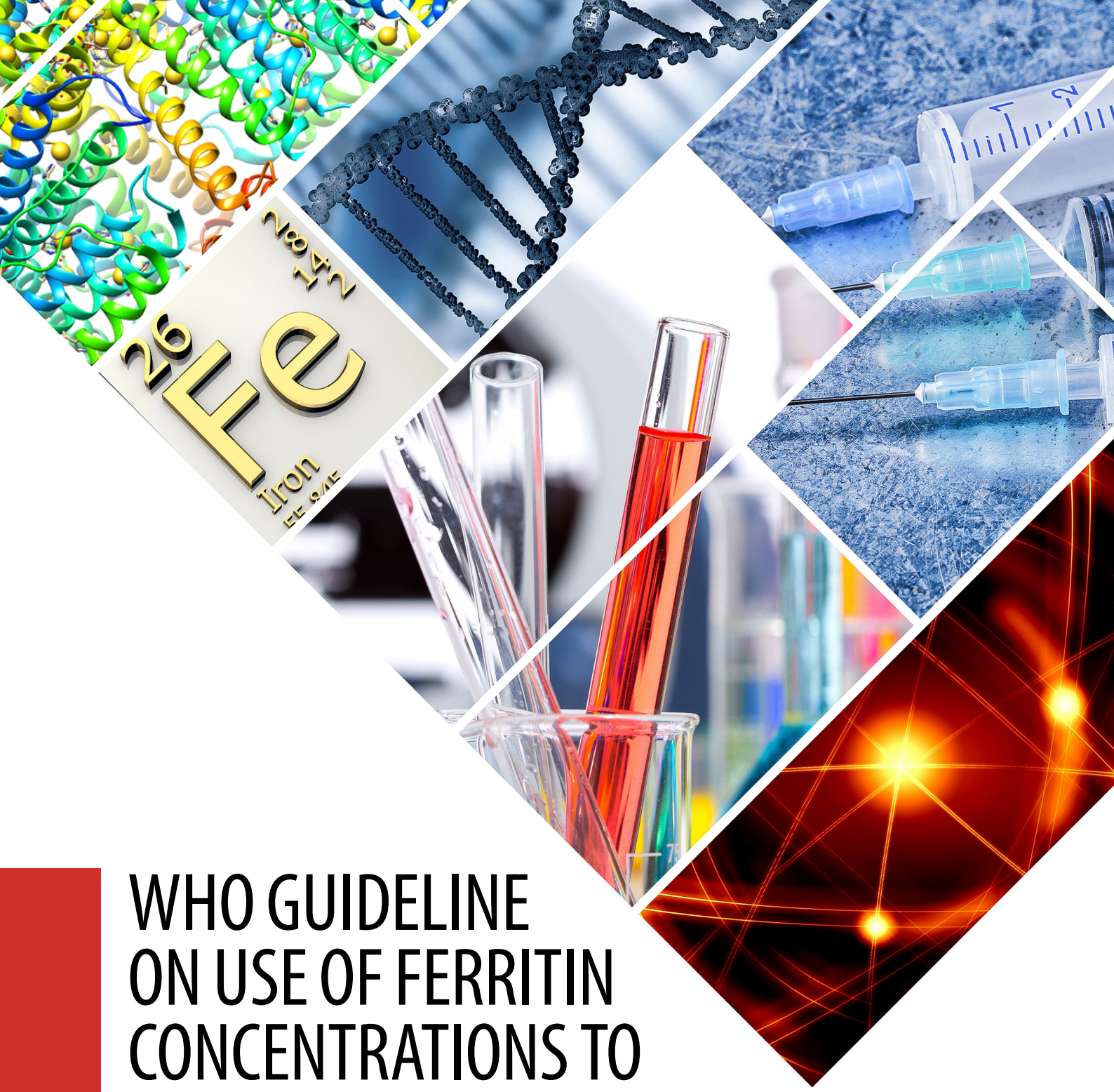

WHO GUIDELINE ON USE OF FERRITIN CONCENTRATIONS TO ASSESS IRON STATUS IN INDIVIDUALS AND POPULATIONS

World Health Organization

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CONTENTS

PUBLICATION HISTORY vii

ACKNOWLEDGEMENTS vii

Financial support vii

EXECUTIVE SUMMARY viii

Purpose of the guideline viii

Guideline development methodology viii

Available evidence ix

Recommendations and remarks ix

QUESTION 1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)? ix

QUESTION 2. Is ferritin an adequate marker for assessing the impact of iron interventions? xi

QUESTION 3. How should ferritin be measured? xi

QUESTION 4. Should ferritin be measured in combination with indicator(s) of infection or inflammation? xii

QUESTION 5. What are the population prevalence ranges for determining a public health problem? xiii

Research gaps xiv

Plans for updating the guideline xiv

INTRODUCTION 1

Objectives 2

Scope 2

Population of interest 2

Priority questions 2

Outcomes of interest 3

Target audience 3

BACKGROUND 5

Iron deficiency and iron overload 6

Iron deficiency 6

Iron overload 6

Assessment of iron status 7

Ferritin 7

Ferritin cut-off values 8

Clinical pathways for iron deficiency and overload 8

Ferritin assays 10

History of the project on the use of ferritin for the assessment of iron status 11

Meetings on the ferritin project 11

Why is it important for WHO to develop this guideline? 13

EVIDENCE AND RECOMMENDATIONS 15

QUESTION 1. **Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)?** 16

Summary of evidence 16

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations 17

Recommendations 18

Rationale 18

Remarks 19

QUESTION 2. **Is ferritin an adequate marker for assessing the impact of iron interventions?** 20

Summary of evidence 20

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations 21

Recommendations 21

Remarks 22

QUESTION 3. **How should ferritin be measured?** 22

Summary of evidence 22

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations 23

Recommendations 24

Remarks 24

QUESTION 4. **Should ferritin be measured in combination with indicator(s) of infection or inflammation?** 25

Summary of evidence 25

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations 25

Recommendations 26

Remarks 26

QUESTION 5. **What are the population prevalence ranges for determining a public health problem?** 27

Summary of evidence 27

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations 28

Remarks 28

IMPLEMENTATION OF THE GUIDELINE 30

Implementation considerations 30

Regulatory considerations 30

Ethical and equity considerations 31

Monitoring and evaluation of guideline implementation 31

RESEARCH GAPS 32

GUIDELINE DEVELOPMENT PROCESS 32

WHO steering group 33

Guideline development group 33

External resource persons 34

Systematic review teams 34

Management of conflicts of interests 34

Identification of priority questions and outcomes 34

Evidence identification and retrieval 35

Quality assessment and grading of evidence 35

Formulation of recommendations 36

Decision-making during the guideline development group meeting 37

Document preparation and peer review 37

DISSEMINATION AND PLANS FOR UPDATING 38

Dissemination 38

Plans for updating the guideline 38

REFERENCES 39

ANNEX 1. QUESTIONS ON THE USE OF FERRITIN TO ASSESS THE IRON STATUS OF POPULATIONS IN POPULATION, INTERVENTION, CONTROL, OUTCOMES (PICO) FORMAT 48

QUESTION 1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)? 48

QUESTION 2. Is ferritin an adequate marker for assessing the impact of iron interventions? 48

QUESTION 3. How should ferritin be measured? 49

QUESTION 4. Should ferritin be measured in combination with indicator(s) of infection or inflammation? 50

QUESTION 5. What are the population prevalence ranges for determining a public health problem? 50

ANNEX 2. GRADE SUMMARY OF FINDINGS TABLES 51

A. QUESTION 1. Estimates of the accuracy of serum ferritin to assess iron deficiency in apparently healthy individuals 51

B. QUESTION 1. Estimates of the accuracy of serum ferritin to assess iron deficiency in non-healthy individuals 52

C. QUESTION 1. Estimates of the accuracy of serum ferritin to assess iron overload in non-healthy in non-healthy individuals 53

ANNEX 3. WHO STEERING GROUP 54

ANNEX 4. WHO GUIDELINE DEVELOPMENT GROUP 55

ANNEX 5. EXTERNAL RESOURCE PERSONS 57

ANNEX 6. SYSTEMATIC REVIEW TEAMS 58

QUESTION 1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)? 58

QUESTION 2. Is ferritin an adequate marker for assessing the impact of iron interventions? 58

QUESTION 3. How should ferritin be measured? 59

QUESTION 4. Should ferritin be measured in combination with indicator(s) of infection or inflammation? 59

QUESTION 5. What are the population prevalence ranges for determining a public health problem? 59

ANNEX 7. PEER-REVIEW 60

ANNEX 8. WHO SECRETARIAT 61

WHO Regional and Country Office 62

PUBLICATION HISTORY

This *WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations* is an update of, and supersedes, previous recommendations in the World Health Organization (WHO)/Centers for Disease Control and Prevention (CDC) publication, *Assessing the iron status of population*, first published in 2004), and recommendations related to ferritin in *Iron deficiency anaemia: assessment, prevention and control, a guide for programme managers* (2001).

In order to produce this guideline, the rigorous procedures described in the *WHO handbook for guideline development* were followed. This document presents the direct and indirect evidence, as well as the qualitative reviews that served to inform the recommendations herein.

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WHO GUIDELINE[1]: USE OF FERRITIN CONCENTRATIONS TO ASSESS IRON STATUS IN INDIVIDUALS AND POPULATIONS

EXECUTIVE SUMMARY

Accurate determination of iron status is crucial for diagnostic and screening purposes in the clinical setting and to guide public health interventions at the population level. In an individual patient, diagnosis of iron deficiency or overload will help guide management, including further investigations and appropriate therapy. At the population level, determination of the magnitude and distribution of iron deficiency can help to prioritize appropriate interventions in settings in which the prevalence is regarded as a severe public health problem, or help to identify populations with hereditary conditions that predispose them to iron overload.

Ferritin is an iron-storage protein present in all cells and can be measured in serum, plasma, liver, red blood cells, and other specimens. Low ferritin concentrations suggest deficient iron stores, whereas elevated ferritin concentrations could suggest iron overload.

Purpose of the guideline

This guideline provides global, evidence-informed recommendations on the use of indicators for assessing a population's iron status and application of the use of ferritin concentrations for monitoring and evaluating iron interventions.

The recommendations in this guideline are intended for a wide audience, including health professionals, clinicians, researchers, managers of nutrition and health programmes, and public health policy-makers, their expert advisers, and technical and programme staff at government institutions and organizations involved in the design and conduct of surveys to assess micronutrient status in all settings.

This guideline aims to help WHO Member States and their partners to make evidence-informed decisions on the appropriate actions in their efforts to achieve the Sustainable Development Goals,[2] and the global targets as put forward in the *Comprehensive implementation plan on maternal, infant and young child nutrition*[3] and the *Global strategy for women's, children's and adolescents' health (2016–2030)*.[4]

Guideline development methodology

WHO developed the present evidence-informed recommendations using the procedures outlined in the *WHO handbook for guideline development*.[5] The steps in this process included: (i) identification of priority questions and outcomes; (ii) retrieval of the evidence; (iii) assessment and synthesis of the evidence; (iv) formulation of recommendations, including research priorities; and planning for (v) dissemination; (vi) implementation, equity and ethical considerations; and (vii) impact evaluation and updating of the guideline. The Grading of Recommendations Assessment, Development and Evaluation (GRADE)[6] methodology was followed to prepare evidence profiles related to preselected topics, based on up-to-date systematic reviews.

The initial scoping of the guideline and the prioritization of the outcomes was done during a meeting on *Priorities in the assessment of vitamin A and iron status in populations*,[7] from 15 to 17 September 2010, in Panama City, Panama and finalized by the guideline development group during a technical meeting held in Atlanta, United States of America from 3 to 5 March 2014. The development and finalization of the evidence-informed recommendations were done by the guideline development group in a meeting held in Geneva, Switzerland, from 15 to 17 June 2016.

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1 This publication is a World Health Organization (WHO) guideline. A WHO guideline is any document, whatever its title, containing WHO recommendations about health interventions, whether they be clinical, public health or policy interventions. A standard guideline is produced in response to a request for guidance in relation to a change in practice, or controversy in a single clinical or policy area, and is not expected to cover the full scope of the condition or public health problem. A recommendation provides information about what policy-makers, health-care providers or patients should do. It implies a choice between different interventions that have an impact on health and that have ramifications for the use of resources. All publications containing WHO recommendations are approved by the WHO Guidelines Review Committee.

2 United Nations Sustainable Development Knowledge Platform. Sustainable Development Goals (https://sustainabledevelopment.un.org/sdgs).

3 Resolution WHA65.6. Comprehensive implementation plan on maternal, infant and young child nutrition. In: Sixty-fifth World Health Assembly, Geneva, 21–26 May 2012. Resolutions and decisions, annexes. Geneva: World Health Organization; 2012:12–13 (WHA65/2012/REC/1; http://www.who.int/nutrition/topics/WHA65.6_resolution_en.pdf).

4 The global strategy for women's, children's and adolescents' health (2016–2023). Survive, thrive transform. Geneva: World Health Organization; 2015 (http://www.who.int/life-course/partners/global-strategy/global-strategy-2016–2030/en/).

5 WHO handbook for guideline development, 2nd ed. Geneva: World Health Organization; 2014 (http://www.who.int/kms/handbook_2nd_ed.pdf?ua=1).

6 GRADE (http://www.gradeworkinggroup.org/).

7 Report: priorities in the assessment of vitamin A and iron status and in populations, Panama City, Panama, 15–17 September 2010. Geneva: World Health Organization; 2012 (https://apps.who.int/iris/bitstream/handle/10665/75334/9789241504225_eng.pdf?sequence=1&isAllowed=y).

Available evidence

The following key questions were posed, based on the policy and programme guidance needs of Member States and their partners. The population, indicator, comparator, outcomes (PICO) format was used, when appropriate.

1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)?
2. Is ferritin an adequate marker for assessing the impact of iron interventions?
3. How should ferritin be measured?
4. Should ferritin be measured in combination with indicator(s) of infection or inflammation?
5. What are the population prevalence ranges for determining a public health problem?

The available evidence for the five questions included: one systematic review that followed the procedures of the *Cochrane handbook for Diagnostic Test Accuracy review*,[1] eight non-Cochrane systematic reviews, two data meta-analyses and two database analyses , as well as one non-Cochrane systematic review submitted for publication. The overall certainty of the available evidence was low to very low for the critical outcomes.[2]

Recommendations and remarks

To ensure that the recommendations are correctly understood and applied in practice, guideline users may want to also refer to the remarks, as well as to the evidence summary, including the considerations on implementation.

This *WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations* is an update of, and supersedes, previous recommendations in the WHO/Centers for Disease Control and Prevention (CDC) publication, *Assessing the iron status of populations*, first published in 2004, and recommendations related to ferritin in *Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers* (2001).

An evidence to decision-making framework was used to lead discussion and decision-making. This included the following considerations: (i) the certainty of the evidence across outcomes critical to decision-making; (ii) the balance of benefits and harms; (iii) values and preferences related to the recommended intervention in different settings and for different stakeholders, including the populations at risk; (iv) the acceptability of the intervention among key stakeholders; (v) resource implications for programme managers; (vi) equity; and (vii) the feasibility of implementation of the intervention.

Question 1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)?

Recommendations

1.1. Ferritin concentration is a good marker of iron stores and should be used to diagnose iron deficiency in otherwise apparently healthy **individuals**[3] (*strong recommendation*,[4] *low certainty of evidence*).

1.2. In **individuals** with infection or inflammation, a ferritin concentration below 30 µg/L in children and 70 µg/L in adults may be used to indicate iron deficiency (*conditional recommendation*,[5] *low certainty of evidence*). In **populations** it is also possible to adjust ferritin values for infection/inflammation by applying correction factors as described for Question 4: Should ferritin be measured in combination with indicator(s) of infection or inflammation?

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[1] Cochrane Methods Screening and Diagnostic Tests. Cochrane handbook for Diagnostic Test Accuracy (DTA) reviews (https://methods.cochrane.org/sdt/handbook-dta-reviews).

[2] According to GRADE, high-certainty evidence indicates that we are very confident that the true effect lies close to that of the estimate of the effect. Moderate-certainty evidence indicates that we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low-certainty evidence indicates that our confidence in the effect estimate is limited: the true effect may be substantially different form the estimate of the effect. Very-low-certainty evidence indicates that we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[3] For the purposes of this guideline, an apparently healthy individual is defined as an individual with physical well-being for their age and physiological status, without detectable diseases or infirmities.

[4] A strong recommendation is one for which the guideline development group is confident that the desirable effects of adherence outweigh the undesirable effects. Implications of a strong recommendation are that most people in these settings would desire the recommended intervention and only a small proportion would not. For policy-makers, a strong recommendation indicates that the recommendation can be adopted as policy in most situations.

[5] A conditional recommendation is one for which the guideline development group concludes that the desirable effects of adherence probably outweigh the undesirable effects, although the trade-offs are uncertain. Implications of a conditional recommendation for populations are that while many people would desire the intervention, a considerable proportion would not. With regard to policy-makers, a conditional recommendation means that there is a need for substantial debate and involvement from stakeholders before considering the adoption of the intervention in each setting.

1.3. A ferritin concentration exceeding 150 µg/L in menstruating women and 200 µg/L in men and non-menstruating women who are otherwise healthy may indicate a risk of iron overload (*conditional recommendation,* based on previous WHO recommendation). In adult, non-healthy **individuals**, a ferritin concentration exceeding 500 µg/L may indicate risk of iron overload (*conditional recommendation, very low certainty of evidence*).

1.4. Ferritin concentration should not be used alone to identify risk of iron overload. Patients with elevated ferritin levels should receive clinical and laboratory evaluation to establish the underlying cause (*strong recommendation, very low certainty of evidence*).

Rationale

The available studies were not sufficient to justify a change in current ferritin cut-off values to define iron deficiency and risk of iron overload by sex or age groups. The recommended cut-off values for ferritin concentrations to define iron deficiency, including previous recommendations and new evidence when available, are presented in Table 1.

Table 1. Recommended cut-off values to define iron deficiency and risk of iron overload in apparently healthy and non-healthy individuals by age group

	Serum ferritin (µg/L)[a,b]			
	Iron deficiency		Risk of iron overload	
	Apparently healthy individuals[c]	Individuals with infection or inflammation	Apparently healthy individuals	Non- healthy individuals
Infants and young children (0–23 months)	<12	<30	—	—
Children under 5 years (24–59 months)	<12	<30	—	—
Children (5 to less than 10 years)	<15	<70	>150 females >200 males	>500[d]
Adolescents (10 to less than 20 years)	<15	<70	>150 females >200 males	>500
Adults (20–59 years)	<15	<70	>150 females >200 males	>500
Older persons (60+ years)	<15	<70	>150 females >200 males	>500
Pregnant women	<15 (first trimester)[e]	—	—	—

[a] From previous WHO recommendations and new evidence.

