# Title: Anaemia, iron deficiency and susceptibility to infection in children in sub-Saharan Africa

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# Abstract

Globally, anaemia, iron deficiency and infections are responsible for a large part of the morbidity and mortality occurring among children. As iron is essential for erythropoiesis and the human immune system as well as a crucial element for many pathogens, these three morbidities often interact. This review considers the question – have the studies conducted so far unraveled the potential complex interaction between these factors sufficiently enough to be able to develop universally applicable guidelines about iron treatment in children. It is imaginable, however, that the area is to complex and diverse, with many sub-populations, and that not universal, but tailor made guidelines are needed based on some agreed principles.

Keywords: Anaemia, iron, iron deficiency, infection risk, iron supplementation, children, iron biomarker, immune system, hepcidin.

# Introduction

Anaemia, iron deficiency and infections are responsible for a large part of the global morbidity and mortality in children less than 5 years of age. This is especially true for resource limited settings, such as sub-Saharan Africa, where the three conditions often interact (Figure I). For example, iron deficiency may, apart from leading to anaemia, increase the susceptibility to infection by suppressing the immunological response to pathogens (Beard et al, 2001). However, iron deficiency may also protect the host against infections such as malaria (Jonker *et al*, 2012, Gwamaka *et al*, 2012). This multidirectional relationship between iron status and infection risk has been subject to many studies. After a short introduction on anaemia, iron metabolism and the relation with infections this article will highlight some interesting research questions which arise from the complex interaction between anaemia, iron deficiency and infections.

## Anaemia

Anaemia is a global public health problem especially affecting young children with prevalence up to 70% in some populations (World Health Organisation, 2005). Increased red blood cell destruction, impaired red blood cell production, and/or acute or chronic blood loss may be the underlying mechanisms leading to the development of anaemia (see Table I). Anaemia should be considered a syndrome rather than a specific disease, because multiple aetiologies may trigger one or more of these three mechanisms leading to severe anaemia (Calis *et al*, 2008). Iron deficiency is often considered the primary cause of severe anaemia. As a result the terms *anaemia*, *iron deficiency* and *iron deficiency anaemia* are often interchanged. This is incorrect as anaemia can occur with sufficient iron stores and iron deficiency in the initial phase does not necessarily lead to anaemia. Other aetiological factors include: infections such as malaria (Menendez *et al*, 2000), hookworm (Jonker *et al*, 2012) and HIV (World Health Organisation, 2005); drugs such as antibiotics (Ahmed & Ibrahim, 2001, Knox-Macaulay, 1992) and anti-retrovirals(Shah, 2006); genetic disorders such as G6PD deficiency, alpha-thalassemia and sickle cell disease(Flint *et al*, 1998); and micronutrient deficiencies (iron, vitamin B12, folic acid and vitamin A) all of which may cause or contribute to the severity of anaemia.

**Iron status**

## *Iron deficiency*

Despite the mechanisms to ensure the integrity of the body’s iron homeostasis, many factors can induce iron deficiency (see Figure II). An increased demand during periods of rapid growth (e.g. first years of life) and/or inadequate diet, are probably the most important causes of childhood iron deficiency in areas like sub-Saharan Africa, with limited iron bioavailability from staple foods (World Health Organisation, 2005). Iron shortage may also be caused by increased blood loss e.g. gastrointestinal blood loss due to enteric parasitic infections including hookworm (Albonico *et al*, 1998; Jonker *et al*, 2012). In addition to true iron shortage in body stores, the physiological systems for iron transport to target tissues may be impaired in the presence of adequate iron stores (World Health Organisation, 2004). This condition is called *functional iron deficiency* and is caused by cytokine release during the acute phase response to infection (Nissenson *et al*, 1999). As depletion of iron stores progresses, iron delivery to the bone marrow becomes insufficient to uphold adequate haemoglobin synthesis, resulting in iron deficiency anaemia. In addition, iron deficiency is a critical problem as it may also effect cognitive development of the child (Beard, 2001; World Health Organisation, 2002).

## *The measurement of iron*

The Perl’s Prussian blue stained bone marrow aspirate is recognised as the ‘gold standard’ for assessing the iron status of a person (Burns *et al*, 1990). Yet it is an invasive method and therefore often not performed. Besides this, the true value of this assessment as reference standard may be questioned as the quantity of stored iron is not necessarily equal to the quantity of *available* of iron. Currently, instead of bone marrow assessment for the determination of iron levels, biomarkers detectable in serum are used. Their predictive value is often limited by infection, as most iron biomarkers act as acute-phase-proteins. To bypass this confounding effect, some studies adjust their cut-off values using inflammatory markers (C-reactive protein or alpha-1-acid glycoprotein) as correcting factors (Le Nguyen Bao *et al*, 2016; Esan *et al*, 2013; Suchdev *et al*, 2016), whereas others have made use of iron biomarkers that are less affected by inflammation (e.g. serum transferrin receptor, zinc protoporphyrin) (Wander *et al*, 2009; Dostal *et al*, 2014). However, even those biomarkers were not free from the influence of infection (Beesley *et al* 2000, Mwangi et al 2014). Several studies compared different iron biomarkers for detection of iron deficiency (anaemia) in the presence of inflammation. However, all of the iron biomarkers tested showed a large number of false positive of false negative detections (Jonker *et a*l 2014, Phiri *et a*l 2009) . The need for a reliable biomarker to asses iron status remains.

