Anaemia, iron deficiency and infections: new perceptions of the interaction between hepcidin, iron biomarkers, anaemia and inflammation in Malawian children
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Chapter eight

Discussion and Recommendations
Anaemia, iron deficiency and infections

Iron deficiency is an important child health problem in sub-Saharan Africa. Although iron supplementation may increase haemoglobin level\(^1\), it has also been associated with an increased malaria risk in certain populations\(^2\), and as a result its use is controversial. Due to the limited quality of the available data on host iron status and infection risk, a consensus on anaemia and iron deficiency management has not yet been reached. The aim of this thesis is to provide reliable data on prevalence, causes and diagnostic tests of iron deficiency in African children and to examine their relation to susceptibility to malaria infection. Hepcidin, a recently discovered key iron regulator, may provide new insights into iron homeostasis; this thesis reports research on its measurement in severely and non-severely anaemic African children. Our data may contribute to improvements in management of iron deficiency, and (severe) anaemia.

Severe anaemia management

To generate novel approaches for treatment and prevention of severe anaemia in Africa, the available literature was reviewed to highlight recent research findings (Chapter 2). New insights concerning the pathophysiological mechanisms and aetiologies of severe anaemia contrast with previously accepted understanding; bacterial infections and hookworm infections, and furthermore deficiencies of vitamin B12 and vitamin A, but not of iron or folic acid, were associated with severe anaemia. In view of these findings, policies and practices concerning treatment and prevention of severe anaemia in this area need to be substantially revised in order to make a significant impact on the huge burden of severe anaemia in Africa.

Severe anaemia management | iron supplementation

Iron supplementation is currently recommended in children with severe anaemia \(^3\); although new insights may question this policy. Firstly in Malawian children a negative association between iron deficiency and severe anaemia has been reported\(^4\), suggesting that iron deficiency may even protect against severe anaemia. This is partly explained by a protective effect against bacterial infections\(^4\).

Secondly in Chapter 6 hepcidin values in severely anaemic children were found to be surprisingly low despite concurrent infections. Low hepcidin values are thought to reflect an ideal and safe condition for iron supplementation \(^5\); since it is associated with increased intestinal iron absorption and a probable absence of inflammation. Normally inflammation will increase hepcidin levels which subsequently will inhibit iron absorption \(^6\), \(^7\), a mechanism which is considered to withhold iron from pathogens during infection (functional iron deficiency). Despite the fact that infection prevalence in our population was high, the elevated erythropoietin levels due to severe anaemia, inhibited hepcidin production, and this influence of erythropoietin exceeded the inflammation stimulus on hepcidin production. Theoretically this may mean that during severe anaemia iron is absorbed, despite the presence of infections which may benefit from the increased iron availability. There is an urgent need for studies investigating iron uptake in severely anaemic children with concurrent infectious diseases, and to assess infection incidence and mortality in children with low hepcidin levels who are receiving iron supplementation. Possibly labelled iron studies may facilitate these future investigations.

Severe anaemia management | *Ancylostoma duodenale*

Hookworm infestation (*Ancylostoma duodenale* and *Necator americanus*) causes intestinal blood loss and through
this mechanism leads to iron deficiency and anaemia. In Chapter 3 hookworm infection with A. duodenale, was substantially associated with severe anaemia in Malawian pre-school children. This association was assessed using a novel and more sensitive test for detection of hookworm, real-time PCR. Hookworm prevalence was much higher than previously had been determined by microscopy, the conventional method (29.2% vs. 5.9%). Since real-time PCR for hookworm allows quantitative measurement we were able to show that even less severe A. duodenale infections contributed to disease burden and thus were of clinical importance. Another surprising finding was the high prevalence of the sub-species, A. duodenale, whereas N. americanus was expected to be the dominant specie in this area. A. duodenale causes 2 to 10 times more blood loss per worm than N. americanus and is therefore more likely to contribute to anaemia and iron deficiency.

This novel highly sensitive test for detection of hookworm enabling differentiation of sub-species as well as quantification of infection is a useful tool to estimate prevalence of hookworm species and their contribution to disease burden in different populations. Data from Mozambique indicated that the ratio between the two hookworm species changed with age (Van Lieshout, unpublished data), suggesting that the contribution of hookworm (A. duodenale) to severe anaemia and iron deficiency may differ per area and/or age group. And since post-treatment data are scarce and extremely little is known about the species-specific effects of mass drug administration further research on treatment effects of the different hookworm species may increase efficacy of hookworm control and thereby contribute to a decrease in prevalence of severe anaemia and iron deficiency.

Iron deficiency and infection

Iron deficiency and infection | Malaria
The relation between host iron status and risk of malaria infections has been the subject of a long standing debate. Studies have described a possible protective effect of iron deficiency against malaria risk. However the available data are inconclusive due to limitations in study designs. In Chapter 4 we have applied modern causal inference methods to analyze the relation between iron deficiency and malaria risk. We found that children who were iron deficient at baseline had a lower incidence of malaria parasitaemia (0.55, 95%-CI 0.41-0.74), and clinical malaria (0.49, 95%-CI 0.33-0.73), during a year of follow-up. These findings suggest that iron deficiency protects against malaria parasitaemia and clinical malaria. However, because we lacked direct measurements of baseline immunity to malaria our results should be confirmed by well conducted randomized controlled trials, or observational birth cohort studies. Until recently these type of studies were lacking, although a well designed birth cohort study has shown a decreased risk of malaria in young children during iron deficient periods.