[b] Markers of inflammation should be assessed along with the ferritin concentration, and ferritin adjusted as necessary.

[c] For the purposes of this guideline, an apparently healthy individual is defined as an individual with physical well-being for their age and physiological status, without detectable diseases or infirmities.

[d] In adult, non-healthy populations, a ferritin concentration exceeding 500 µg/L may indicate risk of iron overload or other disease. This cut-off value indicates the need for further clinical and laboratory evaluation to establish the diagnosis and underlying cause of the ferritin levels

[e] There are several physiological changes occurring in pregnancy that may contribute to the variation in thresholds of iron deficiency in pregnancy as defined by serum ferritin, including a physiological rise in acute phase proteins secondary to pregnancy; second trimester plasma volume expansion; and changes in inflammatory measures in the final trimester of pregnancy.

Remarks

- In the absence of inflammation, the concentration of plasma/serum ferritin is positively correlated with the size of the total body iron stores. Ferritin levels are low in iron-deficient individuals and high in iron-loaded individuals.
- In populations, ferritin testing to ascertain the prevalence of iron deficiency or to determine the risk of iron overload is usually performed along with haemoglobin testing to assess the prevalence of anaemia. Measures of inflammation (e.g. C-reactive protein [CRP] and/or α-1 acid glycoprotein [AGP]) and additional iron indices, such as soluble transferrin receptor, are commonly used.

- The physiological changes occurring in hormones, blood composition and haemodynamics, as well as in inflammatory status during pregnancy, render it difficult to establish a fixed, unique ferritin concentration to define iron deficiency, especially when comparing to an invasive gold standard test such as bone marrow biopsy.
- Ferritin may be elevated due to iron overload or other causes, including liver disease, obesity, inflammation and malignancy. In cases of risk of iron overload, ferritin concentration only indicates the possibility of iron overload and the need for further assessment of the specific diagnosis, and the severity of the problem.
- Liver biopsies have commonly been used to report iron overload, because the liver is the dominant iron-storage organ, liver iron concentration correlates closely with the total iron balance, and the liver is the only organ in which the iron concentration is elevated in all forms of systemic iron overload. Non-invasive methods such as magnetic resonance imaging and computerized tomography are widely used to assess iron content in the liver.

Question 2. Is ferritin an adequate marker for assessing the impact of iron interventions?

Recommendation

2.1. Ferritin concentration increases in response to iron-related interventions and may be used to monitor and assess the impact of interventions on iron status (*strong recommendation, moderate certainty of evidence*).

Remarks

- Iron interventions should be implemented in such a manner as to enable monitoring to be undertaken with the lowest number of blood draws.
- The interval following initiation of an iron intervention at which ferritin should be measured depends on the type of intervention and the amount of iron provided.
- Comprehensive planning, monitoring and evaluation of all simultaneous interventions that increase iron intake and/or utilization and/or reduce iron losses are required to account for the total amount of iron being received by populations that would result in ferritin changes in cases of iron deficiency, and to avoid risk of iron overload.
- Knowledge of the prevalence of infection/inflammation is critical for interpretation of ferritin concentrations in population surveys and to interpret changes after iron interventions.
- The inclusion of markers to diagnose iron-related genetic disorders is valuable, especially in regions where thalassaemias and other haemoglobinopathies are common.
- Cases of iron overload should be treated at individual level, since high ferritin concentrations are not sensitive to the effects of nutrition interventions.
- More research is needed to evaluate the effect of nutrition interventions on ferritin concentration through the life-cycle, especially during pregnancy, owing to changes in concentration, especially the typical decrease in concentration in late pregnancy.

Question 3. How should ferritin be measured?

Recommendations

3.1. Ferritin may be measured using radiometric, nonradiometric and agglutination assays. One method does not appear to be superior to another and all methods are acceptable if a commutable material traceable to the WHO international reference standard is used to calibrate the assay. Once a method has been selected, that same method should be used for the follow-up of individuals and populations (*strong recommendation, moderate/low certainty of evidence*).

3.2. Use of the WHO international reference standard of ferritin is recommended for calibration of all commercial kits and in regular laboratory practice, especially when following up individual cases, for population surveys or to measure the impact of public health interventions (*strong recommendation, moderate certainty of evidence*).

Remarks

- The risks of radioactive contamination and the high cost of equipment are important drawbacks of radiometric assays.
- For follow-up of individuals and populations, the same method for ferritin determination should be used, to minimize variability. It is also important to control other sources of error in laboratory testing related to handling of samples; transport and storage conditions; the use of manual versus automated procedures; and differences in equipment performance and those inherent to the operator.
- Ferritin may be measured in either serum, plasma or other biological fluids, but the same sample matrix should be used when measuring the impact of interventions in individuals and at the population level.
- International reference materials for ferritin have been developed for calibrating working standards in the routine ongoing assays performed in laboratories and also for evaluating and standardizing new tests for quantification of ferritin. A WHO international standard of ferritin from the National Institute for Biological Standards and Control, WHO International Laboratory for Biological Standards, United Kingdom of Great Britain and Northern Ireland (NIBSC code 94/572), is commercially available and recommended for use with all assays.
- It is important that reference materials are commutable and traceable to the WHO reference standard, so the results are equivalent among procedures and to avoid calibration bias.
- Quality controls should be included with every run, or at least daily, on instruments measuring ferritin. The inclusion of quality controls of low, medium and high ferritin concentrations is desirable.
- Laboratories performing ferritin determinations for patient care or for public health assessments should participate in external quality assurance programmes.

Question 4. Should ferritin be measured in combination with indicator(s) of infection or inflammation?

Recommendations

4.1. In areas of widespread infection or inflammation, serum ferritin should be assessed with the concurrent measurement of two acute phase response proteins, CRP and AGP (*strong recommendation, moderate certainty of evidence*).

4.2. The increase in ferritin values caused by inflammation should be accounted for in **individuals** and **populations**. One method is to raise the cut-off value that defines deficiency, to 30 µg/L or 70 µg/L, depending on the age group (see Table 1). Another method is to exclude individuals with elevated concentrations of CRP or AGP from prevalence calculations based on ferritin. Alternatively, arithmetic or regression correction approaches may be used to adjust ferritin concentrations for inflammation and apply the cut-off points recommended for healthy populations. The adjustment that best suits the country reality should be selected and used as long as those conditions prevail (*strong recommendation, moderate certainty of evidence*).

Remarks

- The need for and magnitude of infection/inflammation correction depends on the population group, geographic region and other factors.
- The application of different adjustment approaches will result in a high degree of variability in the estimated prevalence of depleted iron stores. The selected adjustment based on country conditions should be used as long as those conditions prevail.
- Determination of both CRP and AGP concentrations may be important because they reflect different phases of the acute phase response that range from acute infection to chronic inflammation.

- Possible adjustments include the following:
    - the higher ferritin cut-off adjustment approach uses a higher ferritin-concentration cut-off value for individuals with infection/inflammation, e.g. <30 µg/L;
    - the exclusion approach uses the inflammation, malaria-biomarker information, or both, to exclude individuals with elevated inflammation (as defined by a CRP concentration >5 mg/L, AGP concentration >1 g/L, or both) or individuals with malaria infection;
    - the arithmetic correction factor approach applies an arithmetic correction factor by grouping inflammation into groups, e.g. (i) reference (both CRP concentration <5 mg/L and AGP concentration <1 g/L); (ii) incubation (CRP concentration >5 mg/L and AGP concentration <1 g/L); (iii) early convalescence (both CRP concentration >5 mg/L and AGP concentration >1 g/L); and (iv) late convalescence (CRP concentration <5 mg/L and AGP concentration >1 g/L);
    - the regression correction approach uses linear regression to adjust ferritin concentrations by the CRP and AGP concentrations on a continuous scale, and malaria infection as a dichotomous variable. The adjusted ferritin equation is calculated by subtracting the influence of CRP, AGP and malaria as follows:

$$\text{Ferritin}_{\text{adjusted}} = \text{ferritin}_{\text{unadjusted}} - \beta_1(\text{CRP}_{\text{obs}} - \text{CRP}_{\text{ref}}) - \beta_2(\text{AGP}_{\text{obs}} - \text{AGP}_{\text{ref}}) - \beta_3\,\text{malaria}$$

where β_1 is the CRP regression coefficient, β_2 is the AGP regression coefficient, β_3 malaria is the malaria regression coefficient, obs is the observed value, and ref is the external reference value generated to define low inflammation.

Question 5. What are the population prevalence ranges for determining a public health problem?

Remarks

- Owing to the scarcity and dispersion of data, it was not possible to make a recommendation for population prevalence ranges to define the magnitude of iron deficiency as a public health problem using ferritin concentrations.
- The population prevalence ranges established for determining the magnitude of anaemia as a public health problem could be suitable as a guide to determine the prevalence ranges for defining the severity of iron deficiency as a public health problem based on adjusted ferritin concentrations. To classify iron deficiency as severe, moderate, mild or no public health problem (measured by ferritin concentration below the recommended cut-off values), the prevalence of iron deficiency could be ≥40.0%, 20.0–39.9%, 5.0–19.9% or ≤4.9%, respectively (see Table 2).
- Initiating iron interventions in populations with a mild, moderate and/or severe prevalence of iron deficiency could help prevent anaemia, as well as adverse consequences of iron deficiency without anaemia.

Table 2. Population prevalence ranges to define the magnitude of iron deficiency as a public health problem using ferritin concentrations

Magnitude of the public health problem	Prevalence range (%)
High	≥40.0
Moderate	20.0–39.9
Mild	5.0–19.9
No public health problem	≤4.9

Research gaps

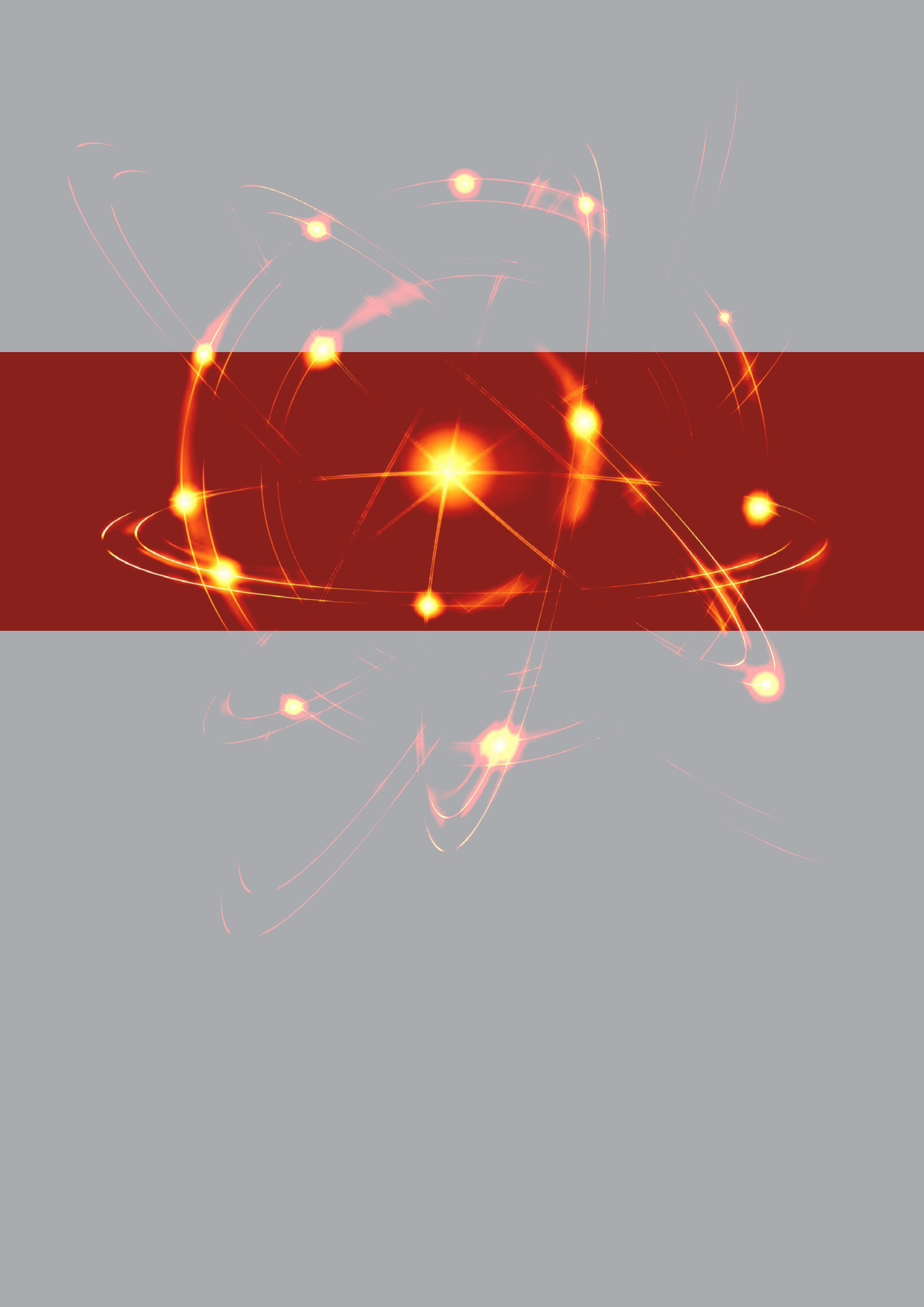
Discussions between the members of the WHO guideline development group and the external resource group highlighted the limited evidence available in some knowledge areas, meriting further research, particularly in the following areas:

- studies comparing ferritin concentrations and bone marrow iron content in healthy, non-infected/non-inflammed populations of all ages;
- studies comparing ferritin and other iron status indicator concentrations and bone marrow iron content in the presence of biomarkers of inflammation, to validate the different approaches to adjusting iron status for inflammation;
- well-designed, population-based longitudinal studies to define the role of other iron indicators and their response to iron interventions, which can be used to monitor the impact of public health programmes;
- studies that clarify cut-off points for iron deficiency and the differential diagnoses when chronic or low-degree inflammation is present in older persons and conditions such as obesity or controlled diabetes;
- changes affecting ferritin concentrations during pregnancy and establishment of cut-off points by trimester;
- factors affecting ferritin levels (fasting, inflammation, infection, liver disase, comorbidities) in the general population, stratified by age, sex and physiological status, living in different settings (malaria, tuberculosis and HIV);
- studies on the utility of ferritin concentrations to diagnose the risk of iron overload and to establish cut-off values for all age groups and settings; and
- the comparability of ferritin concentrations among sample matrices: venous blood collection versus pooled capillary samples or dried serum spot samples.

Plans for updating the guideline

The WHO Secretariat will continue to follow research developments on iron deficiency, particularly on biomarkers to detect it, with emphasis on ferritin determination and cut-off values to define iron deficiency and iron overload in different settings and age groups, and on the influence of infection/inflammation on diagnosis of iron status, particularly for questions in which the certainty of evidence was found to be low or very low. If the guideline merits an update, or if there are concerns about the validity of the guideline, the Department of Nutrition and Food Safety will coordinate the guideline update, following the formal procedures of the *WHO handbook for guideline development*.

As the guideline nears the 10-year review period, the Department of Nutrition and Food Safety at the WHO headquarters in Geneva, Switzerland, along with its internal partners, will be responsible for conducting a search for new evidence.



INTRODUCTION

WHO GUIDELINE[1]: USE OF FERRITIN CONCENTRATIONS TO ASSESS IRON STATUS IN INDIVIDUALS AND POPULATIONS

INTRODUCTION

Objectives

This guideline provides global, evidence-informed recommendations on the use of indicators for assessing a population's iron status and application of the use of ferritin concentrations for monitoring and evaluating iron interventions.

This guideline is intended to contribute to discussions among stakeholders when selecting or prioritizing indicators for use in assessing micronutrient status. This document presents the key recommendations and a summary of the supporting evidence.

It is not intended as a comprehensive operational manual on the assessment of iron status or an implementation tool for the conduct of a micronutrient survey.

Scope

This *WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations* is an update of, and supersedes, previous recommendations in the WHO/United States Centers for Disease Control and Prevention (CDC) publication, *Assessing the iron status of populations (1),* first published in 2004, and recommendations related to ferritin in *Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers* (2001) *(2).*

This guideline aims to help WHO Member States and their partners to make evidence-informed decisions on the appropriate use of indicators for assessing a population's iron status and application of the use of ferritin concentrations for monitoring and evaluating iron intervention programmes, in their efforts to achieve the Sustainable Development Goals *(3)* and the global targets as put forward in the *Comprehensive implementation plan on maternal, infant and young child nutrition (4),* endorsed by the Sixty-fifth World Health Assembly in 2012, in resolution WHA65.6, and the *Global strategy for women's, children's and adolescents' health (2016–2030) (5).*

Population of interest

The guideline will affect all individuals and population groups at the public health level.

Priority questions

The following key questions were posed, based on the policy and programme guidance needs of Member States and their partners. The population, indicator, comparator, outcomes (PICO) format was used, when appropriate.

1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)?
2. Is ferritin an adequate marker for assessing the impact of iron interventions?
3. How should ferritin be measured?
4. Should ferritin be measured in combination with indicator(s) of infection or inflammation?
5. What are the population prevalence rangesfor determining a public health problem?

[1] This publication is a World Health Organization (WHO) guideline. A WHO guideline is any document, whatever its title, containing WHO recommendations about health interventions, whether they be clinical, public health or policy interventions. A standard guideline is produced in response to a request for guidance in relation to a change in practice, or controversy in a single clinical or policy area, and is not expected to cover the full scope of the condition or public health problem. A recommendation provides information about what policy-makers, health-care providers or patients should do. It implies a choice between different interventions that have an impact on health and that have ramifications for the use of resources. All publications containing WHO recommendations are approved by the WHO Guidelines Review Committee.

Outcomes of interest

The outcomes of interest considered critical for decision-making included the following:

- accurate diagnosis of the risk of iron deficiency (using <12 µg/L, <15 µg/L, <30 µg/L and other commonly used cut-off values) (question 1);
- accurate diagnosis of the risk of iron overload (question 1);
- change in ferritin concentrations with iron interventions (question 2);
- change in the prevalence of iron deficiency, iron deficiency anaemia or iron overload with iron interventions (as defined by trialist) (question 2);
- the sensitivity of ferritin methods (question 3);
- the specificity of ferritin methods (question 3);
- the predictive value of ferritin methods (question 3);
- the cost/financial feasibility of ferritin methods (question 3);
- the limit of detection of ferritin methods (question 3);
- the relationship between acute phase proteins and ferritin concentrations (question 4);
- the median difference in the percentage change in prevalence of iron deficiency with different correction factors (question 4);
- the range of prevalence of deficiency when applying different correction factors for inflammation compared to no correction (question 4);
- the validity of different correction factors (question 4);
- the precision of different correction factors (question 4);
- the feasibility of different correction factors (question 4); and
- correlation coefficients of ferritin concentrations with measures of country/individual development, for determining the magnitude of iron deficiency as a public health problem (question 5).

