Iron Status and Associated Malaria Risk Among African Children

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Summary: Decreased ferritin and transferrin saturation are associated with protection against malaria in African children. Hepcidin, soluble transferrin receptor and hemoglobin concentrations are not associated with malaria protection. These findings may reflect differences in parasite iron acquisition.
Abstract

**Background:** It remains unclear whether improving iron status increases malaria risk, and few studies have looked at the effect of host iron status on subsequent malaria infection. We therefore aimed to determine whether a child’s iron status influences their subsequent risk of malaria infection in sub-Saharan Africa.

**Methods:** We assayed iron and inflammatory biomarkers from community-based cohorts of 1309 Kenyan and 1374 Ugandan children aged 0 - 7 years and conducted prospective surveillance for episodes of malaria. Poisson regression models were fitted to determine the effect of iron status on the incidence rate ratio of malaria using longitudinal data covering a period of 6 months. Models were adjusted for age, sex, parasitemia, inflammation and study site.

**Results:** At baseline, the prevalence of iron deficiency (ID) was 36.9% and 34.6% in Kenyan and Ugandan children, respectively. Iron deficiency anemia (IDA) affected 23.6% of Kenyan and 17.6% of Ugandan children. Malaria risk was lower in children with ID (IRR = 0.7; 95% CI: 0.6, 0.8; p<0.001) and IDA (IRR = 0.7; 95% CI: 0.6, 0.9; p=0.006). Low transferrin saturation (<10%) was similarly associated with lower malaria risk (IRR = 0.8; 95% CI: 0.6, 0.9; p=0.016). However, variation in hepcidin, soluble transferrin receptors (sTfR) and hemoglobin / anemia was not associated with altered malaria risk.

**Conclusions:** ID appears to protect against malaria infection in African children when defined using ferritin and transferrin saturation, but not when defined by hepcidin, sTfR or hemoglobin. Further research is required to determine causality.

**Keywords:** Iron status; Iron deficiency; malaria risk; African children
INTRODUCTION

Iron deficiency (ID) and malaria remain important public health problems in African children [1,2]. ID, the most common nutrient deficiency in pre-school African children [3], is associated with poor brain development and long-term behavioral and cognitive impairments [4]. Similarly, malaria has devastating health effects in African children. In 2015, malaria caused an estimated 292,000 deaths in African children under the age of five years [2] and remains a persistent and widespread problem in Africa infecting 24% of the population at any one time [5].

The safety of iron supplementation has been a long-standing concern among policy makers and clinicians in malaria-endemic areas [6,7]. In these areas, the World Health Organization (WHO) recommends iron supplementation in conjunction with effective malaria prevention and treatment strategies [8]. However, randomized controlled trials of iron supplementation have reported conflicting findings [9,10]. Furthermore, it is unclear whether iron supplementation might be unsafe because it improves iron status itself thus resulting in a long-term increase in the risk of malaria.

Few observational studies have investigated the effect of iron status on malaria risk. These studies indicate that ID is associated with a reduced risk of both mild and severe Plasmodium falciparum malaria in African children [11–14], but have largely used ferritin-based definitions of ID. Little is known about whether other indicators of iron status (including hepcidin, hemoglobin, soluble transferrin receptors (sTfR), and transferrin saturation (TSAT)) influence malaria risk in humans. In mouse models, hepcidin has been shown to play a role in preventing superinfection by depriving the
Plasmodium liver stage of iron [15], but studies in children have reported mixed findings [16,17].

Two previous studies have reported that hemoglobin concentrations do not influence malaria risk [18,19] while in vitro culture indicates otherwise [20]. There are no specific reports of the influence of sTfR and TSAT on malaria in humans.

In this study, we aimed to investigate whether a child’s iron status influences their subsequent risk of malaria infection in 2683 Kenyan and Ugandan children, thus making this the largest observational study on iron status and risk of malaria to date with the most comprehensive range of iron markers.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was provided by the Scientific Ethics Review Unit of the Kenya Medical Research Institute for the Kenyan cohort and by the Uganda Virus Research Institute and the London School of Hygiene & Tropical Medicine for the Ugandan cohort.

Study Population

This study used data from two African community-based cohorts of children in Kilifi, Kenya and Entebbe, Uganda.

Kenya cohort. This included three community cohorts, Junju, Ngerenya and RTSS. Junju and Ngerenya are ongoing rolling cohorts evaluating malaria immunity as described elsewhere [21]. The RTSS cohort was the RTS,S/AS01E vaccine trial against malaria that was conducted between 2007 and 2008 with continued active malaria surveillance for 8 years [22]. Within these cohorts, children
are followed-up to a maximum age of 13 years with annual cross-sectional bleeds. The follow-ups involved weekly visits to assess for fever and if the temperature was above 37.5°C, a malaria blood film was taken. Iron biomarkers were measured from a single cross-sectional bleed based on the availability of plasma samples archived at -80°C.

