

A critical analysis of iron status indicators in three independent studies of South African primary school children

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It all starts here™



Preface

This mini-dissertation will be presented in article format. Teresa Harris the *Magister Scientiae* (MSc) student, wrote the manuscript: "A critical analysis of iron status indicators in three independent studies of South African primary school children" in accordance with the authors' instructions of the journal *South African journal of clinical nutrition* to which the manuscript will be submitted.

The co-authors of this manuscript Prof JC Jerling and Prof SM Hanekom provided permission that the article be submitted for examination purposes. The article is still to be submitted to the journal, therefore, no permission was obtained from the editor of the journal.

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Abstract

Background

The potential dire consequences of iron deficiency (ID) and iron deficiency anaemia (IDA) on childhood development are of major public health concern. Many factors contribute to anaemia, ID being only one progressive factor. The prevalence of ID and IDA must be accurately determined before iron intervention strategies can be safely prescribed. There is continued uncertainty regarding the optimal approach to identifying and measuring ID, as indicators have different roles, explore different aspects of iron metabolism and cannot be directly compared. Furthermore, inflammation and infection have a confounding effect on the commonly applied indicator and acute phase reactant, serum ferritin (SF). In the public health setting, a suitable method to assess iron status in developing countries has to be inexpensive, standardised, established, easy to measure and its applications specific to identifying ID.

Aim

We conducted secondary analysis of screening data from three independent iron intervention studies to critically evaluate the indicators used to determine iron status in 6-11-year-old primary school children from three South African provinces.

Study design and methods

A cross-sectional descriptive analysis was performed on the screening data collected in 2009 and 2010 during iron intervention studies in KwaZulu-Natal (n=736), Northern Cape (n= 1045), and North West (n=546). The three distinct study sites were analysed independently and collectively.

Children's haemoglobin (Hb), SF, transferrin receptor (TfR), zinc protoporphyrin (ZPP), and C-reactive protein (CRP) concentrations were measured and body iron calculated. ID prevalence was compared using different methods (namely the single indicators SF, TfR and ZPP, body iron and the multiple criteria model), and the influence of inflammation on SF was considered. Literature suggests that the multiple criteria model provides a more complete assessment of iron status. The performance of single and body iron indicators were compared to the multiple criteria model (by assessing sensitivity, specificity and predictive values).

Results

Significant positive correlations between CRP (indicator of inflammation) and SF existed in all study sites and the combined sample ($p < 0.01$). The mean SF concentration was substantially

higher in subjects with inflammation than those without. A different SF cut-off to identify ID was applied to subjects with inflammation.

The percentage of ID subjects varied using different indicators (4.2 – 26.5% in KwaZulu-Natal; 4.1 – 13.4% in Northern Cape; 7.0 – 24.4% in North West; and 5.4 – 15.2% in the combined sample). The sensitivity, specificity and predictive values of alternate ID indicators varied within and between study sites, compared to the multiple criteria model.

Conclusion

Simply using Hb as an ID indicator is inaccurate. The vast differences between percentages identified as ID by different indicators is reason for concern. No consistent agreement appeared between single ID indicators, body iron and the multiple criteria model for ID identification after correcting for inflammation in primary school children. The global view of the multiple criteria model as the gold standard for estimating ID is debatable and potentially impractical at a public health level. Current evidence cautions against overestimating the prevalence of ID, as there is more associated harm than deficiency underestimation. This critical analysis has confirmed a need for research to identify a suitable, accurate and precise alternative to Hb as a tool in the South African public health setting. Furthermore, the impact of inflammation on iron status indicators, in particular SF, should be assessed in context to clearly set parameters for its use in nationally-representative nutrition surveys, the cornerstone of iron intervention strategies.

Key Words

Iron deficiency; Primary school children; Iron status indicators; Inflammation; Serum ferritin; Transferrin receptor; Zinc protoporphyrin; Body iron; Multiple criteria model

Opsomming

Agtergrond

Die moontlike ernstige gevolge wat ystertekort (YT) en ystertekort-anemie (YTA) vir ontwikkeling gedurende die kinderjare inhou is 'n groot openbare gesondheidsbepoornis. Baie faktore dra by tot anemie, waarvan YT net een progressiewe faktor is. Die voorkoms van YT en YTA moet presies bepaal word voor yster-intervensiestrategieë met veiligheid voorgeskryf kan word. Daar is voortslepende onsekerheid oor die optimale benadering tot die identifisering en meting van YT, aangesien die verskillende indikatore verskillende rolle vervul en verskillende aspekte van ystermetabolisme ondersoek en gevolglik nie direk vergelykbaar is nie. Wat meer is, inflammasie en infeksie het 'n strengelinge effek op die mees algemeen gebruikte indikator en akute fase reaktant, serum ferritien (SF). In die openbare gesondheidsomgewing sal 'n toepaslike metode om ystertekort te meet in ontwikkelende lande goedkoop, gestandaardiseer en gevestig moet wees, maklik moet wees om te meet en die toepassing sal spesifiek moet wees tot die identifisering van YT.

Doelstelling

Ons het 'n sekondêre analise gedoen van die siftingsdata van drie onafhanklike yster-intervensiestudies om die indikatore te evalueer wat gebruik is om die ysterstatus in 6-11-jaaroue laerskoolkinders uit drie provinsies van Suid-Afrika te meet.

Studie-ontwerp en metodes

'n Deursnee beskrywende analise is uitgevoer op die siftingsdata wat ingesamel is in 2009 en 2010 gedurende yster-intervensiestudies in KwaZulu-Natal (n=736), Noord-Kaap (n=1045), en Noordwes (n=546). Die verskillende studieterreine is onafhanklik en gesamentlik geanaliseer.

Kinders se hemoglobien (Hb), SF, transferrien reseptor (TfR), sink protoporfirien (ZPP), en C-reaktiwe proteïen (CRP) konsentrasies is gemeet en liggaamsyster is bereken. Die voorkoms van YT soos uitgewys deur die gebruik van verskillende metodes (naamlik die enkelindikatore SF, TfR en ZPP, liggaamsyster en die veelvoudige kriteria-model) is vergelyk, en die invloed van inflammasie op SF is in berekening gebring. Die literatuur voer aan dat die veelvoudige kriteria-model 'n meer volledige beoordeling van ysterstatus bied. Die prestasie van enkel- en liggaamsysterindikatore is vergelyk met die veelvoudige kriteria-model (deur die sensitiviteit, spesifisiteit en voorspellingswaarde te meet).

Resultate

Daar was betekenisvolle positiewe korrelasies tussen CRP (aanduiders van inflammasie) en SF by al die studieterrine en die gesamentlike steekproef ($p < 0.01$). Die gemiddelde SF konsentrasie is wesenlik hoër in proefpersone met inflammasie as by die daarsonder. 'n Ander SF-afsnypunt vir YT is geïdentifiseer en toegepas op proefpersone met inflammasie.

Die persentasie van proefpersone met YT het gevarieer na aanleiding van die verskillende indikatore (4.2 – 26.5% in KwaZulu-Natal; 4.1 – 13.4% in die Noord-Kaap; 7.0 – 24.4% in Noordwes; en 5.4 – 15.2% in die gesamentlike steekproef). Die sensitiviteit-, spesifisiteit- en voorspellingswaardes van die verskillende indikatore het gevarieer binne en tussen studieterrine in vergelyking met die veelvoudige kriteria-model.

Gevolgtrekking

Die blote gebruik van Hb as indikator vir YT is onakkuraat. Die groot verskille tussen die persentasies wat geïdentifiseer is as YT by die verskillende indikatore is verdere rede tot kommer. Geen konsekwente ooreenkoms het tussen die verskillende enkel YT-indikatore, liggaamsyster en die veelvoudige kriteria-model vir YT identifisering na korrigerings vir inflammasie in laerskoolkinders voorgekom nie. Die wêreldwye agting van die veelvoudige kriteria-model as die goudstandaard vir die skatting van YT is debatteerbaar en potensieel onprakties op 'n openbare gesondheidsvlak. Huidige bewyslewering waarsku teen die oorskating van die voorkoms van YT, aangesien daar meer geassosieerde skade is as wat daar tekort onderskatting is. Hierdie kritiese analise bevestig die behoefte aan navorsing wat streef om 'n toepaslike, akkurate en presiese alternatief vir Hb as metode binne die Suid-Afrikaanse openbare gesondheidsorgomgewing te vind. Verder behoort die impak van inflammasie op ysterstatusindikatore, veral SF, nagegaan te word binne hierdie konteks om duidelike aanduiders daar te stel vir die gebruik daarvan in nasionaal-verteenvoerdigende voedingsopnames, die hoeksteen van ysterintervensie-strategieë.

Sleutelwoorde

Ystertekort; Laerskoolkinders; Ysterstatusindikatore; Inflammasie; Serum Ferritien; Transferritien reseptor; Sink protoporfirien; Liggaamsyster; Veelvoudige kriteria-model

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Abbreviations

ACD	Anaemia of Chronic Disease
AGP	α -1-glycoprotein
APP	Acute phase proteins
CEN	Centre of Excellence for Nutrition
CRP	C-reactive protein
Hb	Haemoglobin
ID	Iron deficiency
IDA	Iron deficiency anaemia
IDE	Iron-deficient erythropoiesis
KZN	KwaZulu-Natal
MRC	Medical Research Council
NC	Northern Cape
NPV	Negative predictive value
NW	North West
PPV	Positive predictive value
SANHANES-1	South African National Health and Nutrition Examination Survey-1
Se	Sensitivity
SF	Serum ferritin
Sp	Specificity
TfR	Serum transferrin receptor
WHO	World Health Organization
ZPP	Zinc protoporphyrin

Description of terms and conditions

Several important terms that are used in this dissertation will be delineated to promote clarity.

Term / condition	Description
Acute phase proteins	Positive or negative secretory proteins in the liver that are altered (increased or decreased) in response to injury or infection (Mahan, & Escott-Stump, 2012).
Anaemia	A deficiency in the amount of haemoglobin red blood cells transport or the number or size of the red blood cells (Mahan, & Escott-Stump, 2012).
Body iron	A quantitative estimate of total body iron; the logarithm of this ratio is directly proportional to the amount of stored iron in iron-replete patients and the tissue iron deficit in iron deficiency (Cook <i>et al.</i> , 2003)
Bone marrow aspirate	This direct measure of iron stores is regarded as the gold standard and involves the microscopic examination of Perl's Prussian blue stained bone marrow aspirate (Aguilar <i>et al.</i> , 2011).
C-reactive protein	A positive acute phase that is synthesised in the liver in response to infection, systemic inflammation or tissue damage. It increases rapidly, reaching a maximum concentration within 24 – 48 hours, and falls very soon after the infection (Davis <i>et al.</i> , 2012:178; Thurnham <i>et al.</i> , 2010:546).
Ferritin	The main protein in which iron is stored (WHO, 2011).
Haemoglobin	A conjugated protein that is the oxygen-carrying pigment of the erythrocytes (Mahan, & Escott-Stump, 2012). The highest concentration of iron (approximately 60% of body iron) is bound to haemoglobin (Crichton <i>et al.</i> , 2008).
Hepcidin	A major iron-regulating hormone secreted by the liver which reduces the absorption of iron by the duodenum and the release of iron by macrophages (Ganz, 2013:1721; Pasricha <i>et al.</i> , 2011:1099).
Inflammation	The purpose of inflammation is “protective and designed to neutralize and remove the invader and repair the damage caused directly by the invader and indirectly” (WHO, 2012).
Iron	Iron is an essential mineral for all organisms and most commonly occurs in the forms of ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron (Pantopoulos <i>et al.</i> , 2012:5705).
Iron deficiency	Iron deficiency, which is also referred to as early functional iron deficiency, occurs when iron transport is diminished. Iron deficient erythropoiesis occurs while Hb levels remain within range (Lynch, 2011a:673S).
Iron deficiency anaemia	Functional iron deficiency with anaemia (IDA) occurs when haemoglobin levels decrease to below the normal range (Lynch, 2011a:673S). It occurs when there is a defect in haemoglobin synthesis, resulting in the formation of smaller erythrocytes with a lower haemoglobin content, as well as negative effects on

	other functional iron-containing proteins and enzymes (Johnson, 1990:1486; Tussing-Humphreys <i>et al.</i> , 2012:391) .
Iron status indicators	Indirect determinants of the iron status of a population include serum ferritin, transferrin receptor and zinc protoporphyrin.
Multiple criteria model	The use of two or three abnormal indicators are indicative of iron deficiency (Cook <i>et al.</i> , 1976; Lynch, 2012).
Negative predictive value	The negative predictive value refers to the probability that a subject that was diagnosed as not having condition was correctly diagnosed (Altman, 1999:409).
Positive predictive value	The positive predictive value refers to the probability that a subject that was diagnosed with a condition was correctly diagnosed (Altman, 1999:409).
Sensitivity	Sensitivity is the true positive rate and is defined as “the proportion of positive cases that are correctly identified by the test” (Altman, 1999:409).
Specificity	Specificity is the true negative rate, defined as “the proportion of negatives cases that are correctly identified by the test” (Altman, 1999:409).
Storage iron depletion	The iron storage compartment is in the form of ferritin or haemosiderin (Crichton <i>et al.</i> , 2008).
Transferrin receptor	Cells express more transferrin receptors in response to iron deficiency; this is an indirect measure of adequacy of iron supply (WHO, 2012).
Zinc protoporphyrin	During iron deficiency and when the supply of iron is low, zinc is substituted for iron in protoporphyrin; this is an indirect measure of the adequacy of iron supply (WHO, 2012).

1 Chapter 1: Introduction

1.1 Background

It is estimated that 300 million preschool- and school-age children worldwide are anaemic as a result of iron deficiency (ID) (de Benoist *et al.*, 2008). The potential dire consequences of ID and iron deficiency anaemia (IDA) on childhood development are of major concern (WHO, 2001). Access to robust, high quality health indicators for children would assist in prioritising interventions and programmes for this vulnerable age group. Malnutrition, including ID, during school-aged years may have a negative influence on the health and survival of future generations due to the nutritionally disadvantaged position (Best *et al.*, 2010:400; Engle-Stone *et al.*, 2013:369). Individuals have to be accurately diagnosed with ID or IDA before iron intervention strategies can be safely prescribed to individuals and populations.

Haemoglobin (Hb) is an inexpensive practical indicator and is used at a public health level as a proxy for ID, although it has low specificity and sensitivity with regard to identifying ID (Cameron, B. M. & Neufeld, L. M., 2011:S49; Cook, 2005:319; Northrop-Clewes, C.A. & Thurnham, D.I., 2013:11). The prevalence of ID is more accurately described using other indicators, namely SF, however this is affected by inflammation and infection, meaning that a person with acute or chronic inflammation has a higher concentration of SF than an individual without inflammation, independent of iron status (Beard *et al.*, 2006:1498). The consequential alternative approaches that have been used in previous studies in order to adjust for inflammation include: 1) exclude subjects with inflammation; 2) adjust the cut-off to define a low SF concentration in individuals with inflammation; or 3) apply a correction factor to SF (Beard *et al.*, 2006:1498; Thurnham, D.I. & McCabe, G.P., 2012; Thurnham *et al.*, 2010:546).

It is not advisable to exclude subjects with elevated acute phase proteins (APPs), as subjects with ID may be more susceptible to infection and inflammation. Their exclusion may therefore result in bias and may substantially reduce sample sizes in areas with a high prevalence of inflammation, resulting in an underestimation of ID (Engle-Stone *et al.*, 2013:369; Thurnham *et al.*, 2010; WHO & CDC, 2007). An alternative method suggested by Thurnham *et al.* (2010) proposes a viable technique, which mathematically adjusts individual observations by interpreting the two APPs, C-reactive protein (CRP) and α_1 -acid glycoprotein (AGP). The most useful alternative approach to consider the effect of inflammation on SF concentrations is to adjust the cut-off value for ID (as mentioned above). For individuals ≥ 5 years of age, this means applying SF cut-off of 19 $\mu\text{g/l}$, instead of 15 $\mu\text{g/l}$ to those individuals with elevated CRP (Thurnham, D.I. & McCabe, G.P., 2012).

Additional iron status indicators include transferrin receptor (TfR) and zinc protoporphyrin (ZPP). However, each have strengths and weaknesses. Elevated TfR is an indicator of ID within the tissues, but the method lacks standardisation, is confounded by factors other than iron that affect erythropoiesis and relies on test kit reference ranges (Cook, 2005:319; Northrop-Clewes, C.A. & Thurnham, D.I., 2013:11). It is essential to test levels of ZPP on washed red blood cells, which makes the required methodology time consuming. The test lacks specificity, it still requires consensus regarding threshold cut-off values and ZPP levels are confounded by environmental lead exposure (Cook, 2005:319; Thomas *et al.*, 2013:639).

An additional indicator of ID is the calculation of body iron (Cook *et al.*, 2003:3359). The advantage of this method is that it provides a measure of iron status that is independent of Hb and does not rely on cut-off values (Lynch, 2011b). However, the calculation incorporates SF and TfR and their limitations are mentioned above.

The various single indicators have different roles, explore different aspects of iron metabolism and cannot be directly compared (Lynch, 2011a:673S). It is assumed that evaluating a combination of these indicators by using the multiple indicator model, may provide a more complete assessment (Pasricha *et al.*, 2011:1099).

1.2 Motivation for study and study design

This mini-dissertation provides an opportunity to critically evaluate iron status indicators by analysing data from iron intervention studies. It is inaccurate to use Hb as a proxy for ID, which is currently the practice at a public health level. Therefore, suitable alternatives that are practical and realistic have to be investigated. The suggestion that SF is the preferred ID indicator needs to be explored in the context of inflammation as this influences SF concentrations, independent of iron status. In addition to SF, promising indicators that are to be investigated include TfR, ZPP, body iron and the multiple criteria model.

We used screening data from three independent iron intervention studies and conducted secondary analysis to enable a critical evaluation of the indicators used to determine iron status in 6-11-year-old primary school children from three different South African provinces. Subjects were recruited and samples collected in 2009 and 2010 from primary schools in the rural area of the Valley of a Thousand Hills in KwaZulu-Natal (n=736) (Baumgartner *et al.*, 2012b:1327) and peri-urban areas in Kimberley, Northern Cape (n= 1045) (Troesch *et al.*, 2011:237) and Klerksdorp, North West (n=546) (Taljaard *et al.*, 2013:2271). The distinct study sites were analysed independently and collectively.

Each of the three intervention studies that provided the data for this secondary analysis obtained ethical approval. Principal researchers from the three independent studies were approached and their permission was requested to utilise the screening data from the studies. Principal researchers were invited to be co-authors of a published journal article. The screening data was made available for further exploration by Prof Johann Jerling (principle supervisor of this mini-dissertation and data owner of the North West study (2010)); Prof Marius Smuts (principal investigator and data owner of the KwaZulu-Natal study (2009); and Dr Lize van Stuijvenberg (principal investigator and data owner of the Northern Cape study (2009)).

The screening data was provided in Excel spread sheets and no direct contact was made with subjects. The subjects remained anonymous and were only referred to by their subject number when specific queries relating to an indicator value arose. I managed the data on my personal computer, which was access controlled with a password and all data sheets will be returned to the principle supervisor once the final dissertation has been submitted. All data sheets will be deleted from my computer on completion of the research.

1.3 Basic hypothesis and study objectives

Each indicator used to describe iron status has its own advantages, disadvantages and limitations. The hypothesis for this study is that the even though the prevalence of ID will differ when various indicators are applied after correcting for inflammation, there will be a definitive superior indicator to determine ID as compared to the multiple criteria model.

The secondary analysis of screening data from the three iron intervention trials provides an opportunity to critically analyse iron status indicators and to document the influence of inflammation in these South African primary school children from sites in KwaZulu-Natal, Northern Cape and North West. This information could provide a more accurate assessment of the iron status of individuals at a public health level and of populations, thereby inform nationally-representative nutrition surveys.

Furthermore, it may assist in the compilation of appropriate guidelines to apply at a primary health care level to accurately identify ID and IDA, and appropriately prescribe treatment, as opposed to Hb being used as an inaccurate indicator of ID.

The study objectives are therefore to:

- Investigate the influence of inflammation on SF concentrations;
- Describe the prevalence of anaemia, ID and IDA using different indicators; and

- Investigate a superior indicator to determine ID as compared with the multiple criteria model.

1.4 Structure of this mini-dissertation

This dissertation is written in article format according to the postgraduate guidelines of the North-West University. The overall structure of the study takes the form of four chapters, including this introductory chapter.

Chapter 2 begins by discussing the available published literature, complementing the title of the dissertation, a critical analysis of iron status indicators in three independent studies of South African primary school children.

The third chapter presents an article entitled, “Comparison of indicators of iron deficiency in three independent studies of South African primary school children”. This chapter is written following the authors’ guidelines of *The South African journal of clinical nutrition*.

The word limit stipulated by *The South African journal of clinical nutrition* has not been adhered to due to the complex nature of the subject explored, but all parts that have been elaborated upon will be shortened or referred to in short when submitting the article for publication.

The fourth and final chapter presents an elaboration on the main findings of the research documented in Chapter 3, focusing on the three key objectives that have been identified earlier. It draws upon the entire dissertation, tying up the various theoretical strands in order to come to a final conclusion that explains the implications of the findings, and to identify further research areas.

Decimal numbers are used to ensure that the headings follow a logical sequence, except for Chapter 3, where headings are given without numbering according to the instructions for authors of *The South African journal of clinical nutrition*. One combined reference list has been compiled for chapters 1, 2 and 4 and is presented after Chapter 4, followed by the addenda. Chapter 3 has a reference list according to the Vancouver reference style, as directed by the instructions for authors of *The South African journal of clinical nutrition*.

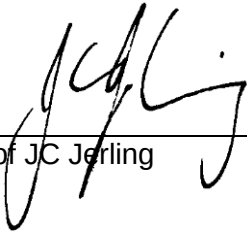
1.5 Contributions of the research team

Table 1-1: Level of involvement of the student in the exploration of the screening data from iron intervention studies in KwaZulu-Natal, Northern Cape and North West, and authors' contributions to the article to be submitted

Team member	Institution	Role
Prof Johann Jerling	CEN, NWU* Potchefstroom campus	Supervisor who fulfilled an advisory role on all the content of this mini-dissertation and data owner of the Klerksdorp, North West study (2010).
Prof SM Hanekom	CEN, NWU* Potchefstroom campus	Co-supervisor in an advisory role on all the content of this mini-dissertation.
Prof Marius Smuts	CEN, NWU* Potchefstroom campus	Principal Investigator and data owner of the Hillcrest, KwaZulu-Natal study (2009).
Dr Lize van Stuijvenberg	Nutritional Intervention Research Unit, Medical Research Council, South Africa	Principal Investigator and data owner of the Kimberley, Northern Cape study (2009).
Mrs Marike Cockeran	Medicine Usage in South Africa, North West University, Potchefstroom campus	Provided guidance with and conducted part of the statistical analysis.
Dr Jeannine Baumgartner	Post-doctoral fellow, CEN, NWU* Potchefstroom campus	Completed her PhD on the Hillcrest, KwaZulu-Natal study and provided feedback on the article in this mini-dissertation.
Dr Christine Taljaard	Post-doctoral fellow, CEN, NWU* Potchefstroom campus	Completed her PhD on the Klerksdorp, North West study and provided feedback on the article in this mini-dissertation.
Mrs Teresa Harris	MSc candidate, CEN, NWU* Potchefstroom campus	Developed and formulated the research questions. Conducted the statistical analysis and was the primary writer of the article and sole writer of the rest of the mini-dissertation.

