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Delayed iron does not alter cognition or behavior among children with severe malaria and iron deficiency

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Abstract

Background—Malaria and iron deficiency (ID) in childhood are both associated with cognitive and behavioral dysfunction. The current standard of care for children with malaria and ID is concurrent antimalarial and iron therapy. Delaying iron therapy until inflammation subsides could increase iron absorption but also impair cognition.

Methods—In this study, Ugandan children 18 months to 5 years old with cerebral malaria (CM, n=79), severe malarial anemia (SMA, n=77) or community children (CC, n=83) were enrolled and

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Author Contributions: Mr. Ssemata was responsible for IRB application, conduct of the study and data collection, interpretation of data, and drafting the initial manuscript. Dr. Hickson carried out the statistical analyses, interpreted the data, and drafted the initial manuscript. Dr. Bangirana directed the conduct of the study, interpreted the data, reviewed and revised the manuscript, and approved the final manuscript as submitted. Dr. Ssenkusu carried out statistical analyses and interpretation of data. Dr. Cusick conceptualized and designed the study, was responsible for IRB application, conduct of the study and data collection, statistical analyses, and interpretation of data. Dr. Opoka directed the conduct of the study and interpreted the data. Drs. Kroupina and Georgieff helped to conceptualize and design the study, and interpreted the data. Dr. John conceptualized and designed the study, directed the conduct of the study, and interpreted the data. All authors contributed significantly to the intellectual design, provided critical revisions to the manuscript, and approved the final version.

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Written informed consent was required from the study participant's legal guardian for participation in this study.

Data Sharing Statement

Deidentified individual participant data (with data dictionaries) will be made available will be made available upon publication to researchers who provide a methodologically sound proposal for data analysis that is consistent with the goals of the approved protocol. The study protocol, statistical analysis plan, and informed consent form will be provided with the participant data. Proposals should be submitted to Debbie Bennett, debabenn@iupui.edu.

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tested for ID. Children with ID were randomized to immediate vs. 28-day delayed iron therapy. Cognitive and neurobehavioral outcomes were assessed at baseline and 6 and 12 months (primary endpoint) after enrollment.

Results—All children with CM or SMA and 35 CC had ID (zinc protoporphyrin concentration ≥80 μmol/mol heme). No significant differences were seen at 12-month follow-up in overall cognitive ability, attention, associative memory, or behavioral outcomes between immediate and delayed iron treatment (mean difference (standard error of mean) ranged from −0.2 (0.39) to 0.98 (0.5), all $P \geq 0.06$).

Conclusion—Children with CM or SMA and ID who received immediate vs. delayed iron therapy had similar cognitive and neurobehavioral outcomes at 12-month follow-up.

INTRODUCTION

Malaria is a major contributor to the developmental gap faced by children in sub-Saharan Africa relative to other regions (1). Prior studies by our group demonstrated that two manifestations of severe malaria (SM), cerebral malaria (CM) and severe malarial anemia (SMA), both contribute to cognitive impairment in children (2,3). In addition, we recently demonstrated that children exhibit persistent neurobehavioral problems following SM, including more anxious and depressed symptoms in children with SMA, and more somatic complaints and aggressive behavior in children with either SMA or CM (4). Iron deficiency (ID), also common in much of sub-Saharan Africa, often coexists with *Plasmodium falciparum* malaria (5), and has well-established consequences on cognition and child behavior (6). Young children with ID exhibit a more fearful, withdrawn affect than their iron-sufficient peers (7), and chronic ID can lead to more internalizing symptoms and problems with attention that persist into adolescence even when iron is given on diagnosis of ID (7). Additionally, ID can affect multiple cognitive functions, including motor control, memory, and attention resulting, in poorer scholastic achievement, and problem-solving skills (8).

There is a complex interplay between iron status, malaria risk, and neurobehavioral development in malaria endemic areas. Studies show that iron therapy at the time of malaria treatment may lead to delayed parasite clearance and increased susceptibility to clinically significant *P. falciparum* malaria (9). Conversely, malaria can lead to anemia of inflammation. Levels of hepcidin and ferritin, an acute phase reactant implicated in iron metabolism, rise as a result of the inflammatory response to malaria, leading to sequestration of body iron and impaired iron absorption in the gut (10,11). Impaired iron absorption and sequestration of body iron can prevent utilization of iron for hematopoiesis and brain development (9). Studies of dietary ID (7,12–15) and studies of children with SM (2,4) have both shown impaired attention, internalizing, and externalizing behaviors, suggesting that dietary ID and malaria-related anemia of inflammation with reduced bioavailability of iron may act on the developing brain through similar and potentially additive pathways. Effective management of ID in SM could represent an important opportunity to limit the neurobehavioral toll of malaria. However, it is difficult to know the relative contributions of dietary ID and anemia of inflammation with reduced bioavailability of iron in a child

presenting with SM, as the profound inflammation that characterizes SM alters currently available iron biomarkers.