**Uganda cohort.** The Entebbe Mother and Baby Study (EMaBS) is a prospective birth cohort study that was originally designed as a randomized double-blind placebo-controlled trial [ISRCTN32849447] to determine whether anti-helminthic treatment during pregnancy and early childhood was associated with differential responses to vaccination or incidence of infections such as pneumonia, diarrhea or malaria [23]. Blood samples were collected at birth and at subsequent birthdays up to five years of age. Markers of iron status were assayed from a single birthday based on the availability of stored samples. The study included longitudinal active surveillance of malaria and other infections during fortnightly home visits and quarterly clinic visits.

Longitudinal parasitemia data were obtained from active surveillance during the six months following measurement of iron biomarkers. 94% of Kenyan and 90% of Ugandan children were followed up for 6 months while the length of follow-up for the remainder ranged from 1-5 months. We chose a follow-up period of not more than six months since iron status may change over a longer follow-up period. Secondary analyses included a one-year period of follow-up. Clinical malaria data included microscopy confirmed density of asexual *P. falciparum* parasitemia and temperature. Genotyping of hemoglobin types was conducted by PCR [24] using DNA extracted by Qiagen DNA Blood Mini Kit (Qiagen, West Sussex, United Kingdom).

**Measurement of Iron and Inflammatory Biomarkers**
The assayed biomarkers of iron status included plasma ferritin (Chemiluminescent Microparticle Immunoassay (CMI), Abbott Architect, USA), hepcidin (DRG Hepcidin 25 [bioactive] HS ELISA Kit, DRG Diagnostics), sTfR (Human sTfR ELISA, BioVendor, CZ), iron (MULTIGENT iron calorimetric assay, Abbott Architect, USA), transferrin (CMI, Abbott Architect, USA), and hemoglobin (Medonic CA 530 Hemoglobinometer). Since biomarkers of iron are influenced by inflammation, C-reactive protein (CRP) (MULTIGENT CRP Vario assay, Abbott Architect, USA) was assayed to adjust for inflammation [25].

Definitions

Two definitions of ID were used: 1) based on low ferritin defined as plasma ferritin < 12µg/L or < 30µg/L in the presence of inflammation (CRP > 5mg/L) in children < 5 years or < 15µg/L in children ≥ 5 years; [25] and 2) TSAT < 10% (calculated as iron in µmol/L / (transferrin in g/L x 25.1) x 100) [26]. TSAT was calculated in Kenya only because Ugandan plasma samples were stored in ethylenediaminetetraacetic acid (EDTA), which chelates iron. We did not define ID by hepcidin or sTfR since there are no internationally established cut-offs. Anemia was defined as hemoglobin < 11g/dL in children aged 0 to 4 years or hemoglobin < 11.5 g/dL in children above 4 years while iron deficiency anemia (IDA) was defined as low ferritin and anemia [27]. A malaria episode was defined as parasitemia and temperature > 37.5°C. All malaria episodes occurring during the follow-up period were included except those occurring within 14 days of an initial presentation, which were regarded as recrudescence.

Statistical Analyses

All analyses were conducted using STATA 13.0 (StataCorp., College Station, TX). Iron biomarkers (except hemoglobin) were log_e-transformed to normalize their distributions. Geometric means of
iron biomarkers and proportions of ID and anemia were computed. Two-tailed Student’s $t$-tests were used to test for difference in means between groups. Poisson regression models of counts of malaria episodes were fitted as predicted by iron status (ID / anemia / individual iron biomarkers) and were adjusted for age, sex, parasitemia, inflammation and study site. Difference in individual length of follow-up was accounted for in the model by including the length of follow-up as “exposure” in the model. We accounted for multiple episodes using robust cluster variance estimation which takes into account correlations between multiple events. Secondary analyses involved excluding children with parasitemia or inflammation at baseline to mitigate the effects of concurrent infection on iron status [28]. We used Cox proportional hazards analyses to evaluate the temporal effect of iron status on malaria risk. A p-value of < 0.05 was considered significant.

We searched the databases PubMed and Google Scholar with search terms that included “ID OR ferritin OR hepcidin OR sTfR OR TSAT OR hemoglobin OR anemia AND malaria children”. We found four longitudinal studies investigating the effect of ID on malaria risk. A meta-analysis of the current study and previous longitudinal studies investigating the relationship between ID and malaria risk was performed using the “metan” command in STATA.