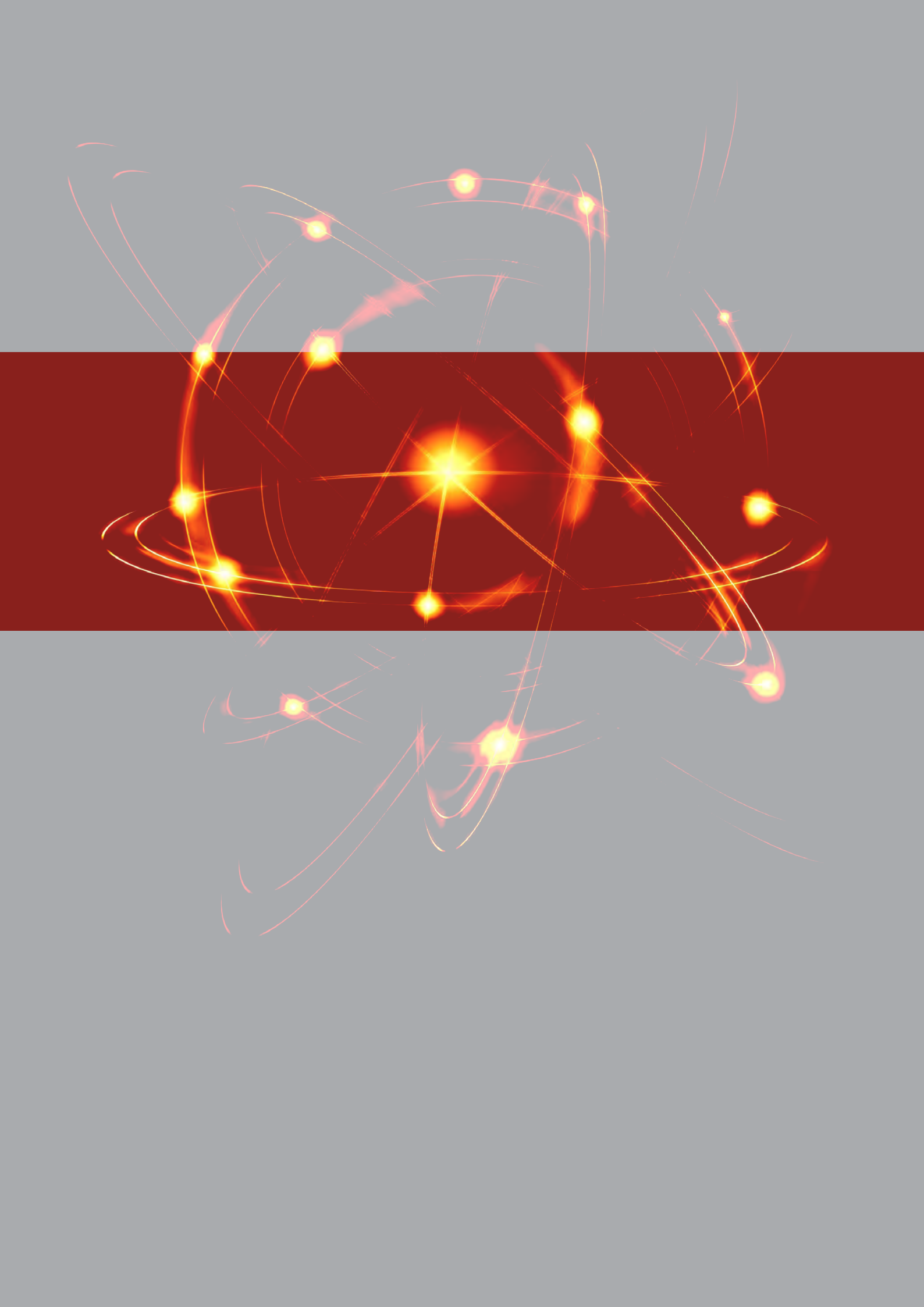
The key questions and outcomes guiding the evidence review and synthesis for the recommendations in this guideline are listed in Annex 1.

Target audience

The recommendations in this guideline are intended for a wide audience, including health professionals, clinicians, researchers, managers of nutrition and health programmes, and public health policy-makers, their expert advisers, and technical and programme staff at government institutions and organizations involved in the design and conduct of surveys to assess micronutrient status in all settings.

The end-users of this guideline are:

- national and local policy-makers;
- implementers and managers of national and local nutrition programmes;
- nongovernmental and other organizations and professional societies involved in the planning and management of nutrition actions and the surveillance of nutrition status; and
- health professionals, including clinicians, researchers, managers of nutrition and health programmes and public health policy-makers in all settings.



BACKGROUND

BACKGROUND

Iron deficiency and iron overload

Iron is an essential element with important functions such as oxygen transport, DNA synthesis and muscle metabolism *(6, 7)*. Iron deficiency is the main cause of anaemia, which is the most prevalent nutritional deficiency worldwide, affecting 29% of non-pregnant women, 38% of pregnant women, and 43% of children worldwide *(8)*. On the other hand, iron overload (i.e. accumulation of iron in the body from any cause) is generally the result of disorders such as hereditary haemochromatosis, thalassaemias, repeated blood transfusions or other conditions that affect iron absorption or regulation and can also have important deleterious consequences on health if left untreated *(9)*. Owing to its high reactivity, iron is always bound to a protein, depending on its role and location in the body. Iron circulates in the plasma bound to transferrin, is stored in cells as ferritin, and functions as part of haemoglobin or myoglobin molecules.

Iron deficiency

Iron deficiency exists when body iron stores are inadequate to meet the needs for metabolism. Progressive iron deficiency can result in iron-deficient erythropoiesis (formation of red blood cells) and, eventually, iron deficiency anaemia *(10)*. However, even in the absence of anaemia, iron deficiency appears to be associated with clinical signs and symptoms including fatigue *(11)*, impaired physical performance *(12)*, decreased work productivity *(13)* and suboptimal brain development *(14)*. Iron deficiency may result from physiological, environmental, pathological, drug-related, genetic or iron-restricted erythropoietic causes *(15)*. Iron deficiency may be caused by inadequate iron intake, excess iron (i.e. blood) loss, or excess iron utilization. Inadequate iron intake may result from a diet that is poor in iron, and/or that contains iron in a biologically inaccessible form. Iron may also fail to be absorbed in individuals with intestinal disorders such as coeliac disease, and perhaps *Helicobacter pylori* infection *(16)*. Inflammation can also impair iron absorption, which may mediate iron deficiency in athletes *(17)*. The most common cause of blood loss is menstruation, which is the primary reason that iron deficiency is more common in women. In low-income settings, other important causes include chronic blood loss from hookworm and schistosomiasis. In all settings, blood donation *(18)* and bleeding from intestinal lesions *(19)* must be considered. Iron requirements are increased during growth (especially in infants and children under 5 years of age) and adolescence, while iron requirements during pregnancy are increased due to iron needs for maternal and fetal erythropoiesis and fetal growth.

When considering these factors, it is not surprising that iron deficiency and iron deficiency anaemia are most common in preschool-age children and women of reproductive age, and that, overall, iron deficiency is most common in low-income settings where dietary iron content and availability are low and parasitic infections are highly prevalent. It is estimated that approximately 33% of the world's population has anaemia, with iron deficiency considered to be the leading cause, and that anaemia accounts for almost 9% of the world's years lived with a disability burden *(20)*. It has also been estimated that, worldwide, 273 million preschool-age children have anaemia (43% of all children, 42% of cases responsive to iron); 32 million pregnant women have anaemia (38% of all pregnant women, 50% of cases responsive to iron); and 496 million non-pregnant women have anaemia (29% of all non-pregnant women, 50% of cases responsive to iron). Anaemia is most prevalent in central and west Africa and south Asia *(8)*.

Iron overload

Because elemental iron is toxic to the body (due to its propensity to initiate redox reactions and generate free radicals, causing tissue damage), it must be chaperoned and stored in the body by binding proteins (i.e. transferrin, ferritin). There is no physiological mechanism to excrete iron, hence homeostatic regulation of iron stores is entirely mediated by changes in iron absorption (mainly via modulation of the hepatic hormone, hepcidin) *(9)*. Iron overload results from excess iron absorption, generally caused by autosomal dominant genetic conditions (hereditary haemochromatosis, caused chiefly by mutations in the *HFE* gene, but also less commonly by mutations in genes that encode haemojuvelin [*HJV*], transferrin receptor 2 [*TFR2*] and ferroportin [*SLC40A1*]); conditions associated with ineffective erythropoiesis (for example thalassaemia intermedia and haemoglobin E-beta thalassaemia); and iron accumulation from repeated red cell transfusions, usually to treat inherited (e.g. thalassaemia and other congenital conditions) or acquired (e.g. aplastic anaemia, myelodysplasia) anaemia *(21, 22)*.

Over time, iron overload results in excess iron accumulation in organs, especially the liver (resulting in cirrhosis, liver failure and hepatocellular carcinoma), endocrine organs (causing pituitary and gonadal failure), pancreas

(causing diabetes), skin (causing pigmentation) and heart (resulting in cardiomyopathy, heart failure and arrhythmia). Untreated, most patients with severe iron overload succumb to cardiac or hepatic complications. Thus, early diagnosis and non-invasive tests for monitoring treatment are essential to optimal management of iron overload *(23)*. The global burden of iron overload is uncertain and varies by population (ethnicity, age and sex), as well as the methodology used for estimation (screening with ferritin, transferrin saturation or genetic testing). The prevalence of hereditary haemochromatosis in Caucasian populations has been estimated to be 3.5 to 4.5 per 1000 population with clinical expression, being greater in males than females *(24, 25)*. Annually and worldwide, approximately 21 000 children are born with haemoglobin E-beta thalassaemia (about half of whom are transfusion dependent) and approximately 23 000 are born with thalassaemia major; a further 14 000 are born with haemoglobin H disease. Thus, genetic conditions associated with risk of iron overload are prevalent conditions worldwide *(26)*.

Assessment of iron status

Indicators for the study of iron status in populations are important for determining its magnitude and distribution, for deciding on intervention options, and for monitoring and evaluating the impact of implemented public health programmes. The strategy may include one or more direct or indirect interventions affecting iron status, such as nutrition education or counselling; universal or targeted provision of iron supplements; point-of-use fortification of foods with micronutrient powders containing iron; fortification of staple foods or condiments with iron and other micronutrients; deworming; and water, sanitation and hygiene.

The assessment of iron status is not precise, since proteins reflect the status of different compartments in the body. For example, measurement of serum ferritin assesses storage iron *(27–32),* while measurements of serum iron and the percentage of transferrin saturation reflect the iron supply to tissues. Serum transferrin receptor (sTfR), erythrocyte ferritin and red cell zinc protoporphyrin are indicators of the iron supply to bone marrow. The use of iron by the bone marrow can be assessed by the percentage of hypochromic red blood cells, mean corpuscular volume and reticulocyte haemoglobin content. Risk of iron overload is usually studied by liver biopsy or magnetic resonance imaging (MRI).

As these biomarkers are affected by other conditions such as age, sex, disease, smoking, infection and inflammation, it may be difficult to identify a unique indicator of iron status.

Ferritin

Ferritin is the primary iron-storage protein and is critical to iron homeostasis *(33)*. The ferritin molecule is an intracellular hollow protein shell, composed of 24 subunits surrounding an iron core that may contain as many as 4000–4500 iron atoms. In the body, small amounts of ferritin are secreted into the blood circulation. In the absence of inflammation, the concentration of this plasma (or serum) ferritin is positively correlated with the size of the total body iron stores *(10, 33, 34)*. A low serum ferritin concentration reflects depleted iron stores, but not necessarily the severity of the depletion as it progresses *(34)*.

To determine its usefulness to detect low iron reserves or iron deficiency in populations, the concentration of ferritin can be compared to iron contained in the bone marrow *(35)*. The absence of stainable iron on a bone marrow aspirate that contains spicules is diagnostic of iron deficiency. In some studies, however, bone marrow aspirates have failed to detect iron deficiency, suggesting methodological and interpretation limitations *(36, 37)*. Although bone marrow is the appropriate tissue to assess iron deposits, aspirations or biopsies are invasive and costly procedures that are not free of methodological difficulties. For these reasons, they have been largely replaced by other determinations such as ferritin, serum iron and total iron-binding capacity to diagnose iron deficiency *(38)*.

On the other side of the spectrum, liver biopsies have commonly been used to detect iron overload, because the liver is the dominant iron-storage organ; liver iron concentration correlates closely with the total iron balance; and the liver is the only organ in which the iron concentration is elevated in all forms of systemic iron overload *(39)*. Non-invasive methods such as MRI have become established in diagnosis and quantitation of iron overload. Other methods, including superconducting quantum interference device biomagnetic susceptometry (SQUID) and computerized tomography (CT), are being used to assess iron content in the liver. An advantage of MRI over other methods is that it includes a low variability between measures and can detect the iron load in the liver, heart and endocrine tissues *(40–42)*.

Ferritin cut-off values

Definitive identification of iron status for a patient requires invasive or expensive studies and is generally not available in public health or primary care settings. Iron deficiency is considered to exist when bone marrow iron staining is absent, but bone marrow aspiration cannot be routinely used in the population health context and is often unacceptable to patients in routine clinical practice. Iron overload can be assessed by histological assessment of accumulation of iron in tissues (usually the liver), or measurement of tissue iron content, either directly (i.e. by biochemical assessment of biopsied liver samples) or indirectly with MRI. These investigations can only be done in selected patients and the determination of iron status in patients or populations therefore relies on measurement of indices in peripheral blood *(43)*.

Appropriate cut-off values for ferritin need to be characterized to define pathology (for both iron deficiency and iron overload). However, ferritin concentrations are also raised in inflammation with or without infection, liver disease, obesity, and some rare haematological conditions. Inflammation can distort interpretation of ferritin concentrations, obscure the diagnosis of iron deficiency and be misleading in the diagnosis of iron overload *(44)*. Clinical or biochemical assessment for concomitant inflammation is therefore essential, but optimal adjustments of ferritin measures to account for inflammation remain uncertain *(43)*.

The existing cut-off points for serum ferritin as a measure of iron stores have been summarized by WHO *(34)*. Although widely implemented and cited, these cut-off values are based on qualitative expert opinion and not a systematic appraisal of the published literature *(45)*, and have not been universally adopted. A review of the literature revealed use of a broad range of cut-off values and approaches for obtaining those values *(44)*.

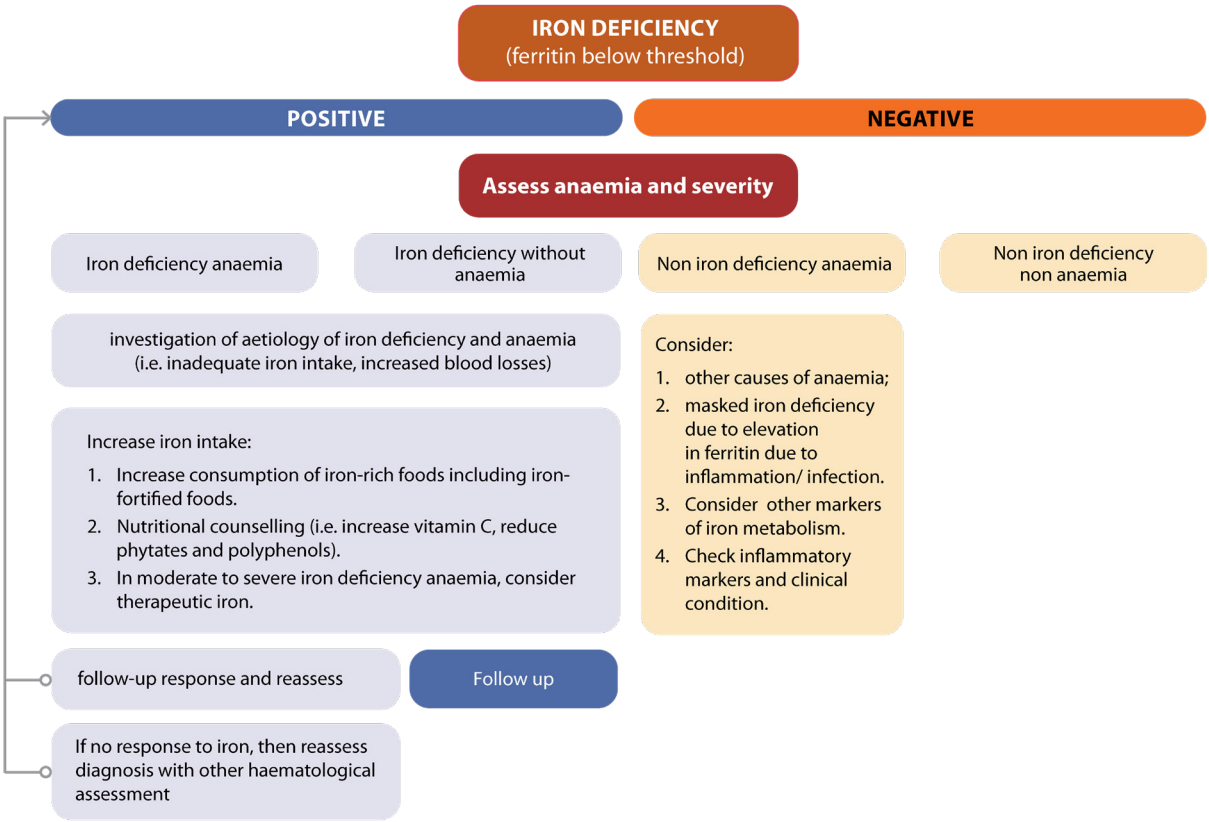
Selection of appropriate cut-off values for an index that yields continuous data to indicate the dichotomous presence or absence of disease necessitates trade-offs between sensitivity and specificity. The optimal cut-off point depends on the task of the test: for example, for a screening test, or when the outcome of a false-positive result is not severe, a cut-off value that provides a higher sensitivity would be appropriate. Conversely, if a false-positive outcome puts the patient or population at risk, a cut-off value with higher specificity is preferable. The diagnostic properties of selected thresholds are useful to the end-user to enable appropriate interpretation *(43)*.

Clinical pathways for iron deficiency and overload

Evaluation of iron status may be performed clinically, for individual patients, or across a population. Measurement of iron status in individuals is important to correctly define iron status and provide appropriate treatment for iron deficiency; to prompt further testing and management if iron overload is suspected; and to monitor interventions for both iron deficiency and iron overload. Measurement of iron status in populations is important to determine the prevalence and distribution of iron deficiency and risk of iron overload in the population, and thus to decide appropriate interventions, and to monitor and evaluate the impact and safety of implemented public health programmes *(46)*.