World Health Organization (WHO) guidelines recommend starting iron and antimalarial therapies concurrently when ID (defined as anemia or an abnormal value for any iron biomarker) and malaria occur concurrently (16). However, recent studies have demonstrated that the relative timing of antimalarial and iron therapies is important and that concurrent administration may not facilitate optimal iron intake into hemoglobin. A study of Gambian children recovering from malarial anemia demonstrated that iron incorporation into hemoglobin after iron supplementation remained significantly reduced two weeks after starting antimalarials, while iron incorporation in children recovering from iron deficiency anemia alone was excellent at baseline and two weeks later (11). In addition, a recent study of Ugandan children with malaria and anemia showed that iron incorporation into hemoglobin was twice as great when the start of iron therapy was delayed by four weeks vs. given concurrently (17). Together these findings suggest that delaying iron therapy until after parasite-induced inflammation and the resultant disruptions in iron metabolism are resolved may permit greater iron absorption and incorporation, and that four weeks may be the most appropriate delay. However, whether delaying iron supplementation constitutes a risk to the developing brain is unknown. We conducted a clinical trial in which children with SMA and CM who were iron-deficient (as defined by elevated zinc protoporphyrin), and iron-deficient community children, were randomized to start iron therapy either immediately or after 28 days and were assessed for malaria incidence, hemoglobin level, and cognitive and neurobehavioral outcomes over 12 months of follow-up. In this manuscript, we describe the results of immediate vs. 28-day delayed iron therapy on general cognitive ability and neurobehavioral outcomes at the 6- and 12-month follow-up visits.

METHODS

Participant Enrollment, Randomization, Iron Treatment and Follow-up

Children 18 months to 4.9 years of age with CM, SMA, or community children were enrolled at Mulago Hospital, Kampala, Uganda between June 2010 and September 2014. CM was defined as: 1) coma (Blantyre Coma Score ≤2); 2) *Plasmodium falciparum* on blood smear; and 3) no other known cause of coma. SMA was defined as presence of Plasmodium falciparum on blood smear in children with a hemoglobin (Hb) level ≤5 g/dL.

Community children (CC) 18 months to 4.9 years of age who were currently healthy with no symptoms of illness and no history of chronic illness were recruited from the extended family or household compound area of children with severe malaria. CC were enrolled to be in the same age range as children with CM or SMA, but were not matched to specific children with CM or SMA. Mean age and weight-for-age z-scores did not differ between children with CM or SMA and CC (18). Exclusion criteria for all children included 1) known chronic illness requiring medical care; 2) known developmental delay; or 3) history of coma, head trauma, hospitalization for malnutrition, or cerebral palsy.

Children with iron deficiency, defined as a zinc protoporphyrin (ZPP) level ≥ 80 μmol/mol heme, were randomized to receive a three-month course of daily oral ferrous sulfate

(2mg/kg/day) either concurrently with antimalarial treatment (immediate group) or 28 days after receiving antimalarial treatment (delayed group).

Complete details on participant enrollment, randomization, study iron treatment, and follow-up are provided in our manuscript on the effect of immediate vs. delayed iron treatment in this study population on iron biomarkers, malaria incidence, and hemoglobin levels (18), and in Supplemental Methods S1 (online). The results showed that children with SMA in the delayed compared to immediate treatment group had lower mean soluble transferrin receptor (6.1 vs. 7.8 mg/L, p=0.03) and zinc protoporphyrin (83 vs. 106 μmol/mol heme, p=0.02) after 12 months and showed a non-significant trend toward fewer episodes of severe malaria (incidence rate ratio [95% confidence interval), 0.39 [0.14, 1.12]) (18).

Cognitive Testing

Children were assessed for general cognitive ability, sustained attention, and associative memory a week after discharge (CM or SMA) or at enrollment (CC). These assessments were repeated at 6 and 12 months after enrollment. The assessments were conducted from the cognitive testing rooms on the pediatric ward at Mulago National Referral Hospital. Assessments were carried out by neuropsychological testers with undergraduate degrees in Psychology. They were blinded to the participant's study group and treatment arms and not involved in the randomization or clinical procedures. The Mullen Scales of Early Learning (MSEL) (19), which assesses motor, visual, and language ability, were used to measure general cognitive ability. Scores from fine motor, visual reception, receptive language, and expressive language scales were summed to give the early learning composite score, a measure of general cognitive ability. Ability to sustain attention was assessed using the Early Childhood Vigilance Test (ECVT) (20), where a child is asked to focus his or her gaze on cartoons screened on a computer for approximately 7 minutes; the measure of sustained attention is the percentage of time the child spends gazing at the screen. Associative memory was assessed using the Color Object Association Test (COAT) (21), in which children are required to associate toys with specific color-coded boxes and scored on the total number of toys placed in the correct boxes. The MSEL, COAT and ECVT have been successfully used to assess cognition, memory and attention in multiple studies of children with HIV or malaria in Uganda (2,22). The tests used designed to assess children in the study's age range and have been used and validated in a number of studies and cross-cultural settings in Uganda and sub-Saharan Africa (4,23–26).