RESULTS

Baseline Characteristics of Study Population

A total of 1309 Kenyan and 1374 Ugandan children aged between 0 - 7 years and 1 - 5 years respectively were included in the analyses. Table 1 shows the characteristics of study participants. At baseline, the prevalence of ID and IDA were 36.9% and 23.6% in Kenyan and 34.6% and 17.6% in Ugandan children respectively. The prevalence of ID based on TSAT (measured in Kenya only) was 52.4%. The prevalence of malaria parasitemia was higher in Kenyan (20.1%) compared to Ugandan
children (6.7%). During the six-month follow-up, 31.1% of Kenyan and 14.3% of Ugandan children experienced at least one episode of malaria infection. Malaria incidence rate per child-year of follow-up was 0.6 in Kenya and 0.3 in Uganda.

Higher Ferritin Concentrations and TSAT are Positively Associated with Malaria Infection

Concentrations of ferritin and TSAT, but not other iron markers, were higher in children that subsequently developed a malaria episode (Figure 1A). Similarly, a unit increase in log ferritin was associated with an increased incidence rate ratio for malaria overall (IRR = 1.3; 95% CI: 1.2, 1.4; p < 0.001) and in each cohort individually (Figure 1B). A unit increase in log TSAT was also associated with a 20% increased risk of malaria in Kenyan children (IRR = 1.2; 95% CI: 1.05, 1.4); p = 0.009). However, hepcidin, sTfR and hemoglobin concentrations were not associated with subsequent risk of malaria (Figure 1B).

ID Defined Using Ferritin or TSAT Protects Against Malaria Risk

ID defined by low ferritin concentrations and IDA were associated with a 30% reduction in the incidence of malaria infection (IRR = 0.7; 95% CI: 0.6, 0.8; p < 0.001 and IRR = 0.7; 95% CI: 0.6, 0.9; p = 0.006, respectively). These findings were consistent for the individual cohorts (Table 2). Likewise, ID defined by low TSAT reduced the risk of malaria in Kenyan children (IRR = 0.8; 95% CI: 0.6, 0.9; p = 0.016). However, anemia itself was not significantly associated with variation in malaria risk (Table 2).

In Cox proportional hazards models, ID defined by low ferritin, ID defined by low TSAT, and IDA were associated with 40%, 20% and 30% reduced risk of malaria respectively (Figure 2A-C) for the six
months of follow-up compared to iron replete children. However, anemia was not associated with malaria risk (Figure 2D and Supplementary Table 1).

We observed similar results regardless of whether the follow-up period was extended to one year, the children had malaria parasitemia or inflammation at baseline, age, or following adjustment for sickle cell trait (Supplementary Figures 1-5).

A meta-analysis of observational studies examining the influence of ID on malaria risk is shown in Figure 3. All the studies report that ID, using a ferritin-based definition, protects against malaria infection, despite differences in study site, length of follow-up, and definition of ID (Supplementary Table 2). The overall estimate indicates that ID is associated with a 34% lower risk of malaria infection. We report the largest study to date.

**DISCUSSION**

In this study, we report an observational analysis of the influence of iron status on subsequent malaria risk in 2683 Kenyan and Ugandan children. We found that ID, defined using either ferritin or TSAT, and IDA were associated with a lower risk of subsequent malaria infection. However, anemia (or hemoglobin concentrations), hepcidin and sTfR were not significantly associated with variation in malaria risk.

Consistent with our findings, previous observational studies have reported that ID based on low ferritin concentrations confers protection against malaria infection in African children [11–14]. Nyakeriga et al reported a 30% reduction in clinical malaria during a six month follow-up of Kenyan children aged 8 months to 8 years [11]. Similarly, studies in Malawi (aged 6 - 60 months with one
year of follow-up) [12] and Tanzania (birth to 3 years follow-up) [13] reported reduced malaria risk of 45\% and 23\% respectively. Recently, a study in Zambian children aged 4 - 8 years followed-up for six months reported an increased risk of malaria in children with high ferritin concentrations [14]. These estimates are similar to our finding of a 30\% reduction in malaria risk in iron deficient children (0 - 7 years).

We further found that ID defined by TSAT was associated with a 20\% reduction in the subsequent risk of malaria and TSAT was positively associated with malaria incidence. Using a combination of low TSAT and low ferritin, Nyakeriga et al reported a 30\% reduction in clinical malaria among iron deficient children [11]. In support of our findings, *P. falciparum* has been demonstrated to obtain iron from transferrin using *in vitro* parasite culture [29]. Furthermore, Clark et al demonstrated that parasitized red blood cells utilize serum iron [30]. These studies indicate that increasing bioavailable transferrin-bound iron may predispose an individual to increased risk of malaria. And indeed, we show that higher TSAT may increase malaria risk in children.