The risks of a missed case of iron deficiency in infants and young children may portend irreversible consequences of iron deficiency and anaemia in this critical period. A false-negative test in adulthood may leave fatigue, lethargy and reduced exercise performance untreated; impaired pregnancy outcomes (anaemia, reduced birth weight, reduced gestation) in pregnant women unresolved; and underlying potentially serious causes of iron deficiency undetected. A misdiagnosed case of iron deficiency in a non-iron-deficient individual would cause unnecessary side-effects from iron therapy (e.g. constipation, abdominal pain, metallic taste); potential increased risk of infection; or a risk of toxicity in overdose. In the case of a negative test for iron deficiency with anaemia, it would be critical to search for other causes of anaemia and consider masked iron deficiency due to elevation in ferritin as a result of infection/inflammation. In addition to inflammatory markers, the clinician should also consider other markers of iron metabolism and clinical conditions. A clinical pathway for investigation of iron deficiency is proposed in Fig. 1.

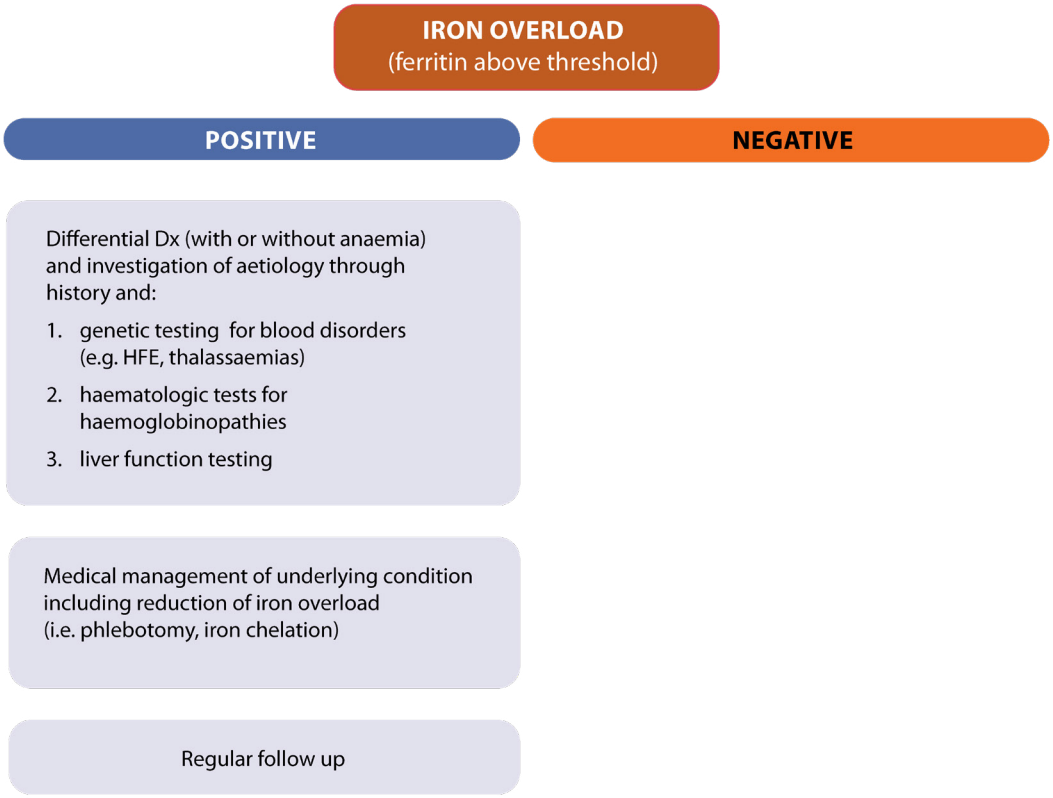
Fig. 1. Clinical pathway for iron deficiency



Source: Garcia-Casal MN, Pasricha S-R, Martinez RX, Lopez-Perez L, Peña-Rosas JP. Serum or plasma ferritin concentration as an index of iron deficiency and overload. Cochrane Database Syst Rev. 2015;(7):CD011817. doi:10.1002/14651858.CD011817 *(46)*.

For iron overload, a positive ferritin test (above a determined threshold) would trigger additional testing to assess differential diagnoses (with or without anaemia), with investigation of aetiology through history and genetic testing for blood disorders (e.g. the *HFE* gene, thalassaemia); haematological tests for haemoglobinopathies; and liver function testing. After confirmation of the diagnosis of iron overload, medical management of the underlying condition, including reduction of iron overload (i.e. phlebotomy, iron chelation), would follow. Misdiagnosis of iron overload would be a missed opportunity to treat iron overload (liver disease, cardiomyopathy, diabetes, pituitary failure, hypothyroidism, arthropathy), as well as a missed opportunity to detect affected family members with similar conditions. False-positive test results would entail unnecessary exposure to additional tests (e.g. genetic studies, MRI) and, potentially, unnecessary treatment with phlebotomy or chelators. The clinical pathway for iron overload with ferritin is depicted in Fig. 2.

Fig. 2. Clinical pathway for iron overload



Dx: diagnosis; HFE: hereditary haemochromatosis.

Source: Garcia-Casal MN, Pasricha S-R, Martinez RX, Lopez-Perez L, Peña-Rosas JP. Serum or plasma ferritin concentration as an index of iron deficiency and overload. Cochrane Database Syst Rev. 2015;(7):CD011817. doi:10.1002/14651858.CD011817 *(46)*.

Ferritin assays

Interpretation of and comparison between studies that have been undertaken in various laboratories at different times in the last decades may be compounded by variation and evolution in assay techniques and platforms, as well as the limited use of WHO reference materials *(47, 48)*.

The WHO Expert Committee on Biological Standardization has established international reference materials to develop tests or to evaluate inter-laboratory performance. These reference materials for ferritin have been developed for calibrating working/secondary standards in routine assays performed in laboratories and also for evaluating and standardizing new assays for ferritin quantification. At least three international reference materials have been developed: first (liver), second (spleen) and third (recombinant) *(49–51)*.

Since ferritin concentration is widely used as marker of iron stores and status, it is important to determine whether all commonly used methods can detect and discriminate the full range of iron status (deficiency, repletion and overload), and to assess the comparability of methods across measurement systems.

History of the project on the use of ferritin for the assessment of iron status

There are previous WHO documents on the use of ferritin for the assessment of iron status in populations from consultations held in 1987 and 1993 *(1, 2, 51)*.

In 2004, a joint WHO/CDC technical consultation was held on assessment of the iron status of populations *(1)* and an analysis of indicators of iron status and acute phase proteins was undertaken. In preparation for this consultation, a non-systematic review sought to identify the most efficient indicators to evaluate the impact of interventions to control iron deficiency and detect a true change in iron status of a population, using the fewest and simplest tests *(52)*. Several iron indicators were reviewed to assess their ability to measure change in iron status due to an iron intervention. Based on the data analysis and the consultation, participants concluded that the concentration of haemoglobin should be measured for the assessment of iron status, even though not all anaemia is caused by iron deficiency, and that the assessment of serum ferritin and soluble transferrin receptor would be the best approach for measuring the iron status of populations. In evaluating the impact of interventions to control iron deficiency in populations, it was recommended to use serum ferritin as the indicator of a response to an intervention to control iron deficiency and to measure it along with the haemoglobin concentration in all programme evaluations. Additionally, the consultation concluded that if funding was available, it may also be useful to measure the concentration of one or both of the acute phase proteins, C-reactive protein (CRP) or α-1 acid glycoprotein (AGP), to account for a high serum ferritin caused by inflammation, as well as measuring transferrin receptor during repeated surveys.

Meetings on the ferritin project

WHO guideline development group meeting: Use of ferritin concentrations to assess iron status in populations, Panama City, Panama 15–17 September 2010

WHO convened the first guideline development group meeting in Panama City, Panama in 2010, on priorities in the assessment of vitamin A and iron status in populations *(53)*, to discuss and initiate the work of updating WHO guidelines on indicators for the assessment of vitamin A and iron status. With regard to the assessment of iron status, serum ferritin and soluble transferrin receptor were ranked of highest priority for undergoing a thorough review.

Starting in 2013, WHO developed a project for retrieving, summarizing and assessing the evidence to inform WHO recommendations on the use and interpretation of serum/plasma ferritin concentrations for assessing iron status in populations. Five protocols were initially developed to answer specific topics, through systematic reviews of published data and analysis of raw data from international databases. These protocols are listed next.

1. *Serum/plasma ferritin for assessing iron status in populations*. This protocol examined whether ferritin concentration reflects all possible iron statuses (deficiency, repletion and overload) and what the cut-off points are to define each iron status. This protocol searched for the association of serum/plasma ferritin with other measures of iron stores, as indicated by bone marrow aspirates, haemoglobin, blood smears and liver biopsies.
2. *How ferritin concentration responds to nutrition interventions*. The aim was to summarize systematic reviews that assess the effects of nutrition-specific and nutrition-sensitive interventions on ferritin concentrations, particularly in children aged 6–59 months, school-age children and pregnant and non-pregnant women of reproductive age.
3. *The accuracy and comparability of methods for measuring ferritin concentration*. This protocol searched the different laboratory methods of assessing ferritin concentration and aimed to estimate between-methods adjustments if necessary.
4. *The influence of inflammation on ferritin concentrations*. A methodological approach was used to adjust values in population-based surveys. This protocol aimed to describe and compare different approaches to account for inflammation, using the measurement of acute phase proteins. The reviews aimed to identify a single "best" approach that accurately describes the prevalence of low ferritin, is easy to use, and can be applied in most populations.

5. *The use of ferritin concentrations in defining levels of public health concern with respect to the iron status of populations*. The objective was to describe the magnitude and distribution of iron deficiency as measured by ferritin concentration in different regions of the world, to inform guidelines and to define ranges, severities and proportions of iron deficiency at national or regional levels.

WHO/CDC technical consultation: Ferritin concentrations to assess iron status in populations, Emory University, Atlanta, United States of America (USA) 3–5 March 2014

WHO, in collaboration with CDC, convened a technical consultation to validate the methodological approach to retrieve, summarize and assess the evidence to inform future guidelines on the use of ferritin as an indicator of iron status in populations, including cut-off points to define iron deficiency, repletion and overload in different population groups. Experts in the areas of ferritin methodology, immunology, nutrition and programme implementation met with authors of the meta-analyses, to discuss the draft review protocols and any preliminary results.

Meeting: Review of evidence to inform WHO/CDC guidelines on the use of ferritin concentrations to assess iron status in populations, Geneva, Switzerland 4–5 December 2014

WHO, in collaboration with CDC, convened a meeting with the authors of the reviews and invited guests to discuss the outcomes of the reviews and draft recommendations in preparation for a meeting of the WHO guideline development group. The objectives were to describe the WHO guideline development process; present results of each of the reviews for informing the WHO/CDC guideline on the use of ferritin concentrations to assess iron status in populations; and discuss results and develop a draft guideline for consideration by the WHO guideline development group.

Meeting: Review of evidence to inform WHO/CDC recommendations on the use of ferritin concentrations to assess iron status in populations, Bethesda, USA 6–8 May 2015

This meeting was held in collaboration with CDC and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). The objectives were to present preliminary results of each of the five protocols, retrieving evidence for informing the WHO/CDC recommendations on the use of ferritin concentrations to assess iron status in populations, and to discuss the results and main conclusions of the findings *(54)*.

Meeting: Review of evidence to inform WHO recommendations on the use of ferritin concentrations to assess iron status in populations, Geneva, Switzerland 3–4 March 2016

WHO, in collaboration CDC, convened a review group meeting to present the final results of each of the reviews for informing the WHO recommendations on the use of ferritin concentrations to assess iron status in populations; discuss results and main conclusions of the findings; and draft recommendations to put forward to the WHO guideline development group *(55)*.

WHO guideline development group meeting: Use of ferritin concentrations to assess iron status in populations, Geneva, Switzerland 15–17 June 2016

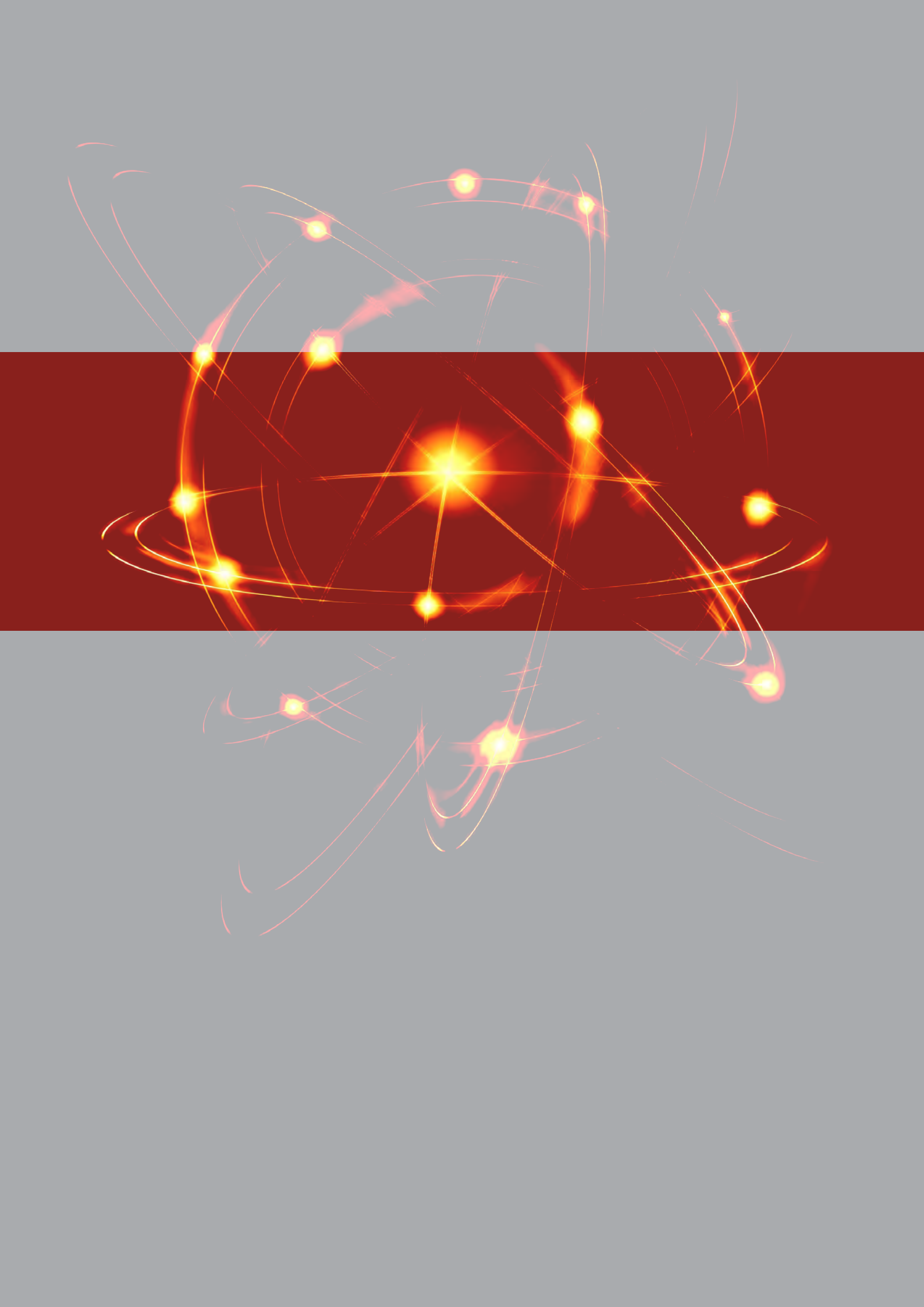
The WHO Department of Nutrition and Food Safety established the WHO guideline development group – ferritin, to present and discuss evidence to finalize a recommendation on the use of ferritin concentrations to assess iron status in populations; determine the strength of the recommendations, considering costs, values and preferences; define implications for further research; and discus challenges for implementation of the guideline *(56)*.

Why is it important for WHO to develop this guideline?

The WHO 13th General Programme of Work (GPW13) 2019–2023 *(57)* focuses on delivering impact for the people at the country level, in all countries – low, middle and high income, and is based on the United Nations Sustainable Development Goals (SDGs) *(58)*. The three strategic priorities set out in GPW13, referred to as the triple billion goals, include achieving universal health coverage, addressing health emergencies and promoting healthier populations. Nutrition, as a cross-cutting area in the health and development sectors, is an integral part of these goals *(57, 59)*. Accurate determination of iron status is crucial for diagnostic and screening purposes in the clinical setting, and to guide public health interventions at the population level. In an individual patient, diagnosis of iron deficiency or overload will help guide management, including further investigations and appropriate therapy. At the population level, determination of the magnitude and distribution of iron deficiency can help prioritize appropriate interventions in settings in which the prevalence is regarded as a severe public health problem, or help identify populations with hereditary conditions that predispose them to iron overload.

In support of WHO GPW13, the 2030 SDG agenda, particularly SDG2 and SDG3, and in concert with the *United Nations Decade of Action on Nutrition (2016–2025) (60)*, WHO's *Ambition and action in nutrition 2016–2025 (61)* aims for "A world free from all forms of malnutrition where all people achieve health and well-being". It defines the unique value of WHO for advancing nutrition – the provision of leadership, guidance and monitoring – and proposes a theory of change. In line with these three core functions, WHO's work in nutrition will build on the outcomes of the efforts to accelerate progress towards achieving the Global Nutrition Targets *(60, 61)*, as well as WHO's triple billion goals, in the key areas of guidance, policy, surveillance and engagement for achieving universal health coverage, addressing health emergencies and promoting healthier populations *(57, 59)*.

This guideline is in line with GPW13, particularly in outcomes 1.1: Improved access to essential nutrition actions as part of quality essential health services; 3.1: Determinants of health addressed; 3.2: Risk factors reduced through multi-sectoral action; and 4.1: Strengthened country capacity in data and innovation *(57)*.



EVIDENCE AND RECOMMENDATIONS

EVIDENCE AND RECOMMENDATIONS

To ensure that the recommendations are correctly understood and applied in practice, guideline users may want to also refer to the remarks, as well as to the evidence summary, including the considerations on implementation.

Question 1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)?

Summary of evidence

The evidence that informed the recommendation on ferritin as a marker of iron stores is based on three systematic reviews. The key question and outcomes guiding the evidence review and synthesis for the recommendations on this question are listed in Annex 1.

Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)?

a. What is the diagnostic accuracy of ferritin compared to the gold standard in free-living populations?

b. What should the cut-off value be to define deficiency/excess?