Our findings support the theory of nutritional adaptation to infectious diseases: Iron deficiency, highly prevalent in African children, seems to be associated with an increased chance of survival as it may protect against malaria, a major morbidity and mortality factor in these settings. Treatment of iron deficiency should occur concurrently with sustained control and surveillance of prevalent infections to avoid “neutralisation” of the protective effect of iron deficiency and increasing malaria risk. We recommend that, prevention and treatment of iron deficiency occurs within the context of the Integrated Management of Childhood Illness. Prevention and treatment of infections such as malaria and hookworm may at the same time prevent iron deficiency and decrease the need for iron supplementation.
Iron deficiency and infection | HIV
Iron supplementation is often provided to children with HIV as iron deficiency is considered the most important micronutrient deficiency causing HIV-associated anaemia. The prevalence of iron deficiency in HIV-infection is little studied, and iron supplementation may even be detrimental in these children who are particularly vulnerable to infectious diseases. In Chapter 5 the literature on studies investigating iron status in HIV-infected children was systematically reviewed to assess prevalence of iron deficiency. Pooled analyses of studies that included an HIV-uninfected control population suggested that HIV-infected children were less likely to be iron deficient compared with HIV-uninfected children (OR 0.50; 95%CI 0.27-0.94). Nevertheless iron deficiency was still common in HIV-infected children with a prevalence of 34%, and like other iron deficient children, may require intervention. Our systematic review further showed that iron deficiency prevalence was lower in HIV-infected children on HAART compared with HIV-infected children not on HAART (24% vs. 37%). Although confounding factors such as disease stage may partly explain this difference, this observation makes a case for research examining the effects of HAART on iron status, and of its role as a potential intervention for iron deficiency in HIV-infected children.

Especially in HIV-infected children, studies are needed to determine the safety (and benefit) of iron supplementation. Furthermore, as for other African children, alternative strategies to control iron deficiency should be considered, such de-worming, malaria control and HAART.

Assessment of iron deficiency in African children
Determination of iron status is difficult in sub-Saharan Africa as prevalent infections and other nutritional deficiencies interfere with the accuracy and validity of iron biomarkers. The extent to which this affects our current knowledge on iron status assessments, is unknown as these iron biomarkers have not been validated against iron staining of bone marrow aspirate, the reference standard of iron status, in African children.

In Chapter 7 we therefore tested the diagnostic accuracy of the currently used iron biomarkers against bone marrow iron assessment in Malawian children. Two new potential iron markers, hepcidin and Red cell Size Factor (RSF) (haemoglobin content) were included. No iron marker in this study showed both a high sensitivity and specificity; which confirms the difficulty of assessing status in these settings. Ferritin, the widely criticized marker seemed to be one of the most accurate markers in discriminating children with bone marrow iron deficiency from children with iron replete stores; a newly defined cut-off for ferritin (<18 µg/L) resulted in a maximal accuracy of 73.7% sensitivity and 77.1% specificity. sTfR-F, the ratio from sTfR over log-ferritin, performed in an equivalent way to serum ferritin; a new cut-off (≥1.85) showed 72.5% sensitivity and 75.0% specificity. It must be noted these assays for sTfR are not uniformly standardized and the latter cut-off should not be universally applied. Hepcidin, sTfR and MCV moderately; and RSF and MCHC poorly performed as iron predictive biomarkers.

Unlike previous data in African populations the sTfR-F had no added value over ferritin alone. However those studies included hospitalized populations whereas our study population consisted of “healthy” preschool children and may better represent the average population of African children.

Our data can be used to guide diagnostic testing, depending on the purpose of the determination. For ‘estimating prevalence’ of iron deficiency, a sensitive marker such as ferritin (cut-off <30 µg/L) could be used. For ‘research purposes’ a high accuracy test (optimal sensitivity and specificity) would be preferred to avoid classification bias (ferritin cut-off <18 µg/L). For ‘interventional purposes’ other aspects appear to be more
relevant than identifying the iron deficient population, for which this cross-sectional study was not designed. The effect of iron supplementation is a dynamic process which requires a longitudinal study design. As hepcidin regulates intestinal absorption of oral iron, it is considered to play an important role in this process and may function as guide for iron supplementation\(^5\) which is shortly discussed below.

**Hepcidin**

Hepcidin, a recently discovered key iron regulator\(^{25-27}\), inhibits iron absorption in iron loaded conditions whilst decreased levels stimulate iron absorption during iron deficiency\(^7, 28, 29\). In Chapter 7 compared to other iron markers, hepcidin was a not an accurate predictor of bone marrow iron deficiency in healthy children. Also in severely anaemic children hepcidin poorly predicted bone marrow iron deficiency (Chapter 6). This was probably explained by concurrent conditions influencing the other signalling pathways for hepcidin, such as anaemia and (even low grade) inflammation.

As a key regulator of iron, hepcidin also already been proposed as a predictor of the effect and safety of iron supplementation; in Gambian children hepcidin strongly predicted red blood cell iron incorporation after oral iron supplementation\(^3\). Yet since this was not fully confirmed in another study that demonstrated that regulation of iron absorption, but not the actual incorporation of iron into the erythrocyte, associated with hepcidin vlues\(^{30}\). In addition we described low hepcidin levels in severely anaemic children (Chapter 6) suggesting that iron absorption may even occur in children with severe infections. Further longitudinal research is needed to determine the potential role of serum hepcidin for guiding iron therapy.
References

3. WHO. Conclusions and recommendations of the WHO Consultation on prevention and control of iron deficiency in infants and young children in malariacendemic areas. 2007.