So how might ID protect against malaria infection? In *in vitro* parasite cultures iron deficient human erythrocytes are poorly infected by *P. falciparum* compared to those that are iron replete and this protective effect is reversed by iron supplementation [31]. In mouse models, Matsuzaki-Moriya et al. showed that during ID, macrophages cleared parasitized erythrocytes more efficiently suggesting that either erythrocytes produced under iron deficient conditions are easily phagocytized by macrophages or that there is an enhancement of macrophage function during ID [32]. Furthermore, ID has also been shown to up-regulate nitric oxide which has anti-parasitic properties against *Plasmodium* [33]. Another possible explanation is that during ID, zinc is incorporated in place of iron.
during heme synthesis leading to formation of zinc protoporphyrin that in turn is thought to inhibit formation of hemozoin (the parasite survival pigment) in a manner similar to quinolines [34].

We further report that anemia (or hemoglobin concentration) does not influence subsequent malaria risk. Similar observations have been reported by two previous studies. A recent longitudinal study in Papua New Guinean infants aged 3 months and followed-up for one year reported a non-significant association between lower hemoglobin concentrations and subsequent malaria infection [18]. A similar observation was made by Ghosh et al in Indian children [19]. Indeed, both studies and ours report that anemic children have a non-significant increase in malaria risk rather than protection from malaria. In contrast, Goheen et al reported that anemia was associated with decreased in vitro growth rate of *P. falciparum* [20]. However, it is possible that in vitro parasite growth rate might not mimic direct malaria susceptibility for children. Moreover, hemoglobin has low sensitivity and specificity in determining body iron status due to the overlap of values in iron deficient and replete individuals [35] and the multiple overlapping causes of low hemoglobin concentrations in African children [36]. It further remains unclear whether the malaria parasite utilizes heme iron in hemoglobin or has other sources and mechanisms of iron acquisition. There are suggestions that the parasite may utilize storage iron since bioavailable iron content increases in parasitized red blood cells as the parasite develops from ring stage to schizont [30].

We further hypothesized that raised hepcidin concentrations may reduce malaria risk through sequestering iron within macrophages and enterocytes [37], thereby starving liver-stage *Plasmodium* [15]. Since the parasite requires iron for growth, it has been suggested that withholding iron from hepatocytes inhibits the development of malaria [15]. Furthermore, high cord blood hepcidin has been associated with decreased risk of clinical malaria although not parasitemia or
severe malaria in Tanzanian infants [17]. However, in agreement with our study in an independent cohort of Kenyan children [16], we found no association between hepcidin concentrations and clinical malaria episodes. Differences in age or environmental factors may account for the different findings. Moreover, the high prevalence of ID, which is normally associated with decreased hepcidin, and reduces the risk of malaria in our participants, may counter a possible protective role of hepcidin.

Our data indicate that a child’s erythropoietic drive (as measured by sTfR) does not influence their subsequent risk of malaria infection. The expression of sTfR increases with both ID and expanded erythropoiesis (with the latter being more influential) [38], factors that might have opposing effects on malaria risk. For example, increased erythropoiesis may increase the risk of malaria since *Plasmodium* parasites preferentially infect young red blood cells [39], whereas ID may be protective. These opposing effects could explain why sTfR concentrations were not associated with malaria risk in our study. It is also known that malaria itself causes increased sTfR concentrations [38,40].

A major challenge in this study is that iron biomarkers are themselves influenced by infections and inflammatory processes which may confound the effect of iron status on malaria infection [28]. To mitigate the potential confounding effects of infection on iron biomarkers, we excluded children with inflammation or malaria parasitemia at the time of iron measurement in secondary analyses and observed similar results (Supplementary Figure 2). Additionally, other potential confounders such as age, sex, length of follow-up and study site were adjusted for in regression models. Limitations of the study were lack of TSAT concentrations in Ugandan children and that only febrile malaria was included for the malaria episodes. Strengths of our study included its large size (n=2,683 children) across two study sites and that we used multiple iron biomarkers in order to determine
their individual effects on malaria risk making it the largest and most definitive observational study to address the question of iron status and risk of malaria infection.