A Cochrane Diagnostic Test Accuracy systematic review on serum or plasma ferritin concentration as an index of iron deficiency and overload *(46)*, and a descriptive article detailing results from this review *(62)* aimed to determine the diagnostic accuracy of ferritin concentrations (serum or plasma) for detecting iron deficiency and risk of iron overload.

All study designs in any language seeking to evaluate serum or plasma ferritin concentrations measured by any method in individuals of any age, sex, clinical or physiological status from any country were selected, if they compared against bone marrow iron stores for iron deficiency and against liver iron content for iron overload. Risk of bias and applicability was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool. Grading of Recommendation Assessment, Development and Evaluation (GRADE) *(63)* was used to assess the certainty of the evidence and the strength of evidence for conclusions.

Seventy-two studies on iron deficiency and 36 on iron overload were included in the quantitative analysis. For iron deficiency, the mean ferritin concentration in healthy individuals was 15.1 mg/L (9 studies, 390 participants) when bone marrow iron content was undetectable, and 70.4 mg/L (3 studies, 151 participants) when bone marrow iron was detectable. In non-healthy populations, mean ferritin concentrations were 82.43 mg/L for iron depletion (38 studies, 1023 participants) and 381.61 mg/L for iron sufficiency (38 studies, 1549 participants), with wide variations depending on the pathology. For iron overload, the results point to a cut-off value close to 500 mg/L, although the data were very limited.

Only three studies included pregnant women and none of the reported values were below 30 mg/L. In non-pregnant women (2 studies) mean ferritin concentrations when bone marrow iron content was undetectable were similar, although with a great dispersion of values: 12.5 ± 11.2 mg/L and 13.21 ± 22.41 mg/L. Accuracy estimates of the serum ferritin concentration to detect iron deficiency in healthy populations and in non-healthy populations are presented in Annex 2A and 2B. The GRADE certainty of evidence of these results is very low in both cases, owing to risk of bias, serious indirectness, and imprecision. Estimates of the accuracy of serum ferritin concentration to assess iron overload in non-healthy individuals are shown in Annex 2C. The GRADE certainty of evidence of these results is very low due to risk of bias, serious indirectness, and serious inconsistency.

The authors concluded that ferritin concentration is low in iron-deficient individuals and high in iron-loaded individuals, regardless of confounding clinical conditions. Current WHO thresholds for healthy populations appear valid but the data are limited for different age groups or physiological conditions. For iron overload, ferritin concentrations would only help in the presumptive diagnosis and indicate the need for further assessment.

A previous systematic review included 55 studies that compared laboratory tests with histological examination of the bone marrow in adults with anaemia and concluded that measurement of serum ferritin was a useful test for the diagnosis of iron deficiency anaemia, with an area under the receiver operating characteristic curve (AUROC) of 0.95; the authors found that a ferritin concentration <15 µg/L had a positive likelihood ratio for iron deficiency of 51.9 *(64)*.

In 2017, a systematic review of the most commonly used ferritin cut-off values to define iron deficiency in pregnant women, and the origin of those cut-off values, was undertaken. Seventy-six studies were included in the systematic review.

The most commonly used thresholds of serum ferritin concentration to diagnose iron deficiency in studies of iron and/or micronutrient supplementation in pregnant women were <12 µg/L (35 studies) and <15 µg/L (17 studies). Other commonly used thresholds defining iron deficiency in pregnancy included serum ferritin concentration <20 µg/L (8 studies) and <30 µg/L (6 studies). All the studies included in the review provided an a priori threshold of serum ferritin concentration defining iron deficiency. Only 47% (36 studies) provided a reference to the source from which the threshold was derived, and in only 5 studies (7%) the ferritin threshold was based on a published primary study comparing serum ferritin measurements with measurements in bone marrow aspirates.

The authors concluded that the most commonly used thresholds of serum ferritin concentration for the diagnosis of iron deficiency in pregnancy were <12 µg/L and <15 µg/L (68%) and that most of the studies provided no justification for the choice of serum ferritin threshold used; they also highlighted the need for unified international thresholds of iron deficiency for women throughout pregnancy *(65)*.

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations

The guideline development group, with the support of the steering group, formulated recommendations informed by the evidence presented and with explicit consideration of the factors listed next.

Certainty of evidence

The overall certainty of evidence for the systematic reviews of this section is low to very low.

Balance of benefits and harms

Selection of appropriate cut-off values: the risks of a missed case of iron deficiency in infants and young children could mean irreversible consequences of iron deficiency and anaemia in this critical period. On the other hand, a misdiagnosed case of iron deficiency in a non-iron-deficient individual would cause unnecessary side-effects from iron therapy.

The group also discussed considerations about the benefits of knowing the population prevalence of iron deficiency and risk of iron overload, in order to decide in favour of or against iron interventions.

Values and preferences

The guideline development group discussed the relative importance of knowing iron status, from the viewpoint of the individual being assessed and inquiring about the rejection rate for blood draws in surveys for women in general, pregnant women and children under 2 years of age.

The discussion addressed iron overload and its significance as a public health problem. The group also discussed the importance of knowing whether populations are willing to receive iron interventions without knowing the risk of iron deficiency in that population.

Acceptability

The group discussed considerations about the acceptability for participants of providing a blood sample for a population survey, and also the acceptability for care providers or national survey coordinators of including ferritin and other markers of inflammation in the assessment.

Resource implications

Resource implications raised included analysis of the costs of reaching populations in a survey, drawing blood, transport to a laboratory, supplies, assays and human resources. Analysis of the cost of missing a case versus the cost of treating someone who does not need it (as highlighted in balance of benefits and harms) was also discussed. Considerations about giving results to participants and useful timelines to deliver them were also raised.

Equity and human rights

The importance of equal access to and utilization of testing and results was discussed, as well as considerations on the right to receive results and be informed: survey participants should receive the results and the explanation about them in a fashion that is adapted to the individual's characteristics, such as their own language or non-scientific language. There was also discussion about the next steps after receiving results from a survey: referral or treatment.

Feasibility

At the population level, assessment is generally done as part of a large national survey. Considerations about the availability of laboratories in the network for assessing both ferritin and indicators of infection were addressed, and the group was informed of the WHO Global Laboratory Directory.

The requirement for a comprehensive regulatory framework and appropriately trained health workers was discussed. The need to facilitate the training was highlighted.

Recommendations

1.1. Ferritin concentration is a good marker of iron stores and should be used to diagnose iron deficiency in otherwise apparently healthy **individuals**[1] (*strong recommendation*,[2] *low certainty of evidence*).

1.2. In **individuals** with infection or inflammation, a ferritin concentration below 30 µg/L in children and 70 µg/L in adults may be used to indicate iron deficiency (*conditional recommendation,*[3] *low certainty of evidence*). In **populations** it is also possible to adjust ferritin values for infection/inflammation by applying correction factors as described for Question 4: Should ferritin be measured in combination with indicator(s) of infection or inflammation?

1.3. A ferritin concentration exceeding 150 µg/L in menstruating women and 200 µg/L in men and non-menstruating women who are otherwise healthy may indicate a risk of iron overload (*conditional recommendation,* based on previous WHO recommendation). In adult, non-healthy **individuals**, a ferritin concentration exceeding 500 µg/L may indicate risk of iron overload (*conditional recommendation, very low certainty of evidence*).

1.4. Ferritin concentration should not be used alone to identify risk of iron overload. Patients with elevated ferritin levels should receive clinical and laboratory evaluation to establish the underlying cause (*strong recommendation, very low certainty of evidence*).

Rationale

The available studies were not sufficient to justify a change in current ferritin cut-off values to define iron deficiency and risk of iron overload by sex or age groups. The recommended cut-off values for ferritin concentrations to define iron deficiency, including previous recommendations and new evidence when available, are presented in Table 1.

1 For the purposes of this guideline, an apparently healthy individual is defined as an individual with physical well-being for their age and physiological status, without detectable diseases or infirmities.

2 A strong recommendation is one for which the guideline development group is confident that the desirable effects of adherence outweigh the undesirable effects. Implications of a strong recommendation are that most people in these settings would desire the recommended intervention and only a small proportion would not. For policy-makers, a strong recommendation indicates that the recommendation can be adopted as policy in most situations.

3 A conditional recommendation is one for which the guideline development group concludes that the desirable effects of adherence probably outweigh the undesirable effects, although the trade-offs are uncertain. Implications of a conditional recommendation for populations are that while many people would desire the intervention, a considerable proportion would not. With regard to policy-makers, a conditional recommendation means that there is a need for substantial debate and involvement from stakeholders before considering the adoption of the intervention in each setting.

Table 1. Recommended cut-off values to define iron deficiency and risk of iron overload in apparently healthy and non-healthy individuals by age group

	Serum ferritin (µg/L)[a,b]			
	Iron deficiency		Risk of iron overload	
	Apparently healthy individuals[c]	Individuals with infection or inflammation	Apparently healthy individuals	Non- healthy individuals
Infants and young children (0–23 months)	<12	<30	—	
Children under 5 years (24–59 months)	<12	<30	—	
Children (5 to less than 10 years)	<15	<70	>150 females >200 males	>500[d]
Adolescents (10 to less than 20 years)	<15	<70	>150 females >200 males	>500
Adults (20–59 years)	<15	<70	>150 females >200 males	>500
Older persons (60+ years)	<15	<70	>150 females >200 males	>500
Pregnant women	<15 (first trimester)[e]	—	—	

[a] From previous WHO recommendations and new evidence.

[b] Markers of inflammation should be assessed along with the ferritin concentration, and ferritin adjusted as necessary.

[c] For the purposes of this guideline, an apparently healthy individual is defined as an individual with physical well-being for their age and physiological status, without detectable diseases or infirmities.

[d] In adult, non-healthy populations, a ferritin concentration exceeding 500 µg/L may indicate risk of iron overload or other disease. This cut-off value indicates the need for further clinical and laboratory evaluation to establish the diagnosis and underlying cause of the ferritin levels.

[e] There are several physiological changes occurring in pregnancy that may contribute to the variation in thresholds of iron deficiency in pregnancy as defined by serum ferritin, including a physiological rise in acute phase proteins secondary to pregnancy; second trimester plasma volume expansion; and changes in inflammatory measures in the final trimester of pregnancy.

Remarks

The remarks in this guideline are intended to give some considerations for implementation of the recommendations, based on the discussion of the guideline development group.

- In the absence of inflammation, the concentration of plasma/serum ferritin is positively correlated with the size of the total body iron stores. Ferritin levels are low in iron-deficient individuals and high in iron-loaded individuals.
- In populations, ferritin testing to ascertain the prevalence of iron deficiency or to determine the risk of iron overload is usually performed along with haemoglobin testing to assess the prevalence of anaemia. Measures of inflammation (e.g. C-reactive protein [CRP] and/or α-1 acid glycoprotein [AGP]) and additional iron indices, such as soluble transferrin receptor, are commonly used.
- The physiological changes occurring in hormones, blood composition and haemodynamics, as well as in inflammatory status during pregnancy, render it difficult to establish a fixed, unique ferritin concentration to define iron deficiency, especially when comparing to an invasive gold standard test such as bone marrow biopsy.
- Ferritin may be elevated due to iron overload or other causes, including liver disease, obesity, inflammation and malignancy. In cases of risk of iron overload, ferritin concentration only indicates the possibility of iron overload and the need for further assessment of the specific diagnosis, and the severity of the problem.

- Liver biopsies have commonly been used to report iron overload, because the liver is the dominant iron-storage organ, liver iron concentration correlates closely with the total iron balance, and the liver is the only organ in which the iron concentration is elevated in all forms of systemic iron overload. Non-invasive methods such as magnetic resonance imaging and computerized tomography are widely used to assess iron content in the liver.

Question 2. Is ferritin an adequate marker for assessing the impact of iron interventions?

Summary of evidence

The evidence that informed the recommendation on ferritin response to nutrition interventions is basesd on one overview of systematic reviews and one systematic review. The key question and outcomes guiding the evidence review and synthesis for the recommendations on this question are listed in Annex 1.

Is ferritin an adequate marker for assessing the impact of iron interventions?

a. If yes, when should it be measured?

An overview of systematic reviews was commissioned to address the question on how ferritin concentration responds to nutrition interventions. The objective was to summarize the effects of nutrition-specific and nutrition-sensitive iron interventions on serum ferritin concentration among relatively healthy populations of children aged 6 months and older and women of reproductive age and to explore the consistency in results across environments and intervention designs, following Cochrane methodology for an overview of reviews. The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO), number CRD42014008926 *(66)*.

The search included peer-reviewed systematic reviews with meta-analyses without date, geographic or language restrictions, on apparently healthy populations that included children aged 6 months to adolescence and/or women of reproductive age who received nutrition-specific and/or nutrition-related interventions. The methodological quality of each meta-analysis was measured using the Assessment of Multiple Systematic Reviews (AMSTAR) tool. This review did not include individual studies or assessments of eligibility or of risk of bias, and did not involve additional meta-analyses of the results extracted from the systematic reviews.

Ten meta-analyses were selected, covering four types of interventions: supplementation (6 studies); food fortification (1 study); supplementation + fortification (1 study); late versus early cord clamping (1 study); and anthelmintic treatment (1 study). In all cases, serum ferritin concentration consistently increased in response to interventions.

This overview is limited by the fact that results were extracted from available systematic reviews, and no additional analyses were performed. Therefore, while this overview provides support that ferritin concentration consistently responds positively to interventions designed to improve iron status, it is not possible to describe the differential impact of various intervention designs, such as iron supplementation dose or duration, on the change in ferritin status (*66;* Merrill R, Mei Z, personal communication, 10 February 2020).

In 2015, a systematic review of studies on iron-deficient but non-anaemic endurance athletes investigated the effects of iron supplementation on serum ferritin, serum iron, transferrin saturation and haemoglobin concentration. A meta-analysis of 17 eligible studies indicated that iron treatments had a large effect on improving serum ferritin (Hedges' g = 1.088, 95% confidence interval [CI]: 0.914–1.263, $P < 0.001$), serum iron (Hedges' g = 1.004, 95% CI: 0.828–1.181, $P < 0.001$) and transferrin saturation (Hedges' g = 0.741, 95% CI: 0.564–0.919, $P < 0.001$) and a moderate effect on improving haemoglobin concentration (Hedges' g = 0.695, 95% CI: 0.533–0.836, $P < 0.001$). Regression analysis revealed a significant interaction between the effect of iron treatment on serum ferritin and treatment duration, suggesting treatments that lasted beyond 80 days had the least effect on serum ferritin. The authors concluded that iron treatments improve the iron status and aerobic capacity of iron-deficient non-anaemic endurance athletes *(67)*.

In 2019, a systematic review and meta-analysis to determine the impact of fortification programmes on micronutrient status (vitamin A, iodine, iron and folic acid) in low- and middle-income countries, demonstrated

measurable improvements in micronutrient status *(68)*. The effect on ferritin concentration of fortification programmes providing at least iron included six studies (n = 6893). Serum ferritin increased by 0.39 µg/L (95% CI: 0.34–0.44 µg/L), indicating a significant improvement in iron stores for combined age groups following iron fortification. The certainty of the evidence was moderate, by Child Health Epidemiology Reference group (CHERG) score. Iron deficiency was analysed in seven studies (n = 7249). The prevalence of iron deficiency declined by 58% among all population subsets (relative risk [RR]: 0.42; 95% CI: 0.32–0.56). The certainty of the evidence for this outcome was low by CHERG score.

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations

The guideline development group, with the support of the steering group, formulated recommendations informed by the evidence presented and with explicit consideration of the factors listed next.

Certainty of evidence

The overall certainty of evidence for the systematic reviews in the overview of reviews in children and women is low by AMSTAR tool.

Balance of benefits and harms

For this question, the guideline development group discussed the benefits of knowing whether there is a biological response (or not) to an intervention versus the possible harms of drawing blood. Considerations included the many iron interventions being recommended in various settings and the observation that iron deficiency is still a global public health problem.

Values and preferences

The guideline development group discussed considerations about how countries, health-care providers or individuals value/prefer a biological measure over a functional outcome: would they prefer questions regarding intake (diet, supplementation, fortified food) or blood sampling?

Acceptability

The group raised considerations about sampling, especially considering that more than one blood sample would be required from each individual.

Resource implications

The guideline development group agreed that, overall, the implementation of nutrition interventions requires important resources (surveys, drawing blood, transport to laboratory, supplies, assays, human resources, supplements), but the impact is worth the costs.

Equity

The guideline development group agreed that the impact of iron interventions could be easily analysed in different population groups (e.g. using stratifiers such as age, income group, urban/rural residence, or ethnicity).

Feasibility

Nutrition interventions are feasible and, at the population level, assessment is generally done as part of a large national survey.

Recommendation

2.1 Ferritin concentration increases in response to iron-related interventions and may be used to monitor and assess the impact of interventions on iron status (*strong recommendation, moderate certainty of evidence*).

Remarks

- Iron interventions should be implemented in such a manner as to enable monitoring to be undertaken with the lowest number of blood draws.
- The interval following initiation of an iron intervention at which ferritin should be measured depends on the type of intervention and the amount of iron provided.
- Comprehensive planning, monitoring and evaluation of all simultaneous interventions that increase iron intake and/or utilization and/or reduce iron losses are required to account for the total amount of iron being received by populations that would result in ferritin changes in cases of iron deficiency, and to avoid risk of iron overload.
- Knowledge of the prevalence of infection/inflammation is critical for interpretation of ferritin concentrations in population surveys and to interpret changes after iron interventions.
- The inclusion of markers to diagnose iron-related genetic disorders is valuable, especially in regions where thalassaemias and other haemoglobinopathies are common.
- Cases of iron overload should be treated at individual level, since high ferritin concentrations are not sensitive to the effects of nutrition interventions.
- More research is needed to evaluate the effect of nutrition interventions on ferritin concentration through the life-cycle, especially during pregnancy, owing to changes in concentration, especially the typical decrease in concentration in late pregnancy.

Question 3. How should ferritin be measured?

Summary of evidence

The evidence that informed the recommendation on methods to measure ferritin is basesd on one systematic review. The key question and outcomes guiding the evidence review and synthesis for the recommendations on this question are listed in Annex 1.

How should ferritin be measured?

a. What assays are available?

b. What biological samples are acceptable for analysis (plasma/serum/dried blood spots from venous or capillary blood)?

A systematic review analysed the performance and comparability of the most common laboratory methods used for determination of serum or plasma ferritin concentration to detect iron deficiency, repletion or overload. The protocol was registered in PROSPERO, under the number CRD42016036222 *(69)*. The objectives were to determine the sensitivity, specificity and predictive value among ferritin methods; to assess the variability of serum or plasma ferritin concentrations using different laboratory methods of detection; and to review the use of international standard materials of ferritin for calibration purposes and in global public health surveillance *(70)*.