* CEN, Centre of Excellence for Nutrition, NWU, North-West University

The following statement and signatures confirm the co-authors' role in the article, and their permission to include the article (Chapter 3) into this dissertation: "I declare that I have approved the above-mentioned article, and that my role in the study, as indicated above, is representative of my actual contribution. I hereby give my consent that the article may be published as part of the MSc (Nutrition) dissertation of Mrs T Harris."



Prof JC Jerling



Prof SM Hanekom



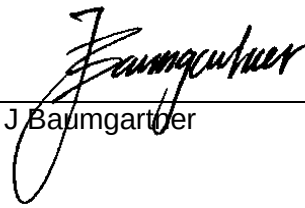
Prof CM Smuts



Dr ME van Stuijvenberg



Dr M Cockeran



Dr J Baumgartner



Dr C Taljaard

2 Chapter 2: Literature review

2.1 Introduction

More than two billion people worldwide suffer from micronutrient deficiencies, which is an immense concern from a socioeconomic and public health point of view. The most prevalent micronutrient deficiency is of iron, which results in suboptimal health and functioning and in severe cases, even death (Tulchinsky, 2010:243). Iron is a vitally important micronutrient required by every cell and organ of the body (Hartfield, 2010:347). A sustained negative iron balance caused by inadequate dietary intake, utilisation and / or absorption, increased requirements or blood loss results in iron deficiency (ID) and can advance to iron deficiency anaemia (IDA) when there is insufficient iron to support normal erythrocyte production (WHO, 2011a). It is estimated that 300 million preschool- and school-age children worldwide are anaemic as a result of ID (de Benoist *et al.*, 2008).

The potential dire consequences of ID and IDA on childhood development, both cognitive and physical, are of major concern to the nutrition community (WHO, 2001). Infants and young children may experience adverse effects on cognitive performance and behaviour, physical growth and the immune system as a result of ID (WHO, 2001). Pollitt (1997:133) has reported on the association between ID and impaired learning ability and scholastic performance.

The WHO (2012) confirms that there is an urgent need to address the selection and standardisation of iron status indicators. Currently the most promising indicators include serum ferritin (SF), transferrin receptor (TfR) and zinc protoporphyrin (ZPP), but attention must be paid to making these indicators affordable, their assays appropriate to use in developing countries and their applications specific (WHO, 2012). Individuals need to be accurately diagnosed with ID or IDA before iron intervention strategies can be safely prescribed to individuals and populations, as there are risks associated with supplementing individuals with a normal iron status who were incorrectly identified as having ID (Engle-Stone *et al.*, 2013:369; Lynch, 2012:55) .

As a foundation to exploring iron status, how it is assessed and the factors that affect iron status, it is critical to first understand iron metabolism and the key influences on iron homeostasis.

2.2 Iron physiology and homeostasis

2.2.1 Iron transport

Iron is an essential mineral for all organisms and most commonly occurs in the forms of ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron (Pantopoulos *et al.*, 2012:5705). The importance of iron to the body is evident from the fact that it is incorporated into many enzymatic and non-enzymatic proteins that play crucial roles in physiological functions (Pantopoulos *et al.*, 2012:5705). Such functions include oxygen transport, storage and homeostasis, electron transport and energy production, DNA synthesis, anti- and beneficial pro-oxidant functions, as well as metabolism (Beard, 2001:568S). An adequate amount of iron is imperative for normal functioning of the immune system as changes in the immune system are associated with both iron overload and iron deficiency (Mahan, L.K, & Escott-Stump, S., 2012).

Free iron is toxic and highly reactive and unbound iron causes cellular damage, therefore it is always bound to proteins (Ganz, 2013:1721). Iron may be bound to functional proteins, which make up the function iron compartment (comprising of haemoglobin, myoglobin, haem- and non-haem enzymes), bound to the iron transport protein transferrin, or incorporated into the iron storage compartment in the form of ferritin or haemosiderin (Crichton *et al.*, 2008). The highest concentration of iron (approximately 60% of body iron) is bound to haemoglobin (Crichton *et al.*, 2008).

2.2.2 Iron absorption

Iron homeostasis is critical to ensure that iron levels remain optimal to prevent ID or, on the other extreme, damage from excess iron. Haem iron, found in animal foods, is more absorbable (15%) than non-haem dietary iron found in animal and plant foods (3-8%) (Mahan, & Escott-Stump, 2012). An individual's iron status determines the amount of iron they absorb, with absorption decreasing during iron overload and increasing during deficiency (Ganz, 2013:1721; Mahan, L.K, & Escott-Stump, S., 2012). Absorption and transport take place in two stages - the jejunum and duodenum are the major sites for iron absorption, and post-absorption iron is enzymatically converted to ferritin (the intracellular store). Iron is then moved into the plasma via an active transport mechanism iron. There are different routes for the absorption of haem and non-haem iron. Haem iron is transported across the first cellular membrane (brush border) through vesicle formation. Non-haem iron is absorbed in three stages to cross the brush border membrane via the iron transporter divalent metal transporter 1 (DMT1) (Mahan, & Escott-Stump, 2012).

Humans require relatively little iron as it is highly conserved. Only 10% of total body iron is excreted in bile and the balance is recovered and reused by the body every day (Mahan, L.K, & Escott-Stump, S., 2012). The various causes of ID include inadequate absorption or utilisation, increased requirements, blood loss or excretion, inadequate intake or an increased destruction resulting in decreased release from stores (Mahan, L.K, & Escott-Stump, S., 2012). There are four cell types involved in iron homeostasis, namely enterocytes in the duodenum that absorb dietary iron; hepatocytes that store and release iron; erythrocytes manufactured in the bone marrow and macrophages that incorporate recycled iron into erythrocytes (Ganz, 2013:1721).

The cellular uptake of iron is well controlled and its free movement is restricted by tight junctions between cells. The liver secretes the transport protein transferrin, which binds plasma iron and which is the source of iron for most cells (Pantopoulos *et al.*, 2012:5705). A common measure of iron status is total binding capacity; i.e. the amount of iron that can be bound to transferrin. Iron is released into the cell in three stages: 1) transferrin receptors bind the transferrin-iron complex on cell membranes (serum transferrin receptors are present in serum at a concentration proportional to their cellular counterpart); 2) the complex is then internalised by endocytes; and 3) the transferrin-iron complex is dissolved, thereby releasing iron into the cell (Pantopoulos *et al.*, 2012:5705). Any cellular iron that is not required immediately, is assimilated into ferritin and stored in this form in the spleen or in bone marrow. Iron is also secreted into plasma, depending on the amount of cellular iron. Lastly, iron is stored in the form of haemosiderin (Horl, 2013:291; Hunt, 2005:82; Pantopoulos *et al.*, 2012:5705).

Iron bound to transferrin in the plasma is approximately 3-4g with 1-2mg of iron being lost daily, 1-2mg absorbed and 8-13mg not being absorbed (Pantopoulos *et al.*, 2012:5705). Between 200-1500 mg of iron is stored in the body as ferritin and haemosiderin. One third is stored in the liver, an additional third in bone marrow and the balance in muscles and the spleen (Mahan, L.K, & Escott-Stump, S., 2012). It is evident from examining the movement of iron at a cellular level that promising indicators to assess iron status include ferritin, reflecting iron stores; and the number of transferrin receptors in the cell membrane, reflecting the amount of iron available at a tissue level. These, in addition to other indicators, will be explored in more detail at a later stage.

Iron loss (through blood loss and shedding of skin and intestinal cells) is not actively controlled, and therefore optimal levels are achieved by controlling intestinal iron absorption. Hepcidin, the hormone secreted by the liver, acts as a major regulator of iron metabolism by reducing the absorption of iron by the duodenum and the release of iron by macrophages (Ganz,

2013:1721; Pasricha *et al.*, 2011:1099). Hepcidin levels decline during ID, IDA and anaemia, yet increase during inflammation or when body levels of iron are high (Tussing-Humphreys *et al.*, 2012:391).

A review by Ganz (2013:1721) illustrates that the synthesis of hepcidin in the liver is determined by three influencers. Firstly, synthesis is increased by inflammatory cytokines and iron released from stores and decreased in response to erythropoiesis, as bone marrow produces a substance to suppress hepcidin. Secondly, hepcidin is regulated by inflammatory signals, although the mechanism of action remains unclear (Ganz, 2013:1721). Thirdly, inflammatory cytokines stimulate hepcidin production and when circulating concentrations of hepcidin are high, it reduces efflux of iron from enterocytes, which decreases dietary iron absorption (Aeberli *et al.*, 2009:1111; Amato A, *et al.*, 2010:1772; Baumgartner *et al.*, 2012a:24; Tussing-Humphreys *et al.*, 2010:2010; Tussing-Humphreys *et al.*, 2009:297; Viatte & Vaulont, 2009:1223; Zimmermann *et al.*, 2008:1098). This further aggravates iron transport to physiological tissues even though iron stores remain normal. Even though iron homeostasis is altered by inadequate dietary iron intake, inflammation and obesity (Aeberli *et al.*, 2009:1111; Tussing-Humphreys *et al.*, 2012:391), it is useful to measure the factors related to this movement when investigating iron status. The majority of body iron is present in the functional iron compartment as haem in haemoglobin molecules or myoglobin in muscles (Pantopoulos *et al.*, 2012:5705).

Even though iron physiology and homeostasis is complex and the transport and absorption of iron are tightly regulated, there remain confounders influencing iron status that need to be addressed.

2.3 Factors altering iron homeostasis and iron status indicators

Even though it may not be possible to always measure and accurately consider the impact of inflammation, vitamin A deficiency, weight status and exposure to environmental lead on individuals, it is important to acknowledge them as potential confounders when considering iron homeostasis and interpreting results from iron status indicators.

2.3.1 Inflammation

Persons with inflammation have altered iron status indicators, which will be discussed in detail at a later stage, compared to those without inflammation, and inflammation causes the body to handle iron differently (Nel, 2013; Thomas, C. & Thomas, L., 2005:14). As a protective mechanism to prevent free iron from being available to stimulate parasitic and bacterial

growth, acute phase proteins (APPs) reduce systemic iron by transferring it to hepatic stores (Zimmermann *et al.*, 2010:1406).

The prevalence of ID is described using biomarkers that are affected by inflammation and infection, meaning that a person with an acute or chronic infection has a higher concentration of SF than an individual without inflammation, independent of iron status (Beard *et al.*, 2006:1498). The consequential alternative approaches that have been used in previous studies in order to adjust for inflammation include: 1) exclude subjects with inflammation; 2) adjust the cut-off to define a low SF concentration in individuals with inflammation; or 3) apply a correction factor to SF (Beard *et al.*, 2006:1498; Thurnham, D.I. & McCabe, G.P., 2012:63; Thurnham *et al.*, 2010:546).

The effect of inflammation or infection on APP, namely C-reactive protein (CRP) and α_1 -acid glycoprotein (AGP), and SF is not synchronised. CRP is synthesised in the liver in response to infection, systemic inflammation or tissue damage (Davis *et al.*, 2012:178). At the onset of infection, CRP increases rapidly, reaching a maximum concentration within 24 – 48 hours, and falls very soon after the infection (Thurnham *et al.*, 2010:546). The extent of the underlying pathology is reflected in the rate of synthesis and concentration of CRP (Davis *et al.*, 2012:178). The AGP concentration on the other hand increases for 4-5 days and remains elevated even after infection subsides. Table 2-1 below summarises the effect of inflammation on biomarkers. Serum ferritin follows a different pattern to AGP and CRP: it increases rapidly within a few hours of inflammation and remains elevated until after CRP has subsided and while AGP is still increasing (Thurnham *et al.*, 2010:546).

It is not advisable to exclude subjects with elevated APPs, as subjects with ID may be more susceptible to infection and inflammation. Their exclusion may therefore result in bias and may substantially reduce sample sizes in areas with a high prevalence of inflammation, resulting in an underestimation of ID (WHO & CDC 2007; Thurnham *et al.*, 2010:546; (Engle-Stone *et al.*, 2013:369). A useful alternative approach to consider the effect of inflammation on SF concentrations is to adjust the cut-off value for ID (as mentioned above). For individuals ≥ 5 years of age, this means applying a calculated SF of 19 $\mu\text{g/l}$ instead of 15 $\mu\text{g/l}$ to those individuals with elevated CRP (Thurnham, D.I. & McCabe, G.P., 2012:63). An alternative method suggested by Thurnham *et al.* (2010:546) propose a viable technique, which mathematically adjusts individual observations by interpreting the two APPs, namely CRP and AGP.

Table 2-1: Impact of inflammation on indicators of iron status and anaemia (Thurnham *et al.*, 2010:546; Thurnham & McCabe, 2012:63; WHO, 2012)

Indicator	Impact of inflammation
Haemoglobin	Short-term inflammation has a minor impact on red cell mass and iron loss inhibits Hb synthesis, but continuous or frequent inflammation results in a decline in red cell mass and anaemia of chronic disease.
Serum ferritin	Inflammation increases SF, but it is possible to apply correction factors related to CRP and AGP. SF appears to be raised even after 7 days of trauma, possibly as a result of SF having a longer half-life than CRP or the persistent depression of erythropoiesis.
Transferrin receptor	Studies confirmed that TfR increases in response to ID. However, research shows a decline in TfR as a result of impaired erythroblast iron availability, and TfR concentrations remained low for up to 7 days following limb surgery.
Zinc protoporphyrin	Inflammation has a limiting effect on circulating iron and therefore increases ZPP concentrations.
Body iron	This calculation relies on SF and is therefore influenced by inflammation. However, it is possible to correct for inflammation by applying the correction factors related to CRP and AGP.

Each method proposed has positive and negative elements. SF and the APPs, CRP and AGP increase and decrease at different rates. If a higher cut-off value was applied to all individuals in a population with a high prevalence of inflammation, this method may result in ID being underestimated in subjects with inflammation and overestimated in subjects with adequate iron stores. It is challenging to select and apply a single cut-off SF value to populations as the presence of inflammation differs from one individual to the next (Ayoya *et al.*, 2010:1784; Engle-Stone *et al.*, 2013:369; Grant *et al.*, 2012a:105; Kung'u *et al.*, 2009:2124; Thurnham *et al.*, 2010:546).

Correction factors applicable to other studies were introduced by Thurnham *et al.* (2010), who recommend that CRP and AGP be measured. This allows individuals to be categorised according to the presence and stage of inflammation based on the concentration of the APPs. The four categories and correction factors include absence of inflammation (normal CRP and AGP): incubation (raised CRP) correction factor of 0.77; early convalesces (raised AGP and CRP) correction factor 0.53; and late convalescence (raised AGP) correction factor 0.75. These correction factors adjust SF for inflammation, thereby allowing the standard WHO cut-off value of 15 µg/L for children > 5 years to be used to diagnose the presence of ID.

2.3.2 Obesity

Anaemia is more common in obese individuals than normal weight individuals. Obesity appears to alter iron homeostasis (Tussing-Humphreys *et al.*, 2012:391). In a randomised controlled intervention trial, Baumgartner *et al.* (2012a:24) found that iron supplementation was less effective in overweight children than normal weight children. Even though the exact process is unknown, researchers suspect that the proposed mechanism suggests that inflammation increases levels of hepcidin because excess adipose tissue produces inflammatory cytokines, which stimulate hepatic secretion of hepcidin (Aeberli *et al.*, 2009; Tussing-Humphreys *et al.*, 2009:297; Tussing-Humphreys *et al.*, 2010:2010; Tussing-Humphreys *et al.*, 2012:391; Viatte & Vaulont, 2009:1223; Zimmermann *et al.*, 2008:1098). More research to confirm causality and the health implications of concurrent ID and obesity is needed. However, studies in this field are challenging and may be confounded by obesity-induced inflammation, as inflammation affects certain ID indicators (namely SF) independent of ID (Baumgartner *et al.*, 2012a:24; Tussing-Humphreys *et al.*, 2012:391; Zimmermann & Hurrell, 2007:511).

2.3.3 Malaria

Malaria and iron have a complex relationship related to iron metabolism. Malaria causes profound disturbances in iron utilisation and distribution (Spottiswoode *et al.*, 2014:1). While this is acknowledged as significant, it is not the focus of this particular mini-dissertation as the study sites for data collection (as identified later) were specifically selected to be free of malaria. Therefore this factor will not be addressed in detail.

2.3.4 Environmental lead

When exposure to environmental lead is elevated, for example in urban settings, the specificity of ZPP may be reduced as lead poisoning can elevate ZPP levels independently of iron status. Individuals with ID are more susceptible to long-term lead poisoning after exposure to environmental lead (Zimmermann, 2008).

The exposure of children to lead results in poor school performance. Research has been conducted to explore the heavy metal contamination of school vegetable gardens in Johannesburg, South Africa. Even though levels of lead were within acceptable intake limits, further research is needed in areas where schools are in close proximity to sources of pollution such as mining areas to assess the extent of exposure the public has to heavy metals contained in vegetables (Kootbodien *et al.*, 2012:234). Exposure to environmental lead is a public health concern in resource-poor South Africa as there is a lack of surveillance and

screening, as well as resources to alleviate the negative effects. There is extensive exposure to environmental lead in urban South African areas and childhood poverty is a major determinant of lead exposure (Naicker *et al.*, 2013). Meaningful approaches to reduce lead exposure include the introduction of legislation to control the use of lead in paint and the phasing out of leaded petrol (Mathee, 2012). Previous research has found that the domestic energy course, household hygiene and overcrowding were lead exposure risk factors for children living in proximity to lead mines in the Northern Cape, as well as children from informal settlements in KwaZulu-Natal (Mathee, 2012).

2.3.5 Vitamin A deficiency

Iron deficiency and IDA may also be caused by vitamin A deficiency as this inhibits the normal metabolism of iron (WHO, 2001). It was found that both vitamin A and iron deficiencies result in anaemia in preschool children. Vitamin A deficiency may contribute to ID as a result of increasing one's risk of infection, haematopoiesis (the formation of blood cellular components) and by affecting iron metabolism (Gamble *et al.*, 2004). Hanekom (2003) confirms that subjects have an increased risk of IDA if they have a low vitamin A status.

2.3.6 Other factors

Various factors that could influence iron metabolism include intestinal worms, age, ethnicity and altitude. Gastrointestinal parasites, such as worms, increase blood loss, which contributes to dietary deficiencies and negatively influences iron status by causing blood loss (Zimmermann & Hurrell, 2007:511). Their influence on anaemia and Hb is well-documented (Taljaard *et al.*, 2013). Literature refers to race-specific criteria for anaemia. Long-term exposure to higher altitude living increases Hb and thus the altitude of study sites should be considered (Johnson-Spear & Yip, 1994; Perry *et al.*, 1992; WHO, 2001). However, this is only relevant for individuals identified as anaemic and with IDA. Cook *et al.* (2005) found an increase in body iron after the age of two years, even with an increase in body size – a potential area for further research in the selected studies would be to evaluate the relationship between body iron and age.

The complexity of iron metabolism, the critical role that iron plays, as well as the factors that affect iron status, have been explained. Before the consequences of insufficient iron are described, differentiating between anaemia and a poor iron status will be explored. It is necessary to do so because anaemia is in fact often used as a proxy for ID. However, this is enormously inaccurate because a multitude of disorders, other than ID, can result in anaemia (Lynch, 2011a:673S), as illustrated in Figure 2-1.

2.4 Differentiating between anaemia and poor iron status

Anaemia is a public health priority in developing countries. ID, together with other causes, result in this problem, which leads to insufficient mass of circulating red blood cells (de Benoist *et al.*, 2008; WHO & CDC, 2007). Anaemia is diagnosed when Hb concentration falls below a specified cut-off (11.5 g/dL in children 5 – 11 years of age) (WHO, 2001). Even though it is impractical at a public health level to only confirm true IDA if Hb concentrations improve in response to treatment, it would prevent inaccurate assumptions. An example of such an inaccurate assumption, which is based on anaemia prevalence surveys, is that 50% of anaemia is a result of IDA (McLean *et al.*, 2008; WHO, 2012).

Iron deficiency is a progressive condition. Sustained negative iron balance caused by a combination or single-handedly by increased requirements, inadequate dietary intake, utilisation and / or absorption or blood loss - results in compromised synthesis of iron-containing proteins, namely Hb. The transition from ID to IDA happens when the negative iron balance is so severe that Hb falls below a specified cut-off value (Haas & Brownlie, 2001:676S; WHO, 2011b). There is no consistent or linear relationship between anaemia and IDA; anaemia can occur without ID and not all ID results in IDA (WHO & CDC 2007).

A dated review by Stoltzfus (2001) highlights an important point: “We have relied too much on anaemia prevalence as our sole indicator for assessing and monitoring iron deficiency”. This appears to still be relevant in public health settings at a provincial level in South Africa. In the 2014 manual for Integrated Management of Childhood Illnesses issued by the Department of Health, instructions refer to “give iron for anaemia” and if Hb 7 – 10 g/dL (which is indicative of anaemia), “give iron and counsel on iron-rich foods”. The assumption is that all anaemia indicated by a low Hb should be treated with iron.

2.4.1 Anaemia

Anaemia is a broad term used to describe a deficiency in the amount of Hb that red blood cells contain or the size of the red blood cells and therefore their oxygen-carrying capacity (Mahan, L.K, & Escott-Stump, S., 2012:). Hb is simple to measure with a single drop of capillary blood and is an important indicator of health status (Cameron, B. M. & Neufeld, L. M., 2011:S49). Even though it is thought that ID is the most relevant contributor to anaemia, there are many factors that contribute to this condition (Figure 2-1) (Northrop-Clewes, C.A. & Thurnham, D.I., 2013:11). Other causes of anaemia include other nutritional deficiencies (vitamins B₁₂, B₆ and A, folate and riboflavin), malaria helminth infection, chronic infection, intestinal and gastric disease and haemoglobinopathies (Northrop-Clewes, C.A. & Thurnham, D.I., 2013:11).

Factors such as age, altitude and genetics (ethnicity) influence Hb distribution and therefore cut-off values used to diagnose anaemia (WHO, 2001). Micronutrient deficiencies usually occur in combination with other deficiencies, because poor diet and lack of access to food are the common underlying causes of malnutrition (Best *et al.*, 2010:400).

Anaemia of chronic disease (ACD), which occurs in individuals suffering from an acute or chronic inflammatory condition, infection or trauma, develops as a result of altered iron metabolism and the retention of iron within body stores, not as a result of inadequate iron intake. This results in insufficient supply of iron to erythroid marrow (Thomas *et al.*, 2013:639) Weiss, 2002). It can co-exist with ID or IDA and indicators need to be carefully selected and interpreted to differentiate between these conditions (Tussing-Humphreys *et al.*, 2012:391).

Figure 2-1 below illustrates that Hb levels decrease as a result of nutritional and non-nutritional causes, for example micronutrient deficiencies, parasitic infections, genetic disorders and anaemia of chronic disease.

