technical support and quality control procedures. However, practical considerations will limit the use of such equipment, particularly during field studies in remote regions, because it may be difficult to take blood samples to a laboratory on the day of collection. It is therefore appropriate to focus on the use of instruments such as the Hemocue (Hemocue AB, Ängelholm, Sweden). It is possible to get highly reproducible results when the Hemocue is used to measure the haemoglobin concentrations of venous blood samples, but there may be considerable variability when the Hemocue is used to measure the haemoglobin concentrations of capillary blood samples. This variability is caused by poor capillary blood sampling techniques. The appropriate use of the Hemocue and capillary sampling techniques is currently a very important issue that is dealt with in a recent manual published by Helen Keller International (31). A manual from the International Nutritional Anemia Consultative Group on "Measurements of Iron Status" (32) also provides useful information.

3.2 Hematocrit or packed cell volume

The measurement of hematocrit does not supply any information about anaemia that cannot be obtained from measuring the haemoglobin concentration. The hematocrit is a derived value on particle counters. Values measured directly by centrifuging blood samples in the field tend to have poor reproducibility unless a stable power supply can be assured and the equipment is well standardized.

3.3 Mean cell volume and mean cell haemoglobin

Mean cell volume (MCV) and mean cell haemoglobin (MCH) provide identical information and are interchangeable in terms of their value in identifying nutritional iron deficiency. MCV is the value that has been used most widely for the evaluation of nutritional iron deficiency.

A low MCV is not specific to iron deficiency. Low values are encountered in thalassaemia (2 or 3 gene deletions for alpha thalassaemia, beta thalassaemia including heterozygotes) and in about 50% of people with anaemia due to inflammation.

Reliable measurements of MCV require a particle counter and are therefore unlikely to provide an advantage over specific measurements of iron status in terms of cost and technical support.

3.4 Red cell distribution width

The initial enthusiasm for the use of red cell distribution width (RDW) to distinguish between iron deficiency anaemia and the anaemia of inflammatory disorders has not been supported by subsequent studies (33). Measurements of RDW are made using a particle counter.

3.5 Reticulocyte haemoglobin concentration and percentage of hypochromic red cells

Reticulocyte haemoglobin concentration has been shown to be a reliable method for identifying iron deficiency and has been recommended for the evaluation of the adequacy of the iron supply in patients undergoing dialysis for renal failure who are receiving erythropoietin (34–38). The measurement has been compared to the “gold standard” for iron deficiency, which is stainable bone marrow iron, and found to be reliable. While it promises to be a useful tool for evaluating iron supply even in clinically complex settings, it currently requires access to a specific brand of flow cytometer. It is therefore unlikely to be helpful in developing countries. Moreover reticulocyte haemoglobin concentrations seem to be unsuitable for evaluating iron status in alpha and beta thalassaemias, including carrier states.

4. Role of haemoglobin as a screening indicator for iron deficiency

It is generally assumed that worldwide “at least half the anaemia is due to nutritional iron deficiency” (39) and that “up to a prevalence of iron deficiency anaemia of 40%, the prevalence of iron deficiency will be about 2.5 times that of anaemia” (10). However, it is evident from the foregoing discussion that there are considerable variations in both of these ratios depending on the age and sex of the people being studied, the region of the world in which they live, and the prevalence rates of other causes of anaemia. The prevalence of anaemia alone can therefore give only a very rough estimate of the likely prevalence of iron deficiency anaemia. Attempts to analyse the variation in the relationship between prevalence rates for iron deficiency and anaemia are confounded by the absence of a consistent standard for identifying iron deficiency. There are very few studies that have used bone marrow iron, which is clearly too invasive an indicator to be applied routinely in field studies. Various combinations of iron indicators have been used, but the thresholds for iron deficiency vary.

For example: the threshold at which ferritin indicates iron deficiency may be set as high as 30 µg/l in some developing countries because of the presence of infectious diseases (40,41); thresholds for zinc protoporphyrin vary between 40 and 70 µmol/mol haem depending on whether the cells have been washed before the assay or not (42,43); and there is a lack of standardization between different commercial kits for measuring the concentration of transferrin receptor.

The response of the haemoglobin concentration to treatment has long been the primary indicator for evaluating the efficacy or effectiveness of intervention programs. It remains a satisfactory indicator for this purpose, but lacks sensitivity when the prevalence of anaemia is low. Furthermore, if there is a significant residual prevalence of anaemia at the end of the intervention (perhaps despite a considerable reduction when compared with the baseline values) it may be impossible to determine whether the intervention was suboptimal or not as the residual anaemia may be unrelated to iron deficiency. Despite the drawbacks outlined above it has been suggested that the prevalence of anaemia may be the best epidemiological indicator of iron status in infants and young children because of the poor performance of the other currently available indicators (14).

5. Conclusions

1. Anaemia is an inevitable consequence of iron deficiency of sufficient severity.
2. Nutritional iron deficiency anaemia is a mild anaemia because iron loss is reduced to some extent in iron deficiency (44).
3. A significant overlap in the distributions of haemoglobin concentration of iron deficient and iron sufficient individuals has been demonstrated in children, non-pregnant women, and during pregnancy.
4. A low haemoglobin concentration is not a specific indicator of iron deficiency anaemia. Other epidemiologically important causative factors include: infectious and inflammatory disorders, the prevalence of genes for thalassaemia, vitamin A deficiency, and deficiencies of folic acid and vitamin B₁₂ in certain settings.
5. The current WHO thresholds provide the best separation between iron deficient and iron sufficient individuals in a population. They should be adjusted for altitude, smoking and, where information is available, ethnic origin.
6. Haemoglobin concentration can be used as an initial screening indicator. However, because of variations in the relationships between iron deficiency, iron deficiency anaemia and anaemia from other causes, the prevalence of iron deficiency cannot be predicted with any degree of accuracy. Further evaluation using specific iron indicators is necessary. It may be possible to agree on an anaemia prevalence below which further evaluation for iron deficiency would not be warranted in certain age or sex groups.
7. The change in the haemoglobin concentration and the prevalence of anaemia can be used to provide a qualitative or a semi-quantitative assessment of the efficacy of intervention strategies, but the residual prevalence of iron deficiency cannot be predicted.
8. Anaemia is a useful clinical and experimental criterion for defining the severity of iron deficiency. It may be less useful in epidemiological surveys. Consideration should be given to developing a new set of criteria, based on specific laboratory indicators of iron status, for defining the severity of iron deficiency in epidemiological surveys.

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