Socio-Emotional Behavioral Testing

Baseline neurobehavioral testing was performed one-week post-discharge for children with CM or SMA and at enrollment for CC. Assessments were repeated 6 and 12 months after enrollment. Primary outcomes were age-adjusted *z*-scores at 12-month follow-up for: 1) internalizing, externalizing, and total behavioral problems, assessed with the parent-report preschool Child Behavioral Checklist (CBCL) (27); 2) global executive function, evaluated using the parent-report Behavior Rating Inventory of Executive Functioning, Preschool edition (BRIEF-P) (28); and age-adjusted scores for 3) observer-rated affect and behaviors, coded using the Behavior Rating Scales (BRS), developed by Lozoff and colleagues to capture behavioral domains associated with ID (7), adapted by author MK for use with older

children (29), and assessed from videotaped cognitive testing sessions by an experienced BRS coder at the University of Minnesota. The CBCL is a parent report tool focused on indicators of externalizing and internalizing behavior. The BRIEF is a parent report tool focused on indicators of emotional regulation and executive functioning. Literate parents completed printed versions of the two tools themselves; illiterate parents were read all items in each tool verbatim in a language of their choosing (English or Luganda), and their verbal responses were recorded by a trained tester.

The CBCL and BRIEF have previously been adapted for use in Uganda (25), while the BRS was adapted for this study by author MK (details in Supplemental Methods S2 [online]) and coded by an experienced coder (intra-class correlation coefficient >0.8). For the CBCL, children were scored on the syndrome scales, which are: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, attention problems, aggressive behavior, and sleep problems. These were summarized into internalizing problems (emotionally reactive, anxious/depressed, somatic complaints, withdrawn), externalizing problems (attention problems, aggressive behavior) and total problems, which included both internalizing and externalizing problems as well as 2 more scales, sleep problems and other problems (4,23).

Secondary outcomes included 12-month follow-up sub-scale scores of the BRIEF-P and CBCL; and baseline and 6-month follow-up global executive function, internalizing behavior, externalizing behavior, total behavioral problems, and observer-rated behavior scores. For CBCL and BRIEF scores and subscales, higher scores indicate more problematic behavior or diminished function. For the BRS, higher raw scores on Fearful/Wary Affect, Negative Affect and Hyperactivity/Over-activity, and lower raw scores on Positive Affect, Adaptation to Change, and Exploration and Activity Level, indicated more problematic behavior.

Statistical Analysis

Age-adjusted z-scores for general cognitive and neurobehavioral outcomes were computed using the scores of community children (CC) age 18 months – 4.9 years enrolled in a parallel prospective longitudinal cohort study of children with CM, SMA or CC that did not include iron treatment (2). Scores from asymptomatic community children were used to generate age-adjusted z-scores because this provided population-specific normative values for cognitive and behavioral outcomes, as opposed to using the US-based norms given with most tests, which would be inappropriate for this population.

For each outcome, the z score was computed as (actual score – mean score for child's age)/standard deviation (SD), where the mean score for age and SD were computed by fitting a mixed linear model to data from all available visits for CC in the parallel study (2) (allowing correlated errors for a child's multiple visits); z scores have a mean of 0 and SD 1 in the reference population (CC) over all time points.

Five of seven BRS outcomes were heavily skewed and could not be converted to a *z*-score. These skewed scales were converted to categorical outcomes that best matched the scale distribution. For Fearful/Wary Affect, Negative Affect, Positive Affect, Overall Hyperactivity/Over-activity, any expression was termed "present" and no expression was

termed “absent.” For Overall Adaptation to Change/Cooperation, scores equal to or above the median were termed “present,” and a score below the median “absent.”