Our findings, in agreement with other studies, suggest that ID protects children against malaria infection and thus that improving iron status may predispose African children to infection. Interestingly, of the iron biomarkers, only higher concentrations of ferritin and TSAT were predictive of increased rates of subsequent malaria, perhaps reflecting differences in their relationship to parasite mechanisms of iron acquisition. Although WHO recommends iron supplementation coupled with malaria treatment and prevention strategies in malaria endemic areas [8], these strategies remain difficult to implement. Thus, it is important to establish whether improved iron status increases malaria risk since this would necessitate long-term malaria prevention and treatment programs. However, our findings and that of other studies do not necessarily imply causality since observational studies may be subject to confounding and reverse causation, for example, prior malaria exposure might lead to both ID and the acquisition of protective immunity against malaria, while malaria itself increases ferritin levels. Since ID prevents children from reaching their developmental potential it is important to establish causality in the iron-malaria relationship. Thus, these data warrant further large-scale studies, including studies which utilize genetic variants associated with iron status to infer causality (Mendelian randomization), and prospective interventional trials.
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Conflict of Interests

All authors: No conflicts of interest.
References

1. Kyu HH, Pinho C, Wagner JA, Brown JC, Bertozzi-Villa A, Charlson FJ, et al. Global and National Burden of Diseases and Injuries Among Children and Adolescents Between 1990 and 2013. JAMA Pediatr. 2016;170:267.

2. World Health Organization. World Malaria Report 2015. WHO. 2015;

3. Petry N, Olofin I, Hurrell R, Boy E, Wirth J, Moursi M, et al. The Proportion of Anemia Associated with Iron Deficiency in Low, Medium, and High Human Development Index Countries: A Systematic Analysis of National Surveys. Nutrients. 2016;8:693.

4. Lozoff B. Early Iron Deficiency Has Brain and Behavior Effects Consistent with Dopaminergic Dysfunction. J Nutr. 2011;141:740–6.

5. Snow RW, Sartorius B, Kyalo D, Maina J, Amratia P, Mundia CW, et al. The prevalence of Plasmodium falciparum in sub-Saharan Africa since 1900. Nature. 2017;550:515–8.

6. Suchdev P, Leeds I, McFarland D, Flores R. Is it time to change guidelines for iron supplementation in malarial areas? J Nutr. 2010;140:875–6.

7. Brittenham GM. Safety of Iron Fortification and Supplementation in Malaria-Endemic Areas. Nestle Nutr Inst Work Ser. 2012;70:117–27.

8. World Health Organization. Guideline: Daily iron supplementation in infants and children. WHO. 2016.

9. Sazawal S, Black R, Ramsan M, Chwaya H, Stoltzfus R, Dutta A, et al. Effect of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community based, randomised, placebo-controlled trial. Lancet. 2006;367:133–43.
10. Zlotkin S, Newton S, Aimone AM, Azindow I, Amenga-Etego S, Tchum K, et al. Effect of iron fortification on malaria incidence in infants and young children in Ghana: a randomized trial. JAMA. 2013;310:938–47.

11. Nyakeriga AM, Troye-blomberg M, Chemtai AK, Marsh K, Williams TN. Iron Deficiency and Malaria among Children Living on the Coast of Kenya. J Infect Dis. 2004;190:439–47.

12. Jonker FAM, Calis JCJ, van Hensbroek MB, Phiri K, Geskus RB, Brabin BJ, et al. Iron status predicts malaria risk in Malawian preschool children. PLoS One. 2012;7:1–8.

13. Gwamaka M, Kurtis JD, Sorensen BE, Holte S, Morrison R, Mutabingwa TK, et al. Iron deficiency protects against severe plasmodium falciparum malaria and death in young children. Clin Infect Dis. 2012;54:1137–44.

14. Barffour MA, Schulze KJ, Coles CL, Chileshe J, Kalungwana N, Arguello M, et al. High iron stores in the low malaria season increase malaria risk in the high transmission season in a prospective cohort of rural Zambian children. J Nutr. 2017;147:1531–6.

15. Portugal S, Carret C, Recker M, Armitage AE, Gonçalves L a, Epiphanio S, et al. Host-mediated regulation of superinfection in malaria. Nat Med. 2011;17:732–7.

16. Atkinson SH, Uyoga SM, Armitage AE, Khandwala S, Mugyenyi CK, Bejon P, et al. Malaria and Age Variably but Critically Control Hepcidin Throughout Childhood in Kenya. EBIOM. 2015;2:1478–86.

17. Brickley EB, Spottiswoode N, Kabyemela E, Morrison R, Kurtis JD, Wood AM, et al. Cord blood hepcidin: Cross-sectional correlates and associations with anemia, malaria, and mortality in a tanzanian birth cohort study. Am J Trop Med Hyg. 2016;95:817–26.