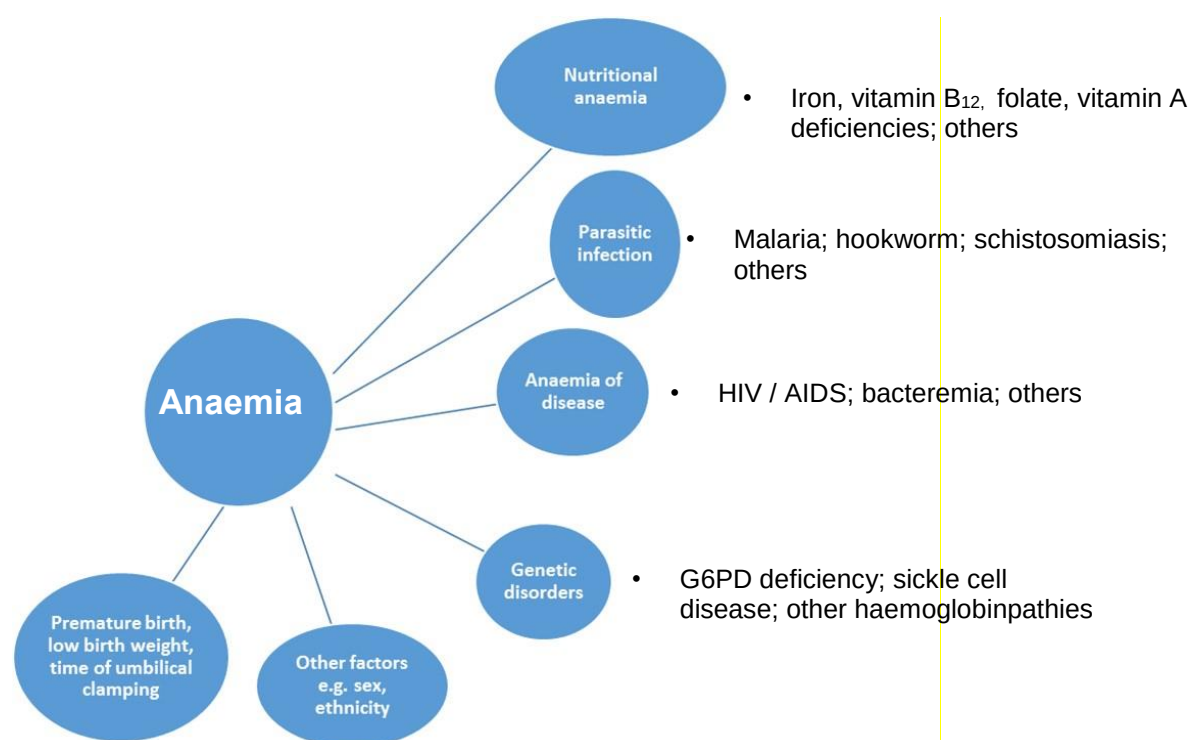


Figure 2-1: Determinants and risks of anaemia (low haemoglobin concentration) in populations (Cameron, B. M. & Neufeld, L. M., 2011:S49)

2.4.2 Iron deficiency and iron deficiency anaemia

When storage iron and transport and function iron are adequate, one's iron status is normal. As iron declines, there is insufficient iron to maintain normal functioning of bodily tissues and this decline has been described as taking place in three stages, as depicted in Figure 2-2

(Tussing-Humphreys *et al.*, 2012:391). It is important to acknowledge that these stages are simply a theoretical model. In practice, the development of ID and IDA is far more complex.

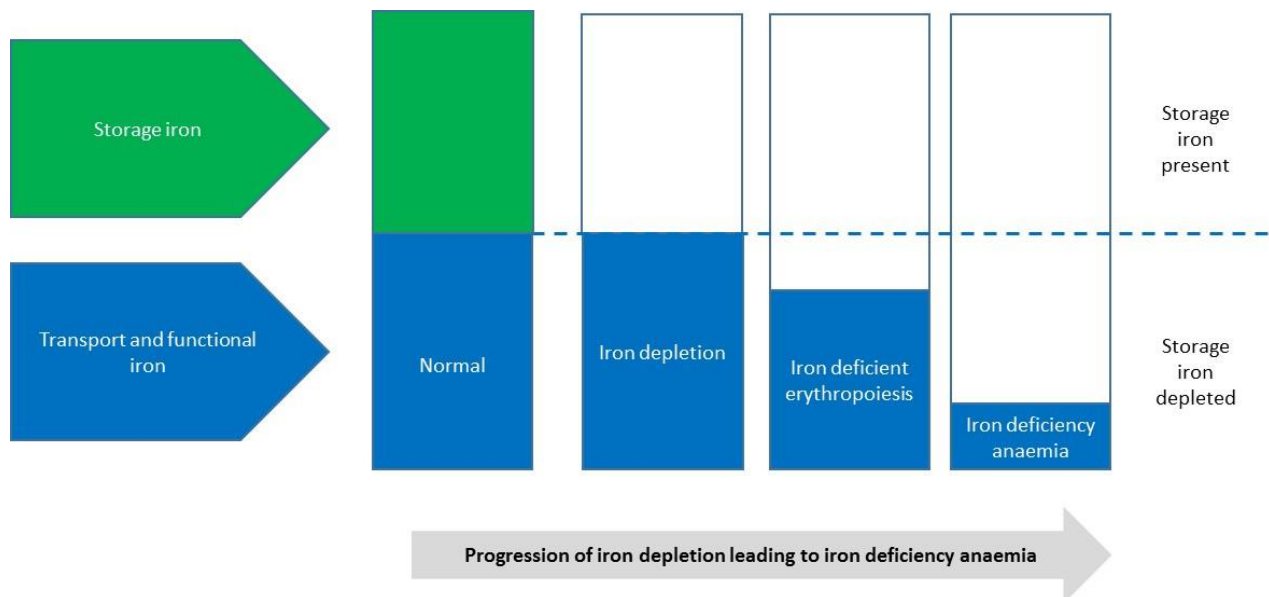


Figure 2-2: The spectrum of iron deficiency (adapted from Australian Red Cross Blood Service, 2014)

Stage 1, termed depletion of storage iron, occurs when the compartment of iron stores is depleted. This is indicated by SF dropping below its threshold value if liver disease and inflammation or infectious disorders are absent (Lynch, 2011a:673S).

Iron deficiency, classified as stage 2, is also referred to as early functional iron deficiency and occurs when iron transport is diminished. Iron deficient erythropoiesis occurs while Hb levels remain within range (Lynch, 2011a:673S). This is indicated by an upregulation of TfR as the additional receptors are expressed on the cell surfaces to increase iron uptake (Lynch, 2011a:673S). This stage may also be identified by an increase in ZPP. Due to the insufficient amount of available iron for haeme production, zinc replaces iron when the erythrocyte protoporphyrin is synthesised (Lynch, 2011a:673S). A more detailed insight into the formation of ZPP reveals that in the final step of Hb synthesis, iron (as Fe^{2+}) is inserted into a protoporphyrin by the enzyme ferrochetalase, which is then incorporated into the newly synthesised globin to form Hb. However, when there is insufficient iron in bone marrow to be incorporated into the new molecule during the early stages of iron deficient erythropoiesis, trace amounts of zinc are incorporated into protoporphyrin instead of iron resulting in an increase in ZPP, which is unable to transport oxygen (Labbe *et al.*, 1999:146). Once there is insufficient iron to maintain normal physiological functions of tissues such as the brain, muscles and blood, ID is confirmed (WHO, 2001).

As depicted in Figure 2-2, the third and final stage is defined as functional iron deficiency with anaemia (IDA) as Hb levels decrease to below the normal range (Lynch, 2011a:673S). IDA occurs when there is a defect in Hb synthesis, resulting in the formation of smaller erythrocytes with a lower Hb content, as well as negative effects on other functional iron-containing proteins and enzymes (Johnson, 1990:1486; Tussing-Humphreys *et al.*, 2012:391) .

The economic and health implications of ID and IDA are substantial and the consequences of such deficiencies will now be explored in more detail.

2.5 Consequences of poor iron deficiency and iron deficiency anaemia

Severe iron deficiency anaemia can result from various causes and has a negative impact on a child's cognitive development, intellectual performance and work capacity (Best *et al.*, 2010:400). Access to robust, high quality health indicators for children would assist to prioritise interventions and programmes for this vulnerable age group. Malnutrition, including ID, during school-aged years may have a negative influence on the health and survival of future generations due to the nutritionally disadvantaged position (Best *et al.*, 2010:400; Engle-Stone *et al.*, 2013:369).

Research has found that ID and IDA results in lethargy and suboptimal functioning of the brain and muscles as a result of a reduced oxygen-carrying capacity of the blood. This has a negative impact on work performance, endurance and economic productivity (Haas & Brownlie, 2001:676S; Olney *et al.*, 2007:2756). Research involving preschool children found that IDA had similar negative effects on behaviour as was observed in infants with IDA. However, the challenge with investigating such an age group is that one is uncertain of the exact point at which IDA occurred. IDA may have occurred for an extended period of time, and the negative effects observed may not be related directly to this specific age group (Lozoff *et al.*, 2007:683). A limited amount of research has been conducted with children to investigate the relationship between ID and cognitive performance and behaviour.

Beard (2003) explored the mechanism by which ID has an irreversible effect on the infant's brain and he found that it is related to changes in the biology and chemistry of the nervous system and that the movement of iron into specific areas of the brain is tightly controlled and age-related.

In a Lancet seminar, Zimmermann & Hurrell (2007) summarised the harsh effects of ID and IDA. From an economic and health system point of view, the mean value of physical productivity losses per year due to ID, in ten developing countries, was almost 0.6% of gross domestic profit. Untreated IDA during pregnancy results in low birthweight ID infants and

increases the risk of preterm labour. IDA has been shown to increase the frequency, duration and severity of respiratory tract infections in children. It results in negative effects on iodine and vitamin A status; and ID children are at a higher risk of environmental lead poisoning. The literature is inconclusive regarding the effects of ID on infants and children's cognitive and motor development, yet a systematic review confirmed that there are in fact negative effects of ID and IDA on school children (Grantham-McGregor & Ani, 2001:649S). School children with low Hb levels had poorer school performance, social attention and decreased motor activity, yet researchers are uncertain as to whether the negative effects are a result of low haemoglobin or low iron levels.

Research by Pollitt (1997) looked at the effect of ID on education. Studies in Indonesia, Thailand and Egypt all showed a difference in educational achievement in children with and without IDA. However, different results were seen in Guatemala and this raises the question of whether the degree and severity of ID and IDA influence cognition. Such studies are challenging, as it is difficult to distinguish between the effect of ID and other possible nutritional deficiencies as the determinants of school performance are multifactorial.

Aside from studies looking at the consequences of ID and IDA, it is critical to note that all of this research hinges on different methodologies to diagnose ID and IDA, which remain questionable.

2.6 Assessing iron status

Public health nutrition addresses the needs of individuals or population groups that are at risk for micronutrient deficiencies (Tulchinsky, 2010:243). In order to assess a population's or an individual's risk of ID, it is necessary to investigate the adequacy of an individual's iron supply, as well as the size of iron stores (Lynch, 2011a:673S).

Bone marrow aspirate examination is the only method available to directly assess iron status. However, it is invasive, prone to error and costly. Therefore alternative, reliable iron indicators must be established to assess the prevalence of ID and IDA, identify iron-deficient populations and monitor the impact of intervention strategies (Engle-Stone *et al.*, 2013:369; Lynch, 2012). Table 2-2 (Hanekom, 2003) below summarises the progression of ID to IDA and the changes in the various indicators / laboratory tests during the three stages.

There is continued uncertainty regarding the optimal epidemiological approach to identifying and measuring the severity of ID at a population level (Cook *et al.*, 2003) and the WHO (2012) confirmed that there is an urgent need to address the selection and standardisation of iron status indicators. However, attention must be paid to making these indicators affordable, their

assays and cut-offs standardised and appropriate to use in developing countries; and their applications specific to identifying ID at a population level (WHO, 2012).

Table 2-2: The progression of iron deficiency reflected in laboratory test results

Laboratory test	Depletion of storage iron	Iron-deficient erythropoiesis	IDA
Serum ferritin	↓	↓	↓
Serum transferrin receptor	↔	↑	↑
Serum iron	↔	↓	↓
Total iron binding capacity	↔	↑	↑
Transferrin saturation	↔	↓	↓
Erythrocyte protoporphyrin	↔	↑	↑
Mean cell volume	↔	↔	↓
Red cell distribution width	↔	↔	↑
Haemoglobin	↔	↔	↓
Haematocrit	↔	↔	↓

Within normal limits ↔; Decrease ↓; Increase ↑

2.7 Indicators of iron deficiency

Currently the most promising indicators to determine the iron status of a population and identify high-risk populations in need of intervention strategies include SF, TfR and ZPP. These are supplemented by the introduction of the multiple criteria model by Cook *et al.* (1976), whereby the use of two or three abnormal indicators are indicative of ID (Lynch, 2012), and the calculation of body iron by Cook *et al.* (2003) incorporating SF and TfR as a reflection of body iron stores.

The WHO (2012) recommends that in order to accurately assess the iron status of a population the following indicators should be used: SF to reflect iron stores; TfR as a measure of the adequacy of iron supply (with an elevated level indicating a deficiency); and Hb applied in conjunction with SF to indicate IDA. Zinc protoporphyrin should be considered as an indicator of ID as it measures adequacy of iron supply. If budget permits, the inflammatory markers CRP and AGP should be included in the assessment. Below Table 2-3 summarises the recommended indicators to detect a depletion in storage iron as the condition progresses from depletion of storage iron to iron deficient erythropoiesis, and lastly to iron deficient with anaemia.

Table 2-3: Recommended indicators to address anaemia and the different stages of ID and IDA (Lynch, 2011a:673S)

	Storage iron depletion	Early functional iron deficiency (iron deficient erythropoiesis)	Established iron deficiency with anaemia	Anaemia
Indicators and methods of assessment	Low SF	Elevated TfR or ZPP Multiple indicator model Body iron	Low SF and Hb	Low Hb

Each indicator will be explored in detail as the features of these indicators need to be considered when deciding which indicator to use in assessing a particular population. Such features include specificity and sensitivity, cost and logistics involved in collecting the required samples and performing the analysis (WHO, 2012).

2.7.1 Serum ferritin

The World Health Organization had previously identified SF to be the most preferred indicator to detect ID as it correlates with low body iron stores, but this is only in the absence of infection (WHO, 2001). Serum ferritin is a positive APP, meaning that it is elevated during infection or inflammation independent of iron status (Thurnham *et al.*, 2010:546). A further limitation in the use of SF is its inability to quantitatively reveal a further decline of the tissue iron pool once iron stores are depleted (Yang *et al.*, 2008:1892).

In order to standardise assay results, the WHO introduced International Standards in 1985 with the third and most recent one being released in 1997 (Ferraro *et al.*, 2012:1911). In a review by Ferraro *et al.* (2012), concern was raised regarding the fact that the studies used to validate the use of SF as a diagnostic indicator for ID and to determine the recommended cut-offs were all carried out before the standardised assays were introduced. There is a need for current studies that apply the WHO International Standards to optimise the clinical use of the SF as an indicator of ID and the cut-off values to accurately diagnose ID.

2.7.2 Transferrin receptor

Soluble transferrin receptor can be used to detect early changes in iron status. It is an indicator of erythropoietic activity, and elevated levels indicate ID only when iron stores are depleted. An advantage of using TfR is that it is able to estimate the extent of the functional iron deficit once iron stores are depleted, indicated by SF (Yang *et al.*, 2008:1892). Even though elevated TfR is an indication of ID within the tissues, the method lacks standardisation, is confounded

by factors other than iron that affect erythropoiesis and relies on test kit reference ranges (Tussing-Humphreys *et al.*, 2012; WHO, 2012).

2.7.3 Zinc protoporphyrin

Red cell zinc protoporphyrin: haem ratio is a measure of the adequacy of iron supply because zinc is substituted for iron in protoporphyrin when iron supply is inadequate (Lynch, 2012). Elevated ZPP reflects the iron status in the bone marrow, as this is the site where the substitution of zinc for iron occurs. It is essential to test levels of ZPP on washed red blood cells, which makes the required methodology time consuming. The test lacks specificity, it still requires consensus regarding threshold cut-off values; and ZPP levels are confounded by environmental lead exposure (Cook, 2005:319; Thomas *et al.*, 2013:639).

2.7.4 Hepcidin

Hepcidin is a peptide hormone, and the principal regulator of systemic iron homeostasis plasma and urinary assays are available and have been shown to provide information about iron status metabolism. There is considerable enthusiasm for its potential role as an iron status indicator. However, more research is needed to evaluate its role as an ID indicator. Hepcidin performed well as a diagnostic tool when compared to the TfR – SF index. However, these findings have to be confirmed in populations other than the non-anaemic females of reproductive age that are blood donor tested (Pasricha *et al.*, 2011).

2.8 Methods of assessing iron deficiency

In 2001 the WHO stated that the best indicator for detecting ID is SF (in the absence of infection) and when assessing populations, multiple indicators (namely Hb, SF and TfR) are useful. The multiple indicators reflect the complex iron scenario, with Hb reflecting functional impairment. Tissue avidity for iron is reflected by TfR and SF reflects iron storage. The latest WHO recommendation, similar to that in the earlier 2007 document, states that in order to assess the iron status of a population, Hb should be measured together with SF and TfR (WHO & CDC, 2007; WHO, 2012).

2.8.1 Single indicators

It is possible to assess the iron status of a population using SF on its own. However, this may underestimate the prevalence of ID in populations with a high prevalence of infection and inflammation, since SF concentrations are upregulated and falsely interpreted as adequate in these circumstances (WHO & CDC, 2007). In a study using data from the US National Health

and Nutrition Examination Survey 2003–2006, which involved children 3-5 years and females 12-49 years of age, agreement between body iron and the multiple criteria model to determine the prevalence of ID was fair to good. It was concluded that body iron was the preferred method to determine the functional consequences of ID (Cogswell *et al.*, 2009:1334). However, Grant *et al.* (2012) assessed ID in preschool Kenyan children and found that TfR better estimated the prevalence of ID compared to SF, ZPP and the TfR: ferritin ratio reflecting body iron.

It is debatable which cut-off value to apply when describing the prevalence of ID and IDA. Table 2-4 below summarises the cut-off values selected to assess the iron status of children 6-11 years old in previous studies. To be classified as ID, an individual has to have a value less than the cut-off, as those with values equal to or greater than the cut-off are not classified as being deficient in iron. The dilemma is that the purpose of an indicator is to diagnose a condition, rather than to indicate a point of minimum risk. Stoltzfus (1997) draws on a useful analogy by referring to the definition of low birth weight and malnutrition. A child 1 standard deviation (SD) below the reference mean is still malnourished and will experience adverse health effects even though moderate to severe stunting or wasting is only diagnosed at 2 SD below the reference mean. Similarly, an infant with a birth weight of 2.6 kg still has an increased risk of mortality even though low birth weight is only diagnosed at less than 2.5 kg. It is important to intensify efforts to monitor the prevalence of ID and IDA and it is proposed that simply a change in cut-off values could address this need (Stoltzfus, 1997:1764).

2.8.2 Body iron

An additional indicator of ID is the calculation of body iron whereby iron replete subjects are identified by a positive value and iron deficiency by a negative value: $\text{Body iron (mg/kg)} = -(\log_{10} (\text{TfR} \times 1000/\text{SF}) - 2.8229) / 0.1207$ (Cook *et al.*, 2003:3359). The advantage of this method is that it provides a measure of iron status that is independent of Hb and does not rely on cut-off values (Lynch, 2011). However, the limitations are that the calculation relies on SF, which is influenced by inflammation, and the method to determine TfR lacks standardisation; is confounded by factors other than iron that affect erythropoiesis; and relies on test kit reference ranges (Cook, 2005:319; Northrop-Clewes, C.A. & Thurnham, D.I., 2013:11). Even though this calculation to estimate body iron stores has only been validated in adults, it has been used as an iron status indicator in other studies (Engle-Stone *et al.*, 2013:369; Grant *et al.*, 2012a:105). Body iron actually replaced the multiple indicator method for iron status evaluations in US National Health and Nutrition Examination Surveys (NHANES) evaluations (Cogswell *et al.*, 2009).

Table 2-4 below summarises the indicators of iron status discussed above and includes the cut-off values appropriate for subjects in the research that will be conducted in three independent samples from primary schools in South Africa.

Table 2-4: Iron status indicators and selected cut-off values for children 6-11 years of age

Indicator	Cut-off value
Hb (WHO, 2001)	Less than 11.5 g/dL indicates anaemia
SF (Thurnham <i>et al.</i> , 2010: 546)	Less than 15 µg/L in subjects without inflammation and < 19 µg/L in subjects with inflammation indicates ID
ZPP (Metzgeroth <i>et al.</i> , 2005)	Greater than 70 mmol/mol haem indicates ID
TfR (Ramco Laboratories)	Greater than 8.3mg/L indicates ID
Body iron (Cook <i>et al.</i> , 2003: 3359; Skikne <i>et al.</i> , 1990: 1870)	A ratio of < 0 mg/kg indicates depleted iron stores

2.8.3 Multiple indicator model

The “multiple indicator model” also termed the “ferritin model” for iron status was introduced by Cook *et al.* in 1976 (Cook *et al.*, 1976; Lynch, 2012). Originally the use of two or three abnormal indicators (namely SF, erythrocyte protoporphyrin and transferrin saturation) was used to diagnose ID. Serum iron combined with total iron binding capacity can be used to calculate transferrin saturation (Cameron, B. M. & Neufeld, L. M., 2011:S49). However, Grant *et al.* (2012) applied the multiple indicator model, which consisted of two or more abnormal values for SF, TfR and ZPP. A cause for concern is that inconsistent use of the multiple criteria model has been reported, yet Cogswell *et al.* (2009) found reasonably good agreement between the prevalence of ID estimated by body iron stores and the multiple indicator model. An aim of this research is similar to this exercise whereby the agreement between the multiple criteria model and single indicators as well as body iron will be explored.

2.8.4 Optimal approach

If the inappropriate indicators are applied to an individual, ID and IDA may be misdiagnosed with anaemia or anaemia of chronic disease, as these four conditions exhibit common elements and in fact often co-exist (Tussing-Humphreys *et al.*, 2012:391). Each indicator reflects different aspects of iron metabolism and therefore different stages of deficiency. It is proposed that evaluating a combination of these indicators, namely the multiple indicator

model, may provide a more complete assessment (Pasricha *et al.*, 2011:1099). In order to adequately assess one's iron status it is necessary to use a combination of indicators, as each indicator evaluates a different aspect of iron metabolism and single indicators often disagree when compared to each other (Tussing-Humphreys *et al.*, 2012:391).

The review by Tussing-Humphreys *et al.* (2012) summarises the indicators that distinguish ID from IDA and ACD, as well as the abnormalities in iron status indicators during obesity, which are discussed above. It is useful to consolidate the biochemical features that differentiate ID and IDA from ACD and the changes to iron status indicators as a result of obesity.