To estimate the mean age-adjusted z-score differences (I – D) at baseline, 6 and 12 months, linear regression models were fitted separately for each study group (CM, SMA, CC). Categorical BRS outcomes, adjusted for age and sex, were compared by logistic regression. The three primary outcomes at 12 months were pre-specified and no adjustment for multiple testing was made to P values. For outcomes at baseline and 6 months, we adjusted for multiple testing using Holm’s sequential Bonferroni procedure (30). Hemoglobin at baseline and malaria incidence over the 12-month follow-up period did not differ between I and D treatment arms and so were not adjusted for in analysis. Data was analyzed using STATA version 12.1 (Stata Corp, Texas). Sample sizes of 50 CC, 80 CM and 100 SMA, equally allocated between delayed and immediate iron treatment groups were estimated a priori to give 89% power ($\alpha = 0.05$) to detect a 1 SD difference in z scores between the CM group with the immediate treatment strategy and other malaria-iron treatment groups

RESULTS

Study cohort characteristics

The numbers of children tested for general cognitive and neurobehavioral outcomes at each time point are shown in Supplemental Figure S1 (online). All tests of cognition and behavior used were designed for children <5 years, so testing numbers decreased as some children became >5 years old.

Baseline demographic, clinical and laboratory findings are summarized in the accompanying manuscript on iron biomarkers and malaria incidence and in Supplemental Table S1a for the children who received iron treatment (online). All variables, including home environment and socioeconomic status, were similar in each disease group in the I vs. D treatment arms.

Cognitive outcomes at 12-month follow-up

For the primary cognitive outcome endpoint (cognitive outcomes at 12-month follow-up), there were no statistically significant differences between children in the immediate compared to delayed treatment arm in any study group (CM, SMA or CC) for any cognitive outcome (general cognitive ability, sustained attention, associative memory, Table 1 and Figure 1).

Effect sizes for differences in immediate and delayed treatment arms were small (<0.5) for all cognitive outcomes for children with severe malaria (CM or SMA, Table 1). Effect size was larger for associative memory for CC, with a mean difference (standard error of mean (SE) of 0.98 (0.50), p=0.06, favoring immediate therapy. Overall, scores for cognitive ability were lower in children with CM or SMA were lower than CC, but the study was not designed or powered to assess statistical differences between these study groups.

Cognitive outcomes at baseline and 6-month follow-up

No significant differences between immediate and delayed iron treatment arms were seen for any cognitive z-score at baseline or 6 month follow-up testing for children with SMA, but

modestly higher z-scores in general cognitive ability were seen at baseline in CC in the immediate treatment arm (mean difference (SE), +0.77 (0.33), p=0.03) and at 6-month follow-up in children with CM in the immediate (+0.70 (0.28), p=0.01 (Table 2 and Figure 1).

Socioemotional behavior at 12-month follow-up

For the primary behavioral endpoint (neurobehavioral outcomes at 12-month follow-up), no significant differences in internalizing, externalizing, or total problem behaviors on the CBCL were seen for I vs. D iron therapy for any study group (Table 3, S3), nor were any significant differences seen in the CBCL sub-scales (Table 5, S5). Similarly, no significant differences were seen in BRIEF global executive composite (Table 3, S3) or subscale z-scores (Table 5, S5). Analyzing observed behavior, no differences were seen in the Behavior Rating Scales outcomes between the I vs. D iron treatment arms for children in any study group (Tables 4a and 4b).

Socioemotional behavior at baseline and 6-month follow-up

No significant differences were seen in socioemotional, executive function or observed behavior scores between I and D treatment groups in any study group at baseline or at 6-month follow-up (Supplemental Tables S2 a–d [online]).

DISCUSSION

In this randomized clinical trial of immediate vs. 28-day delayed iron therapy for children with severe malaria and iron deficiency, general cognitive and neurobehavioral outcomes were similar at 12 months in children who started iron 28 days after antimalarial treatment as compared to those who started iron at the time of antimalarial treatment. The study was powered to detect a fairly large difference in cognitive or behavioral z-scores (one standard deviation [SD]), so it is possible that smaller differences were present and not detected. Future studies with larger sample sizes are needed to clarify the current study findings, and the studies may also need longer follow-up time, as the effects of iron supplementation may take additional time to evaluate. Interestingly, the only large effect sizes seen for cognition (>0.5 z-score) were in the community children, with a mean difference of 0.98 (standard error 0.5) for associative memory, favoring the immediate treatment arm (p=0.06). This finding suggests that in the absence of inflammation, children with ID as defined by ZPP may do better with immediate treatment.

Iron plays a role in the regulation of dopaminergic activity where dopamine, a key neurotransmitter in the extrapyramidal system of the brain, acts as a powerful regulator of many aspects of cognitive brain function (31), and modulates externally focused and internally directed attention, concentration, motivational processes and working memory (32). Numerous studies have demonstrated that iron deficiency is associated with an impairment in multiple cognitive domains, including general cognitive ability, executive function, recognition memory, inhibitory control, and sustained attention (7,15,33,34) and impairment in multiple areas of behavior (7,12,13,15).