Two hundred and fifty-two studies were assessed, including 187 studies in the qualitative analysis and 148 in the meta-analysis. The most used methods for determination of ferritin in plasma or serum included radiometric, nonradiometric and agglutination assays. The overall within-run imprecision for the most reported ferritin methods was 6.2 ± 3.4% (95% CI: 5.69–6.70%; $n = 171$), between-run imprecision 8.9 ± 8.7% (95% CI: 7.44–10.35%; $n = 136$) and recovery rate 95.6% (95% CI: 91.5–99.7%; $n = 94$). The pooled regression coefficient was 0.985 among all methods analysed, and 0.984 when comparing nonradiometric and radiometric methods, without statistical differences in ferritin concentration ranging from 2.3 µg/L to 1454 µg/L.

There was no significant difference in within-run imprecision, between-run imprecision, limit of detection, recovery rate or linearity between commercial and home-made assays, or between automated multiple-analytes

detection equipment and single laboratory apparatus, showing, overall, that the methods used in the included studies, whether commercial or home-made assays, and the use of automated or single apparatus equipment for detection, made no difference for ferritin determinations.

Several studies reported calibration of ferritin assays to WHO or other international reference materials, although calibration data were not presented in the articles. There were no studies reporting the use of these materials on a routine laboratory basis. Only one study reported results from a serum pool "spiked" with either the first or second international standard for ferritin. Those sera were measured by 52 laboratories, using five automated methods, and the recovery of the target values was calculated. The recovery of the first international reference materials by three of the methods was between 104% and 129%; and of the second reference material was between 99% and 125%. The authors recommended manufacturers to calibrate their methods against the third international standard (recombinant), and to periodically assess their methods relative to this standard as a means of avoiding assay drift over time *(71, 72)*. The authors concluded that the laboratory methods most used to determine ferritin concentrations have comparable accuracy and performance and suggested calibration of methods against the third international standard (recombinant).

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations

The guideline development group, with the support of the steering group, formulated recommendations informed by the evidence presented and with explicit consideration of the factors listed next.

Certainty of evidence

The quality of publications, especially for automated and commercial assays, is low. In many cases, they are only reports or abstracts from scientific meetings that did not result in formal, complete publications.

The use of a Diagnostic Test Accuracy approach was not possible, due to the difficulty in unequivocally identifying a gold standard method for ferritin assays to perform comparisons.

Balance of benefits and harms

The guideline development group discussed the benefits and drawbacks of each assay. Is there a practical benefit for laboratory personnel when performing radioactive methods? Is there a clear or immediate harm if they are used? Consequences for participants were also considered, and the group concluded that with small blood volumes and a limited number of samplings there are no haematological consequences from repeated blood sampling, especially for children or women.

Values and preferences

Discussions were centred on what laboratories, health workers and laboratory personnel prefer and also about blood sampling from participants in a survey or nutrition intervention.

Acceptability

The group discussed considerations from laboratory personnel about preferences in methods (less time to perform, easier or more convenient) or concerns about being exposed to radioactivity. From the participants' point of view, there were considerations about sampling, especially if more than one blood sample would be required from each individual.

Resource implications

The group identified the likelihood of wide variation in the costs of measurements (assay, time, supplies, equipment) but not in the cost of the international reference standard (although the cost could be more significant for some laboratories than for others).

Implications of health workers' training were also discussed. If additional training is needed, is this factored in the cost estimates? (see also Feasibility considerations).

Equity

Although methods are comparable and could be used interchangeably, providing they are standardized to a WHO international standard and performed by trained personnel, the group discussed differences in access to all methods and what mechanisms are in place so that those groups with limited access can be more easily reached.

Feasibility

The guideline development group agreed that feasibility will not be affected by differences in methods or equipment used in different laboratories, but that the worldwide availability of the WHO international reference standard (NIBSC code 94/572) *(50)*, as well as properly trained health workers and laboratory personnel, would affect feasibility.

Recommendations

3.1. Ferritin may be measured using radiometric, nonradiometric and agglutination assays. One method does not appear to be superior to another and all methods are acceptable if a commutable material traceable to the WHO international reference standard is used to calibrate the assay. Once a method has been selected, that same method should be used for the follow-up of individuals and populations (*strong recommendation, moderate/low certainty of evidence*).

3.1. Use of the WHO international reference standard of ferritin is recommended for calibration of all commercial kits and in regular laboratory practice, especially when following up individual cases, for population surveys or to measure the impact of public health interventions (*strong recommendation, moderate certainty of evidence*).

Remarks

- The risks of radioactive contamination and the high cost of equipment are important drawbacks of radiometric assays.
- For follow-up of individuals and populations, the same method for ferritin determination should be used, to minimize variability. It is also important to control other sources of error in laboratory testing related to handling of samples; transport and storage conditions; the use of manual versus automated procedures; and differences in equipment performance and those inherent to the operator.
- Ferritin may be measured in either serum, plasma or other biological fluids, but the same sample matrix should be used when measuring the impact of interventions in individuals and at the population level.
- International reference materials for ferritin have been developed for calibrating working standards in the routine ongoing assays performed in laboratories and also for evaluating and standardizing new tests for quantification of ferritin. A WHO international standard of ferritin from the National Institute for Biological Standards and Control, WHO International Laboratory for Biological Standards, United Kingdom of Great Britain and Northern Ireland (NIBSC code 94/572) *(50)*, is commercially available and recommended for use with all assays.
- It is important that reference materials are commutable and traceable to the WHO reference standard, so the results are equivalent among procedures and to avoid calibration bias.
- Quality controls should be included with every run, or at least daily, on instruments measuring ferritin. The inclusion of quality controls of low, medium and high ferritin concentrations is desirable.
- Laboratories performing ferritin determinations for patient care or for public health assessments should participate in external quality assurance programmes.

Question 4. Should ferritin be measured in combination with indicator(s) of infection or inflammation?

Summary of evidence

The evidence that informed the recommendation on adjustments of ferritin concentration for inflammation is based on two meta-analyses of data. The key question and outcomes guiding the evidence review and synthesis for the recommendations on this question are listed in Annex 1.

Should ferritin be measured in combination with indicator(s) of infection or inflammation?

a. If yes, what indicators (AGP alone, CRP alone, both AGP and CRP? Provide prioritization) and how should they be interpreted?

b. How should ferritin be adjusted for inflammation?

A meta-analysis estimated the increase in ferritin in 32 studies of apparently healthy persons by using CRP and AGP, individually and in combination, to calculate factors to remove the influence of inflammation from ferritin concentrations. The studies in the meta-analysis included infants (5 studies), children (7 studies), men (4 studies) and women (16 studies) (n = 8796 subjects). Inflammation increased ferritin by 49.6% (CRP) or 38.2% (AGP; both $P < 0.001$). In 4-group analysis, ferritin was 30%, 90% and 36% (all $P < 0.001$) higher in the incubation, early convalescence and late convalescence subgroups, respectively, with corresponding correction factors of 0.77, 0.53 and 0.75. Overall, inflammation increased ferritin by approximately 30% and was associated with a 14% (95% CI: 7–21%) underestimation of iron deficiency. The authors concluded that measures of both APP and CRP are needed to estimate the full effect of inflammation and can be used to correct ferritin concentrations. Few differences were observed between age and sex subgroups *(73)*.

Another meta-analysis assessed the relation between ferritin concentrations and inflammation and malaria in preschool children (6–59 months) and women of reproductive age (15–49 years) and investigated adjustment algorithms to account for these effects. Cross-sectional data from 15 surveys for preschool children (n = 27 865) and 8 surveys for women of reproductive age (n = 24 844), from the Biomarkers Reflecting the Inflammation and Nutritional Determinants of Anemia project (BRINDA) were analysed. Several approaches were explored to estimate depleted iron stores (ferritin concentration <12 mg/L in preschool children and <15 mg/L in women of reproductive age) in inflammation and malaria settings as follows: (i) increase the cut-off value for ferritin concentration to <30 mg/L; (ii) exclude individuals with CRP concentrations >5 mg/L or AGP concentrations >1 g/L; and (iii) apply arithmetic correction factors; and (iv) use a regression correction approach.

Results show that estimates of depleted iron stores incrementally increased as CRP and AGP deciles decreased (4% compared with 30%, and 6% compared with 29% from the highest compared with lowest CRP deciles for pooled preschool children and women of reproductive age, respectively, with similar results for AGP). Depending on the approach used to adjust for inflammation (CRP plus AGP), the estimated prevalence of depleted iron stores increased by 7–25 and 2–8 absolute median percentage points for preschool children and women of reproductive age, respectively, compared with unadjusted values. Adjustment for malaria in addition to CRP and AGP did not substantially change the estimated prevalence of depleted iron stores.

The authors concluded the value of using internal regression correction to estimate the prevalence of depleted iron stores in regions with inflammation. More research is warranted to validate the proposed approaches, but this study contributes to the evidence base to guide decisions about how and when to adjust ferritin for inflammation *(74)*.

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations

The guideline development group, with the support of the steering group, formulated recommendations informed by the evidence presented and with explicit consideration of the factors listed next.

Certainty of evidence

Analysis was not available.

Balance of benefits and harms

The guideline development group discussed considerations on the benefits and drawbacks of knowing the population prevalence of inflammation/infection and on adjusting ferritin concentrations for infection/inflammation.

Values and preferences

The group discussed aspects related to the added value of knowing the population prevalence of infection/inflammation; preferences of the population with regard to performing extra tests depending on the prevalence of inflammation; preferences of laboratories for the measurement of inflammation; and preferences of survey organizers/statisticians with regard to "adjusting" data for inflammation.

Acceptability

There was discussion on the number of samples needed. If the same blood sample taken for the assessment of ferritin is used to assess infection/inflammation, that would increase acceptability from patients and from health workers.

Considerations about the use of either capillary or venous blood were also discussed. Is the use of dried serum spot samples possible?

Resource implications

Resource implications include the cost of each approach for accounting for infection/inflammation, as well as extra costs incurred for assessing CRP and/or AGP.

Equity and human rights

In settings with endemic infection/inflammation, combined measurement of ferritin and indicator(s) of infection or inflammation should be the standard procedure.

Feasibility

Feasibility depends on the availability of CRP and/or AGP tests in all countries. Considerations about special training for sampling or quantifying those acute phase reactants were also discussed.

Recommendations

4.1. In areas of widespread infection or inflammation, serum ferritin should be assessed with the concurrent measurement of two acute phase response proteins, CRP and AGP (*strong recommendation, moderate certainty of evidence*).

4.2. The increase in ferritin values caused by inflammation should be accounted for in **individuals** and **populations**. One method is to raise the cut-off value that defines deficiency, to 30 µg/L or 70 µg/L, depending on the age group (see Table 1). Another method is to exclude individuals with elevated concentrations of CRP or AGP from prevalence calculations based on ferritin. Alternatively, arithmetic or regression correction approaches may be used to adjust ferritin concentrations for inflammation and apply the cut-off points recommended for healthy populations. The adjustment that best suits the country reality should be selected and used as long as those conditions prevail (strong recommendation, moderate certainty of evidence).

Remarks

- The need for and magnitude of infection/inflammation correction depends on the population group, geographic region and other factors.
- The application of different adjustment approaches will result in a high degree of variability in the estimated prevalence of depleted iron stores. The selected adjustment based on country conditions should be used as long as those conditions prevail.

- Determination of both CRP and AGP concentrations may be important because they reflect different phases of the acute phase response that range from acute infection to chronic inflammation.
- Possible adjustments include the following:
    - the higher ferritin cut-off adjustment approach uses a higher ferritin-concentration cut-off value for individuals with infection/inflammation, e.g. <30 µg/L;
    - the exclusion approach uses the inflammation, malaria-biomarker information, or both, to exclude individuals with elevated inflammation (as defined by a CRP concentration >5 mg/L, AGP concentration >1 g/L, or both) or individuals with malaria infection;
    - the arithmetic correction factor approach applies an arithmetic correction factor by grouping inflammation into groups, e.g. (i) reference (both CRP concentration <5 mg/L and AGP concentration <1 g/L); (ii) incubation (CRP concentration >5 mg/L and AGP concentration <1 g/L); (iii) early convalescence (both CRP concentration >5 mg/L and AGP concentration >1 g/L); and (iv) late convalescence (CRP concentration <5 mg/L and AGP concentration >1 g/L);
    - the regression correction approach uses linear regression to adjust ferritin concentrations by the CRP and AGP concentrations on a continuous scale, and malaria infection as a dichotomous variable. The adjusted ferritin equation is calculated by subtracting the influence of CRP, AGP and malaria as follows:

$$\text{Ferritin}_{\text{adjusted}} = \text{ferritin}_{\text{unadjusted}} - \beta_1(\text{CRP}_{\text{obs}} - \text{CRP}_{\text{ref}}) - \beta_2(\text{AGP}_{\text{obs}} - \text{AGP}_{\text{ref}}) - \beta_3\,\text{malaria}$$

where β_1 is the CRP regression coefficient, β_2 is the AGP regression coefficient, β_3 malaria is the malaria regression coefficient, obs is the observed value, and ref is the external reference value generated to define low inflammation.

Question 5. What are the population prevalence ranges for determining a public health problem?

Summary of evidence

The key question guiding the evidence review and synthesis for the recommendations on this question is listed in Annex 1.

What are the population thresholds for determining a public health problem?

There is a WHO classification of anaemia as a problem of public health significance *(10)*. To classify anaemia as severe, moderate, mild or no public health problem, the prevalence of anaemia should be ≥40.0%, 20.0–39.9%, 5.0–19.9% or ≤4.9%, respectively. However, there are no details on how these cut-off values were determined.

There are currently no population prevalence ranges for defining a public health problem using ferritin concentrations. A review (*The magnitude and distribution across countries of iron deficiency using serum/plasma ferritin*) was commissioned to describe the magnitude and distribution of iron deficiency as measured by serum/plasma ferritin in different regions of the world, to define ranges in the proportion of a given population below a specific cut-off level of serum/plasma ferritin for iron deficiency associated with the severity as a public health problem *(75)*.

Two data sources were analysed: BRINDA *(74)* and the WHO Vitamin and Mineral Nutrition Information System (VMNIS) *(76)*. Data on preschool children (<5 years) and non-pregnant women of reproductive age (15–49 years) were examined. The relationships between the prevalence of low ferritin and country gross domestic product, infant mortality rate, anaemia, maternal mortality rate (for women only), and stunting rate (for preschool children only) were examined. The authors reported that correlations between the prevalence of low ferritin and socioeconomic or health characteristics of the country were weak and not statistically significant in non-pregnant women. Correlations were stronger and statistically significant if the inflammation-adjusted prevalence of low ferritin was used in children. The authors concluded that the quartile values of prevalence of low ferritin for children and non-pregnant women could be used to define the severity of ferritin as a public health problem.

Summary of the considerations of the members of guideline development group for determining the direction and strength of the recommendations

The guideline development group, with the support of the steering group, formulated recommendations informed by the evidence presented and with explicit consideration of the factors listed next.

Certainty of evidence

There was no systematic review associated with this question.

Balance of benefits and harms

The group discussed the benefits and drawbacks of categorizing populations by the prevalence of iron deficiency/risk of iron overload and whether it would help in prioritizing countries in need of assistance. Further, they discussed the benefit of initiating nutrition programmes and having a baseline to measure the impact of the intervention.

Values and preferences

The discussions were centred on the added value of categorizing populations by the prevalence of iron deficiency. The group considered that from the global and national perspectives this could help countries to gauge whether they need an intervention or not, and global organizations to know where priority countries/areas for intervention are.

Acceptability

The group discussed the acceptability and convenience of the global nutrition community categorizing countries.

Resource implications

There were no extra costs identified for this calculation.

Equity and human rights

When determining the population prevalence ranges, the group discussed that an analysis of demographic and social stratifiers should be used to identify national variability across the social gradient (e.g. by using stratifiers such as age, income group, urban/rural residence, or ethnicity). This analysis should help to target the most affected population groups for specific measures.

Feasibility

The group concluded that it is feasible to determine population thresholds.

Remarks

- Owing to the scarcity and dispersion of data, it was not possible to make a recommendation for population prevalence ranges to define the magnitude of iron deficiency as a public health problem using ferritin concentrations.
- The population prevalence ranges established for determining the magnitude of anaemia as a public health problem could be suitable as a guide to determine the prevalence ranges for defining the severity of iron deficiency as a public health problem based on adjusted ferritin concentrations. To classify iron deficiency as severe, moderate, mild or no public health problem (measured by ferritin concentration below the recommended cut-off values), the prevalence of iron deficiency could be ≥40.0%, 20.0–39.9%, 5.0–19.9% or ≤4.9%, respectively (see Table 2).
- Initiating iron interventions in populations with a mild, moderate and/or severe prevalence of iron deficiency could help prevent anaemia, as well as adverse consequences of iron deficiency without anaemia.

Table 2. Population prevalence ranges to define the magnitude of iron deficiency as a public health problem using ferritin concentrations.

Magnitude of the public health problem	Prevalence range (%)
High	≥40.0
Moderate	20.0–39.9
Mild	5.0–19.9
No public health problem	≤4.9

IMPLEMENTATION OF THE GUIDELINE

Implementation considerations

As this is a global guideline, Member States are expected to adapt the recommendation according to their setting and its feasibility. Public health nutrition, and health programmes for children, women and older persons that include nutrition interventions, anaemia-control programmes and healthy diet promotion and support, require supportive policies and health-care services that enable the proper access to quality services. Other related WHO documents and manuals could be created to help with the implementation of this guideline. WHO regional and country offices assist with these processes.

Engaging with multiple stakeholders and partners will be critical in strengthening implementation and sustaining gains in diagnostics of iron deficiency and anaemia control. Working in collaboration with sectors involved in child, adolescent and women well-being; reproductive health; water, sanitation and hygiene; early childhood development and education; social marketing; and others can help ensure a comprehensive, cross-sectoral and more sustainable approach to knowledge of the iron staus of populations and implementation of measures to control iron deficiency or iron overload.

Particular attention will be given to improving access to these guidelines for stakeholders that face more, or specific, barriers in access to information, or to those who play a crucial role in the implementation of the guideline recommendations, for example, policy-makers and decision-makers at subnational level that disseminate the contents of the guideline, and health workers and education staff that contribute to the delivery of the intervention. Disseminated information may emphasize the benefits of the intervention in populations or regions presenting an important risk of suboptimal nutrition and health. This is particularly important in rural communities or highly isolated settings where seeking health care is less frequent or obtaining health care is more difficult, because of distance or transport barriers or because of other factors affecting the availability, accessibility and acceptability of care (e.g. social norms, language barriers, or social discrimination of specific population groups). In addition, these guidelines and the information contained therein should be accessible to the nongovernmental organizations working in coordination with national authorities on the implementation of nutrition interventions, especially those related to the prevention and control of malnutrition in infants and children.