Table 2-5: Features that distinguish the iron status parameters between iron deficiency (ID), iron deficiency anaemia (IDA), anaemia of chronic disease (ACD) and obesity (Tussing-Humphreys *et al.*, 2012:391)

Iron status parameter	ID	IDA	ACD	ACD + ID / IDA	Obesity
Hb concentration	↔	↓	↓	↑↔↓	↔
Mean cell volume	↔	↓	↔ ↓	↔ ↓	↔
SF	↓	↓	↑ ↔	↑ ↔	↑ ↔
Serum iron	↓	↓	↓	↓	↓
Transferrin saturation	↓	↓	↔ ↓	↓	↓
Total iron binding capacity	↑	↑	↔ ↓	↔ ↑	↔ ↑
Soluble TfR	↑	↑	↔ ↓	↑	↑
Inflammatory markers	↔	↔	↑	↑	↑
Serum hepcidin	↓	↓	↑	↔ ↑	↔ ↑

Within normal limits ↔; Decrease ↓; Increase ↑

It is important to meaningfully assess the quality of diagnostic information and also essential to look beyond assessing the accuracy of a diagnostic test (Meltz, 1978). Even though accuracy is a single and simple method to describe an indicator's diagnostic performance, it is preferable to look in more detail at an indicator's performance in terms of sensitivity and specificity (Meltz, 1978). The statistical method involves evaluating the proportion of subjects diagnosed with ID according to the single indicators and body iron, compared to the multiple criteria model and calculating the sensitivity, specificity, predictive values and percentage of subjects misclassified (Altman, 1999:409) to identify a better indicator of determining ID than the multiple criteria model. Sensitivity is the true positive rate and is defined as "the proportion of positives that are correctly identified by the test". Specificity is the true negative rate, defined as "the proportion of negatives that are correctly identified by the test" (Altman, 1999:409). The disadvantage of these calculations is that they are not clinically useful, but the advantage is that they are not affected by the prevalence of ID. In addition to sensitivity and specificity, one also has to assess the probability of the indicator correctly diagnosing ID. The positive

predictive value refers to the probability that a subject that was diagnosed with ID was correctly diagnosed; the negative predictive value refers to the probability that a subject that was diagnosed as not having ID was correctly diagnosed (Altman, 1999:409). Even though these calculations are clinically useful, it must be noted though that the predictive values observed cannot be universally applied, as they are strongly influenced by the prevalence of ID.

The optimal approach should also take into account the laboratory costs involved in testing the selected indicators. Grant *et al.* (2012) reported that there is a relatively similar laboratory cost to test SF, TfR and ZPP, and therefore the overall expense is related to the number of individuals in the sample as it is more costly to test all three indicators than testing a single indicator. The laboratory assessment costs in South Africa (according to AMPATH Laboratories, 2014) are similar for SF and TfR (approximately ZAR 166 and ZAR 156 per sample, respectively) and introducing the third indicator (ZPP) for the multiple criteria model adds approximately ZAR 90 per sample. Hb is by far the most affordable indicator (approximately ZAR 24 per sample), but does not accurately identify ID (as discussed above). Testing CRP in order to correct for inflammation adds approximately an additional ZAR 145 to the budget. Consequently, the overall expense is related to the number of individuals to be tested, the number of indicators tested and whether inflammation is identified.

Consensus is necessary regarding the use of appropriate iron status indicators; which cut-off values to apply; and the appropriate correction for inflammation, in order to compare effects of interventions and the prevalence of ID and IDA across populations and over time.

2.9 Epidemiology of iron deficiency in South Africa

In order to ensure that the potential negative consequences of ID and IDA are not realised for public health purposes, it is essential to ascertain the current prevalence and severity of ID in South African children. Within the South African context, surveys conducted in 2005 and 2013 explored the iron status at a national level. Table 2-6 below summarises the prevalence of ID, IDA and anaemia of South African children. In 2005 the prevalence of ID and IDA in children 7-9 years of age was 4.4 % and 2.0%, respectively. In 2013 the prevalence of ID and IDA in children under five years of age was 8.1% and 1.9%, respectively (Labadarios, D. 2007; Shisana *et al.*, 2013a). The potential limitations of these local national surveys relate to the methodology and the use of a single indicator to assess ID. SF was the indicator selected, but it is affected by inflammation and infection independent of iron status, and the cut-off value selected did not correct for the confounding effect of inflammation and infection on SF. This may have masked inadequate iron status in the 2013 survey. In Cameroonian children, it was found that when a particular cut-off value was applied, it appeared to underestimate and

overestimate the prevalence of ID in children with and without inflammation respectively (Engle-Stone *et al.*, 2013:369).

Table 2-6: Summary of national South African surveys describing the iron status of children

National survey	Age; sample size	Prevalence of iron deficiency (%)	Prevalence of iron deficiency anaemia (%)	Diagnostic cut-off values applied	Age-specific cut-off values (Thurnham <i>et al.</i> , 2010:546; WHO, 2001:)
NFCS-FB-I 2005 (Labadarios, 2007)	7-9 years; n = 499	4.4	2.0	ID: SF < 12 µg/L IDA: SF < 12 µg/L and Hb < 11.5 g/dL Inflammation: CRP > 10 mg/L	Hb: < 11 g/dL < 5 years < 11.5 g/dL 5 – 11 years SF: < 12 µg/L < 5 years
SANHANES-1 (Shisana <i>et al.</i> , 2013)	0 – 5 years; n = 349	8.1	1.9	ID: SF < 12 µg/L IDA: SF < 12 µg/L and Hb < 11 g/dL No inflammatory markers tested	< 15 µg/L > 5 years with corrective methods for individuals with inflammation

It is helpful to also consider smaller studies which, even though they not nationally representative, highlight interesting findings and involve children of the same age as those in the samples used in this research. A review by Taljaard *et al.* (2013) identified four independent studies in South Africa that involved primary school children 5-11 years of age that investigated the iron status and anaemia prevalence and were conducted between the 2005 and 2013 national surveys. The provinces and years when the four studies were performed include: Western Cape, 2006 (van Stuijvenberg *et al.*, April 2008:782); Northern Cape, 2009 (Troesch *et al.*, 2011:237); KwaZulu-Natal, 2009 (Baumgartner *et al.*, 2012b:1327) ; and North West, 2010 (Taljaard *et al.*, 2013). These four intervention studies selected children with a poor iron status and the prevalence is therefore not representative of the study population. After screening and selecting suitable subjects, anaemia prevalence was 17.2% in the Western Cape, 7.3% in the Northern Cape, 20.9% in KwaZulu-Natal and 7.1% in the North West. ID prevalence was 23.1% in the Western Cape, 20.6% in KwaZulu-Natal and 15.7% in the North West (data was not available for the Northern Cape). Subjects with inflammation (as determined by either CRP > 10 or > 5 mg/L) were excluded and the single indicator SF was selected to diagnose ID. ID was recalculated based on a SF cut-off of 12 µg/L across all four samples.

2.10 Conclusion

It is recommended that in order to address IDA, policy and strategy issues have to be addressed and researchers, communication specialists and programme operators need a collaborative approach (Yip, 2002: 802S). However, to imply that there is sufficient knowledge and evidence regarding the prevalence of ID is unfounded, as the optimal approach to assess ID and IDA in populations remains debatable. It is evident that iron is an essential mineral for the human body and because of the negative consequences associated with poor iron status, it is important to survey and monitor the iron status of populations. Indicators that estimate iron stores and the extent of the deficiency have to be evaluated in terms of their reliability to determine ID and IDA and to distinguish between other causes of anaemia and ACD. It is also necessary to evaluate the methodology and costs involved in the sample collection and laboratory testing. It is imperative that a reliable and accurate diagnosis of ID be made as there are dangers associated with inappropriate supplementation if ID is overestimated, such as increased susceptibility to infection and poor growth as free iron stimulates parasitic and bacterial growth (Nel, 2013).

The reality is that haemoglobin is currently used as a proxy for iron deficiency in the public health setting in South Africa. The intention of this mini-dissertation is to adhere to the World Health Organization's recommendation to evaluate the current knowledge and evidence to establish the best possible approach to identifying and measuring the severity of iron deficiency in South African primary school children.

3 Chapter 3: Article

Comparison of indicators of iron deficiency in three independent studies of South African primary school children

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Key words: South Africa; Children; Iron deficiency; Iron status indicators; Inflammation; Serum ferritin; Transferrin receptor; Zinc protoporphyrin; Body iron; Multiple criteria model

The word limit stipulated by *South African journal of clinical nutrition* has not been adhered to, but all parts that have been elaborated upon will be shortened or referred to in short when submitting the article for publication.

3.1 Abstract

This research aims to critically evaluate the indicators used to determine iron status and potentially identify an indicator superior to the multiple criteria model. A cross-sectional descriptive analysis was performed on the 2009 and 2010 screening data of primary school children aged 6-11 from three independent iron intervention studies in KwaZulu-Natal, the Northern Cape and North West. Haemoglobin (Hb), serum ferritin (SF), transferrin receptor (TfR), zinc protoporphyrin (ZPP), and C-reactive protein (CRP) concentrations (inflammatory marker) were measured and body iron calculated. Iron deficiency (ID) prevalence using single indicator methods, body iron and the multiple criteria model was compared, and the influence of inflammation on SF considered.

Significant positive small correlations between CRP and SF existed at all sites. The mean SF concentration was substantially higher in subjects with inflammation than those without. A different SF cut-off to identify ID was applied to subjects with inflammation. The percentage of ID subjects varied using different indicators. The sensitivity, specificity and predictive values of ID indicators varied within and between sites.

The vast differences in ID identification by different indicators are concerning as no consistence emerged. The global view of the multiple criteria model as the gold standard for estimating ID is debatable and potentially impractical at a public health level. Current evidence cautions against the harm of overestimating the prevalence of ID. This analysis confirms a need for research to identify a suitable, accurate and precise alternative to Hb to use in the South African public health setting.

3.2 Abstract 2

We conducted a secondary analysis of screening data from iron intervention studies to critically evaluate ID indicators applied to 6-11-year-old primary school children from three different South African provinces. No consistent agreement appeared between single indicators, body iron and the multiple criteria model, for ID identification after correcting for inflammation. This analysis confirms a need for research to identify a suitable, accurate and precise alternative to Hb to use in the South African public health setting.

3.3 Introduction

It is estimated that 300 million preschool- and school-age children worldwide are anaemic as a result of iron deficiency (ID) ¹. South African national surveys found that in 2005 the prevalence of ID and iron deficiency anaemia (IDA) in children 7-9 years of age was 4.4 % and 2.0% respectively ². In 2013 the prevalence of ID and IDA in children under 5 years of age was 8.1% and 1.9% respectively ³. The potential dire consequences of ID and IDA on childhood development are of major concern ⁴. Individuals have to be accurately diagnosed with ID or IDA before iron intervention strategies can be safely prescribed to individuals and populations, as there are risks associated with supplementing individuals with a normal iron status who were incorrectly identified as having ID ^{5,6}.

There are many factors that contribute to anaemia, ID being only one such factor ⁷. When there is inadequate iron to maintain normal function of bodily tissues, ID occurs. Research suggests that this process occurs in three stages ^{8,9}. The initial stage, termed storage iron depletion, is indicated by serum ferritin (SF) dropping below its threshold value if liver disease and inflammation or infectious disorders are absent ¹⁰. The second stage occurs when functional and transport Fe diminishes and Fe deficient erythropoiesis occur, yet haemoglobin (Hb) levels remain within range. This is indicated by an upregulation of transferrin receptors (TfR), as the additional receptors are expressed on the cell surfaces to increase iron uptake, and zinc protoporphyrin (ZPP) increases as a result of zinc being incorporated into protoporphyrin due to the lack of iron in bone marrow. The third and final stage is defined as IDA as Hb levels decrease to below the normal range, together with abnormal SF, ZPP and TfR levels ¹⁰.

Bone marrow aspirate examination is the only direct measure to diagnose ID, however, it is invasive, prone to error and costly ⁶². The development of alternative, reliable, accurate indicators to assess ID and IDA is therefore urgent. Consequently, indirect measures are relied upon to determine anaemia, ID and IDA. There is continued uncertainty regarding the optimal epidemiological approach to identifying and measuring the severity of ID at a population level ¹¹ and the WHO ¹² confirmed that there is an urgent need to address the selection and standardisation of Fe status indicators. Currently the most promising indicators include SF, TfR and ZPP and the introduction of the multiple criteria model of Cook *et al.* ¹³ according to which two or three abnormal indicators are indicative of ID ⁶. The calculation of body iron by Cook *et al.* ¹¹ incorporates SF and TfR as a reflection of body iron stores. In the public health setting, a suitable method to assess iron status has to be inexpensive, the analysis standardised and established, easy to measure and appropriate to use in developing countries and their applications specific to identifying ID at a population level ^{12, 14}. There should be

consensus regarding the use of appropriate iron status indicators, the cut-off values to apply and the approach to correction for inflammation to compare effects of interventions and the prevalence of ID and IDA across populations and over time.

In the 2005 and 2013 local national surveys, the potential problems with the methodology included the use of a single indicator to assess ID^{2,3}. SF was the indicator selected, but it is affected by inflammation and infection, independent of iron status. The cut-off value selected did not correct for the confounding effect of inflammation and infection on SF, which may have masked inadequate iron status in the 2013 survey. In Cameroonian children, it was found that when a particular cut-off value was applied, it appeared to underestimate and overestimate the prevalence of ID in children with and without inflammation respectively⁵.

Even though it has been suggested that the only way to accurately confirm IDA is if Hb levels increase in response to treatment, this is not practical at a population level¹⁴. Hb is an inexpensive practical indicator and is used at a public health level as a proxy for ID. However, it has low specificity and sensitivity with regard to identifying ID^{7,14,15}. SF, provides insight into iron store status and is a well standardised indicator, but is also an acute phase protein (APP), and therefore it must be interpreted together with C-reactive protein (CRP), which is an inflammatory marker as inflammation increases SF independent of iron status. Elevated TfR is an indicator of ID within the tissues, but the method lacks standardisation, is confounded by factors other than iron that affect erythropoiesis and relies on test kit reference ranges^{7,15}. It is essential to test levels of ZPP on washed red blood cells, which makes the required methodology time consuming. The test lacks specificity and still requires consensus regarding threshold cut-off values. Also, ZPP levels are confounded by environmental lead exposure¹⁵.¹⁶ Hepcidin, a hormone secreted by hepatocytes, is involved in systemic iron homeostasis¹⁷. Even though quantifying this hormone has many advantages and it is promising as an alternative ID indicator, uncertainty regarding its use as a diagnostic tool remains¹⁶.

The various single indicators have different roles, explore different aspects of iron metabolism and cannot be directly compared¹⁰. It is assumed that by evaluating a combination of ID indicators at the same time, the multiple criteria model may provide a more complete assessment¹⁸. However this assumption is challenged by the fact that the various indicators reflect different aspects of iron metabolism. The stages of ID do not follow along a continuum, which adds a further complication and makes it challenging to directly compare iron status diagnosed using different indicators within population groups¹⁰.

Different approaches have been suggested and applied to address the confounding effect of inflammation^{19,20,21,22,23}. The various approaches include exclusion of subjects with

inflammation, (which may introduce bias as subjects with ID may be more susceptible to infection and inflammation); the use of adjusted cut-off values to diagnose ID in subjects with inflammation ranging from 12 µg/L to 20 µg/L; or the application of correction factors to remove the influence of inflammation. This does make it challenging to compare iron status studies when different approaches are applied.

A key recommendation by the WHO regarding iron surveys and which prompted the aim of this research is to “use the best possible approach based on current knowledge” ²³. The aim of this research was to investigate a superior iron status indicator as compared to the multiple criteria model after correcting for inflammation, in three independent studies and in a combined sample involving primary school children from three separate and independent study sites in South Africa.

3.4 Method

3.4.1 Study design

A cross-sectional descriptive research design was used, which incorporated secondary analysis of data from three different study sites in South Africa.

3.4.2 Study population

The data used was generated from independent iron intervention studies in primary schools in three different provinces of South Africa and are described below in Table 3-1.

All parents provided written informed consent and blood samples were obtained from the children selected for the baseline screening. The data was collected in 2009 and 2010 and the following relevant indicators were analysed: age, Hb, SF, TfR, ZPP and the inflammatory marker, C-reactive protein (CRP). The relevant ethical clearance from the Ethics Committee of the South African Medical Research Council was obtained for the Northern Cape (NC) study. The Research and Ethics Committee of the North-West University granted ethical approval for the KwaZulu-Natal (KZN) and North West (NW) studies, as well as this study.

3.4.3 Data handling

The methods to collect the blood samples of subjects and the laboratory analysis performed to analyse each indicator are presented in Table 3-2.

Table 3-1: Geographic and demographic characteristics of study sites

Published intervention trials	KwaZulu-Natal ²²	Northern Cape ²⁴	North West ²⁵
Nearest town / city	Bothas Hill	Kimberley	Klerksdorp
Original screening sample size	n = 926	n = 1153	n = 574
Selection process; Primary study design	Children from four primary schools were invited to provide consent (from parents and children) to participate; randomised control trial	All children present in two selected primary schools whose parents provided consent; randomised control trial	Children from three primary schools were invited to provide consent (from parents and children) to participate; randomised control trial
Socioeconomic area	Low	Low	Low
Malaria	Free	Free	Free
Age range	6-11 years	6-10 years	5-11 years
Dewormed	Post-baseline measurements	Post-baseline measurements	1 week pre-baseline measurements
Demographics	Rural village	Peri-urban	Peri-urban
Geographical characteristics ²⁶	683 m elevation; 673 mm annual rainfall; 16 °C average minimum and maximum annual temperatures	1184 m elevation; 414 mm annual rainfall; 18 °C average minimum and maximum annual temperatures	1322 m elevation; 482 mm annual rainfall; 18 °C average minimum and maximum annual temperatures
Dietary intake ³			
*Fat score	Provincial - 6.9; Rural - 5.6	Provincial - 6.3; Urban informal - 6.7	Provincial - 6.2; Urban informal - 6.7
*Sugar score			
*Fruit and vegetable score	Provincial - 3.0; Rural - 2.3 – 2.5	Provincial - 3.0; Urban informal - 2.8	Provincial - 2.6; Urban informal - 2.8
	Provincial - 3.5; Rural - 3.2 – 3.3	Provincial - 3.1; Urban informal - 3.4	Provincial - 3.6; Urban informal - 3.4

* Dietary intake was reported in SANHANES-1 (2013) by detailing fat, sugar and fruit and vegetable scores. Fat score describes the use of fat in the diet and the inclusion of fatty meat, high fat snacks and fried food with 0-7 being a low score, 8-14 moderate and 15-20 high. Sugar score describes the use of sugar in the diet and the inclusion of sugary drinks and snacks and confectionary with 0-2 being a low score, 3-5 moderate and 6-8 high. Fruit and vegetable score reflects the reported daily intake of fruit and vegetables with 0-2 being a low score, 3-5 moderate and 6-8 high.

Iron deficiency was defined using single indicators, body iron and the multiple criteria model for the reporting of prevalence and statistical analyses by applying cut-off values:

- SF: < 15 µg/L for children > 5 years old 4 or < 19 µg/L in subjects with inflammation ²⁰
- TfR: > 8.3 mg/L (test kit reference value)
- ZPP: > 70 mmol per mol heme ^{23, 27}

- Body iron: < 0 mg/L ^{11, 28}
- Multiple criteria model: ID was considered present if subjects have ≥ 2 abnormal values according to the cut-off values above from among SF, TfR and ZPP ^{13, 6, 29}

Table 3-2: Blood sampling and analysis described in the three published papers from the intervention trials using data from the various study sites

	KwaZulu-Natal ²²	Northern Cape ²⁴	North West ²⁵
Blood sampling	Venous blood samples were drawn into EDTA-coated and trace-element free evacuated tubes. Samples were centrifuged and serum and plasma were separated into aliquots, placed on ice and transported to the laboratory, where samples were frozen until analysis (-80 °C in KZN and NW and -20 °C in NC).		
Hb method	Haemoglobin concentrations were measured on site on an aliquot of whole blood by using the direct cyanmethemoglobin method (Ames Mini-Pak haemoglobin test pack and Ames Minilab, Bio Rad Laboratories (Pty) Ltd), using Drabkin's solution and a standard miniphotometer.	Hb was measured in whole blood on the day of the blood draw at the Department of Haematology of the National Health Laboratory Services at the Kimberley hospital (Kimberley, South Africa) using a Sysmex32 2000i (Roche Diagnostics).	Hb was determined from whole blood. Hb was measured using an AcT 5Diff Cap Pierce Hematology Analyzer (Beckam Coulter, Miami, Florida, USA).
SF method	SF was measured using an automated chemiluminescent immunoassay system (IMMULITE; DPC Bühlmann GmbH).	SF was measured on an IMMULITE automatic system (DPC Bühlmann).	SF was determined from serum. Enzyme-linked immunosorbent assays were used to measure SF (Ramco Laboratories Inc.)
ZPP method	ZPP was measured on washed red blood cells by using a hematofluorometer (Aviv Biomedical) and 3-level control material provided by the manufacturer on the same day of blood sampling.	ZPP was measured on red blood cells from frozen samples.	ZPP was determined from washed red blood cells and measured with a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA).
TfR method	Serum TfR was measured using an enzyme immunoassay (Ramco Laboratories Inc.).	TfR was measured using enzyme immunoassays (Ramco Laboratories Inc.).	TfR was determined from serum. Enzyme-linked immunosorbent assays were used to measure TfR (Ramco Laboratories Inc.).
High-sensitivity CRP method	C-reactive protein (CRP) was measured using an automated chemiluminescent immunoassay system (IMMULITE; DPC Bühlmann GmbH).	CRP was measured on an IMMULITE automatic system (DPC Bühlmann).	CRP was determined from serum. Serum CRP was measured through immunoturbidimetric test (Human Biochemical and Diagnostic Laboratories, South Africa).

The cut-off value to select for ZPP is debatable, as various iron indicator studies and reports apply different cut-off values. Beard³⁰ recommended a cut-off value of 70 µmol/mol. This same cut-off value was applied to the KZN intervention trial²⁷ and to the NW intervention trial (test kit reference value). Grant *et al.*¹⁹ applied a cut-off of 80 µmol/mol heme to children 6-35 months. Zimmermann and Hurrell³¹ recommend a cut-off of 40 µmol/mol heme for children > 5 years and Troesch *et al.*³² refer to a cut-off value of 40 µmol/mol heme for fresh samples. The intervention trial in the NC was the only sample to analyse ZPP on frozen samples and the suggested cut-off for ZPP under these circumstances is 68.9 µmol/mol heme³². Therefore, based on literature and for consistency purposes, a ZPP cut-off value of 70 µmol/mol heme was selected for this secondary analysis.

Anaemia was defined as Hb < 11.5 g/dL for children 5-11 years old⁴ and IDA defined as both Hb < 11.5 g/dL and SF < 15 or 19 µg/L³³. CRP was the only APP available. Subjects were defined as having inflammation if CRP > 5 mg/L, as this cut-off value enabled comparison with other studies²⁰. Three approaches were investigated regarding diagnosing ID based on SF:

- 1) Individuals were classified into two groups based on their CRP concentration – an apparently healthy reference group (CRP ≤ 5 mg/L) and an incubation group (CRP > 5 mg/L). Two different SF cut-offs were applied, as the SF cut-off was raised in subjects with inflammation from 15 µg/L to < 19 µg/L^{20, 34}.
- 2) Individuals were classified into two groups based on their CRP concentration – an apparently healthy reference group (CRP ≤ 5 mg/L) and an incubation group (CRP > 5 mg/L). The SF concentration of individuals with inflammation was adjusted using a correction factor of 0.77^{20, 34}.
- 3) In order for the results to be comparable to SANHANES-1³, SF was not corrected for inflammation.