18. Lombardo P, Vaucher P, Rarau P, Mueller I, Favrat B, Senn N. Hemoglobin Levels and the Risk of Malaria in Papua New Guinean Infants: A Nested Cohort Study. Am J Trop Med Hyg.
19. Ghosh SK, Yadav RS, Das BS, Sharma VP. Influence of Nutritional and Haemoglobin Status on Malaria Infection in Children. Indian J Pediatr. 1995;62:321–6.

20. Goheen MM, Wegmüller R, Bah A, Darboe B, Danso E, Affara M, et al. Anemia Offers Stronger Protection Than Sickle Cell Trait Against the Erythrocytic Stage of Falciparum Malaria and This Protection Is Reversed by Iron Supplementation. EBIOM. 2016;

21. Mwangi TW, Ross A, Snow RW, Marsh K. Case definitions of clinical malaria under different transmission conditions in Kilifi District, Kenya. J Infect Dis. 2005;191:1932–9.

22. Bejon P, Ph D, Lusingu J, Ph D, Olotu A, Ch B, et al. Efficacy of RTS, S / AS01E Vaccine against Malaria in Children 5 to 17 Months of Age. N Engl J Med. 2008;359:2521–32.

23. Elliott AM, Kizza M, Quigley M a, Ndibazza J, Nampijja M, Muhangi L, et al. The impact of helminths on the response to immunization and on the incidence of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and e. Clin Trials. 2007;4:42–57.

24. Waterfall CM, Cobb BD. Single tube genotyping of sickle cell anaemia using PCR-based SNP analysis. Nucleic Acids Res. 2001;29:E119.

25. World Health Organization. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Vitamin and Mineral Nutrition Information System. WHO. 2011.

26. Yamanishi H, Iyama S, Yamaguchi Y, Kanakura Y, Iwatani Y. Total iron-binding capacity calculated from serum transferrin concentration or serum iron concentration and unsaturated iron-binding capacity. Clin Chem. 2003;49:175–8.

27. World Health Organization. Iron Deficiency Anaemia: Assessment, Prevention, and Control. A
28. Aguilar R, Moraleda C, Quintó L, Renom M, Mussacate L, Macete E, et al. Challenges in the Diagnosis of Iron Deficiency in Children Exposed to High Prevalence of Infections. PLoS One. 2012;7:3–9.

29. Pollack S, Fleming J. Plasmodium falciparum takes up iron from transferrin. Br J Haematol. 1984;58:289–93.

30. Clark M, Fisher NC, Kasthuri R, Cerami Hand C. Parasite maturation and host serum iron influence the labile iron pool of erythrocyte stage Plasmodium falciparum. Br J Haematol. 2013;161:262–9.

31. Clark MA, Goheen MM, Fulford A, Prentice AM, Elnagheeb MA, Patel J, et al. Host iron status and iron supplementation mediate susceptibility to erythrocytic stage Plasmodium falciparum. Nat Commun. 2014;5:4446.

32. Matsuzaki-moriya C, Tu L, Ishida H, Imai T, Suzue K, Hirai M, et al. A critical role for phagocytosis in resistance to malaria in iron-deficient mice. Eur J Immunol 2011. 2011;1365–75.

33. Fritsche G, Larcher C, Schennach H, Weiss G. Regulatory interactions between iron and nitric oxide metabolism for immune defense against Plasmodium falciparum infection. J Infect Dis. 2001;183:1388–94.

34. Iyer JK, Shi L, Shankar AH, Sullivan DJ. Zinc Protoporphyrin IX Binds Heme Crystals to Inhibit the Process of Crystallization in Plasmodium falciparum. Mol Med. 2003;9:175–82.

35. Cook D, Smith J. Evaluation of the iron status of a population. Blood. 1976;48:449–55.

36. Foote EM, Sullivan KM, Ruth LJ, Oremo J, Sadumah I, Williams TN, et al. Determinants of Anemia among Preschool Children in Rural, Western Kenya. Am J Trop Med Hyg.
2013;88:757–64.

37. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin Regulates Cellular Iron Efflux by Binding to Ferroportin and Inducing Its Internalization. Science (80- ). 2004;306:2090–3.

38. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta. 2003;329:9–22.

39. Pasvol G, Weatherall DJ, Wilson RJ. The increased susceptibility of young red cells to invasion by the malarial parasite Plasmodium falciparum. Br J Haematol. 1980;45:285–95.