Accessing hard-to-reach population groups is extremely important during implementation stages, as it contributes to preventing or tackling health inequities and to furthering the realization of children's rights to health. Appropriate surveillance and monitoring systems can thus provide information on the impact of the disseminated guidelines and their implementation. Dissemination of the guidelines and information on the benefits of the intervention in contexts where malnutrition, anaemia and/or iron deficiency are of public health significance helps to empower communities and thus contributes to creating demand for services and care.

Regulatory considerations

As this is a global guideline, it should be adapted to the context of each Member State. Prior to implementation, a public health programme should have well-defined objectives that take into account available resources, existing policies, suitable delivery platforms and suppliers, communication channels, and potential stakeholders. Ideally, the intervention should be implemented as part of an integrated programme on nutrition and health that includes addressing iron deficiency and anaemia.

This recommendation should be framed under the existing national strategy on prevention and control of iron deficiency and anaemia and/or micronutrient deficiencies. The choice of an intervention to prevent micronutrient deficiencies should be considered in the context of that strategy, including consideration of the costs, feasibility, accessibility and acceptability among the different stakeholders (e.g. decision-makers, law-makers, programme managers, manufacturers, industry organizations, importers, exporters, retailers, consumers' organizations, organizations with opposing views). A mapping exercise of the different stakeholders and their interests and form of involvement in the intervention is a useful practice *(77)*.

Once the programme is defined for public health and for individual treatment, the *WHO Model List of Essential Medicines* (EML) compiles medicines that satisfy the priority health-care needs and are selected with due regard to disease prevalence, evidence on efficacy and safety, and comparative cost effectiveness *(78)*.

Ethical and equity considerations

Ethical principles lead to consideration of whether an intervention is producing benefits to individuals and communities; preventing harms, also at the individual and societal levels; and distributing health benefits across social groups, that is, how much an intervention is contributing to health equity; and respecting and promoting the exercise of human rights.

An assessment of the accessibility and acceptability of a nutrition programme or survey and ferritin determinations can inform programme design and development, in order to increase therapeutic adherence to iron supplementation and the understanding and acceptance of ferritin determinations to evaluate the impact of the programme. This is particularly relevant in settings where the prevailing social norms and determinants may set unequal conditions and opportunities for different groups. For instance, in some settings, gender norms may create unequal opportunities for girls and boys at any age, both within and out of school; in other settings, social perceptions around ethnicity and race intervene in how certain population groups access and use an intervention. A comprehensive identification and assessment of the social determinants of health affecting the target groups is suggested at an early stage of implementation.

Furthermore, intersectoral action is fundamental in those setting where the intervention is delivered in coordination with the education, social development or other sectors. Linking the implementation of these recommendations with other intersectoral interventions will benefit the sustainability and scale-up of the intervention.

Monitoring and evaluation of guideline implementation

A plan for monitoring and evaluation with appropriate indicators, including equity-oriented indicators, is encouraged at all stages. The impact of this guideline can be evaluated within countries (i.e. monitoring and evaluation of the programmes implemented at national or regional scale) and across countries (i.e. adoption and adaptation of the guideline globally).

For evaluation at the global level, the WHO Department of Nutrition and Food Safety has developed a centralized platform for sharing information on nutrition actions in public health practice implemented around the world. By sharing programmatic details, specific country adaptations and lessons learnt, this platform will provide examples of how guidelines are being translated into actions. The Global database on the Implementation of Nutrition Action (GINA) *(79)* provides valuable information on the implementation of numerous nutrition policies and interventions.

An efficient system for the routine collection of relevant data, including relevant determinants of health, therapeutic adherence and measures of programme performance, is critical to ensure programmes are effective and sustained and drivers to the achievement of the right to health for all population groups. Monitoring differences across groups in terms of accessibility, availability, acceptability and the quality of the interventions contributes to the design of better public health programmes. Creation of indicators for monitoring can be informed by social determinants of health approaches, so that inequities can be identified and tackled. It is particularly important to design sound implementation strategies to serve as the basis for scaling-up efforts. Appropriate monitoring requires suitable data, so attempts towards collecting and organizing information on the implementation are also fundamental.

RESEARCH GAPS

Discussions between the members of the WHO guideline development group and the external resource group highlighted the limited evidence available in some knowledge areas, meriting further research, particularly in the following areas:

- studies comparing ferritin concentrations and bone marrow iron content in healthy, non-infected/non-inflammed populations of all ages;
- studies comparing ferritin and other iron status indicator concentrations and bone marrow iron content in the presence of biomarkers of inflammation, to validate the different approaches to adjusting iron status for inflammation;
- well-designed, population-based longitudinal studies to define the role of other iron indicators and their response to iron interventions, which can be used to monitor the impact of public health programmes;
- studies that clarify cut-off points for iron deficiency and the differential diagnoses when chronic or low-degree inflammation is present in older persons and conditions, such as obesity or controlled diabetes;
- changes affecting ferritin concentrations during pregnancy and establishment of cut-off points by trimester;
- factors affecting ferritin levels (fasting, inflammation, infection, liver disase, co-morbidities) in the general population, stratified by age, sex and physiological status, living in different settings (malaria, tuberculosis and HIV);
- studies on the utility of ferritin concentrations to diagnose the risk of iron overload and to establish cut-off values for all age groups and settings; and
- the comparability of ferritin concentrations among sample matrices: venous blood collection versus pooled capillary samples or dried serum spot samples.

GUIDELINE DEVELOPMENT PROCESS

This guideline was developed in accordance with the WHO evidence-informed guideline-development procedures, as outlined in the *WHO handbook for guideline development (80)*.

The inclusion of various sources and qualities of data improved the guideline development process by using different methods for summarizing and assessing programmatic experiences; by translating the solutions or evidence into policy, practice and products that will improve implementation; and by addressing values and preferences that will result in better strategies for communicating the guidelines to managers and policy-makers to scale-up operations.

High-quality information on the iron status of populations is required to enable the right interventions to be chosen for combating both iron deficiency and anaemia, and then, once programmes are in place, to have the right indicators to monitor their impact *(53)*. It is also necessary to address the risk of iron overload at individual and at population levels and to identify a suitable indicator to detect the risk.

Clinically, the heterogeneity in recommended and used ferritin thresholds causes confusion for clinicians and patients, while for public health, differences in thresholds impair comparison between surveys, slow development of global estimates of the prevalence of iron deficiency, and obscure estimates of the effectiveness and safety of nutrition interventions. Modern processes for guideline development require a rigorous, systematic assessment of the literature, incorporating an assessment of the certainty of evidence *(80)*. Thus, the evidence supporting the present ferritin thresholds requires a systematic approach to inform the recommendations on the use and interpretation of ferritin levels in clinical and public health settings.

A rigorous and transparent approach should also facilitate international harmonization of ferritin thresholds *(80)*, or at least enable different institutions to select appropriate thresholds on the basis of an interpretation of the best available evidence. Moreover, this approach can serve as an example for the development of evidence-informed thresholds for other biomarkers for health and development.

WHO steering group

A WHO steering group (see Annex 3), led by the Department of Nutrition and Food Safety, was established in 2009 with representatives from the Departments of Global Capacities, Alert and Response; Essential Medicines and Health Products; Knowledge, Ethics and Research; Health Statistics and Information Systems; and Maternal Perinatal Health. The steering group guided the overall guideline development process, as well as the retrieval, assessment and summary of the evidence.

The steering group drafted the scope of the guideline and key questions in PICO format; identified the systematic review teams and guideline methodologist; developed and finalized the planning proposal; helped with selection of the guideline development group and the external resource persons; oversaw the evidence retrieval, assessment and synthesis; collected and assessed disclosures of interest; and managed conflicts in consultation with the Office of Compliance, Risk Management and Ethics. The steering group drafted the recommendation, based on the decisions of the guideline development group; drafted the final guideline, including management of the peer-review process; and oversaw the dissemination of the guideline. Regional advisers from the WHO regions also participated in the meetings of the guideline development group.

Guideline development group

The Guideline development group – ferritin was established with 12 experts with a range of technical skills, diverse perspectives, wide geographic representation and gender balance. They consisted of content experts, methodologists, and representatives of potential stakeholders and beneficiaries. The list of members of the guideline development group came from suggestions from all WHO departments with an interest in the provision of scientific nutrition advice, WHO expert advisory panels, previous guideline development group memberships and those identified through a call for experts published on the WHO Nutrition website and distributed to the WHO Nutrition mailing list.

The WHO guideline development group – ferritin advised WHO on: (i) the scope of the guidelines and priority questions for which systematic reviews of evidence will be commissioned; (ii) the choice of important outcomes for decision-making and developing recommendations; (iii) the interpretation of evidence with explicit consideration of the overall balance of risks and benefits; and (iv) the formulation of final drafting of recommendations, taking into account existing evidence as well as diverse values and preferences.

The initial guideline development group meeting was held in 2010 in Panama City, Panama, where the group agreed on the general scope, key questions (including the ranking of prioritization of the outcomes) and the target audience of the guideline *(53)*. In preparation for this meeting, four background papers were commissioned, one of which was on the rationale for selecting and standardizing indicators of iron status. The meeting report summarizes the discussions that occurred during the meeting and presents the background papers *(53)*.

In a second meeting of the guideline development group in Geneva, Switzerland on 15–17 June 2016, the group examined the evidence used to inform the recommendation and appraised its certainty using the GRADE evidence profiles *(63, 81, 82)*. It interpreted the evidence, taking in consideration the Developing and Evaluating Communication Strategies to support Informed Decisions and Practice based on Evidence (DECIDE) framework *(83)*, an evidence-to-decision tool that includes intervention effects, values, resources, equity, acceptability and feasibility criteria, to guide the formulation of the recommendations *(84, 85)*. The list of the guideline development group members and their areas of expertise appears in Annex 4. Regional advisers from the WHO regions participated in the meetings of the guideline development group.

External resource persons

The external resource persons for this guideline were six individuals identified by the steering group who could provide valuable insights to the guideline development group on issues relevant to the topic. Their expertise included iron metabolism, micronutrient deficiencies, programme evaluation and development of systematic reviews. The external resource persons participated in technical presentations and in discussions related to those presentations, providing factual information, feedback and clarification when required. They were not present in closed-session deliberations of the guideline development group. That is, they participated in general discussions on the evidence and factors to consider for the crafting of the recommendations but did not contribute to the decision on the recommendation wording, direction or strength. The six external resource persons are listed in Annex 5.

Systematic review teams

The systematic review teams provided comprehensive, objective syntheses of the evidence for each of the key questions to inform the recommendations. Advances from systematic reviews were presented in the meetings described in the section History of the project on the use of ferritin for the assessment of iron status. The final results from these systematic reviews were presented at the guideline development group meeting in Geneva in June 2016. The list of systematic reviews and authors is presented in Annex 6.

Management of conflicts of interests

The steering group, in compliance with the WHO guidelines for *Declaration of interests for WHO experts (86)* and in collaboration with the Department of Compliance and Risk Management and Ethics, managed the potential conflicts of interests. All potential guideline development group members were asked to fill in and sign the standard WHO declaration-of-interests and confidentiality undertaking forms. An updated curriculum vitae was also required from each prospective member of the guideline development group, as they engage in their individual capacity and not as institutional representatives.

The steering group reviewed the declarations-of-interest statements in conjunction with the curriculum vitae for all guideline development group members. Information from the internet or media were gathered, in order to identify any public statements made or positions held by the prospective guideline development group members and experts on the issue of iron metabolism and cut-off values for ferritin concentrations to determine iron deficiency or risk of iron overload. These were assessed for intellectual bias that may be perceived to, or actually, affect impartiality. All concerns or potential issues were discussed with the WHO Office of Compliance, Risk Management and Ethics. All potential conflicts of interest were managed on a case-by-case basis.

All members of the guideline development group were assessed to have no perceived or real conflicts of interests on the topic. At the beginning of both guideline development meetings, the members were asked to verbally declare their research and programme experiences and sources of funding.

The names of the guideline development group members, along with a description of the objectives of the meeting, were published on the WHO website, for public notice and comment. No additional information on any interests or biases relating to the individuals being considered for membership of the guideline development group were brought to light from the public notice.

Identification of priority questions and outcomes

An initial set of questions to be addressed in the guidelines was the starting point for formulating the recommendation. The questions were drafted by technical staff at WHO, based on the policy and programme guidance needs of Member States and their partners. The questions were discussed and reviewed by the steering group.

A meeting of the guideline development group on 15–17 September 2010 in Panama City, Panama, was held to finalize the scope of the questions and to rank the outcomes and populations of interest for the recommendation on indicators for the assessment of iron status. Serum ferritin and transferrin receptor were ranked of highest priority for undergoing a thorough review.

The guideline development group discussed the relevance of the questions and modified them as needed. The group scored the relative importance of each outcome from 1 to 9 (where 7–9 indicated that the outcome was critical for a decision, 4–6 indicated that it was important and 1–3 indicated that it was not important). The final key questions on this intervention, along with the outcomes that were identified as critical for decision-making, are listed in PICO format in Annex 1.

The population of interest included participants of any sex, age (i.e. infants, children, adults), pregnancy status or hospitalization status, in any country, although some of the questions focused on vulnerable populations, e.g. children and pregnant women. The main comparators were the risk of iron deficiency, as defined by absent iron stores in bone marrow; the risk of iron overload, as defined by excess liver iron content; the response to nutrition-specific or nutrition-sensitive interventions; the methods of ferritin determination; the role of inflammation on ferritin concentrations; and the public health significance of iron deficiency measured by ferritin. The main outcomes were accurate diagnosis of the risk of iron deficiency; accurate diagnosis of the risk of iron overload; change in ferritin concentrations and in the prevalence of iron deficiency, iron deficiency anaemia or iron overload with nutrition interventions; the sensitivity, specificity and predictive value of ferritin methods; and correlations between acute phase proteins and ferritin concentrations, and between socioeconomic variables and ferritin concentrations.

Evidence identification and retrieval

WHO developed a project for retrieving, summarizing and assessing the evidence to inform WHO recommendations on the use and interpretation of serum/plasma ferritin concentrations for assessing iron status in populations. Five protocols were initially developed to address specific topics through systematic reviews of published data and analysis of raw data from international databases.

The protocols were:

1. serum/plasma ferritin for assessing iron status in populations;
2. how ferritin concentration responds to nutrition interventions;
3. the accuracy and comparability of methods for measuring ferritin concentration;
4. the influence of inflammation on ferritin concentrations; and
5. the use of ferritin concentrations in defining levels of public health concern with respect to the iron status of populations.

Quality assessment and grading of evidence

Systematic reviews based on the PICO questions were used to summarize and appraise the evidence. These reviews followed the procedures of the *Cochrane handbook for systematic reviews of interventions (87) and the Cochrane handbook for Diagnostic Test Accuracy (DTA) reviews (88)*. Each study included in the systematic reviews was assessed for risk of bias. This was recorded and contributed towards the assessment of the overall certainty of the evidence. During the discussion and deliberations, the steering group and the guideline development group carefully reviewed the certainty, scope and study inclusion criteria for the systematic reviews. The relative weight given to the trials and non-randomized studies was taken into account when evaluating the certainty assessment for each study. When possible, the findings were synthesized with a pooled estimate of effect. The results of the systematic reviews were presented to the guideline development group, along with an assessment of the confidence in the estimates of effect for the critical outcomes.

Evidence profiles were prepared according to the GRADE *(63)* approach, to assess the overall certainty of the evidence *(81, 82)*. The certainty of evidence for each outcome was rated as "high", "moderate", "low", or "very low", based on a set of criteria including risk of bias, inconsistency, imprecision, indirectness and publication bias.

Formulation of recommendations

The draft recommendations were discussed by the steering group and in consultation with the guideline development group, in a meeting held on 15–17 June 2016 in Geneva, Switzerland. The systematic reviews and the GRADE evidence profiles for each of the critical outcomes were used for drafting recommendations. An evidence-to-decision framework (based on the DECIDE framework *(83)* was used to lead discussion and decision-making *(84, 85)*. This framework, which considers discussions on key background information, criteria for making a decision and conclusions, was used to help the group to move from evidence to decisions.

For developing the recommendations, the guideline development group considered the certainty of the existing evidence, values and preferences, costs, the baseline prevalence of iron deficiency and/or anaemia and/or other nutritional deficiencies, and the equity and feasibility of implementation. The domains listed next were prepared by the steering group and discussed during the guideline development group meeting for each of the key PICO questions.

Certainty of evidence

The overall degree of confidence in the estimates of effect as presented in the GRADE profile was considered in the drafting of the recommendation. The higher the certainty of evidence across critical outcomes that are relevant to decision-making, the higher the likelihood is of a clear positive recommendation.

Balance of benefits and harms

The guideline development group evaluated the balance between desirable and undesirable consequences, including the magnitude of the effects and relative importance of these consequences. Where benefits clearly outweigh harms or vice versa, the greater the likelihood is of a recommendation in favour of or against the intervention, respectively.

When establishing cut-off values for iron deficiency, it is important to consider what the consequences of a false-positive finding are. If they are not severe, maybe a higher sensitivity is appropriate. If a false-positive result represents a real risk for the patient population, a higher specificity is better. In day-to-day practice, this means that we don't want to deprive pregnant women or children of iron. Likewise, we don't want to give additional iron to infected populations.

Values and preferences

The relative importance of the outcome to the individuals or populations directly affected by the recommendation describes the values and preferences. The steering group performed a review of qualitative information on how end-users perceived interventions to improve iron status to tackle iron deficiency or the risk of iron overload and/or their perception on the importance of knowing their iron status or their ferritin concentration. This was presented during the guideline development group meeting.

Acceptability

Qualitative information on how health-care workers, service providers and end-users perceive interventions to improve iron status and to measure the impact of interventions through multiple blood samplings was analysed during the guideline development group meeting. The higher the acceptability of the intervention among stakeholders, the more likely it is that an intervention will be clearly recommended. When it was deemed necessary to recommend an intervention that is associated with low acceptability, strategies to address concerns about acceptability during implementation were discussed.

Resource implications

This relates to evaluation of how resource intensive and cost effective the intervention is to service users and health systems in different settings. A recommendation in favour of or against the intervention is likely where the resource implications are clearly advantageous or disadvantageous.

Equity

An intervention is likely to be recommended if it will reduce health inequities among different age groups and settings.

Feasibility

The group discussed different scenarios to highlight the feasibility of implementation of ferritin determinations in population surveys, nutrition interventions and/or clinical settings, to identify barriers. For instance, how effectively do ferritin cut-off values identify iron deficiency? Every cut-off value will have some trade-offs. A very high ferritin cut-off value will pick up every single case of iron deficiency, but it will also pick up a lot of false positives. The opposite is true with a very low cut-off value. Based on this, there is a need to confirm the clinical and public health diagnostic accuracy of ferritin as an index of iron deficiency and try to identify the optimal ferritin thresholds. Where there is greater feasibility, the more likely it is that the intervention will be recommended.