The first approach of applying two different SF cut-offs was considered preferable in this research as CRP was the only inflammatory marker available.

3.4.4 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (version 22; IBM Co). The three study sites are geographically and culturally distinct and were analysed independently. They were also analysed collectively as the same indicators were analysed and collective analysis gives a more in-depth assessment of the iron status in South Africa. Subjects with missing data for any one of the ID indicators were excluded from the sample. The cut-off

values applied were not specific for gender, and therefore subjects with missing data for gender were still included. The age range across the three studies was 5 - 11 years of age (Table 3-1), however age was rounded off to a whole number and only subjects 6 – 11 years of age were included in this secondary analysis.

The original datasets reflect subjects with CRP values of 0 mg/L, but an absolute value of 0 mg/L is not physiologically possible. Therefore these values were replaced with half the detection limit for CRP of 0.025 mg/L. Thereafter, box plots were applied to the three data sets for each indicator to flag subjects with outlying values. All of the values for these subjects were inspected further and, in addition the minimum and maximum values of each indicator, were compared to the physiological normal range for the various ID indicators (namely SF 0-300 µg/L; TfR can be eight times below and 20 times above the normal range of 5-8 mg/L; ZPP can rise to above 200 µmol/mol in children; and CRP concentrations can increase 20-1000 fold above the normal concentration of 0.001 g/L ^{12, 23}). Subjects (KZN n = 3; NC n = 2; NW n = 0) with values that were out of the physiological normal range or subjects that did not present with a consistent pattern of either ID or IDA were identified as outliers and deleted. Due to the SF, TfR, ZPP and CRP data not being normally distributed, it was not possible to define outliers as ± 3 SD from the mean.

These deletions resulted in a final sample size of NC n = 1053; KZN n = 736; and NW n = 547. However there was limited data for gender whereby n = 558 in KZN and n = 543 in NW for this variable. Variables that were not found to be normally distributed and presented a positive skewedness (after analysing Q-Q plots and histograms) were log-transformed (namely SF, TfR, ZPP and CRP). Even though the sample sizes are large, these indicators are not normally distributed, and therefore it is recommended to log-transform in terms of statistical norms.

The geometric mean values of SF of the subjects with and without inflammation were calculated. The association between log-transformed CRP and the log-transformed single indicators, SF, TfR and ZPP, were investigated by Pearson's correlations (with a significance level of $p < 0.01$).

The frequencies were calculated to identify the percentage of subjects with ID as identified by the various methods.

The proportion of subjects diagnosed with ID according to the single indicators and body iron were compared to the multiple criteria model using the SPSS descriptive analysis crosstabs method. The sensitivity, specificity, predictive values and percentage of subjects misclassified

were calculated to identify a better indicator to determine ID as compared with the multiple criteria model. Sensitivity is the true positive rate and is defined as “the proportion of positives that are correctly identified by the test”. Specificity is the true negative rate, defined as “the proportion of negatives that are correctly identified by the test” ³⁵. In addition to sensitivity and specificity, one also has to assess the probability of the indicator correctly diagnosing ID. The positive predictive value refers to the probability that a subject that was diagnosed with ID was correctly diagnosed; the negative predictive value refers to the probability that a subject that was diagnosed as not having ID was correctly diagnosed ³⁵.

3.5 Results

3.5.1 Characteristics of the subjects and study sites

The three study sites were in socio-economically poor areas. The general characteristics and demographic areas are described in Table 3-1.

3.5.2 Influence of inflammation on SF

Table 3-3 illustrates subjects grouped according to those with inflammation and those without inflammation and the geometric means of these two groups were explored ^{20, 34}.

Table 3-3: The mean concentrations of serum ferritin in subjects with and without inflammation in the three study sites and the combined sample

Sample	Mean serum ferritin concentration (µg/L) (SD) ¹	
	Inflammation (CRP ² >5 mg/L)	No inflammation (CRP ² ≤5 mg/L)
KZN ³ (n = 88; n = 648) ⁴	46.44 (38.23)	28.09 (18.54)
NC ³ (n = 53; n = 992) ⁴	50.68 (34.74)	36.68 (23.95)
NW ³ (n = 138; n = 408) ⁴	27.05 (22.58)	24.13 (21.96)
Combined (n = 279; n = 2043) ⁴	36.14 (32.62)	31.01 (21.51)

¹ Geometric mean of unadjusted serum ferritin (standard deviation); normal value for serum ferritin > 19 µg/L (inflammation present) or > 15 µg/L (no inflammation present).

² CRP, C-reactive protein.

³ KZN, KwaZulu-Natal; NC, Northern Cape; NW, North West.

⁴ Number of subjects with inflammation; number of subjects without inflammation.

The difference in mean SF was found to be substantially higher in the group with inflammation in KZN (18.35 µg/L) and NC (14 µg/L) compared to the group without inflammation. This difference was only marginal in the group with inflammation in NW and the combined sample compared to the group without inflammation (2.92 and 5.13 µg/L respectively).

In order to investigate the influence of inflammation on SF levels, the association (as correlations) between CRP and SF, TfR and ZPP was explored; see Table 3-4.

Table 3-4: Correlations for C-reactive protein with iron status indicators

Sample	SF		TfR		ZPP	
	r-value*	p	r-value*	p	r-value*	p
KZN ¹	0.226	0.000**	-0.043	0.240	-0.026	0.489
NC ¹	0.161	0.000**	0.099	0.01**	0.100	0.001**
NW ¹	0.181	0.000**	-0.039	0.361	-0.022	0.611
Combined	0.069	0.01**	-0.010	0.621	0.104	0.000**

SF: serum ferritin; ZPP, Zinc protoporphyrin; TfR, Transferrin receptor; r-value, correlation coefficient; p, p-value.

¹ KZN, KwaZulu-Natal; NC, Northern Cape; NW, North West.

* Correlation coefficient interpreted as an effect size whereby 0.1 – small effect, 0.3 – medium effect; and 0.5 – large effect;

**Significant at p<0.01 level.

There were significant positive small correlations between CRP and SF at all study sites and the combined sample (p<0.01). There were small negative, but not significant correlations between CRP and TfR in KZN, NW and the combined sample and a significant positive correlation in NC. A significant small positive correlation was found between CRP and ZPP in NC and the combined sample, yet a negative, but not significant small association in KZN and NW.

3.5.3 Percentage of subjects with anaemia, ID and IDA

Approximately equal proportions of boys (51-53%) and girls (47-49%) 6-11 years of age were present at the different study sites (Table 3-5). The percentage of subjects with anaemia ranged from 5.5% in NC to 17.2% in KZN, compared to the percentage of subjects with IDA ranging from 0.9% to 3.5% respectively in the same study sites. The percentage of children with inflammation was 5% in NC, 12% in KZN and the combined sample and highest in NW at 25.3%. However, many of the CRP values in the NW sample fell just above the cut-off value of 5 mg/L. Of the 25.3% of subjects, 76% had CRP values > 5 but < 9 mg/L. Of the 11.7% of subjects with inflammation in KZN, 55% had values > 9 mg/L; in NC, of the 5% with inflammation, 57% had CRP values >9 mg/L and in the combined sample, of the 11.8% with inflammation, 40% had CRP values > 9mg/L.

In KZN, ZPP identified the highest percentage of children with ID (26.5%) and body iron the lowest (4.2%). In NC, TfR identified the highest percentage of subjects with ID (13.4%) and body iron the lowest (4.1%). The highest percentage of children with ID was identified by SF

in NW (24.4%) and the lowest (7.0%) by TfR. In the combined sample, ZPP identified the highest percentage (15.2%) and body iron the lowest percentage (5.4%) of children with ID.

The percentage of children identified with ID was very similar when SF was not adjusted for inflammation, compared to when a correction factor was applied to SF or when a different SF cut-off was applied to subjects with inflammation.

There is no consistent pattern of the indicators with abnormal values (below the cut-off threshold) that determines ID according to the multiple criteria model in the various study sites (Figure 3-1). In KZN, 6.1% of the total sample had ID according to the multiple criteria model. Of all these subjects, 3.3% had abnormal ZPP and SF values, 0.3% had abnormal SF and TfR values, 1.1% had abnormal TfR and ZPP, and 1.5% had abnormal levels for all three indicators. In NC, 4.6% of the total sample had ID according to the multiple criteria model. Of all these subjects, 0.2% had abnormal ZPP and SF values, 2.3% had abnormal SF and TfR values, 1.1% had abnormal TfR and ZPP, and 1.1% had abnormal levels for all three indicators. In NW, 10.1% of the total sample had ID according to the multiple criteria model. Of all these subjects, 4.8% had abnormal ZPP and SF values, 1.3% had abnormal SF and TfR values, 0.9% had abnormal TfR and ZPP, and 3.1% had abnormal levels for all three indicators. In the combined sample, 6.3% of the total sample had ID according to the multiple criteria model. Of all subjects in combination, 2.2% had abnormal ZPP and SF values, 1.4% had abnormal SF and TfR values, 1.0% had abnormal TfR and ZPP, and 1.6% had abnormal levels for all three indicators.

The total number of subjects in KZN with ID according to ZPP was 26.5% (see Table 3-5) and 20.7% of subjects did not have further abnormal indicators in addition to ZPP. Also, in KZN, the total number of subjects with ID according to SF was 10.5% (according to the preferred approach of applying SF cut-offs of 19 or 15 µg/L in the presence of inflammation) and 5.4% of subjects did not have further additional abnormal indicators in addition to SF. The total number of subjects in KZN with ID according to TfR was 5.2%, and 2.3% did not have further abnormal indicators in addition to TfR. Details for additional study sites are illustrated in Figure 3-1.

3.5.4 Comparison of the accuracy of single indicators and body iron to the multiple criteria model to diagnose ID

Table 3-6 details the sensitivity (Se), specificity (Sp), positive predictive values (PPV) and negative predictive values (NPV) at the three separate study sites and the combined sample when the single indicators and body iron are compared to the multiple criteria model. Results

from each study site will be interpreted individually to assess a pattern. In KZN, when body iron was used, subjects had the highest probability (PPV 0.70) of having ID, yet with the largest confidence interval (CI). It had the most promising ability to identify subjects without ID (Sp 0.99), and it misclassified the smallest percentage of subjects (4.4%) when compared to the multiple criteria model. ZPP had a 100% probability (NPV 1.0) (smallest CI) that the subjects do not have ID and it had the most promising ability to identify subjects with ID (Se 0.95), compared to the multiple criteria model.

In NC, when body iron was used, subjects had the highest probability (77%) that they had ID as diagnosed by the multiple criteria model, it had a superior ability to identify subjects without ID (Sp 0.99), and it misclassified the smallest percentage of subjects (2.4%) compared to the multiple criteria model. TfR had a 100% probability that the subjects did not have ID and had the most promising ability to identify subjects with ID (Se 0.96).

In NW, when TfR was used, subjects had the highest probability (76%) that they have ID as diagnosed by the multiple criteria model. It had the most promising ability to identify subjects without ID (Sp 0.98) and it misclassified the smallest percentage of subjects (6.4%). SF had a probability of 99% that the subject do not have ID and had the best ability to identify subjects with ID (Se 0.91).

With regards to the combined sample, when body iron was used subjects had the highest probability (68%, PPV 0.68) that they do indeed have ID as diagnosed by the multiple criteria model. Body iron also had the best ability to identify subjects without ID (Sp 0.98) and it misclassified the smallest percentage of subjects (4.3%). When SF was used, it had a probability of 99% (NPV 0.99) that the subjects do not have ID, and had the best ability to identify subjects with ID (Se 0.84).

3.6 Discussion

The main findings of this cross-sectional analysis highlight that there is an association between inflammation and SF. The percentage of subjects identified as ID in the three study sites and the combined sample differ when different indicators are applied, and the sensitivity, specificity and predictive values of the alternative indicators compared to the multiple criteria model varies between study sites. There appears to be no consistently best substitute to the multiple criteria model.

3.6.1 Inflammation

SF increases rapidly in response to infection (within 8 hours) as inflammatory cytokines stimulate SF production³⁷. This association was evident in the three cohorts and the combined sample (small positive significant correlation). In a study involving Chilean children 19-72 months, a positive correlation was found between CRP and SF³⁸ and similar associations were shown in Kenyan children¹⁹.

The increase in ZPP is related to chronic inflammation and ZPP increases slowly as a result of limited circulating iron and reduced Hb production³⁹. There was a small positive significant association between CRP and ZPP in NC and the combined sample. Mean concentrations were comparable across cohorts and the combined sample for Hb and NC had the highest mean concentrations for TfR and SF. This contradicts what one would expect to see if anaemia of chronic inflammation was present and responsible for the increase in ZPP (decrease in Hb, increase or no change in SF and decrease or no change in TfR)⁹. There were negative, but not significant associations between CRP and ZPP in KZN and NW, which may be as a result of the possible absence of inflammatory disease in these samples.

TfR decreases (within 24 hours) as a result of impaired erythroid growth in response to inflammatory cytokines³⁷. Even though negative correlations were found in KZN, NW and the combined sample, this association was not significant and a positive significant association was found in NC. Even though TfR actually increases in response to a decrease in SF once $SF < 15 \mu g/L$, TfR decreases in response to inflammation⁴⁰. These conflicting findings may be because subjects were not followed over time and a single blood sample was used to determine all iron status indicators and CRP concentrations. The influence of the different response rates to inflammation cannot be underemphasised. Even though the WHO¹² documents the changes in CRP in response to surgery, the influence of inflammation on an indicator may differ according to the source and severity of inflammation or infection.

It is, however, possible to accurately assess the iron status of a population using SF, if inflammation or infection is taken into account²⁰. At the three study sites, SF levels were raised in subjects with inflammation. However, the difference in the mean SF values between those with and without inflammation differed between study sites. Even though NW had the highest prevalence of inflammation (25.3%), the difference in SF concentrations between subjects with and without inflammation was the smallest (2.92 $\mu g/L$). The mean concentration of CRP in the group with inflammation was 9.67 mg/L compared to 2.98 mg/L in those without inflammation. The trend described as a rapid rise in SF within 8 hours, and a decrease in TfR within 24 hours in response to inflammation³⁴, appears apparent in the observation that TfR

identified the smallest percentage of subjects with ID and SF identified the largest percentage in NW.

Intestinal worms negatively influence iron status as they cause blood loss and their influence on anaemia and Hb is well-documented ³¹. Only subjects in NW received deworming medication the week prior to blood sample collection, compared to subjects receiving the medication post-blood sampling in the other two samples. This may have influenced CRP levels. Hurrell ⁴¹ reported that deworming alone did not influence TfR and SF values. In a trial conducted in anaemic Vietnamese children using iron-fortified noodles and de-worming to assess the changes in iron and anaemia status, the proportion of children with elevated CRP decreased (not significantly) in all treatment groups, but increased in the placebo group ⁴². This does not support the speculation that deworming was a contributing factor to the elevated CRP in the NW group post-deworming. However, key elements to note in this study are that the prevalence of ID was low (1.2-1.3% according to SF < 12 µg/L and 3.5-8.5% according to TfR > 8.5 mg/L) and the definition of elevated CRP was ≥8 mg/L. It is speculated that deworming prior to taking blood samples may be an influencing factor, as the parasitic death which occurs within a few days of administering medication may have resulted in an increase in CRP because the purpose of inflammation as defined by WHO ¹² is “protective and designed to neutralize and remove the invader and repair the damage caused directly by the invader and indirectly”. There are periods of time when inflammation may only be detected biochemically (before clinical symptoms appear and during convalescence) and one may speculate that this is the reason for the highest prevalence of inflammation in NC.

With regard to the optimal approach to correct for inflammation, Beard *et al.* ⁴³ suggest that in large samples when the prevalence of clinically defined inflammation is less than 10% (low), inflammation has little influence on the distribution of indicators of ID. According to this suggestion and as depicted in Table 3-3, NC was the only study site (5.1% of subjects had CRP > 5 mg/L) where inflammation would have little influence on ID indicator distribution.

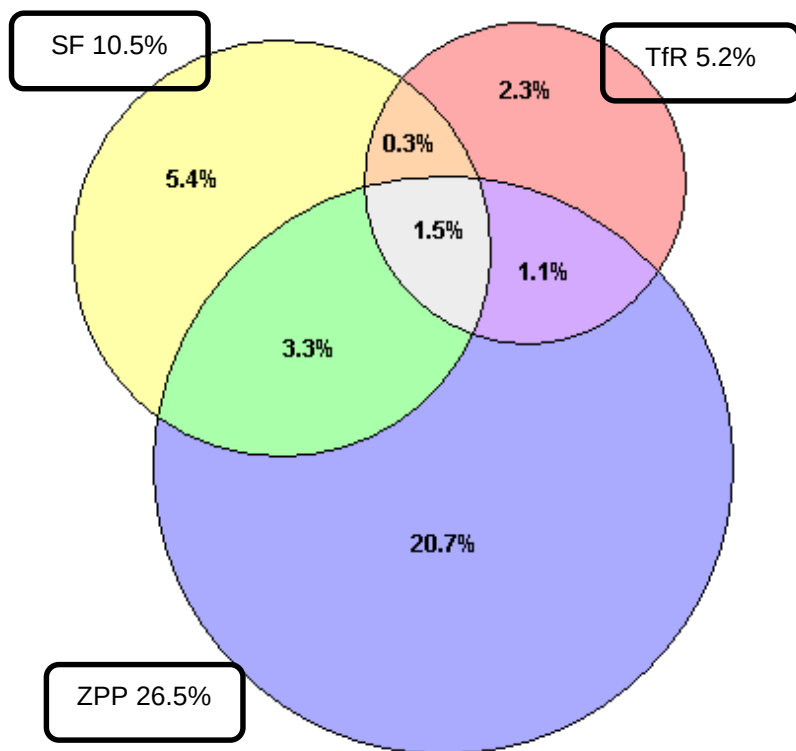
3.6.2 Prevalence of ID using various indicators

If one was to draw conclusions and develop a public health intervention based on the percentage of subjects with ID according to different single indicators, one would draw very different conclusions. This is apparent from the wide range of the percentage of subjects identified with ID occurring within in the same sample of children (Table 3-5).

Table 3-5: Characteristics of subjects at each study site

Characteristic	KZN ¹ (n = 736)	NC ¹ (n = 1045)	NW ¹ (n = 546)	Combined ¹ (n = 2 322)
Gender ratio boys : girls (%) ²	52: 48	53: 47	51: 49	52: 48
Hb (g/dL) ³	12.21 (0.90)	12.74 (0.86)	12.63 (0.93)	12.55 (0.90)
SF (µg/L) ⁴	29.83 (23.02)	37.29 (24.86)	24.84 (22.16)	31.59 (23.30)
TfR (mg/L) ⁴	5.24 (2.59)	6.65 (1.61)	5.65 (1.70)	5.94 (1.73)
ZPP (µmol/mol heme) ⁴	59.84 (35.06)	33.75 (20.39)	56.09 (28.10)	45.56 (27.17)
Body iron (< 0 mg/kg) ³	4.79 (2.69)	4.73 (2.46)	3.86 (3.06)	4.55 (2.67)
CRP (mg/L) ⁴	1.86 (8.22)	0.53 (4.41)	3.64 (5.47)	0.69 (6.33)
Deficiencies % (n)⁵				
Anaemia (< 11.5g haemoglobin / dL)	17.1 (126)	5.5 (57)	7.0 (38)	9.5 (220)
Iron deficiency based on SF ⁶ three scenarios: 1. Cut-off of <15 µg/L applied to subjects without inflammation, and <19 µg/L applied to subjects with inflammation. 2. Corrections factor of 0.77 was applied to subjects with inflammation, and cut-off of < 15 µg/L applied. 3. Cut-off of < 15 µg/L applied to all subjects	10.5 (77)	5.2 (54)	24.4 (133)	11.3 (262)
	10.5 (77)	5.2 (54)	24.5 (134)	11.3 (263)
	10.3 (76)	5.0 (52)	22.7 (124)	10.8 (250)
Iron deficiency based on TfR (> 8.3 mg/L)	5.2 (38)	13.4 (140)	7.0 (38)	9.3 (215)
Iron deficiency based on ZPP (> 70 µmol/mol heme)	26.5 (195)	4.9 (51)	19.8 (108)	15.2 (352)
Iron deficiency based on a shortage of body iron (< 0 mg/kg) ⁷	4.2 (31)	4.1 (43)	9.7 (53)	5.4 (126)
Iron deficiency based on multiple criteria model ⁸	6.1 (45)	4.6 (48)	10.1 (55)	6.3 (146)
Iron deficiency anaemia (< 11.5g haemoglobin / dL and < 15 or 19 µg SF/L) ⁶	3.5 (26)	0.9 (9)	2.4 (13))	2.0 (47)
Acute-phase protein % (n)				
C-reactive protein (> 5 mg/L)	12.0 (88)	5.1 (53)	25.3 (138)	12.0 (279)

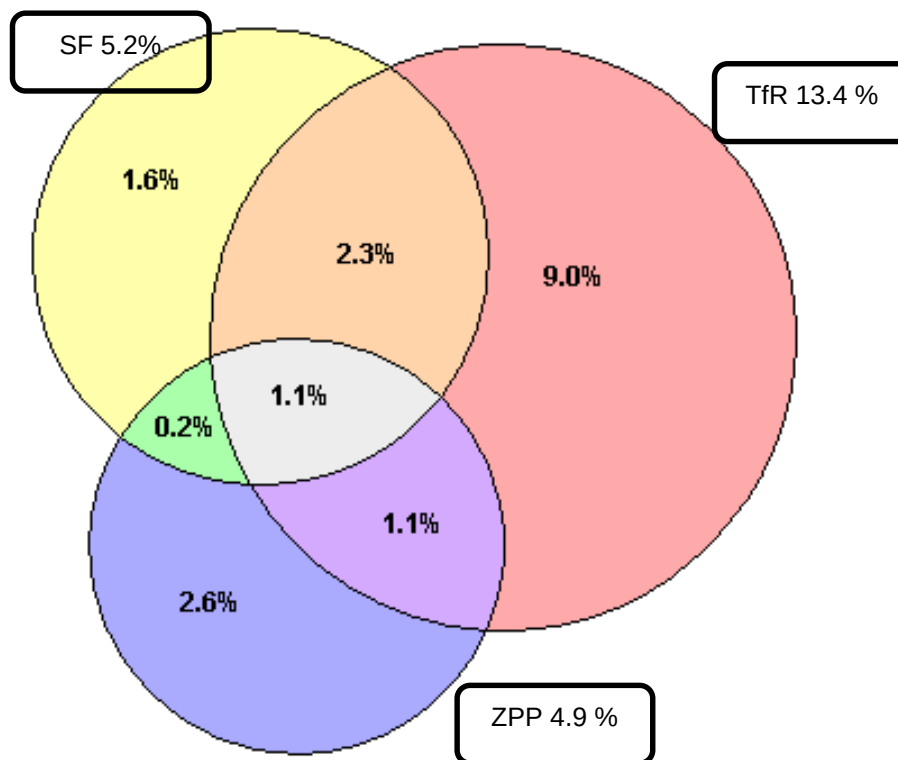
¹ KZN, KwaZulu-Natal; NC, Northern Cape; NW, North West; SF, serum ferritin; TfR, plasma soluble transferrin receptor; ZPP, whole-blood zinc protoporphyrin. ² Gender KZN n = 558; NC n = 1045; NW n = 542; combined n = 2140. ³ Arithmetic mean (standard deviation). ⁴ Geometric mean (standard deviation). ⁵ Percentage of subjects (number of subjects). ⁶ WHO ⁴ cut-off of 15 µg/L for SF was replaced with 19 µg/L to define ID in subjects with inflammation (indicated by CRP >5 mg/L) ^{20, 34}. The corrections factor of 0.77 was applied to subjects with inflammation. ⁷ Body iron was calculated on the basis of Cook *et al.*, ¹¹ followed by the use of the WHO cut-off of 15 µg/L ^{20, 34}. SF was not corrected for inflammation as a comparison. ⁸ Multiple criteria model identified the presence of iron deficiency if ≥ 2 abnormal values from among SF, TfR and ZPP ^{6, 13, 28, 29}.