Detection of dietary ID during an episode of severe malaria is problematic, as virtually all measures of ID are affected by inflammation and/or hemolysis that occurs in severe malaria. Studies of dietary ID and development have used composite measures, which often include one or more of: ZPP, mean corpuscular volume (MCV), transferrin saturation, and levels of serum ferritin, hepcidin, and soluble transferrin receptor (34). ZPP, ferritin and hepcidin (35,36) levels are strongly affected by inflammation; transferrin saturation depends on serum iron levels, which are an unreliable marker of iron deficiency; MCV lacks sensitivity; and serum transferrin receptor levels are altered by the hemolysis seen in malaria (5,37,38). Thus, there is no ideal marker for dietary ID during acute severe malaria. We chose ZPP because it is a point-of-care test for ID and because children in the Pemba study (39) who had an elevated ZPP level and received iron therapy experienced fewer hospitalizations and deaths than children with elevated ZPP who did not receive iron. It is possible, however, that a biomarker that predicts subsequent mortality and morbidity risk may not be the same as one that predicts improved neurobehavioral outcomes. The elevated blood concentrations of ZPP, CRP and ferritin at baseline among children with severe malaria suggest sequestered iron and therefore reduced iron bioavailability, with iron not going to the sites most needed (blood cells, brain).

The resolution of inflammation and consequent release of sequestered iron may be sufficient to correct disruptions in iron absorption and utilization alone, without additional iron therapy. Without a no-iron treatment arm in children with severe malaria, this study could not answer the question of whether iron therapy was required for all of these children.

However, starting supplementation earlier in life and/or more prolonged follow-up may be required to determine if delayed iron treatment has a long-term effect on cognition. Previous studies by our group showed that delayed iron resulted in improved iron incorporation and lower hepcidin after 28 days (17,40). In the present study, we report elsewhere that children who received delayed iron treatment also had improved iron status at 12 month follow-up (18). These findings suggest that assessment of cognitive outcomes at longer follow-up (e.g., 2 years) in this population may be important to fully address the question of whether delayed iron treatment determine if delayed iron treatment has a long-term effect on cognition in children with severe malaria and reduced iron bioavailability.

In addition, prior studies of iron therapy to improve neurobehavioral outcomes in children under 2 years with dietary ID, including one that followed subjects into adolescence, have not demonstrated a clear long-term neurobehavioral benefit to iron treatment once dietary ID is established. Conversely, the dopamine signaling effects of acute, dietary ID in preschool- and school-aged children have been shown to resolve in less than 8 weeks with iron therapy (13,15). Thus, the benefits of iron treatment in children ≥2 years of age with previous dietary ID requires further study. In addition, possible benefits of acute vs. delayed iron in children with reduced iron bioavailability who are <2 years also requires further study, since children <2 years may be at the highest risk of brain injury from iron deficiency, but only a small proportion of the children enrolled in the present study were <2 years of age.

Assessment of behavior is a complex, culturally dependent process. The CBCL and BRIEF have been used and validated in numerous countries and cultural settings (41,42), including

in Uganda (4,25). The Behavior Rating Scales (BRS) have been used in other countries and were adapted and piloted in Uganda prior to study implementation (7,41–43). Luganda-proficient neuropsychology testers were available on site for questions on verbal responses. This access was important because although some behaviors, like fear and hyperactivity, can be assessed based on facial cues and body language with relative accuracy, others, like positive affect and cooperation, may lose nuance without fluency in the language in which the observed interaction is conducted.

Even without general cognitive or neurobehavioral benefits, delayed iron therapy may have clinical and physiologic benefits for children with malaria. In our assessment of iron biomarkers in this same study, we found a significantly lower prevalence of ID based on ZPP in children with SMA in the delayed iron group at 12-month follow-up (18). Additionally, a recent study comparing immediate and delayed iron for ID in uncomplicated malaria suggested that delayed iron was associated with a lower incidence of sick child visits as compared to immediate iron (44). Given the potential benefit to iron status and the reduced infection risk associated with delayed iron therapy, the lack of harmful effects on general cognitive and neurobehavioral outcomes reported in this analysis suggests that delayed iron therapy may be a safe and potentially superior alternative to the current standard of care for children with ID and SM.