Based on the discussions during the meeting, each recommendation was supported by a rationale, implementation considerations and research priorities. Recommendations were defined as "strong" or "conditional", based on the degree to which the guideline development group is confident in the balance between the desirable and undesirable consequences of implementing the recommendation. A strong recommendation is one for which the guideline development group is confident that the desirable effects of adherence outweigh the undesirable effects. A conditional recommendation is one for which the guideline development group concludes that the desirable effects of adherence probably outweigh the undesirable effects, although the trade-offs are uncertain.

Decision-making during the guideline development group meeting

The chairpersons (Dr Lindsey Allen for the first guideline development group meeting in Panama 2010; and Dr Gary Brittenham and Prof Pattanee Winichagoon for the second guideline development group meeting in Switzerland 2016), were nominated at the opening of the consultation and the nominations were approved by the guideline development group.

The procedures for decision-making were established at the beginning of the meetings, including a minimal set of rules for agreement and documentation of decision-making. At least two thirds of the guideline development group members were present for an initial discussion of the evidence and proposed recommendation and remarks. By secret ballot, each member of the guideline development group noted the direction and strength of each of the recommendations, using an online form specifically designed for this purpose. Abstentions were not allowed.

Once voting was complete, subsequent deliberations among the members of the guideline development group could take place. If there was no unanimous consensus (primary decision rule), more time was given for deliberations and a second round of online voting took place. If no unanimous agreement was reached, a two-thirds vote of the guideline development group was required for approval of the proposed recommendation (secondary decision rule). The results from voting forms will be kept on file by WHO for up to 5 years.

Document preparation and peer-review

The responsible technical officer wrote the first draft of the guideline, with comments from the steering group. Technical editing and proofreading were done by a contracted party.

The final draft guideline was peer-reviewed by content experts, to provided technical feedback; identify errors of fact; ensure that there were no important omissions, contradictions or inconsistencies with scientific evidence or programmatic feasibility; and assist with clarifying the language, especially in relation to implementation, adaptation and contextual issues Eight potential peer-reviewers were approached after assessment of the declarations of interests. The details of the peer-reviewer appear in Annex 7.

The steering group reviewed all comments and revised the document, in order to ensure clarity of the recommendation while maintaining consistency with the original meaning.

DISSEMINATION AND PLANS FOR UPDATING

Dissemination

This guideline will be disseminated through electronic media such as slide presentations and the World Wide Web, through the WHO Nutrition mailing list *(89)*, social media, the WHO Nutrition website *(90)* or the WHO e-Library of Evidence for Nutrition Actions (eLENA) *(91)*. eLENA compiles and displays WHO guidelines related to nutrition, along with complementary documents such as systematic reviews and other evidence that informed the guidelines; biological and behavioural rationales; and additional resources produced by Member States and global partners.

This guideline will be disseminated via the *Bulletin of the World Health Organization (92)*, in the public health round-up section. In addition, it will be disseminated through a broad network of international partners, including WHO country and regional offices, ministries of health, WHO collaborating centres, universities, other United Nations agencies and nongovernmental organizations.

Derivative products that are useful for end-users, such as summaries and collation of recommendations related to iron deficiency and anaemia, may be developed. Particular attention will be given to improving access to these guidelines for stakeholders that face more, or specific, barriers in access to information, or to those that play a crucial role in the implementation of the guideline recommendations, for example, policy-makers and decision-makers at subnational level that disseminate the contents of the guideline.

Plans for updating the guideline

The WHO Secretariat will continue to follow research developments on iron deficiency, particularly on biomarkers to detect it, with emphasis on ferritin determination and cut-off values to define iron deficiency and iron overload in different settings and age groups, and on the influence of infection/inflammation on diagnosis of iron status, particularly for questions in which the certainty of evidence was found to be low or very low. If the guideline merits an update, or if there are concerns about the validity of the guideline, the Department of Nutrition and Food Safety will coordinate the guideline update, following the formal procedures of the *WHO handbook for guideline development (80)*.

As the guideline nears the 10-year review period, the Department of Nutrition and Food Safety at the WHO headquarters in Geneva, Switzerland, along with its internal partners, will be responsible for conducting a search for new evidence.

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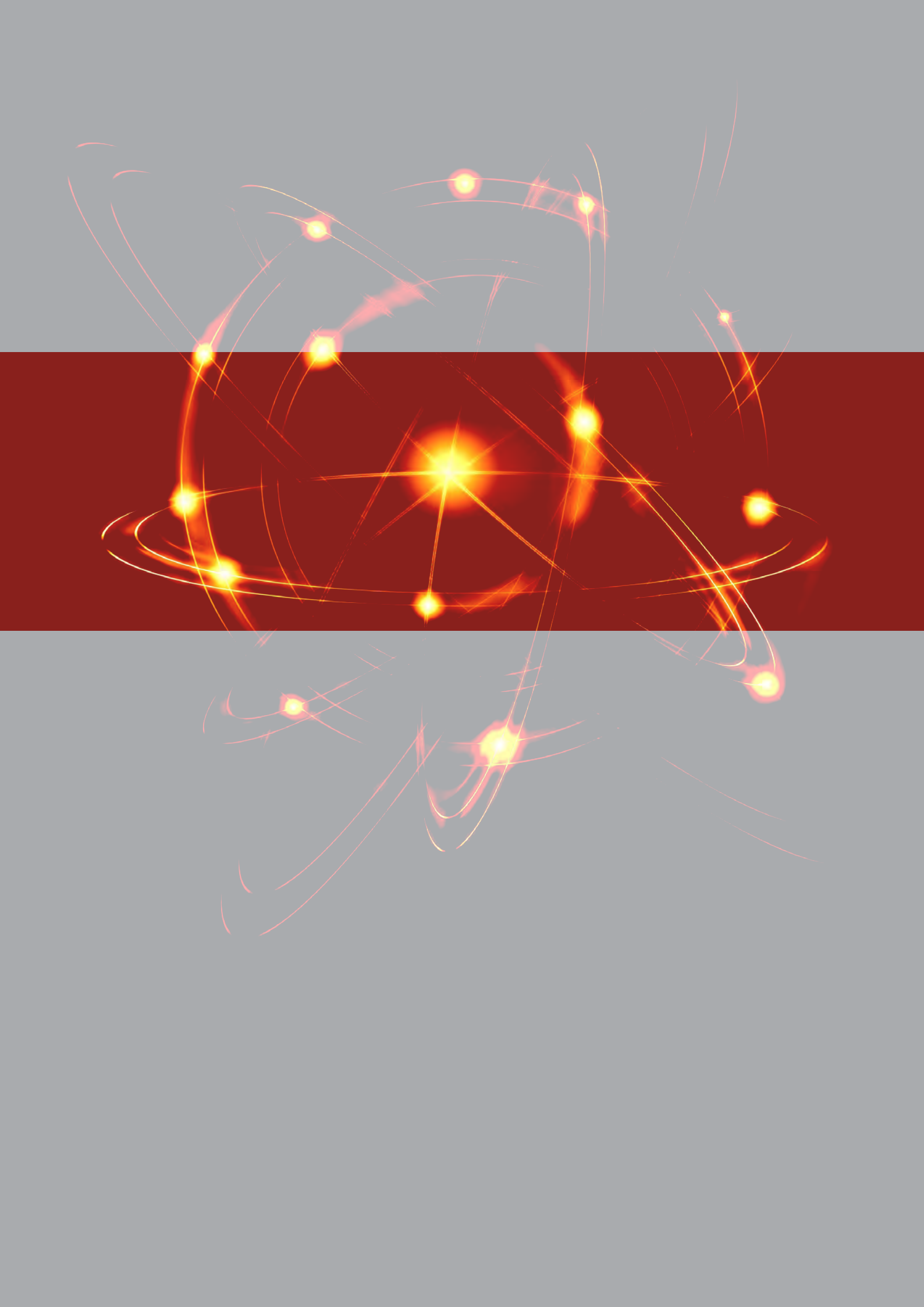

ANNEXES

ANNEX 1. QUESTION ON THE USE OF FERRITIN TO ASSESS THE IRON STATUS OF POPULATIONS IN POPULATION, INTERVENTION, CONTROL, OUTCOMES (PICO) FORMAT

Question 1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)?

a. What is the diagnostic accuracy of ferritin compared to the gold standard in free-living populations?

b. What should the cut-off value be to define deficiency/excess?

Population	Participants of any sex, age (i.e. infants, children, adults), pregnancy status, hospitalization status, in any country
Indicator	Serum or plasma ferritin concentration
Comparator	• Risk of iron deficiency as defined by absent iron stores in bone marrow • Risk of iron overload as defined by excess liver iron content
Outcomes	• Accurate diagnosis of the risk of iron deficiency (using <12 µg/L, <15 µg/L, <30 µg/L and other commonly used cut-off values) • Accurate diagnosis of the risk of iron overload

Question 2. Is ferritin an adequate marker for assessing the impact of iron interventions?

a. If yes, when should it be measured?

Population	Generally healthy populations • Children aged 6 months and older • Non-pregnant women aged 15–49 years • Pregnant women
Indicator	Serum or plasma ferritin before and after receiving an intervention that increases iron intake or decreases iron loss • Nutrition-specific interventions that have a direct impact on the immediate causes of undernutrition • Nutrition-sensitive interventions that address the underlying determinants of nutrition status
Comparator	Serum or plasma ferritin before and after receiving placebo or a similar intervention without an iron-related component • Nutrition-specific interventions that have a direct impact on the immediate causes of undernutrition • Nutrition-sensitive interventions that address the underlying determinants of nutrition status
Outcomes	• Change in ferritin concentrations • Change in the prevalence of iron deficiency, iron deficiency anaemia or iron overload with iron interventions (as defined by trialist)

Question 3. How should ferritin be measured?

a. What assays are available?

b. What biological samples are acceptable for analysis (plasma/serum/dried blood spots from venous or capillary blood)?

Population	Apparently healthy populations • Infants <6 months of age • Infants and young children aged 6–59 months • School-age children (5–11 years) • Adolescents aged 12–14 years • Women aged 15–49 years • Pregnant women • Men • Older persons Sub-analyses • Populations in settings with high rates of inflammation or malaria, and disaster or emergency areas where respiratory and gastrointestinal infections are common • Diabetic, obese, overweight and/or insulin-resistant populations
Index test	Ferritin concentrations measured by any available test
Comparator	Ferritin concentrations measured by enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), nephelometry or chemiluminescence
Outcomes	• The sensitivity of ferritin methods • The specificity of ferritin methods • The predictive value of ferritin methods • The cost/financial feasibility of ferritin methods • The limit of detection of ferritin methods

Question 4. Should ferritin be measured in combination with indicator(s) of infection or inflammation?

a. If yes, what indicators (α-1 acid glycoprotein [AGP] alone, C-reactive protein [CRP] alone, both AGP and CRP? Provide prioritization) and how should they be interpreted?

b. How should ferritin be adjusted for inflammation?

Population	Apparently healthy populations • Children aged 6 months and older • Women aged 15–49 years
Indicator	Serum or plasma ferritin concentrations assessed using current guidance • Perform survey in season of low inflammation • Measure acute phase protein and exclude individuals with elevated CRP or AGP • For individuals (or populations) with inflammation, use higher ferritin cut-off value (<30 µg/L)
Comparator	Serum or plasma ferritin concentrations adjusted for inflammation (AGP alone, CRP alone, both AGP and CRP) using regression modelling
Outcomes	• The relationship between acute phase proteins and ferritin concentrations • The median difference in the percentage change in prevalence of iron deficiency with different correction factors • The range of prevalence of deficiency when applying different correction factors for inflammation compared to no correction • The validity of different correction factors • The precision of different correction factors • The feasibility of different correction factors

Question 5. What are the population prevalence ranges for determining a public health problem?

Population	Apparently healthy populations • Infants and children aged 6–59 months • Non-pregnant women aged 15–49 years
Indicator	Prevalence of low ferritin concentrations (adjusted and unadjusted for inflammation)
Comparator	Social, economic and health indicators (gross domestic product, infant mortality rate, maternal mortality rate, anaemia, stunting)
Outcomes	Correlation coefficients of ferritin concentrations with measures of country/individual development, for determining the magnitude of iron deficiency as a public health problem

A. Question 1. Estimates of the accuracy of serum ferritin to assess iron deficiency in apparently healthy individuals

PICO: Healthy persons at risk of iron deficiency should check their iron status via serum ferritin as replacement of a BM test, to avoid the challenges of an invasive BM procedure and elevated costs, with acceptable rates of false negatives (which increase the risks of undesirable effects of iron deficiency via anaemia, and could lead to more tests), as well as false positives that lead to unnecessary treatment (side-effects, anxiety and costs).

Settings: Community, outpatient, primary health care

			Quality of evidence						Effect per 1000 participants for iron deficiency (prevalence of 40.86%)			
Test accuracy	**Number of studies (number of participants)**	**Study design**	**Risk of bias**	**Indirectness**	**Inconsistency**	**Imprecision**	**Publication bias/other considerations**	**Outcome**	**Specificity = 60%**	**Specificity = 75%**	**Specificity = 90%**	**Certainty of evidence**
Sensitivity	5 (143)	5 prospective/ ambispective cohort	Crucial limitations[1]	Serious[2]	Unclear on how to assess	Serious[3]	Undetected	TPs	203	111	30	⨁⊖⊖⊖ Very low[1,2,3]
								FNs	206	298	378	
Specificity	5 (207)		Crucial limitations[1]	Serious[2]	Unclear on how to assess	Serious[3]	Undetected	FPs	237	148	59	
								TNs	355	444	532	

TPs: true positives; FNs: false negatives; FPs: false positives; TNs: true negatives.

[1] Limitations in participants´ enrolment, selection and flow for most of the studies, sufficient to rate down one level.

[2] Studies done in children, young people and pregnant women; high prevalence of iron deficiency in included studies.

[3] There are very few studies, and the variability is high.

B. Question 1. Estimates of the accuracy of serum ferritin to assess iron deficiency in non-healthy individuals

PICO: Ill patients at risk of iron deficiency should check their iron status via serum ferritin as replacement of a BM test, to avoid the challenges of an invasive BM procedure and elevated costs, with acceptable rates of false negatives (which increase the risks of undesirable effects of iron deficiency via anaemia, and could lead to more tests), as well as false positives that lead to unnecessary treatment (side-effects, anxiety and costs).

Settings: Community, outpatient, primary health care

			Quality of evidence					Effect per 1000 participants for iron deficiency (prevalence of 35.95%)			
Test accuracy	**Number of studies (number of participants)**	**Study design**	**Risk of bias**	**Indirectness**	**Inconsistency**	**Imprecision**	**Publication bias/other considerations**	**Outcome**	**Specificity = 75%**	**Specificity = 85%**	**Specificity = 95%**	**Certainty of evidence**
Sensitivity	70 (2051)	66 prospective / ambispective cohort + 3 restrospective cohort + 1 case-control	Crucial limitations[1]	No	Serious[2]	No	Undetected	TPs	349	338	300	⨁⨁⨀⨀ Low[1,2]
								FNs	10	21	59	
Specificity	70 (3658)		Crucial limitations[1]	No	Serious[2]	No	Undetected	FPs	256	160	64	
								TNs	384	481	577	

TPs: true positives; FNs: false negatives; FPs: false positives; TNs: true negatives.

[1] Limitations in patients' enrolment, selection and final inclusion (ones with all available data) for at least half of the studies, sufficient to rate down one level.

[2] There is unexplained heterogeneity, which may be the cause for the multiple and very diverse thresholds (ranged between 12 µg/L and 273 µg/L).

C. Question 1. Estimates of the accuracy of serum ferritin to assess iron overload in non-healthy individuals

PICO: Ill patients at risk of iron overload should check their iron status via serum ferritin as replacement of liver biopsy test, to avoid the challenges of an invasive biopsy procedure and elevated costs, with low rates of false negatives (which increase the risks of undesirable effects of iron overload, and could lead to more unnecessary tests), as well as acceptable rates of false positives that lead to unnecessary treatment (side-effects, anxiety and costs).

Settings: Community, outpatient, primary health care

			Quality of evidence					Effect per 1000 participants for iron overload (prevalence of 41.7%)				
Test accuracy	**Number of studies (number of participants)**	**Study design**	**Risk of bias**	**Indirectness**	**Inconsistency**	**Imprecision**	**Publication bias/other considerations**	**Outcome**	**Specificity = 65%**	**Specificity = 75%**	**Specificity = 85%**	**Certainty of evidence**
Sensitivity	36 (803)	27 prospective/ ambispective cohort + 2 restrospective cohort + 7 case-control[1]	Very serious[2]	Serious[3]	Serious[4]	No	Undetected	TPs	332	304	258	⨁⊖⊖⊖ Very low[1,2,3,4]
								FNs	85	113	159	
Specificity	36 (1124)		Very serious[2]	Serious[3]	Serious[4]	No	Undetected	FPs	204	146	87	
								TNs	379	437	496	

TPs: true positives; FNs: false negatives; FPs: false positives; TNs: true negatives.

[1] One third of the studies ($n = 9$) are case-control or retrospective, and 20% ($n = 7$) have only diseased population, leading to a very high prevalence of included studies. For the study design, and very high prevalence reasons, we rate down one level.

[2] Very serious risk of bias due to patients' enrolment, flow, selection and final inclusion (ones with all available data) for at least half of the studies, sufficient to rate down one level.

[3] Around half of the studies ($n = 18$) focused on hereditary haemochromatosis, most of them on an outpatient basis. Very high prevalence of iron overload in all included studies.

[4] There is unexplained heterogeneity, which may be the cause for the multiple and very diverse thresholds.

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ANNEX 6. SYSTEMATIC REVIEW TEAMS

Question 1. Is ferritin an adequate marker of iron stores (risk of deficiency and risk of iron overload)?

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McMaster University, Ontario, Canada.

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Queen Mary University of London, London, United Kingdom of Great Britain and Northern Ireland.

Question 2. Is ferritin an adequate marker for assessing the impact of iron interventions?

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Centers for Disease Control and Prevention, Georgia, United States of America.

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Centre for Global Child Health, Hospital for Sick Children, Toronto, Canada.

Question 3. How should ferritin be measured?

Reference *(70)*. Performance and comparability of laboratory methods for measuring ferritin concentrations in human serum or plasma: A systematic review and meta-analysis. PLoS One. 2018;13(5):e0196576. doi:10.1371/journal.pone.0196576.

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Question 4. Should ferritin be measured in combination with indicator(s) of infection or inflammation?

Reference *(73)*. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr. 2010;92:546–55.

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Question 5. What are the population prevalence ranges for determining a public health problem?

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