SF 10.5% comprising of SF alone 5.4%; SF and TfR 0.3%; SF and ZPP 3.3%; and SF, ZPP and TfR 1.5%

ZPP 26.5% comprising of ZPP alone 20.7%; ZPP and TfR 1.1%

KwaZulu-Natal

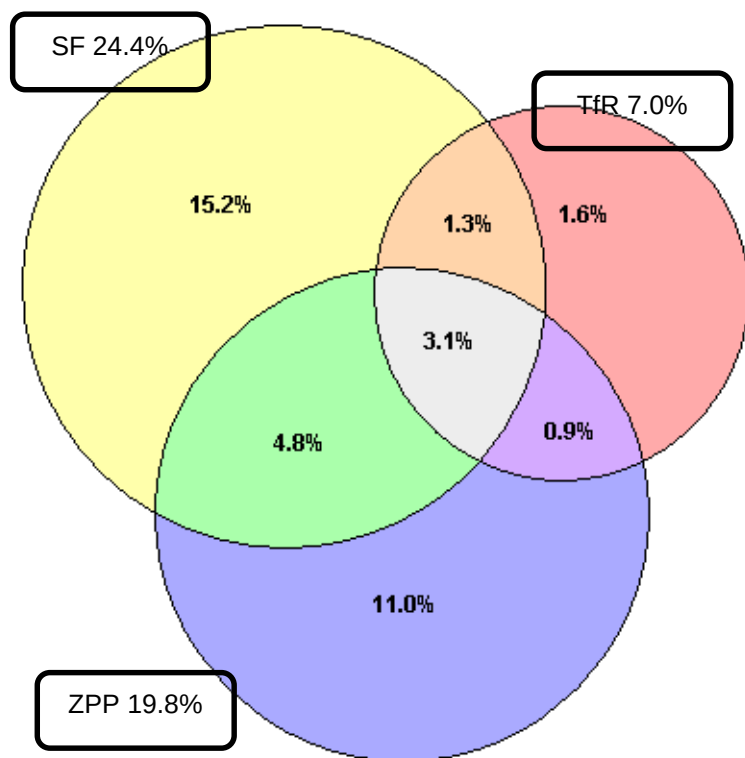


SF 5.2% comprising of SF alone 1.6%; SF and TfR 2.3%; SF and ZPP 0.2%; and SF, ZPP and TfR 1.1%

ZPP 4.9% comprising of ZPP alone 2.6%; ZPP and TfR 1.1%

Northern Cape

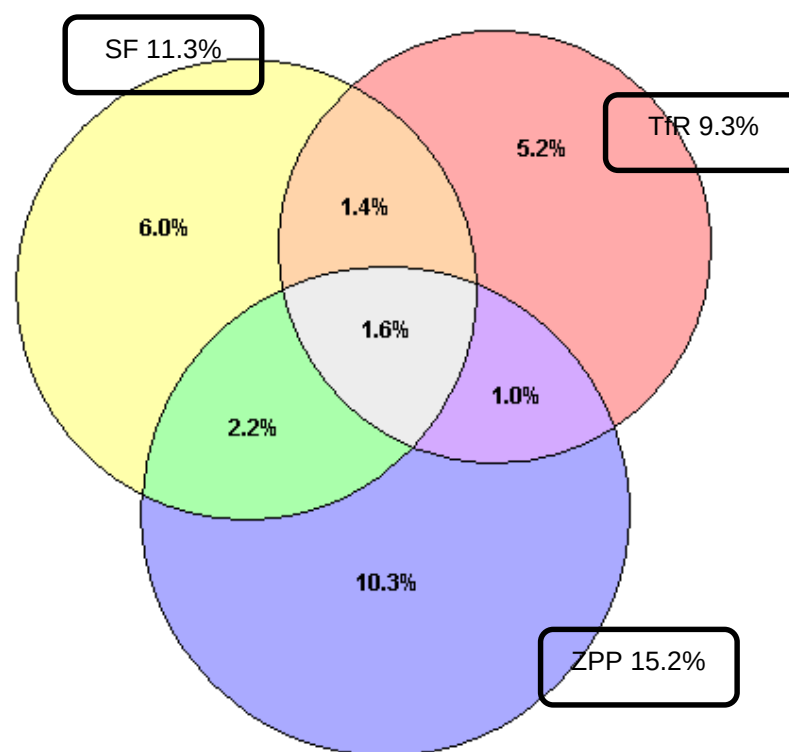
Figure 3-1 : A proportional illustration of subjects with ID as a percentage of the total sample size according to single indicators and the multiple criteria model in the study sites



SF 24.4% comprising of SF alone 15.2%; SF and TfR 1.3%; SF and ZPP 4.8%; and SF, ZPP and TfR 3.1%

ZPP 19.8% comprising of ZPP alone 11.0%; ZPP and TfR 0.9%

North West



SF 11.3% comprising of SF alone 6.0%; SF and TfR 1.4%; SF and ZPP 2.2%; and SF, ZPP and TfR 1.6%

ZPP 15.2% comprising of ZPP alone 10.3%; ZPP and TfR 1.0%

Combined

Figure 3-1: A proportional illustration of subjects with ID as a percentage of the total sample size according to single indicators and the multiple criteria model in the study sites

Table 3-6: Agreement between the multiple criteria model and other indicators used to define iron deficiency and the accuracy and predictability of iron deficiency based on the multiple criteria model by using the other indicators in the study sites

Study site ¹	Iron deficiency ²		Multiple criteria model		Sensitivity ³	Specificity ³	Predictive values ⁴		% misclassified ⁵
			Yes ⁶	No ⁶			PPV	NPV	
KZN	Defined by SF	Yes ⁶	4.77 (35)	5.46 (40)	0.81 (0.78-0.84)	0.94 (0.92-0.96)	0.47 (0.43-0.50)	0.99 (0.98-1.00)	6.6
		No ⁶	1.09 (8)	88.68 (650)					
	Defined by TfR	Yes	2.73 (20)	2.32 (17)	0.47 (0.43 – 0.50)	0.98 (0.96 – 0.99)	0.54 (0.50 – 0.58)	0.97 (0.95 – 0.98)	5.6
		No	3.14 (23)	91.81 (673)					
	Defined by ZPP	Yes	5.59 (41)	20.74 (152)	0.95 (0.94 – 0.97)	0.78 (0.75 – 0.81)	0.21 (0.18 – 0.24)	1.00 (0.99 – 1.00)	21.0
		No	0.27 (2)	73.40 (538)					
	Defined by body iron	Yes	2.86 (21)	1.23 (9)	0.49 (0.45 – 0.52)	0.99 (0.98 – 1.00)	0.70 (0.67 – 0.73)	0.97 (0.96 – 0.98)	4.4
		No	3.00 (22)	92.91 (681)					
NC	Defined by SF	Yes	3.55 (37)	1.63 (17)	0.77 (0.75 – 0.80)	0.98 (0.98 – 0.99)	0.69 (0.66 – 0.71)	0.99 (0.98 – 1.00)	2.7
		No	1.05 (11)	93.77 (978)					
	Defined by TfR	Yes	4.41 (46)	9.01 (94)	0.96 (0.95 – 0.97)	0.91 (0.89 – 0.92)	0.33 (0.30 – 0.36)	1.00 (0.99 – 1.00)	9.2
		No	0.19 (2)	86.39 (901)					
	Defined by ZPP	Yes	2.30 (24)	2.59 (27)	0.50 (0.47 – 0.53)	0.97 (0.96 – 0.98)	0.47 (0.44 – 0.50)	0.98 (0.97 – 0.99)	4.9
		No	2.30 (24)	92.81 (968)					
	Defined by body iron	Yes	3.16 (33)	0.96 (10)	0.69 (0.66 – 0.72)	0.99 (0.98 – 1.00)	0.77 (0.74 – 0.79)	0.99 (0.98 – 0.99)	2.4
		No	1.44 (15)	94.44 (985)					
NW	Defined by SF	Yes	9.16 (50)	15.20 (83)	0.91 (0.88 – 0.93)	0.83 (0.8 – 0.86)	0.38 (0.34 – 0.42)	0.99 (0.98 – 1.00)	16.2
		No	0.92 (5)	74.73 (408)					
	Defined by TfR	Yes	5.31 (29)	1.65 (9)	0.53 (0.49 – 0.57)	0.98 (0.97 – 0.99)	0.76 (0.73 – 0.80)	0.95 (0.93 – 0.97)	6.4
		No	4.76 (26)	88.28 (482)					

Study site ¹	Iron deficiency ²		Multiple criteria model		Sensitivity ³	Specificity ³	Predictive values ⁴		% misclassified ⁵
			Yes ⁶	No ⁶			PPV	NPV	
	Defined by ZPP	Yes	8.79 (48)	10.99 (60)	0.87 (0.84 – 0.90)	0.88 (0.85 – 0.91)	0.44 (0.40 – 0.49)	0.98 (0.97 – 0.99)	12.3
		No	1.28 (7)	78.94 (431)					
	Defined by body iron	Yes	5.86 (32)	3.85 (21)	0.58 (0.54 – 0.62)	0.96 (0.94 – 0.97)	0.60 (0.56 – 0.64)	0.95 (0.94 – 0.97)	8.1
		No	4.21 (23)	86.08 (470)					
Combined	Defined by SF	Yes ⁶	5.25 (122)	6.03 (140)	0.84 (0.82 – 0.85)	0.94 (0.93 – 0.95)	0.47 (0.45 – 0.49)	0.99 (0.98 – 0.99)	7.1
		No ⁶	1.03 (24)	87.68 (2036)					
	Defined by TfR	Yes	4.09 (95)	5.17 (120)	0.65 (0.63 – 0.67)	0.94 (0.94 – 0.95)	0.44 (0.42 – 0.46)	0.98 (0.97 – 0.98)	7.4
		No	2.20 (51)	88.54 (2056)					
	Defined by ZPP	Yes	4.87 (133)	10.29 (239)	0.77 (0.76 – 0.79)	0.89 (0.88 – 0.90)	0.32 (0.30 – 0.34)	0.98 (0.98 – 0.99)	11.7
		No	1.42 (33)	83.42 (1937)					
	Defined by body iron	Yes	3.70 (86)	1.72 (40)	0.59 (0.57 – 0.61)	0.98 (0.98 – 0.99)	0.68 (0.66 – 0.70)	0.97 (0.97 – 0.98)	4.3
		No	2.58 (60)	91.99 (2136)					

¹ KZN, KwaZulu-Natal; NC, Northern Cape; NW, North West; TfR, plasma soluble transferrin receptor; ZPP, whole blood zinc protoporphyrin.

² Iron deficiency was considered present if individuals had ≥ 2 abnormal values from among SF, TfR and ZPP^{6, 13, 28, 29}.

³ Se, Sensitivity = true positive / (true positive + false negative); Sp, Specificity = true negative / (false positive + true negative). Values are proportions, confidence interval in parentheses.

⁴ Positive predictive value, PPV = true positive / (true positive + false positive); NPV, negative predictive value = true negative / (false negative + true negative)³⁵. Values are proportions, confidence interval in parentheses.

⁵ Percentage misclassification = (1 - sensitivity) x (percentage ID subjects) + (1 - specificity) x (percentage subjects without ID)

⁶ Values are proportions, n in parentheses

These differences could be a result of each indicator exploring a different aspect of iron metabolism and stage of deficiency, or other factors such as age, gender, ethnicity or environmental elements (namely altitude, climate and environmental lead exposure). Differences in the prevalence of communicable inflammatory conditions, as well as vitamin A deficiency may also be contributing factors. Literature refers to race-specific criteria for anaemia. However, this is not relevant to this research as all children were of black African ethnicity ^{44, 45}.

Even though the WHO ⁴ estimated that 50% of anaemia is a result of ID, Cameron & Neufeld ¹⁴ question the accuracy of this assumption. Of the subjects with anaemia, the percentage that had IDA ranged from only 15.8 to 34.2%. Speculative reasons for the differences in IDA prevalence between KZN, NC and NW include differences in dietary patterns, different incidences of communicable diseases, as well as a different prevalence of vitamin A, B₁₂ or folate deficiencies, anaemia of chronic disease, and even possibly inherited conditions such as thalassemia ³.

Inflammatory communicable diseases such as human immunodeficiency virus (HIV) and tuberculosis (TB) may influence the prevalence of anaemia. In 2012, the prevalence of HIV nationally was 12.2% and highest in KZN (16.9%), compared to 13.3% in NW and 7.4% in NC ⁴⁶. Children who do not receive antiretroviral treatment and acquire HIV vertically through mother-to-child transmission generally have a life expectancy of two years ⁴⁷. Therefore, the sample of 6-11-year-old children in this study are unlikely be infected with HIV, although the impact of HIV on anaemia in children may be more indirect and a result of the loss of caregiver support, in turn resulting in a loss of economic and food security and a negative psychosocial impact ⁴⁸.

Of the 341 165 South Africans with TB in 2006, the highest percentage were in KZN (30.7%). NW and NC were 8.3% and 2.5%, respectively ⁴⁹. SANHANES-1 ³ found that black African children had the highest prevalence of vitamin A deficiency (45.5%) and the highest prevalence occurred in urban informal areas, such as those in this secondary analysis (Table 3-1).

Long-term exposure to higher altitude living increases Hb and the altitude of study sites should be considered. When exploring the influence of altitude on Hb levels, WHO ⁴ suggested that an altitude of < 1000m had no influence on Hb, yet an altitude of 1000-1500m results in a normal Hb increase of 2 g/l. Therefore, altitude influences Hb in NC and NW as the study sites were at a higher elevation (Table 3-1). However, this would influence only the percentage of subjects identified as anaemic and with IDA (where Hb is used as an indicator). These reasons do not explain the differences in ID according to different single indicators. Cook *et al.* ¹⁵ found an increase in body iron after the age of 2 years even with an increase in body size. Even though the distribution of body size was most likely the same in the three cohorts, considering children of the same age were selected, there was a positive small significant association between age

and body iron in NC, NW and the combined sample ($r = 0.101$; $r = 0.179$; $r = 0.110$, respectively; $p < 0.01$, Pearson's correlation), but not in the KZN sample ($r = 0.025$). The gender and age of subjects were comparable (Table 3-3) and are discounted as plausible reasons to explain the differences in ID prevalence.

Dietary scores were comparable across provinces and urban and rural areas (Table 3-1). A cause for concern is the moderate score for vegetables and fruit, which reflects a moderate intake of this nutrient dense food group. In addition, the lower annual rainfall may influence the households' abilities to have home vegetable gardens in NC and NW. Diet quality and the intake of essential nutrients that influence iron status may be at risk in KZN where only 37.3% of households are food secure compared to 56.5% in NC. The percentage of households experiencing hunger in NC is lower (20.7%) than the national average of 26%, yet more households experience hunger in KZN (28.3%) and NW (29.5%) placing them at risk of nutritionally inadequate diets ³.

Elevated ZPP was the dominant indicator out of range in KZN and the combined sample and the combination of SF and ZPP was the most common contributor to the multiple criteria model (evident in KZN, NW and the combined sample) (Figure 3-1). Even though elevated ZPP theoretically indicates iron deficient erythropoiesis, confounding factors have to be considered as exposure to environmental lead, which falsely elevates ZPP, and ID also places children at risk for chronic lead poisoning ⁵⁰.

A previous study conducted found the mean blood lead level of children from an informal settlement in Durban to be 10 $\mu\text{g}/\text{dl}$ (in line with the internationally accepted action level) and 5% of children had blood lead levels $> 25 \mu\text{g}/\text{dl}$. This study identified overcrowding, household hygiene, use of solid fuels as a domestic energy source and distance from tarred roads to be risk factors for elevated blood lead levels ⁵¹. Blood lead levels of children attending schools were investigated and the proportion of children with elevated lead levels was low in certain schools (namely Mitchell's Plain and Soweto), yet unacceptably high in inner city suburb schools in Cape Town (24%) and Johannesburg (54%) ^{52, 53}. These findings suggest that children in the NC and NW samples may have falsely elevated ZPP levels as they were from peri-urban areas, compared to children from a rural area in KZN.

In theory, ID occurs initially with a decrease in iron stores indicated by a decline in SF, followed by functional and transport iron diminishing with abnormal TfR and ZPP levels ¹⁰. All three indicators were abnormal in 1.1 to 3.1% of all subjects within the four groups, which confirms the theoretical progression of ID. However, the progression of ID does not appear to follow a consistent or linear relationship as only 1.1% of all subjects in KZN and NC, 0.9% of all subjects in NW and 1% of all subjects in the combined sample had abnormal ZPP and TfR levels. This

may be explained by the fact that during states of inflammation and chronic disease, iron is stored to remove it from circulation resulting in adequate SF levels while ZPP and TfR will still present as abnormal.

3.6.3 Optimal approach to diagnose ID

Since each single ID indicator has limitations and reflects different aspects of iron metabolism and therefore the stage of deficiency, the literature seems to indicate that the multiple criteria model has been regarded as the most suitable approach to diagnosing ID ¹⁸.

The optimal indicator to diagnose ID at a population level has to be simple to administer and accurate and therefore cost effective ¹⁴(Cameron, B. M. & Neufeld, L. M., 2011:S49). The laboratory assessment costs are similar for SF and TfR (approximately ZAR 166 and ZAR 156 per sample, respectively) and introducing the third indicator (ZPP) for the multiple criteria model adds approximately ZAR 90 per sample. Hb is by far the most affordable indicator (approximately ZAR 24 per sample), but does not accurately identify ID. Testing CRP in order to correct for inflammation adds approximately an additional ZAR 145 to the budget making the total cost per subject in this research project approximately ZAR 584 ⁵⁰. Therefore the overall expense is related to the number of individuals in the sample as it is more costly to test all three indicators compared to testing a single indicator.

Single indicators have been found to have suitable agreement with the multiple criteria model in various studies. Grant *et al.* ¹⁹ assessed ID in preschool Kenyan children and concluded that TfR indicated excellent agreement with the multiple criteria model. Cogswell *et al.* ²⁹ found reasonably good agreement between the prevalence of ID estimated by body iron stores and the multiple criteria model. It is challenging to clearly compare these findings to this research as no consistent pattern of agreement was found across the various study sites.

In the KZN sample, ZPP had the ability to identify the highest proportion of subjects as having ID compared to the multiple criteria model (indicated by the highest sensitivity value). In NC it was TfR and in both NW and the combined sample it was SF. Body iron had the best ability to identify the highest proportion of subjects without ID (indicated by the highest specificity value) compared to the multiple criteria model in KZN, NC and the combined site, and in NW TfR had this ability.

Body iron in KZN, NC and the combined sample, and TfR in NW, had the most promising ability compared to the multiple criteria model to correctly diagnose subjects that had ID (indicated by the highest PPV). TfR was identified to correctly diagnose the most subjects without ID (indicated by the highest NPV) in NC, SF in NW and the combined sample and both SF and ZPP in KZN

when compared to the multiple criteria model. It must be noted that the intention is for the results from the PPV and NPV calculations to be as close to 1.00 as possible as this indicates that the investigated indicators tested are performing well compared to the gold standard ⁵⁵. The PPV for the indicators was as low as 0.31 to 0.39 yet the lowest NPV ranged from 0.94 to 0.97. All four of the alternate indicators appear to perform well compared to the multiple criteria model in terms of identifying how many of the subjects appear not to have ID.

Even though there was no consistent accurate alternative to the multiple criteria model, these results should be interpreted with caution as sensitivity and specificity calculations offer little clinical use. However, they are not influenced by the prevalence of ID in the particular population ³⁵. The results from the calculations for predictive values imply that in general all indicators evaluated had a stronger ability to correctly diagnose subjects without ID as opposed to correctly diagnosing those with ID. Even though these results are useful from a clinical point of view, they cannot be applied universally as they are strongly influenced by the prevalence of ID in the populations being tested ³⁵.

A pattern emerged at each study site and in the combined sample with regard to sensitivity, specificity, predictive values and the percentage of subjects identified as ID whereby the indicator with the highest sensitivity (ability to correctly identify the highest proportion of subjects as having ID compared to the multiple criteria model), had the highest NPV (the ability to correctly diagnose subjects without ID according to the multiple criteria model) and identified the highest prevalence of ID. The converse applied to specificity, PPV and the indicator, which identified the lowest prevalence of ID. In general, all indicators across all study sites had a very good NPV when compared to the multiple criteria model. However, the inconsistent PPV for indicators compared to the multiple criteria model across the sites is problematic as this would identify more children with a normal iron status according to the multiple criteria model, as having ID (false positive). The associated risk of using single indicators or body iron is that the prevalence of ID and the public health concern may be overestimated compared to the multiple criteria model. However, one could argue that is an over-cautious approach. The multiple criteria model was selected as the ideal method to identify ID based on literature. However, it may not be an economically viable, realistic or practical approach in South Africa where SF is currently the only indicator to detect ID in national nutrition surveys, and Hb is the recommended iron status indicator in a public health setting ⁵⁶.

In summary, these various conflicting and inconsistent results make it challenging to compare indicators to identify an accurate, more cost effective alternative to the multiple criteria model. The mean SF concentrations were higher in individuals with inflammation in all four samples.

There was a consistent association between CRP and SF in all study sites and the combined sample (significant positive correlation). The percentage of subjects with anaemia in KZN is more than double the subjects in the other two sites. However, the prevalence of anaemia remains of mild public health significance in all study sites ⁴. The proportion of subjects with IDA is lower compared to the broad assumption that 50% of anaemia is a result of IDA. In KZN, ZPP had the ability to correctly identify the most number of subjects with ID compared to the multiple criteria model and it is likely that the subjects identified as not having ID corresponded to the multiple criteria model. However, in NC it was TfR and in both NW and the combined sample it was SF. ZPP had relatively few false negatives compared to the multiple criteria model, which means that children with ID will not be missed in KZN when ZPP is used as the indicator, but in NC TfR had this ability. In NW and the combined sample SF had this ability. In KZN, NC and the combined sample, it is body iron but in NW it is TfR that had the ability to identify the highest number of subjects that do not have ID compared to the multiple criteria model. Body iron had relatively few false positives, which will result in less subjects receiving unnecessary iron supplementation if applied at a public health level. If ZPP were to be used in KZN and the combined sample, it would result in the most false positives compared to the multiple criteria model and more children will receive unnecessary iron supplementation. TfR would result in this occurrence in NC and SF in NW. In KZN and NC, TfR will identify the most false negatives meaning that children that need supplementation will not be identified. In NC, if ZPP was used as an indicator instead of the multiple criteria model, and in the combined sample if body iron was used as the indicator, this situation will arise.