In conclusion, compared to immediate iron therapy, 28-day delayed iron therapy did not alter general cognitive or neurobehavioral outcomes at 12-month follow-up in children with SM and ZPP-level defined ID. Additional studies are required to answer a number of questions raised by the present study, including how to define ID in SM, what biomarker(s) best predicts neurobehavioral outcomes in the context of ID and SM, the optimal age at which to provide iron therapy for ID, and whether inflammation-related sequestration of iron has the same effects on neurobehavioral outcomes as dietary ID. In addition, studies that overcome some of the limitations of our study, including the small sample size for children <2 years of age, and the ability to detect only relatively large differences in cognitive and behavioral outcomes (one standard deviation or greater), are also required before definitive recommendations can be made on the timing of iron treatment in severe malaria. Iron status and the timing of iron therapy in malaria remain important areas of study because of the potential risks of iron therapy in dietary iron-replete children in malaria endemic areas and because the populations at risk for malaria and iron undernutrition overlap so extensively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Georgieff helped to conceptualize and design the study, and interpreted the data. Dr. John conceptualized and designed the study, directed the conduct of the study, and interpreted the data. All authors contributed significantly to the intellectual design, provided critical revisions to the manuscript, and approved the final version.

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Impact

- The optimal time to provide iron therapy in children with severe malaria is not known. The present study shows that delay of iron treatment to 28 days after the malaria episode, does not lead to worse cognitive or behavioral outcomes at 12-month follow-up.
- The study contributes new data to the ongoing discussion of how best to treat iron deficiency in children with severe malaria.

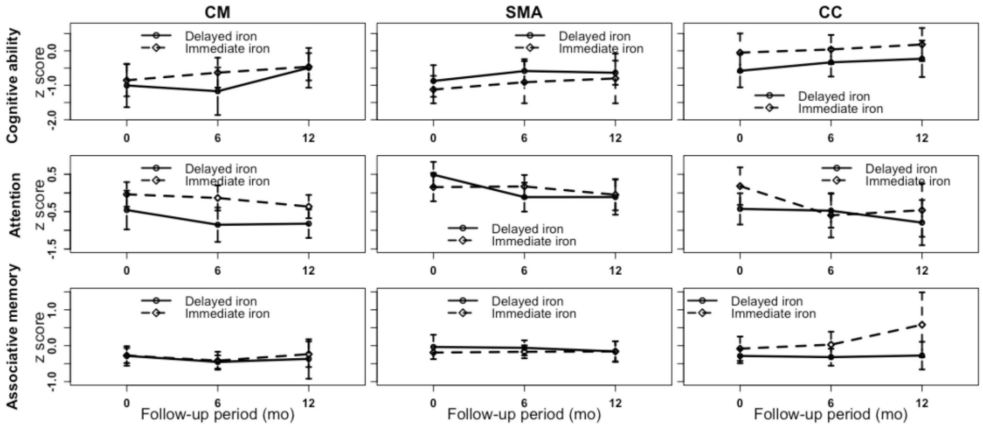

Figure 1.

Age-adjusted neuropsychological scores in children with cerebral malaria (CM), severe malaria anemia (SMA) and community children (CC) at baseline (0), 6 and 12 months for general cognitive ability, sustained attention, and associative memory. Scores are shown as unadjusted means with 95% confidence intervals.

Table 1.

Cognitive ability, attention, and associative memory in children with cerebral malaria, severe malarial anemia, or community children, by treatment group at 12-month follow-up[a]

Score and outcome	Cerebral malaria				Severe malarial anemia				Community controls			
Z score at 12 mo, mean±SE	Immediate (N=30)	Delayed (N=23)	Mean difference (SE), I vs. D	*P*	Immediate (N=29)	Delayed (N=32)	Mean difference (SE), I vs. D	*P*	Immediate (N=16)	Delayed (N=15)	Mean difference (SE), I vs. D	*P*
Cognitive ability	−0.46± 0.22	−0.49 ± 0.25	0.03 (0.33)	0.92	−0.82 ± 0.28	−0.62 ± 0.28	−0.20 (0.39)	0.61	0.29 ± 0.22	−0.34 ± 0.23	0.63 (0.33)	0.07
Attention	−0.38± 0.16	−0.81 ± 0.18	0.43 (0.24)	0.08	−0.04 ± 0.23	−0.12 ± 0.22	0.08 (0.31)	0.81	−0.42 ± 0.32	−0.83 ± 0.33	0.41 (0.47)	0.39
Associative memory	−0.22 ± 0.20	−0.39 ± 0.23	0.16 (0.31)	0.60	−0.18 ± 0.14	−0.14 ± 0.14	−0.04 (0.19)	0.85	0.64 ± 0.34	−0.34 ± 0.35	0.98 (0.50)	0.06

[a]. Z-scores were age-adjusted using community control children as the reference population. Linear regression models were used to compare mean neurocognitive outcomes between the immediate and delayed treatment groups within each study group (CM, SMA, CC) and all analyses were adjusted for sex.