40. Verhoef H, West CE, Ndeto P, Burema J, Beguin Y, Kok FJ. Serum transferrin receptor concentration indicates increased erythropoiesis in Kenyan children with asymptomatic malaria. Am J Clin Nutr. 2001;74:767–75.
| Table 1: Baseline characteristics of study participants |
|-----------------------------------------------|
|                                               |
|                                               |
|                                Kenya, n=1309 | Uganda, n=1374 |
| Mean age in years (range)                    | 2.3 (0.0, 7.1) | 2.3 (1.0, 5.1) |
| Sex, n/total (%)                             |               |
| Males                                          |               |
| 668/1309 (51.0)                               | 696/1374 (51.7)|               |
| Females                                        |               |
| 641/1309 (49.0)                               | 678/1374 (49.3)|               |
| Malaria parasitemia, n/total (%)              | 261/1296 (20.1)| 92/1371 (6.7) |
| Inflammation, n/total (%)^a                   | 334/1264 (26.4)| 316/1337 (23.6)|
| Iron deficiency, n/total (%)^b                |               |
| Low ferritin                                   | 457/1237 (36.9)| 438/1267 (34.6)|
| TSAT<10%                                       | 637/1215 (52.4)| n/a             |

gean, geometric mean; sd, standard deviation; sTfR, soluble transferrin receptors; TSAT, transferrin saturation.

^a Inflammation was defined as C-reactive protein > 5mg/L

^b Iron deficiency was defined using two definitions: 1) Low ferritin defined as plasma ferritin < 12µg/L or < 30µg/L in the presence of inflammation in children < 5 years or < 15µg/L in children ≥ 5 years; and 2) Transferrin saturation < 10% (available in 1215 Kenyan children only and not available (n/a) in Uganda).
|                         | Kenya                      | Uganda                     |
|-------------------------|----------------------------|----------------------------|
| **Anemia, n/total (%)** | 526/765 (68.8)             | 533/1312 (40.6)            |
| **Iron deficiency anemia, n/total (%)** | 172/729 (23.6) | 213/1209 (17.6) |
| **Sickle cell trait, n (%)** | 157/1057 (14.9) | 224/1355 (16.5) |
| **Ferritin, n (gmean±sd) in µg/L** | 1237 (20.8±3.0) | 1267 (20.8±2.9) |
| **Hepcidin, n (gmean±sd) in µg/L** | 1202 (5.6±3.6) | 1333 (6.8±3.3) |
| **sTfR, n (gmean±sd) in mg/L** | 1296 (17.8±1.5) | 1343 (6.7±2.0) |
| **Hemoglobin, n (gmean±sd) in g/dL** | 765 (10.1±1.2) | 1312 (11.0±1.1) |
| **Transferrin saturation, n (gmean±sd) in %** | 1215 (9.3±2.2) | n/a |

*a* Anemia was defined as hemoglobin < 11g/dL in children aged 0 to 4 years or hemoglobin < 11.5 g/dL in children above 4 years. The range of hemoglobin was 5.1-14.7 in Kenya and 5.4-18.5 in Uganda while interquartile range (IQR) was 9.4-11.3 in Kenya and 10.3-12.1 in Uganda. Only 33 (1.6%) had severe anemia (Hb<7g/dL in under 5 years or <8g/dL in over 5 years old).

*b* Iron deficiency anemia was defined as low ferritin and anemia.

Malaria incidence rate per child-year of follow-up was 0.6 in Kenya and 0.3 in Uganda.
### Table 2: Incidence of malaria by iron status and anemia

|            | Kilifi, Kenya | Entebbe, Uganda | Overall |
|------------|---------------|----------------|---------|
|            | No. of episodes | Incidence (95% CI) | p | No. of episodes | Incidence (95% CI) | p | No. of episodes | Incidence (95% CI) | p |
| No ID (iron replete) | 780 | 286 | 0.8 | 1 | 829 | 140 | 0.3 | 1 | 1609 | 426 | 0.6 | 1 |
| ID (low ferritin) | 457 | 93 | 0.4 | 0.8 (0.6, 0.9) | 0.018 | 438 | 45 | 0.2 | 0.5 (0.4, 0.7) | <0.001 | 895 | 138 | 0.3 | 0.7 (0.6, 0.8) | <0.001 |
| No ID (TSAT ≥10%) | 578 | 224 | 0.8 | 1 | n/a | n/a | n/a | n/a | 578 | 224 | 0.8 | 1 |
| ID (TSAT <10%) | 637 | 159 | 0.5 | 0.8 (0.6, 0.9) | 0.016 | n/a | n/a | n/a | n/a | 637 | 159 | 0.5 | 0.8 (0.6, 0.9) | 0.016 |
| No anemia | 239 | 97 | 0.8 | 1 | 779 | 93 | 0.2 | 1 | 1018 | 190 | 0.4 | 1 |
| Anemia | 526 | 219 | 0.9 | 1.0 (0.8, 1.2) | 0.774 | 533 | 93 | 0.4 | 1.2 (0.9, 1.6) | 0.156 | 1059 | 312 | 0.6 | 1.1 (0.9, 1.3) | 0.168 |
| No IDA | 557 | 257 | 1.0 | 1 | 996 | 154 | 0.3 | 1 | 1553 | 411 | 0.5 | 1 |