Sensitivity and specificity are influenced by the prevalence of ID in a particular area, which we see from the fact that the indicator that identifies the lowest prevalence had the highest specificity and the indicator with the lowest prevalence had the highest Se. However, predictive values (namely NPV and PPV) are not influenced by prevalence, yet they follow the same pattern as the measures of sensitivity and specificity.

3.7 Limitations

It must be noted that there was no data available for α_1 -acid glycoprotein (AGP), which may have resulted in some children with inflammation now convalescing, not being detected or controlled for. AGP has a longer half-life than CRP and is able to monitor the later stages of inflammation. It is recommended that both proteins (CRP and AGP) be used to detect inflammation in healthy populations ^{57, 58, 59}. Only one inflammatory marker (namely, CRP) was available and correcting for inflammation using this APP alone may have introduced bias as the effect of inflammation or infection on CRP, AGP and SF is not synchronised and decay rates differ ^{29, 60}.

Hepcidin levels were not available, which has been identified as a potentially superior alternative to traditional ID indicators ¹⁶(Thomas *et al.*, 2013:639) . The weight status of children was not available for inclusion in the analysis and it is important to consider that obesity-induced inflammation confounds ID indicators. Confounders that influence iron status indicators that have to be addressed remain. It may not be possible to always accurately consider the impact of inflammation, prevalence of vitamin A, B₁₂ or folate deficiencies, weight status and exposure to environmental lead on the iron status of subjects.

The limitations of the three indicators used in this research have to be acknowledged. Even though SF is the common indicator used to identify ID, SF is unable to quantitatively reveal a further decline of the tissue iron pool once iron stores are depleted ⁶¹. It is thought premature to use TfR for population-based iron status assessments due to the lack of a standardised TfR assay, the use of toolkit reference values and the potentially prohibitive cost ¹⁴. The limitations regarding the use of ZPP include the lack of consensus regarding the cut-off values, it is a non-specific indicator of ID and lead poisoning can elevate ZPP levels independent of iron status ⁶².

In order to standardise assay results, the WHO introduced International Standards in 1985 with the third and most recent one being released in 1997 ⁶³. In a review by Ferraro *et al.* ⁶³, concern was raised regarding the fact that the studies used to validate the use of SF as a diagnostic indicator for ID and to determine the recommended cut-offs were all carried out before the standardised assays were introduced. There is a need for recent studies that apply the WHO International Standards in order to optimise the clinical use of the SF as an indicator of ID and the cut-off values to accurately diagnose ID.

3.8 Conclusion

In conclusion, our study indicated no consistent agreement between single ID indicators, body iron and the multiple criteria model for identifying the prevalence of ID, after correcting for inflammation, in primary school children. In addition, the percentage of subjects identified as ID differed when different indicators were applied. During the validation process of comparing SF, TfR, ZPP and body iron against the multiple criteria model, no consistent valid alternative was identified that detected the most subjects with ID, excluded the most subjects without ID and had the lowest positive and negative false positive results. The interpretation is that the multiple criteria model as the gold standard for estimating ID is questionable. However, it remains impractical to validate it against bone marrow aspirate, the only direct measure to diagnose ID. Current evidence cautions against overestimating the prevalence of ID, as there is more associated harm compared to underestimating the deficiency. It remains a challenge to select the optimal iron

status indicator for use in the public health setting and nationally representative nutritional surveys. Further studies in this field are needed.

3.9 References

- (1) de Benoist, B., McLean, E., Egli, I. & Cogswell, M., eds. 2008. Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia. http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf: World Health Organization.
 - (2) Labadarios D. 2007. National Food Consumption Survey-Fortification Baseline (NFCS-FB): South Africa, 2005. Tygerberg: University of Stellenbosch.
 - (3) Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Reddy, P., Parker, W., Hoosain, E., Naidoo, P., Hongoro, C., Mchiza, Z., Steyn, N.P., Dwane, N., Makoe, M., Maluleke, T., Ramlagan, S., Zungu, N., Evans, M.G., Jacobs, L. & Faber, M. & SANHANES-1 Team. 2013. South African national health and nutrition examination survey (SANHANES-1). Cape Town: HSRC Press.
- World Health Organization. 2001 Iron deficiency anaemia assessment, prevention, and control. A guide for programme managers. http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf Date of access: 2 January 2013.
- (4) Engle-Stone, R., Nankap, M., Ndjebayi, A.O., Erhardt, J.G. & Brown, K.H. 2013. Plasma ferritin and soluble transferrin receptor concentrations and body iron stores identify similar risk factors for iron deficiency but result in different estimates of the national prevalence of iron deficiency and iron-deficiency anaemia among women and children in Cameroon. *The journal of nutrition*, 143(3):369-377.
 - (5) Lynch, S. 2012. The rationale for selecting and standardizing iron status indicators. in: World Health Organization. Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010. Geneva: World Health Organization.
 - (6) Northrop-Clewes, C.A. & Thurnham, D.I. 2013. Biomarkers for the differentiation of anaemia and their clinical usefulness. *Journal of blood medicine*, 111.

- (7) Suominen, P., Punnonen, K., Rajamäki, A. & Irjala, K. 1998. Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. . *Blood*, 92(8):2934-2939.
- (8) Tussing-Humphreys, L., Pustacioglu, C., Nemeth, E. & Braunschweig, C. 2012. Rethinking iron regulation and assessment in iron deficiency, anaemia of chronic disease, and obesity: Introducing hepcidin. *Journal of the Academy of Nutrition and Dietetics*, 112(3):391-400.
- (9) Lynch, S. 2011. Case studies: iron. *American journal of clinical nutrition*, 94(suppl):673S.
- (10) Cook, J.D., Flowers, C.H. & Skikne, B.S. 2003. The quantitative assessment of body iron. *Blood*, 101(9):3359-3363.
- (11) World Health Organization. 2012. Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010. Geneva, Switzerland: World Health Organization.
- (12) Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. *Blood* 1976; 48:449–55.
- (13) Cameron, B. M. & Neufeld, L. M. 2011. Estimating the prevalence of iron deficiency in the first two years of life: Technical and measurement issues. *Nutrition reviews*, 69(Suppl. 1): S49-S56.
- (14) Cook, J.D. 2005. Diagnosis and management of iron-deficiency anaemia. *Best practice & research clinical haematology*, 18(2):319-332.
- (15) Thomas, D.W., Hinchliffe, R.F., Briggs, C., Macdougall, I.C., Littlewood, T. & Cavill, I. 2013. Guideline for the laboratory diagnosis of functional iron deficiency. *British journal of haematology*, 161(5):639-648.
- (16) Finberg, K.E. 2011. Unravelling mechanisms regulating systemic iron homeostasis. *ASH education program book*, 2011(1):532-537.
- (17) Pasricha, S.R., McQuilten, Z., Westerman, M., Keller, A., Nemeth, E., Ganz, T. & Wood, E. 2011. Serum hepcidin as a diagnostic test of iron deficiency in premenopausal female blood donors. *Haematologica*, 96(8):1099-1105.

- (18) Grant, F.K., Martorell, R., Flores-Ayala, R., Cole, C.R., Ruth, L.J., Ramakrishnan, U. & Suchdev, P.S. 2012. Comparison of indicators of iron deficiency in Kenyan children. *American journal of clinical nutrition*, 95(5):1231-1237.
- (19) Thurnham, D.I., McCabe, L.D., Haldar, S., Wieringa, F.T., Northrop-Clewes, C.A. & McCabe, G.P. 2010. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: A meta-analysis. *American journal of clinical nutrition*, 92(3):546-555.
- (20) Zimmermann, M.B., Molinari, L., Staubli-Asobayire, F., Hess, S.Y., Chaouki, N., Adou, P. & Hurrell, R.F. 2005. Serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children. *American journal of clinical nutrition*, 81(3):615-623.
- (21) Baumgartner, J.; Smuts, C.M., Malan, L., Kvalsvig, J., van Stuijvenberg, M.E., Hurrell, R.F. and Zimmermann, M.B., 2012. Effects of iron and n-3 fatty acid supplementation, alone and in combination, on cognition in school children: a randomized, double-blind, placebo-controlled intervention in South Africa. *American journal of clinical nutrition*, 96(6), pp. 1327-1338.
- (22) World Health Organization and Centres for Disease Control and Prevention, 2007. Assessing the iron status of populations: Report of a joint WHO /CDC Technical Consultation on the assessment of iron status at the population level.
http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107.pdf edn. Geneva, Switzerland: World Health Organization.
- (23) Troesch, B., van Stuijvenberg, M.E., Smuts, C.M., Kruger, H.S., Biebinger, R., Hurrell, R.F., Baumgartner, J. and Zimmermann, M.B., 2011. A Micronutrient Powder with Low Doses of Highly Absorbable Iron and Zinc Reduces Iron and Zinc Deficiency and Improves Weight-For-Age Z-Scores in South African Children. *The Journal of nutrition*, 141(2), pp. 237-242.
- (24) Taljaard, C., Covic, N.M., van Graan, A.E., Kruger, H.S., Smuts, C.M., Baumgartner, J., Kvalsvig, J.D., Wright, H.H., van Stuijvenberg, M.E. & Jerling, J.C. 2013. Effects of a multi-micronutrient-fortified beverage, with and without sugar, on growth and cognition in South African schoolchildren: A randomised, double-blind, controlled intervention. *British journal nutrition*, 110:2271-2284.
- (25) Weather Underground, Weather History & Data Archives – Kimberley, Klerksdorp, Pietermaritzburg <http://www.wunderground.com> Date of access: 17 November 2014

- (26) Metzgeroth, G., Adelberger, V., Dorn-Beineke, A., Kuhn, C., Schatz, M., Maywald, O., Bertsch, T., Wiser, H., Hehlmann, R. and Hastka, J., 2005. Soluble transferrin receptor and zinc protoporphyrin – competitors or efficient partners? *European Journal of Haematology*, 75(4).
- (27) Skikne, B., Flowers, C. & Cook, J. 1990. Serum transferrin receptor: A quantitative measure of tissue iron deficiency. *Blood*, 75(9):1870-1876.
- (28) Cogswell, M.E., Looker, A.C., Pfeifer, C.M., Cook, J.D., Lacher, D.A., Beard, J.L., Lynch, S.R. and Grummer-Strawn, L.M., 2009. *Assessment of iron deficiency in US preschool children and nonpregnant females of childbearing age: National Health and Nutrition Examination Survey 2003–2006*.
- (29) Beard, J. 2007. Indicators of iron status: free erythrocyte protoporphyrin and zinc protoporphyrin; serum and plasma iron, total iron binding capacity and transferrin saturation; and serum transferrin receptor. In: World Health Organization and Centres for Disease Control and Prevention, 2007. *Assessing the iron status of populations: Report of a joint WHO /CDC Technical Consultation on the assessment of iron status at the population level*. http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107.pdf edn. Geneva, Switzerland: World Health Organization.
- (30) Zimmermann, M.B. & Hurrell, R.F. 2007. Nutritional iron deficiency. *Lancet*, 370(9586):511-520.
- (31) Troesch, B. 2013. Erythrocyte zinc protoporphyrin in South African children is not affected by low level lead exposure but by sample preparation (freezing). IUNS: Nutrition Innovation Centre, DSM Nutritional Products, Kaiseraugst, Switzerland. [Artwork].
- (32) World Health Organization. 2011. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. http://www.who.int/vmnis/indicators/serum_ferritin.pdf Date of access: September, 6. 2013.
- (33) Thurnham, D.I. & McCabe, G.P. 2012. Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: World Health Organization. Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010. Geneva: World Health Organization.

- (34) Altman, D.G. 1999. Some common problems in medical research: Diagnostic tests. (In Anon. Practical statistics for medical research. 9th ed. Chapman & Hall / CRC. p. 409-419).
- (35) Statistics South Africa. 2012. Census 2011. <http://www.statssa.gov.za/publications/p03014/p030142011.pdf> Date of access: November. 2014.
- (36) Feelders, R.A., Vreugdenhil, G., Eggermont, A.M., Kuiper-Kramer, P.A., van Eijk, H.G. & Swaak, A.J. 1998. Regulation of iron metabolism in the acute-phase response: interferon-g and tumour necrosis factor-a induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. *European Society for Clinical Investigation*, 28:520–527.
- (37) Brito, A., Hertrampf, E. & Olivares, M. 2013. Iron status biomarkers and C-reactive protein in children aged 19 to 72 months in Chile. *Food and nutrition bulletin*, 34(1):14-20.
- (38) Hastka J et al. Zinc protoporphyrin in anaemia of chronic disorders. 1993. *Blood*, 81:1200–1204.
- (39) Skikne BS, Flowers CH, Cook J. 2008. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood*, 75:1870–1876).
- (40) Hurrell, R.F. 2007. Iron fortification: Its efficacy and safety in relation to infections. *Food and nutrition bulletin*, (Suppl. 4): 585S-594S.
- (41) Le Huong T, Brouwer ID, Nguyen KC, Burema J, Kok FJ. 2007. The effect of iron fortification and de-worming on anaemia and iron status of Vietnamese schoolchildren. *British Journal of Nutrition*, 97(5):955–62.
- (42) Beard, J.L., Murray-Kolb, L.E., Rosales, F.J., Solomons, N.W. & Angelilli, M.A. 2006. Interpretation of serum ferritin concentrations as indicators of total-body iron stores in survey populations: The role of biomarkers for the acute phase response. *American journal clinical nutrition*, 84:1498.
- (43) Johnson, M.A., 1990. Iron: Nutrition Monitoring and Nutrition Status Assessment. *The journal of nutrition*, 120(11 Suppl), pp. 1486-1491.
- (44) Perry, G.G., Byers, T., Yip, R. & Margen, S. 1992. Iron nutrition does not account for the hemoglobin differences between blacks and whites. *The journal of nutrition*, 122:309.

- (45) Shisana, O, Rehle, T, Simbayi LC, Zuma, K, Jooste, S, Zungu N, Labadarios, D, Onoya, D et al. 2014. South African national HIV prevalence, incidence and behaviour survey, 2012. Cape Town: HSRC Press.
- (46) Little K, Thorne C, Luo C, Bunders M, Ngongo N, McDermott P, Newell ML. 2007. Disease progression in children with vertically-acquired HIV infection in sub-Saharan Africa: reviewing the need for HIV treatment. *Current HIV Research*, Mar; 5(2):139-53.
- (47) Richter, L. 2004. The impact of HIV/AIDS on the development of children - A generation at risk. www.issafrica.org Date of access: 8 November 2014.
- (48) South Africa. Department of Health. 2009. National tuberculosis management guidelines.
- (49) Zimmermann MB, Muthayya S, Moretti D, Kurpad A & Hurrell RF. 2006. Iron fortification reduces blood lead levels in children in Bangalore, India. *Pediatrics*; 117: 2014–21.
- (50) Nriagu, J., Jinabhai, C.C., Naidoo, R., & Coutsoodis, A. 1997. Lead poisoning of children in Africa, II. Kwazulu-Natal, South Africa. *Science of the Total Environment*, 197(1- 3), 1-11.
- (51) Mathee, A., Naicker, N., & Barnes, B. 2009a. *Blood lead levels in South African children at the end of the leaded petrol era*. Preliminary Research Report. Johannesburg: Medical Research Council.
- (52) Mathee, A. 2012. Lead poisoning. MRC Environment and Health Research Unit, Faculty of Health Sciences - University of Johannesburg & School of Public Health, University of Witwatersrand. In: van Niekerk A, Suffla S, Seedat M (Eds), ed. *Crime, violence and injury in South Africa: 21st century solutions for child safety*. Psychological Society of South Africa (PsySSA), Houghton, South Africa.
- (53) AMPATH pathology laboratory www.ampath.co.za.
- (54) Parikh, R., Mathai, A., Parikh, S., Sekhar, C. & Thomas, R. 2008. Understanding and using sensitivity, specificity and predictive values. *Indian J ophthalmology*, 56(1):45-50.
- (55) South Africa. Department of Health. 2014. Integrated management of Childhood Illness.
- (56) Northrop-Clewes CA. 2008. Interpreting indicators of iron status during an acute phase response – lessons from malaria and HIV. *Annals of Clinical Biochemistry*, 45:18–32.

- (57) Fleck, A. & Myers, M.A. 1985. Diagnostic and prognostic significance of acute phase proteins. In: Gordon AH, Koj A, eds. *The acute phase response to injury and infection*, 1st ed. Amsterdam, Elsevier Science Publishers, 249–271.
- (58) Koj A. 1985. Catabolism and turnover of acute-phase proteins. In: Gordon AH, Koj A, eds. *The acute-phase response to injury and infection*, 1st ed. Amsterdam, Elsevier Science Publishers, 227–246.)
- (59) Baynes, R., Bezwoda, W., Bothwell, T., Khan, Q. & Mansoor N. 1986. The non-immune inflammatory response: serial changes in plasma iron, iron-binding capacity, lactoferrin, ferritin and C-reactive protein. *Scandinavian Journal of Clinical Laboratory Investigation*, 46:695–704.
- (60) Yang, Z., Dewey, K.G., Lönnerdal, B., Hernell, O., Chaparro, C., Adu-Afarwuah, S., McLean, E.D., Cohen, R.J., Domellöf, M., Allen, L.H. & Brown, K.H. 2008. Comparison of plasma ferritin concentration with the ratio of plasma transferrin receptor to ferritin in estimating body iron stores: Results of 4 intervention trials. *American journal of clinical nutrition*, 87(6):1892-1898.
- (61) Zimmermann, M.B. 2008. Methods to assess iron and iodine status. *British journal of nutrition*, 99(Supplement S3):S2.
- (62) Ferraro, S., Mozzi, R. & Panteghini, M. 2012. Revaluating serum ferritin as a marker of body iron stores in the traceability era. *Clinical chemistry & laboratory medicine*, 50(11):1911-1916.

4 Chapter 4: Summary, conclusions and recommendations

4.1 Introduction

This study set out to conduct a critical analysis of the indicators used to determine the iron status in three previous independent iron intervention studies involving 6-11-year-old primary school children in South Africa in three different provinces (Baumgartner *et al.*, 2012b:1327; Taljaard *et al.*, 2013:2271; Troesch *et al.*, 2011:237). There is continued uncertainty regarding the optimal epidemiological approach to identifying and measuring the severity of iron deficiency (ID) at a population level (Cook *et al.*, 2003), and the WHO (2012) confirmed that there is an urgent need to address the selection and standardisation of iron status indicators. Currently the most promising indicators include serum ferritin (SF), transferrin receptor (TfR) and zinc protoporphyrin (ZPP), the multiple criteria model (Cook *et al.*, 1976) and the calculation of body iron (Cook *et al.*, 2003). The rate of inflammation and infection would need to be taken into account as SF, commonly used as a single indicator to describe the prevalence of ID, is an acute phase protein (APP) that increases independent from iron status (Thurnham *et al.*, 2010:546; WHO, 2011b).

In the public health setting, a suitable method to assess iron status has to be inexpensive, the analysis standardised and established, easy to measure, appropriate to use in developing countries and their applications specific to identifying ID at a population level (Cameron, B. M. & Neufeld, L. M., 2011:S49; WHO, 2012). The review of existing literature revealed a lack of agreement on the indicators to use, as well as a broad range of cut-off values to apply. Consensus on the use of appropriate iron status indicators, which cut-off values to apply and correction for inflammation is necessary in order to compare the effects of interventions and the prevalence of ID and IDA across populations and over time.

Each indicator used to describe iron status has its own advantages, disadvantages and limitations. This chapter therefore firstly synthesises the main findings of the investigation, as discussed in chapter 3. It then presents conclusions based on the results with regard to the given hypothesis, namely that even though the prevalence of ID will differ when various indicators are applied after correcting for inflammation, there will be a definitive superior indicator to determine ID as compared to the multiple criteria model, as evaluating a combination of the single indicators may provide a more complete assessment (Pasricha *et al.*, 2011:1099). This chapter will close by reviewing the research contributions of this mini-dissertation, and including recommendations for future research and guidance to researchers in the field of nationally representative nutrition surveys, which are the cornerstone of iron intervention strategies.

4.2 Main findings and conclusion

In this cross-sectional analysis study involving 736 children in KwaZulu-Natal (KZN), 1045 in the Northern Cape (NC) and 546 children in North West (NW), all aged 6-11 years from various primary schools and almost equal gender distribution, there was an association between inflammation and SF. The percentage of subjects identified as ID in the three study sites and the combined sample differed when different indicators were applied, and the sensitivity, specificity and predictive values of the alternative indicators compared to the multiple criteria model varied between study sites. There appeared to be no consistently best substitute for the multiple criteria model.

The first objective of this research was to investigate the influence of inflammation on SF concentrations. Different approaches have been suggested and applied to address the confounding effect of inflammation. These include the exclusion of subjects with inflammation, the use of adjusted cut-off values to diagnose ID in subjects with inflammation, or the application of correction factors to remove the influence of inflammation (Baumgartner *et al.*, 2012b:1327; Grant *et al.*, 2012b:1231; Thurnham *et al.*, 2010:546; WHO & CDC, 2007: ; Zimmermann *et al.*, 2005:615). However, it is important to keep the influence of inflammation on ID indicators in perspective. When one assesses the difference in the actual number of children identified with ID when considering and not considering inflammation, this difference was small. When inflammation was not taken into account, only marginally fewer children were identified as ID, two in KZN and the NC, nine in NW and twelve in the combined sample. Therefore, according to these results, if one selects SF as the indicator and resources are limited, it does not appear essential to consider the influence of inflammation.

Lynch (2011) discusses a pertinent point. Despite having a thorough understanding of the causes of ID and the physiology of iron absorption, approximately two billion people globally continue to be affected by ID (Zimmermann & Hurrell, 2007:511). The reasons for this disparity between our knowledge of this micronutrient and the continuing deficiency are complex; the accuracy of indirect measures to determine ID prevalence is questionable (Lynch, 2011b:763S). This addresses the additional objectives of the research, namely to describe the prevalence of anaemia, ID and IDA using different indicators, and to investigate a superior indicator to determine ID as compared with the multiple criteria model.

The hypothesis set for this research study is rejected. Despite the expected difference in the prevalence of ID when various indicators were applied after correcting for inflammation, there was no definitive superior indicator to determine ID as compared to the multiple criteria model.

It is well-known that it is not recommended to use Hb as the only indicator for ID, as its sensitivity and specificity are unacceptably low. Nevertheless, this still occurs in developing countries (Lynch, 2011b:763S). Considering the fact that all indirect measures of ID are currently affected by factors other than ID, it would also have been valuable in this study to know additional factors that may have been responsible for ID and anaemia (Lynch, 2011b:763S). This could have been achieved by collecting dietary and anthropometric data, and performing additional laboratory analyses of blood samples for additional micronutrients and inflammatory markers (AGP and hepcidin). Unfortunately, these factors were not collected as part of this study data.