# Iron and infection

## *Iron deficiency and impaired immunity*

Deficiency of iron can lead to an impaired immunity by negatively influencing cell-mediated immunity and components of the human innate immune system, such as the ability of certain phagocytic cells to kill intracellular pathogens. Iron deficiency has also been associated with thymic atrophy, the depression of T-lymphocytes, decreased neutrophil function and a decrease in the microbicidal qualities of macrophages (Kumar & Choudhry, 2010;Drakesmith & Prentice, 2012). Galan and colleagues reported reduced interleukin-2 production by activated lymphocytes in iron-deficient subjects (Galan *et al*, 1992). Furthermore in Malawian HIV-infected anaemic children receiving iron supplementation, an increase in the number of circulating CD-4 positive T-cells was observed (Esan *et al*, 2013).

## *Iron and pathogens*

Besides being an essential element for the human metabolism, iron is also an important nutrient for many pathogens. Microbes may sequester iron from the host depending on the preferred iron source and whether the pathogen adopts a predominately intracellular or extracellular lifestyle (Cassat & Skaar, 2013). For example, some pathogens are able to express siderophores on their membranes; these are, small iron-chelating compounds with a high iron binding affinity (Drakesmith & Prentice, 2012). With these siderophores, pathogens can compete with the iron binding sites of the human host. (Kumar & Choudhry, 2010). Another interesting example is the complex bi-directional interaction between the human host and malaria (*Plasmodium spp).* Studies have shown iron-deficiency or iron deficiency anaemia to be protective against malaria (Clark *et al*, 2014b, Jonker *et al* 2012). In turn, iron deficiency can be a consequence of malaria, partly attributed to its pathophysiology and partly to the host response by up-regulating hepcidin (Clark *et al*, 2014a; Clark *et al*, 2014b; Spottiswoode *et al.,* 2014).

## *Iron deficiency as an immune defense*

As iron may be needed for functioning of pathogens, a way for the host to protect itself from worsening infections is to create a state of iron deficiency in the presence of an infection (Hadley & DeCaro, 2015). This iron withholding response to microbial invasion - *hypoferremia of infection* or *functional iron deficiency* - is primarily induced by hepcidin, a small 20-25 amino acid, primarily synthesized by hepatocytes (Nicolas *et al*, 2001; Park *et al*, 2001; Pigeon *et al,* 2001), as well as by several other cells , although in much lower quantities (Kroot *et al* 2011). It exercises its effect on iron metabolism by binding to the iron transporter ferroportin (FPN), internalizing and degrading it and thereby regulating iron efflux (Nemeth *et al*, 2004). At the site of dietary absorption, hepcidin binds to FPN at the basolateral membrane of enterocytes, preventing iron from entering the bloodstream.Furthermore, hepcidin diverts iron away from the serum by binding to FPN on the surface of macrophages and many other cells, thereby promoting accumulation of intracellular iron (Nemeth *et al*, 2004). Hepcidin is up-regulated in response to increased iron levels, as well as inflammatory stimuli, which generates functional iron deficiency and restricts available iron for pathogenic utilization. Down-regulation of hepcidin occurs in the context of reduced iron levels, hypoxia and increased erythropoiesis (Kroot *et al*, 2011). Moreover, besides systemic effects, at the site of infection the iron binding proteins lactoferrin (e.g. present in breastmilk and released by neutrophils), transferrin and ferritin act as acute-phase proteins as part of the innate immune system, through sequestering iron at the site of infection and thereby withholding iron from pathogens (Drakesmith & Prentice, 2012).

Pathogens have evolved mechanisms to sequester iron from its host, yet at the same time the human body has developed mechanisms that anticipate this scavenge; the answer to the previously mentioned siderophore secretion by pathogens is the protein siderocalin, which binds iron-binding sites of siderophores, interrupting pathogenic iron-acquisition (Holmes *et al*, 2005). Furthermore, the diversion of serum iron into cells like macrophages in response to extracellular pathogen stimuli, could be beneficial for intracellular pathogens, such as Salmonella species (van Santen *et al*, 2013). Subsequently, the host expresses Natural Resistance Associated Macrophage Proteins, or NRAMPs, that target microbe-containing phagosomes within macrophages and monocytes(Kumar & Choudhry, 2010), depleting them of iron which restricts the growth of intracellular pathogens (Burté *et al*, 2013).