ID, iron deficiency; IDA, iron deficiency anemia; TSAT, transferrin saturation

*Iron deficiency (low ferritin) was defined as plasma ferritin < 12µg/L or < 30µg/L in the presence of inflammation (CRP > 5mg/L) in children < 5 years or < 15µg/L in children ≥ 5 years otherwise iron replete.

*Transferrin saturation data were available in 1215 Kenyan children. Not available (n/a) for Uganda.

*Anemia was defined as hemoglobin < 11g/dL in children aged 0 to 4 years or hemoglobin < 11.5 g/dL in children above 4 years.
| IDA | 172 | 47 | 0.6 | 0.8 (0.6, 1.1) | 0.269 | 213 | 23 | 0.2 | 0.5 (0.3, 0.7) | <0.001 | 385 | 70 | 0.4 | 0.7 (0.6, 0.9) | 0.006 |
|-----|-----|----|-----|--------------|-------|-----|----|-----|--------------|-------|-----|----|-----|--------------|-----|

* Iron deficiency anemia was defined as low ferritin and anemia.

Poisson regression models were adjusted for age, sex, parasitemia, inflammation, length of follow-up, and study site. Maximum length of follow-up was 6 months. IRR=Incidence rate ratio. Incidence defined as number of malaria episodes per child-year of follow-up. The number of episodes ranged from 0-5 in Kenya and 0-6 in Uganda. 101 Kenyan and 45 Ugandan children had multiple episodes.
Figure Legends

**Figure 1:** The effect of iron status on subsequent malaria  
A) Scatter plots of iron biomarkers stratified by no subsequent malaria or one or more subsequent malaria episodes. Horizontal red line indicates mean while vertical line indicates standard deviation. P value was derived from Poisson regression model.  
B) Adjusted incidence rate ratios for the effect of iron biomarkers on subsequent malaria episodes. Green indicates overall, red Kenya and blue Uganda. Labels indicate incidence rate ratio and 95% confidence intervals. Poisson regression models were adjusted for age, sex, parasitemia, inflammation, length of follow-up and study site. Maximum length of follow-up was 6 months. sTfR, soluble transferrin receptor; TSAT, transferrin saturation.

**Figure 2:** Kaplan-Meier curves of time to first malaria episode according to  
A) iron deficiency (ID) defined by low ferritin,  
B) ID defined by transferrin saturation (TSAT < 10%),  
C) iron deficiency anemia (IDA), and  
D) anemia. P values were derived from log-rank tests for equality of survivor functions.

**Figure 3:** Meta-analysis of observational studies examining the relationship between iron deficiency (ID) and malaria risk. Study-specific estimates and their relative contribution (percentage weight and sample size) to overall estimates are shown. Definitions of ID varied by study: Nyakeriga et al 2004, ferritin < 12µg/L plus TSAT < 10%; Jonker et al 2012, ferritin < 30µg/L; Gwamaka et al 2012, Ferritin < 30µg/L if CRP < 8.2mg/L or ferritin < 70µg/L if CRP > 8.2mg/L; Barffour et al 2017, ferritin < 12µg/L in children < 5 years or <15µg/L in children ≥ 5 years; and current study, ferritin < 12µg/L or < 30µg/L if CRP > 5mg/L in children < 5 years or < 15µg/L in children ≥ 5 years.
Figure 1.
Figure 2.
Figure 3.

| Study            | Country       | IRR (95% CI)      | % Weight | n   |
|------------------|---------------|-------------------|----------|-----|
| Nyakeriga et al 2004 | Kenya         | 0.70 (0.51, 0.99) | 4.80     | 240 |
| Jonker et al 2012 | Malawi        | 0.49 (0.33, 0.73) | 14.54    | 727 |
| Gwamaka et al 2012 | Tanzania      | 0.77 (0.66, 0.89) | 15.70    | 785 |
| Barffour et al 2017 | Zambia        | 0.63 (0.35, 1.11) | 14.90    | 745 |
| Current study     | Kenya and Uganda | 0.67 (0.56, 0.81) | 50.07    | 2504|
| Overall (I-squared = 40.0%, p = 0.155) |               | 0.66 (0.56, 0.75) | 100.00   | 5001|