Abbreviations: mo., months; P, P-Value; SE, standard error.

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Table 2.

Cognitive ability, attention and associative memory in children with cerebral malaria, severe malarial anemia, or community children by treatment group at baseline and 6-month follow-up[a]

Score and outcome	Cerebral malaria				Severe malarial anemia				Community controls			
	Immediate (N=38)	Delayed (N=32)	Mean difference (SE), I vs. D	*P*	Immediate (N=38)	Delayed (N=36)	Mean difference (SE), I vs. D	*P*	Immediate (N=19)	Delayed (N=16)	Mean difference (SE), I vs. D	*P*
Z score at baseline, mean ±SE												
Cognitive ability	−0.85 ± 0.26	−1.01 ± 0.28	0.16 (0.38)	0.67	−1.14 ± 0.21	−0.86 ± 0.21	−0.28 (0.30)	0.35	0.06 ± 0.22	−0.71 ± 0.24	0.77 (0.33)	0.03
Attention	−0.02 ± 0.19	−0.48 ± 0.22	0.46 (0.29)	0.12	0.18 ± 0.17	0.47 ± 0.18	−0.29 (0.25)	0.24	0.18 ± 0.22	−0.42 ± 0.24	0.60 (0.33)	0.08
Associative memory	−0.27 ± 0.11	−0.29 ± 0.12	0.02 (0.16)	0.93	−0.20 ± 0.12	−0.03 ± 0.13	−0.17 (0.17)	0.34	−0.12 ± 0.13	−0.25 ± 0.14	0.13 (0.19)	0.49
Z score at 6 mo, mean±SE	N=34	N=27			N=33	N=34			N=18	N=15		
Cognitive ability	−0.66 ± 0.26	−1.14 ± 0.29	0.49 (0.39)	0.22	−0.93 ± 0.24	−0.57 ± 0.24	−0.36 (0.34)	0.29	0.10 ± 0.18	−0.41 ± 0.20	0.52 (0.28)	0.07
Attention	−0.14 ± 0.18	−0.84 ± 0.21	0.70 (0.28)	0.01	0.15 ± 0.17	−0.09 ± 0.17	0.24 (0.24)	0.31	−0.61 ± 0.25	−0.46 ± 0.28	−0.16 (0.38)	0.68
Associative memory	−0.44 ± 0.10	−0.43 ± 0.12	0.00 (0.16)	0.98	−0.18 ± 0.10	−0.05 ± 0.09	−0.13 (0.14)	0.34	0.02 ± 0.15	−0.32 ± 0.16	0.34 (0.22)	0.14

a. Age-adjusted z-scores were computed using community control children as the reference population. Linear regression models were used to compare mean neurocognitive outcomes between the immediate and delayed treatment groups within each study group (CM, SMA, CC) and all analyses were adjusted for sex. For baseline and 6-month comparisons, which were secondary comparisons with multiple outcomes, none was significant following Holm's sequential Bonferroni correction for multiple testing.

Abbreviations: mo., months; P, P-Value; SE, standard error.

Table 3.

Socioemotional and Executive Function Outcomes at 12-month Follow-up[a]

	Cerebral Malaria		Severe Malarial Anemia		Community Controls	
	Mean difference (95 % CI), I (n=30) vs. D (n=24)	***P***	**Mean difference (95 % CI), I (n=28) vs. D (n=32)**	***P***	**Mean difference (95 % CI), I (n=16) vs. D (n=15)**	***P***
Child Behavioral Checklist (CBCL)						
Internalizing z-score	−0.08 (−0.55, 0.38)	0.73	0.12 (−0.58, 0.81)	0.74	−0.26 (−1.12, 0.60)	0.54
Externalizing z-score	0.49 (−0.08, 1.06)	0.09	−0.10 (−0.70, 0.50)	0.74	−0.40 (−1.02, 0.22)	0.20
Total Problems z-score	0.22 (−0.33, 0.78)	0.43	−0.04 (−0.71, 0.62)	0.89	−0.42 (−1.18, 0.34)	0.27
Behavior Rating Inventory of Executive Function (BRIEF)						
Global Executive Composite z-score	0.24 (−0.47, 0.95)	0.50	−0.00 (−0.61, 0.60)	1.00	−0.37 (−1.12, 0.38)	0.32

[a]. Linear regression models were performed separately by study group and all analyses were adjusted for age and sex.

Abbreviations: I, Immediate iron; D, Delayed iron; P, P-value; CI, confidence interval.

Table 4a.