## *Iron supplementation and infection*

According to the Global Burden of Disease studies (1990 and 2013) iron-deficiency anaemia is a primary cause of “years lived with disability” among children and adolescents (Global Burden of Disease Pediatrics Collaboration, 2016). Therefore until recently many global guidelines advocated iron supplementation to all children living in regions with a high prevalence of iron deficiency (World Health Organisation, 2002). However, in 2006 a large trial in Tanzania raised concerns that iron supplementation could increase risk of malaria related morbidity and mortality (Sazawal *et al*, 2006). A positive correlation between supplemental iron and subsequent increased infection risk, had already been described in the late 1970s (Murray *et al*, 1978), yet this correlation was never considered to outweigh the benefits of iron supplementation. However, after the Tanzanian trial the WHO advised withholding iron supplementation from iron replete children (World Health Organisation, 2007). Since then several studies have shown conflicting results and a large recently updated Cochrane review did not confirm WHO’s concerns following the Tanzanian trial. Currently the WHO recommends daily iron supplementation for all children in areas with high prevalence of anaemia provided that malaria prevention is guaranteed when malaria is endemic (World Health Organisation, 2016).

# The Dilemma

In most tropical areas where both infections and anemia are highly endemic in children. Iron supplementation plays an important role in these treatment and prevention programs. However iron is not only an essential element of hemoglobin and important for the human immune system, it also affects the function of many pathogens. Therefore maintaining equilibrium between the provision of sufficient iron for optimal function of human biological systems while withholding it from pathogens, is critical. Many studies have examined the relationship between susceptibility to infections and the host iron status and/or iron supplementation. Where some authors found adverse effects of iron supplementation and/or a protective effect of iron deficient status, others reported a decreased risk of infections after iron supplementation. The largest meta-analysis on this subject is the Cochrane review on iron supplementation in children in malaria endemic areas (Neuberger *et al*, 2016). The overall conclusion was that iron supplementation was safe if malaria prevention was guaranteed. A limitation of this review was the lack of studies available with baseline iron status data. In addition, the lack of a suitable screening marker for iron status limits the availability of enough data about the relation between iron status and infection risk of supplemental iron. This data is important since it may enable sub-populations of children with susceptibility to specific infections be identified and offered tailored interventions.

There are other human and pathogen factors which may influence the interplay in iron status. In the Cochrane review children under two years seemed to have less malaria after iron supplementation compared to other age groups; the type of pathogen and prevalence of various type of infections may influence the infection risk (the higher the prevalence, the larger the influence ); confounding by other interventions, for example in the Cochrane review an increase in incidence of diarrhea was found in the iron supplemented group which could later be attributed to the addition of zinc to the iron supplementation (Neuberger *et al*, 2016). Other factors that are important in the relation between iron and infection risk are the host’s genetic background (McDermid, 2006) and the intra- or extracellular location of pathogens and iron in the body. This is shown by studies on Mycobacterium tuberculosis and non-typhoid Salmonella (NTS). For example in cases of enhanced erythrophagocytosis and inflammation, the iron content of macrophages increases and thereby also the survival of intracellular Salmonella spp. (van Santen *et al*, 2013).

Furthermore it has been suggested that oral iron supplementation to treat iron deficiency in the presence of concurrent infection is pointless, as hepcidin up-regulation inhibits the iron uptake in the gut (Casals-Pascual *et al*, 2012). This was supported by a study showing that malaria infection results in 50% reduction in the amount of iron absorbed (Glinz *et al*, 2015). Hepcidin levels could therefore be used to indicate whether or not to use iron supplements. Wegmuller et al planned a promising double blind randomized controlled trial involving mildly anaemic children ages 6-23 months to assess the use of hepcidin as a point-of-care screening test to decide to treat with iron or not (Wegmuller *et al*, 2016). However any findings may not apply to severely anaemic children as in this population the up-regulation during inflammation can be overruled by the down-regulation through erythropoietin, iron would then be absorbed during concurrent infection, possibly increasing the virulence of the present pathogen (Jonker *et al* , 2013).

In conclusion the equilibrium between human iron status and infection risk is complex, and maintaining the optimal balance for human health is critically dependent on population and context specific factors. This makes it very difficult, and possibly inappropriate, to develop and apply generic iron treatment guidelines. A more nuanced approach for iron supplementation will depend being able to predict the safety of iron supplementation which in turn, requires a reliable laboratory test for iron status. Additionally hepcidin as a point-of-care test may help to predict iron absorption, but may not be applicable in sub-populations such as severely anaemic children. Evidence to inform these more nuanced approaches and to determine the limits of their applicability should be research priorities so that the universal guidelines for iron treatment for children in poorer countries may eventually be replaced by a more tailored, personalised approach.

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| **Table I.** Mechanisms and common etiological factors of (severe) anaemia in children in southern Africa |
| **Mechanism** | **Etiological factors** |
| Blood loss  | Through urine, stool or trauma | * Hookworm
* Schistosomiasis
* Shigellosis, Salmonella, Campylobacter, Yersinia.
* Accidents (traffic), crocodile or hippo bite
 |
| Haemolysis | Intra- or extra-vascular | * Malaria
* Sepsis
* Hemoglobinopathies (Sickle cell disease, αThalassemia and G6PD)
 |
| Impaired red blood cell production | Infections | * Inflamatory response as part of a systemic infection
* Parasitic: Malaria,
* Bacteremia: e.g. Non-typhoid Salmonella
* Viral: HIV, Parvovirus, EBV, CMV
 |
| Micronutrient deficiencies | * Iron, folic acid, vitamin B12, vitamin A
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