Even though the WHO (2001) estimated that 50% of anaemia results from ID, of the subjects with anaemia, the percentage that had IDA ranged from only 15.8 to 34.2%. The percentage of children identified as ID using Hb was 11% more in KZN, 0.9% more in NC, 14.3% more in NW and 3.2% in the combined sample. Major factors that influence ID that should be taken into account include differences in dietary patterns as poverty limits dietary diversity, the reliance on cereal-based diets which are low in bioavailable iron, body composition, different incidence of communicable diseases, as well as the prevalence of vitamin A, B₁₂ or folate deficiencies, anaemia of chronic disease and certain relevant inherited conditions (Lynch, 2011b:763S; Shisana *et al.*, 2013b).

4.3 Recommendations

The results of this study showed an association between inflammation and SF, and it is recommended that future studies obtain data for the additional APP, α_1 –acid glycoprotein (AGP), as this would identify children with inflammation now convalescing. AGP has a longer half-life than CRP and is able to monitor the later stages of inflammation. It is recommended that both proteins (CRP and AGP) be used to detect inflammation in healthy populations and it is recommended that both CRP and AGP be used to detect inflammation in healthy populations (Northrop-Clewes, 2008:18).

In light of the varied levels of ID identified by the various single indicators, body iron and the multiple criteria model, another recommendation is to compare the performance of indirect iron indicators to the gold standard, bone marrow aspirate, to identify which indicators identified the highest number of individuals compared to the findings of bone marrow aspirate. Even though bone marrow aspirate is highly specific, it is invasive (samples taken from the iliac crest or tibia under conscious sedation), expensive and requires technical expertise (Aguilar *et al.*, 2012; Lynch, 2011b:763S). It is therefore recommended to conduct power calculations in field settings to identify the smallest sample size required for statistical purposes in both high and low inflammation settings.

In a study conducted with anaemic children in rural Mozambique, researchers found that in a high inflammation setting, no indirect indicator of ID was able to identify an acceptable proportion of children with ID compared to bone marrow aspirate (Aguilar *et al.*, 2012). It is recommended that additional indicators such as ZPP, hepcidin and the multiple criteria model be included in future research, as these indicators may be able to identify more individuals with ID compared to bone marrow aspirate.

Simply using Hb as an ID indicator, per the current recommendation in the public health setting, is inaccurate. As highlighted, a higher percentage of children were identified as ID according to Hb than the multiple criteria model. More reliable, affordable and easy to measure iron markers are needed in resource-poor settings. In order to improve the accuracy of iron indicators, it is recommended that the limitations of each test be addressed by establishing a more controlled environment to test the accuracy of the various indicators (Lynch, 2011:763S).

When investigating the accuracy of SF, it is recommended that both CRP and AGP be tested and that subjects with liver disease be excluded. The methodology for ZPP should be standardised and technology improved. Serum lead should be tested in individuals. TfR methodology has to be standardised and markers of erythropoietic rates should also be tested. Even though cost is a current barrier to these alternative indicators, one should keep in mind the economies of scale when conducting large scale research, as well as the fact that as technology improves over time, the affordability of such tests improves. Even though cost is a current barrier to the multiple criteria model, this may no longer be valid in the near future.

It is recommended that the multiple factors that play a role in selecting the ideal ID indicator for use in field settings such as cost, the type of blood sample required, the infrastructure and facilities required and available for the storage and processing of blood samples, be taken into consideration. ID and IDA continue to have a negative impact on children's cognitive and physical development (Lozoff, 2007).

4.4 Conclusions

The findings from the research indicate that in general, all ID indicators across all study sites had a very good ability to correctly diagnose subjects without ID when compared to the multiple criteria model. However, single indicators and body iron identify more false positives compared to the multiple criteria model. The associated risk of using single indicators or body iron is that the prevalence of ID and the public health concern may be overestimated in comparison to the application of the multiple criteria model. However, one could argue that this is an over-cautious approach. The multiple criteria model was selected as the ideal method to identify ID based on

available literature. However, it may not be an economically viable, realistic or practical approach in South Africa where SF is currently the only indicator to detect ID in national nutrition surveys, and even though it is inaccurate, it is common practice for Hb to be the recommended iron status indicator to perform in a public health setting.

SF is a commonly applied indicator of iron status however it is a positive acute phase protein and therefore increases in response to inflammation and infection, independently of iron status. Literature suggests various ways to address the influence of inflammation and infection on SF (Thurnham *et al.*, 2010:546). However, it is important to note that when inflammation was taken into account using the only available inflammatory marker, CRP, only marginally more children were identified as ID and the influence of inflammation should be assessed in the context of the research environment being explored.

In conclusion, the study indicated no consistent agreement between single ID indicators and body iron and the multiple criteria model for identifying the prevalence of ID, after correcting for inflammation, in the study sites. In addition, the percentage of subjects identified as ID differed when different indicators were applied. Even though the reality with using data collected from individuals is that often more questions are generated than answered, especially in the complex field of iron status, current evidence cautions against overestimating the prevalence of ID, as there is more associated harm compared to underestimating the deficiency. It remains a challenge to select the optimal iron status indicator for use in the public health setting and nationally representative nutritional surveys. Further studies in this field are needed.

Bibliography

Aeberli, I., Hurrell, R.F. & Zimmermann, M.B. 2009. Overweight children have higher circulating hepcidin concentrations and lower iron status but have dietary iron intakes and bioavailability comparable to normal weight children. *International Journal of Obesity*, 33:1111-1117.

Aguilar, R., Moraleta, C., Quinto, L., Renom, M., Mussacate, L. 2012. Challenges in the diagnosis of iron deficiency in children exposed to high prevalence of infections. *PLoS ONE*, 7(11):1-7.

Altman, D.G. 1999. Some common problems in medical research: Diagnostic tests. (In: Practical statistics for medical research. 9th ed. Chapman & Hall/CRC. pp. 409-419).

Amato, A., Santoro, N., Calabro, P., Grandone, A., Swinkels, D.W. & Perrone, L. 2010. Effect of body mass index reduction on serum hepcidin levels and iron status in obese children. *International Journal of Obesity*, 34:1772-1774.

Australian Red Cross Blood Service. 2014. Iron deficiency without anaemia. http://www.transfusion.com.au/anaemia_management/iron_deficiency_without_anaemia Date of Access: 20 September 2014.

Ayoya, M.A., Spiekermann-Brouwer, G.M., Stoltzfus, R.J., Nemeth, E., Habicht, J., Ganz, T., Rawat, R., Traoré, A.K. & Garza, C. 2010. α 1-Acid glycoprotein, hepcidin, C-reactive protein, and serum ferritin are correlated in anemic schoolchildren with *Schistosoma haematobium*. *American journal of clinical nutrition*, 91:1784-1790.

Baumgartner, J., Smuts, C.M., Aeberli, I., Malan, L., Tjalsma, H. & Zimmermann, M.B. 2012a. Overweight impairs efficacy of iron supplementation in iron-deficient South African children: A randomized controlled intervention. *International Journal of Obesity*, 37(1):24-30.

Baumgartner, J., Smuts, C.M., Malan, L., Kvalsvig, J., van Stuijvenberg, M.E., Hurrell, R.F., & Zimmermann, M.B. 2012b. Effects of iron and n-3 fatty acid supplementation, alone and in combination, on cognition in school children: A randomized, double-blind, placebo-controlled intervention in South Africa. *American journal of clinical nutrition*, 96(6):1327-1338.

Beard, J.L., Murray-Kolb, L.E., Rosales, F.J., Solomons, N.W., & Angelilli, M.A. 2006. Interpretation of serum ferritin concentrations as indicators of total-body iron stores in survey populations: The role of biomarkers for the acute phase response. *American journal of clinical nutrition*, 84:1498.

- Beard, J. 2003. Iron deficiency alters brain development and functioning. *The Journal of nutrition*, 133(5):1468S-1472S.
- Beard, J.L. 2001. Iron biology in immune function, muscle metabolism and neuronal functioning. *The Journal of nutrition*, 131(2):568S-580S.
- Best, C., Neufingerl, N., van Geel, L., van den Briel, T. & Osendarp, S. 2010. Nutritional status of school-aged children. *Food and Nutrition Bulletin*, 31(3):400.
- Cameron, B.M. & Neufeld, L.M. 2011. Estimating the prevalence of iron deficiency in the first two years of life: technical and measurement issues. *Nutrition Reviews*, 69(S1):S49-S56.
- Cogswell, M.E., Looker, A.C., Pfeiffer, C.M., Cook, J.D., Lacher, D.A., Beard, J.L., Lynch, S.R. & Grummer-Strawn, L.M. 2009. Assessment of iron deficiency in US preschool children and nonpregnant females of childbearing age: National Health and Nutrition Examination Survey 2003–2006. *American journal of clinical nutrition*, 89: 1334–42.
- Cook, J.D. 2005. Diagnosis and management of iron-deficiency anaemia. *Best Practice & Research Clinical Haematology*, 18(2):319-332.
- Cook, J.D., Flowers, C.H. & Skikne, B.S. 2003. The quantitative assessment of body iron. *Blood*, 101(9):3359-3363.
- Crichton, R.D., Bo, G. & Geisser, P. 2008. *Iron therapy with special emphasis on intravenous administration*. 4th ed. Bremen: Uni-Med.
- Davis, J.F., Vidyassag, S. & Maiya, G.A. 2012. C-reactive protein and coronary heart disease - risk marker or risk factor? *Journal of Clinical Science Research*, 1, pp. 178.
- De Benoist, B., Mclean, E., Egli, I. & Cogswell, M., eds. 2008. *Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia*. http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf: World Health Organization. Date of access: 10 September 2013.
- Engle-Stone, R., Nankap, M., Ndjebayi, A.O., Erhardt, J.G. & Brown, K.H. 2013. Plasma ferritin and soluble transferrin receptor concentrations and body iron stores identify similar risk factors for iron deficiency but result in different estimates of the national prevalence of iron deficiency and iron-deficiency anemia among women and children in Cameroon. *The Journal of nutrition*, 143(3):369-377.

Ferraro, S., Mozzi, R. & Panteghini, M. 2012. Revaluating serum ferritin as a marker of body iron stores in the traceability era. *Clinical Chemistry & Laboratory Medicine*, 50(11):1911-1916.

Ganz, T. 2013. Systemic iron homeostasis. *Physiological Reviews*, 93:1721-1741.

Grant, F.K.E., Suchdev, P.S., Flores-Ayala, R., Cole, C.R., Ramakrishnan, U., Ruth, L.J. & Martorell, R. 2012. Correcting for inflammation changes estimates of iron deficiency among rural Kenyan preschool children. *The Journal of nutrition*, 142(1):105-111.

Grant, F.K., Martorell, R., Flores-Ayala, R., Cole, C.R., Ruth, L.J., Ramakrishnan, U., & Suchdev, P.S. 2012. Comparison of indicators of iron deficiency in Kenyan children. *American journal of clinical nutrition*, 95(5):1231-1237.

Grantham-Mcgregor, S. & Ani, C. 2001. A review of studies on the effect of iron deficiency on cognitive development in children. *The Journal of nutrition*, 131(2):649S-668S.

Haas, J.D. & Brownlie, T. 2001. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *The Journal of nutrition*, 131(2):676S-690S.

Hartfield, D. 2010. Iron deficiency is a public health problem in Canadian infants and children. *Paediatric Child Health*, 15(6):347.

Horl, W.H. 2013. Anaemia management and mortality risk in chronic kidney disease. *Nature Reviews Nephrology*, 9(5):291-301.

Hunt, J.R. 2005. *Encyclopedia of Human Nutrition*. Elsevier.

Johnson, M.A. 1990. Iron: Nutrition Monitoring and Nutrition Status Assessment. *The Journal of nutrition*, 120(S11):1486-1491.

Kootbodien, T., Mathee, A., Naicker, N. & Moodley, N. 2012. Heavy metal contamination in a school vegetable garden in Johannesburg. *South African Medical Journal*, 102:234-235.

Kung'u, J.K., Wright, V.J., Haji, H.J., Ramsan, M., Goodman, D., Tielsch, J.M., Bickle, Q.D., Raynes, J.G. & Stoltzfus, R. 2009. Adjusting for the acute phase response is essential to interpret iron status indicators among young Zanzibari children prone to chronic malaria and helminth infections. *The Journal of nutrition*, 139:2124-2131.

Labbe, R.F., Dewanji, A. & McLaughlin, K. 1999. Observations on the zinc protoporphyrin / heme ratio in whole blood. *Clinical Chemistry*, 45:146-148.

Lozoff, B., Corapci, F., Burden, M.J., Kaciroti, N., Angulo-Barroso, R., Sazawal, S. & Black, M. 2007. Preschool-aged children with iron deficiency anemia show altered affect and behavior. *The Journal of nutrition*, 137(3):683-689.

Lynch, S. 2012. The rationale for selecting and standardizing iron status indicators. (In: WHO, World Health Organization. 2010. Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010. Geneva: World Health Organization).

Lynch, S. 2011a. Case studies: iron¹⁻⁴. *American journal of clinical nutrition*, 94:673S.

Lynch, S.R. 2011b. Why nutritional iron deficiency persists as a worldwide problem. *The Journal of Nutrition*, 141:763S-768S.

Mahan, L.K., & Escott-Stump, S. 2012. *Krause's Food and the Nutrition Care Process*. 13th ed. Elsevier Saunders.

Metzgeroth, G., Adelberger, V., Dorn-Beineke, A., Kuhn, C., Schatz, M., Maywald, O., Bertsch, T., Wisser, H., Hehlmann, R. & Hastka, J. 2005. Soluble transferrin receptor and zinc protoporphyrin – competitors or efficient partners? *European Journal of Haematology*, 75(4):309-317.

Nel, E. 2013. *Iron status, inflammation and anthropometric nutritional status of four-to-thirteen month-old black infants from a rural South African population*. MSc edn. Potchefstroom Campus, North-West University. (Thesis – MSc).

Northrop-Clewes, C.A. 2008. Interpreting indicators of iron status during an acute phase response--lessons from malaria and human immunodeficiency virus. *Annals of Clinical Biochemistry*, 45:18-32.

Northrop-Clewes, C.A. & Thurnham, D.I. 2013. Biomarkers for the differentiation of anaemia and their clinical usefulness. *Journal of Blood Medicine*, 1:11.

Olney, D.K., Pollitt, E., Kariger, P.K., Khalfan, S.S., Ali, N.S., Tielsch, J.M., Sazawal, S., Black, R., Mast, D., Allen, L.H. & Stoltzfus, R.J. 2007. Young Zanzibari children with iron deficiency, iron deficiency anemia, stunting, or malaria have lower motor activity scores and spend less time in locomotion. *The Journal of nutrition*, 137(12):2756-2762.

Pantopoulos, K., Porwal, S.K., Tartakoff, A. & Devireddy, L. 2012. Mechanisms of mammalian iron homeostasis. *Hematology*, 51(29):5705.

- Pasricha, S.R., Mcquilten, Z., Westerman, M., Keller, A., Nemeth, E., Ganz, T. Wood, E. 2011. Serum hepcidin as a diagnostic test of iron deficiency in premenopausal female blood donors. *Haematologica*, 96(8):1099-1105.
- Pollitt, E. 1997. Iron deficiency and educational deficiency. *Nutrition Reviews*, 55(4):133.
- Shisana, O., Labadarios, D., Rehle, T., Simbayi, L., Zuma, K., Dhansay, A., Reddy, P., Parker, W., Hoosain, E., Naidoo, P., Hongoro, C., Mchiza, Z., Steyn, N.P., Dwane, N., Makoe, M., Maluleke, T., Ramlagan, S., Zungu, N., Evans, M.G., Jacobs, L., Faber, M. & Sanhanes-1 Team. 2013. *South African National Health and Nutrition Examination Survey (SANHANES-1)*. Cape Town: HSRC Press.
- Skikne, B., Flowers, C. & Cook, J. 1990. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood*, 75(9):1870-1876.
- Spottiswoode, N., Duffy, P.E. & Drakesmith, H. 2014. Iron, anemia and hepcidin in malaria. *Frontiers in Pharmacology*, 5(125):1-11.
- Stoltzfus, R.J. 1997. Rethinking anaemia surveillance. *Lancet*, 349(9067):1764-1766.
- Taljaard, C., Covic, N.M., van Graan, A.E., Kruger, H.S., Smuts, C.M., Baumgartner, J., Kvalsvig, J.D., Wright, H.H., van Stuijvenberg, M.E. & Jerling, J.C. 2013. Effects of a multi-micronutrient-fortified beverage, with and without sugar, on growth and cognition in South African schoolchildren: a randomised, double-blind, controlled intervention. *British Journal of Nutrition*, 110:2271-2284.
- Thomas, C. & Thomas, L. 2005. Anemia of chronic disease: pathophysiology and laboratory diagnosis. *Laboratory Haematology*, 11:14-23.
- Thomas, D.W., Hinchliffe, R.F., Briggs, C., Macdougall, I.C., Littlewood, T. & Cavill, I. 2013. Guideline for the laboratory diagnosis of functional iron deficiency. *British Journal of Haematology*, 161(5):639-648.
- Thurnham, D.I. & McCabe, G.P. 2012. *Influence of infection and inflammation on biomarkers of nutritional status with an emphasis on vitamin A and iron. In: World Health Organization. Report: Priorities in the assessment of vitamin A and iron status in populations, Panama City, Panama, 15–17 September 2010.* Geneva: World Health Organization.
- Thurnham, D.I., McCabe, L.D., Haldar, S., Wieringa, F.T., Northrop-Clewes, C.A. & McCabe, G.P. 2010. Adjusting plasma ferritin concentrations to remove the effects of subclinical

inflammation in the assessment of iron deficiency: a meta-analysis. *American journal of clinical nutrition*, 92(3):546-555.

Troesch, B., van Stuijvenberg, M.E., Smuts, C.M., Kruger, H.S., Biebinger, R., Hurrell, R.F., Baumgartner, J. & Zimmermann, M.B. 2011. A micronutrient powder with low doses of highly absorbable iron and zinc reduces iron and zinc deficiency and improves weight-for-age z-scores in South African children. *The Journal of nutrition*, 141(2):237-242.

Tulchinsky, T.H. 2010. Micronutrient deficiency conditions: global health issues. *Public Health Reviews*, 32, pp. 243-255.

Tussing-Humphreys L.M., Nemeth E., Fantuzzi, G., Freels, S., Holtermann, A.L., Galvani, C. 2010. Decreased serum hepcidin and improved functional iron status 6 months after restrictive bariatric surgery. *Obesity*, 18:2010-2016.

Tussing-Humphreys, L.M., Liang, H., Nemeth, E., Freels, S. & Braunschweig, C.A. 2009. Excess adiposity, inflammation, and iron-deficiency in female adolescents. *Journal of American Dietetic Association*, 109:297-302.

Tussing-Humphreys, L., Pustacioglu, C., Nemeth, E. & Braunschweig, C. 2012. Rethinking iron regulation and assessment in iron deficiency, anemia of chronic disease, and obesity: Introducing hepcidin. *Journal of the Academy of Nutrition and Dietetics*, 112(3):391-400.

Van Stuijvenberg, M.E., Smuts, C.M., Lombard, C.J. & Dhansay, M.A. 2008. Fortifying brown bread with sodium iron edta, ferrous fumarate, or electrolytic iron does not affect iron status in South African schoolchildren. *The Journal of nutrition*, 138(4):782-786.

Viatte, L. & Vaulont, S. 2009. Hepcidin, the iron watcher. *Biochimie*, 91:1223-1228.

WHO (World Health Organization). 2012. Priorities in the assessment of vitamin a and iron status in populations, Panama City, Panama, 15–17 September 2010. Geneva, Switzerland: World Health Organization.

WHO & CDC (World Health Organization & Centres for Disease Control and Prevention). 2007. Assessing the iron status of populations: Report of a joint WHO /CDC Technical Consultation on the assessment of iron status at the population level.

http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107.pdf
edn. Geneva, Switzerland: World Health Organization.

WHO (World Health Organization). 2011. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. <http://www.who.int/vmnis/indicators/haemoglobin.pdf> Date of access: 6 September, 2013.

WHO (World Health Organization) 2011. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Available: http://www.who.int/vmnis/indicators/serum_ferritin.pdf [September, 6, 2013].

WORLD HEALTH ORGANIZATION, 2001. *Iron Deficiency Anaemia Assessment, Prevention, and Control - A guide for programme managers*. http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf edn.

Yang, Z., Dewey, K.G., Lonnerdal, B., Hernell, O., Chaparro, C., Adu-Afarwuah, S., McLean, E.D., Cohen, R.J., Domellof, M., Allen, L.H. and Brown, K.H., 2008. Comparison of plasma ferritin concentration with the ratio of plasma transferrin receptor to ferritin in estimating body iron stores: results of 4 intervention trials. *American journal of clinical nutrition*, 87(6): 1892-1898.

Yip, R., 2002. Prevention and Control of Iron Deficiency: Policy and Strategy Issues. *The Journal of nutrition*, 132(4): 802S-805S.

Zimmermann, M.B., Chassard, C., Rohner, F., N'Goran, E.K., Nindjin, C., Dostal, A. and ET AL., 2010. The effects of iron fortification on the gut microbiota in African children: a randomized controlled trial in Côte d'Ivoire. *American journal of clinical nutrition*, 92: 1406-1415.

Zimmermann, M.B., Zeder, C., Muthayya, S., Winichagoon, P., Chaouki, N. and Aeberli, I.E.A., 2008. Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and a reduced response to iron fortification. *International Journal of Obesity*, 32: 1098-1104.

Zimmermann, M.B. and Hurrell, R.F., 2007. Nutritional iron deficiency. *The Lancet*, 370(9586): 511-520.

Zimmermann, M.B., Molinari, L., Stabli-Asobayire, F., Hess, S.Y., Chaouki, N., Adou, P. and Hurrell, R.F., 2005. Serum transferrin receptor and zinc protoporphyrin as indicators of iron status in African children. *American journal of clinical nutrition*, 81(3): 615-623.

Annexures

Annexure 1: South African journal of clinical nutrition - Author Guidelines

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Authors must declare all sources of support for the research and any association with the product or subject that may constitute conflict of interest. Protection of patient's rights to privacy. Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives informed written consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. (www.icmje.org)

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Work that is based on or contains reference to ethnic classification must indicate the rationale for this.

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Short items are more likely to appeal to our readers and therefore to be accepted for publication. Manuscript should not exceed 4000 words in total all contents inclusive. Original articles of 4 000 words or less, with up to 6 tables or illustrations, should normally report observations or research of relevance to the field of nutrition. References should preferably be limited to no more than 25. *Short reports or scientific letters*, which include case reports, side effects of nutrient supplements/drugs and brief or negative research findings should be 1000 words or less, with 1 table or illustration and no more than 6 references.

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Research articles should have a structured abstract not exceeding 250 words (50 for short reports) comprising: Objectives, Design, Setting, Subjects, Outcome measures, Results and Conclusions.

A second abstract should be written in simple and clear spoken language highlighting the reason(s) that the research work was undertaken, the key findings and the key recommendations WITHOUT, overtly or covertly implying or containing any claims of whatsoever nature, but rather explaining how the work will help scientists (and/or lay persons) better understand and address the topic of investigation. The abstract should not exceed an absolute maximum of 75 words. In addition, please also include a < 140 character, "strong" message that can be used for social media.

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References should be set out in the Vancouver style and approved abbreviations of journal titles used; consult the List of Journals in Index Medicus for these details.

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