Observed Behavior (Behavior Rating Scales), Categorical Outcomes at 12-month Follow-up[a]

		Cerebral Malaria		Severe Malarial Anemia		Community Controls	
	Value if worse outcome in I arm	**OR (95% CI), I (n=29) vs. D (n=24)**	***P***	**OR (95% CI), I (n=21) vs. D (n=27)**	***P***	**OR (95% CI), I (n=16) vs. D (n=15)**	***P***
Fearful/Wary Affect	>1	2.55 (0.24, 26.62)	0.43	0.62 (0.12, 3.12)	0.57	0.80 (0.03, 19.31)	0.89
Negative Affect	>1	0.58 (0.17, 1.94)	0.37	0.92 (0.24, 3.52)	0.91	9.47 (0.83, 108.47)	0.07
Positive Affect	<1	0.94 (0.28, 3.11)	0.91	0.31 (0.09, 1.09)	0.07	0.81 (0.17, 3.86)	0.79
Overall Hyperactivity/Over-activity	>1	2.20 (0.71, 6.84)	0.17	2.47 (0.73, 8.35)	0.15	1.89 (0.37, 9.77)	0.45
Overall Adaptation to Change/ Cooperation	<1	1.09 (0.31, 3.88)	0.90	0.72 (0.20, 2.60)	0.62	0.23 (0.04, 1.34)	0.10

[a]. Behavior Rating Scale outcomes were compared by logistic regression, adjusted for age and sex

Abbreviations: I, Immediate iron; D, Delayed iron; P, P-value; OR, odds ratio; CI, confidence interval

Table 4b.

Observed Behavior (Behavior Rating Scales), Continuous Outcomes at 12-month Follow-up[a]

		Cerebral Malaria		Severe Malarial Anemia		Community Controls	
	Value if worse outcome in I arm	**Mean difference (95 % CI), I (n=29) vs. D (n=24)**	***P***	**Mean difference (95 % CI), I (n=21) vs. D (n=27)**	***P***	**Mean difference (95 % CI), I (n=16) vs. D (n=15)**	***P***
Exploration	<0	3.09 (−2.91, 9.10)	0.31	−0.66 (−8.65, 7.33)	0.87	−0.23 (−7.97, 7.51)	0.95
Activity Level	<0	0.77 (−2.21, 3.75)	0.61	0.73 (−4.30, 5.75)	0.77	−1.09 (−4.64, 2.45)	0.53

[a]. Linear regression models were performed separately by study group, adjusted for age and sex

Abbreviations: I, Immediate iron; D, Delayed iron; P, P-value; CI, confidence interval

Table 5.

Socioemotional and Executive Function Sub-Scale Outcomes at 12-month Follow-up[a]

	Cerebral Malaria		Severe Malarial Anemia		Community Controls	
	Mean difference (95 % CI), I (n=30) vs. D (n=24)	***P***	**Mean difference (95 % CI), I (n=28) vs. D (n=32)**	***P***	**Mean difference (95 % CI), I (n=16) vs. D (n=15)**	***P***
Child Behavioral Checklist (CBCL)						
Anxious/Depressed z-scores	0.35 (−0.12, 0.82)	0.14	0.11 (−0.50, 0.72)	0.72	0.07 (−0.65, 0.78)	0.85
Somatic Complaints z-scores	−0.16 (−0.62, 0.29)	0.48	−0.05 (−0.50, 0.40)	0.83	0.13 (−0.42, 0.69)	0.63
Withdrawn Behavior z-scores	−0.42 (−0.94, 0.09)	0.11	0.08 (−0.59, 0.76)	0.81	−0.68 (−1.69, 0.33)	0.18
Attention Problems z-scores	0.18 (−0.31, 0.67)	0.47	−0.27 (−0.72, 0.18)	0.23	−0.38 (−0.87, 0.12)	0.13
Aggressive Behavior z-scores	0.51 (−0.05, 1.08)	0.07	−0.03 (−0.62, 0.56)	0.92	−0.34 (−0.96, 0.28)	0.27
Behavioral Rating Inventory of Executive Functioning (BRIEF)						
Meta Cognition Index z-score	0.19 (−0.56, 0.94)	0.61	−0.08 (−0.65, 0.49)	0.79	−0.42 (−1.07, 0.23)	0.19
Flexibility Index z-score	0.29 (−0.27, 0.85)	0.30	−0.05 (−0.64, 0.53)	0.86	−0.11 (−0.95, 0.74)	0.80
Inhibitory Self-Control z-score	0.24 (−0.43, 0.92)	0.48	0.13 (−0.48, 0.73)	0.67	−0.33 (−1.17, 0.51)	0.43

[a] Linear regression models were performed separately by study group (CM, SMA, CC), adjusted for age and sex

Abbreviations: I, Immediate iron; D, Delayed iron; P, P-value; CI, confidence interval