The epidemiology and treatment of childhood anemia in western Kenya
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Efficacy and effectiveness of daily versus twice-weekly iron supplementation for the treatment of childhood anemia in western Kenya

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Abstract

Background: A recent meta-analysis of 14 clinical trials indicated that daily, compared with intermittent iron supplementation resulted in significantly greater hematological improvement in pregnant women. No such definitive beneficial effect was demonstrated in preschool children.

Objective: Compare the efficacy and effectiveness of daily and twice-weekly iron supplementation in the treatment of mild and moderate anemia (hemoglobin 50-109 g/L) in children aged 2-59 months living in a malaria-endemic area of western Kenya.

Design: Un-blinded cluster-randomized trial with factorial design. All children (N=1,049) received a single dose of sulfadoxine-pyrimethamine on enrollment followed by 6 weeks of either: daily supervised iron (3-6 mg/kg/day), twice-weekly supervised iron (6-12 mg/kg/week), daily unsupervised iron, or twice-weekly unsupervised iron.

Results: In the supervised groups hemoglobin concentrations at 6 weeks and 12 weeks (6 weeks post-supplementation) were significantly higher with daily than twice-weekly iron (mean [95% CI] difference at 6-weeks: 4.0 g/L [2.0, 6.0]; 12-weeks: 5.3 g/L [3.1, 7.6]). Among the unsupervised groups, hemoglobin concentrations were not different at 6 weeks (mean [95% CI] difference: 1.1 g/L [-0.9, 3.2]), but higher at 12 weeks for those assigned daily iron (mean [95% CI] difference: 2.5 g/L [0.2, 4.7]), however this difference was not statistically significant after adjustment for multiple testing (P = 0.06).

Conclusions: In this malarious area and following initial antimalarial treatment, six weeks of daily iron results in better hematological responses than twice-weekly iron in the treatment of anemia in preschool children.
Introduction
Fifty to 75% of preschool children in eastern Africa suffer from anemia (1). Iron deficiency is one of the predominant causes (2-5) and may lead to impaired mental development, decreased appetite, decreased resistance to infections and increased risk of HIV infection when blood transfusions are needed (6). For documented iron deficiency anemia, iron supplementation is the treatment of choice; for the prevention of iron deficiency anemia, combined iron supplementation and food based approaches are recommended in developing countries (7). Successful implementation of these programs is limited due to inadequate iron supplies, low coverage, and poor tolerance and adherence to the lengthy duration of required daily dosing (8-12).

In the search for strategies to reduce costs and improve compliance and effectiveness, a series of studies were conducted that demonstrated weekly or twice-weekly iron supplementation was as effective in the prevention (13-16) or treatment (17-24) of mild and moderate anemia as conventional daily iron supplementation, despite a 3- to 7-fold reduction in the cumulative dose. These studies were based on observations of reduced iron absorption and transport with daily exposure to high doses in animal models, explained in part by an apparent inhibitory mucosal block, which can be overcome by giving iron intermittently at intervals of more than 3 days (25). However, studies in humans failed to confirm the existence of such a mucosal block (11, 26). This challenged the earlier conclusion that intermittent iron is as effective as daily iron supplementation (27-30), which resulted in much debate (9, 31). It was suggested that a true difference was missed because efficacy was evaluated only after relatively long intervention periods (≥ 8 weeks) of high dose iron in subjects with predominantly mild iron deficiency and low grade anemia (26).

A recent meta-analysis of 14 clinical trials demonstrated that while the beneficial effect of daily dosing over intermittent iron was indisputable in pregnant women, large inter-study variations make such evidence inconclusive in adolescents and pre-school children (32). It also indicated that the degree of supervision was an important predictor of post-intervention anemia prevalence. A more recent study demonstrated that six weeks of supervised twice-weekly iron for treatment of anemia in children is superior to unsupervised daily iron supplementation in improving hemoglobin concentrations (19).

We compared the therapeutic efficacy of a short six-week course of twice-weekly versus daily iron supplementation in children with mild to moderate anemia in western Kenya. We also compared the impact of supervised versus unsupervised iron supplementation to evaluate the role of adherence and to determine the potential benefits of directly observed therapy. The current study is part of a series of studies conducted to optimize the treatment guidelines for anemia in this area of intense malaria transmission and to address the concern that long courses of iron may increase the risk of malaria (33).
Subjects and methods

**Area and population.** This study was conducted in 14 villages in Asembo, Bondo district, Nyanza Province, western Kenya. The study site has been described in detail elsewhere (34, 35). Over 95% of the people are Luo and predominantly subsistence farmers with limited animal husbandry made up of a few heads of cattle, goats, or chicken. The people mainly cultivate maize, sorghum, cassava and millet, and a few other vegetables. Activities off the farm include fishing in Lake Victoria and management of retail and hotel outlets and vegetable and grain stalls. Families in this polygamous society live in compounds constituting a main house surrounded by several houses for women and children. There are two rainy seasons: the ‘long rains’ from March to May and the ‘short rains’ from October to December. Planting season is in February-March, before the long rains, and the main harvest season is in July-August. Malaria transmission is intense and occurs throughout the year (36) with peaks in June-August and November-December. Recent area-wide deployment of insecticide-treated bednets (ITNs) however has substantially reduced the transmission pressure (37). Prior cross-sectional surveys have shown the prevalence of infection with hookworm, *Schistosoma mansoni* and *Ascaris lumbricoides* to be 11.8%, 0.2% and 25.4% respectively in children <5 years of age (38). Weaning occurs in 70% of children by 18 months and in all children by 3 years of age. Infant and under-five year mortality rates are high (176/1000 and 257/1000 live births, respectively) (39). A quarter of all children admitted to the hospital have severe anemia (hemoglobin concentration of <50 g/L) and account for over half of pediatric hospital deaths (40).

Despite its high public health burden, most local clinics lack standardized guidelines for the use of iron supplementation in the treatment or prevention of anemia. Clinic based surveillance in this area has shown that iron supplementation was not routinely given to children with mild and moderate anemia, and prescribed for only 12% of the children less than five years of age with clinically diagnosed severe anemia, while all received presumptive antimalarial treatment (41). The clinics that prescribe iron for severe anemia in children use short courses of relatively high doses of iron (3-6 mg/kg per day for 14 days). This is combined with presumptive antimalarial treatment to treat the malaria attributable component of anemia, while providing partial protection against potential adverse effects on malaria associated with iron (33, 42). This 2-week regimen combined with antimalarial treatment is also used in other areas with similar intense malaria transmission (2) reflecting the controversy on the safety of longer iron supplementation regimens in these malaria endemic areas (33). The efficacy with this 2-week regimen is unknown, but the short duration of supplementation is likely to result in inadequate restoration of hemoglobin levels (which requires a minimum of 4-6 weeks) (43) and particularly iron stores, which may require iron supplementation for 12 weeks or longer (44).
**Interventions.** All study children were given a single treatment dose of sulfadoxine-pyrimethamine (SP, Fansidar® Hoffman La Roche, Basel, Switzerland) on enrollment dosed for bodyweight as crushed tablets mixed with water (42). In addition, children received 6 weeks of daily or twice-weekly iron. This was the shortest regimen recommended in the treatment of anemia (43) that, combined with SP, was expected to result in clinically significant improvement in hemoglobin concentrations in the majority of patients (19). It also ensured that children in the twice-weekly group received a similar cumulative dose of iron as with the conventional 2-weekly regimen. Folic acid was not considered because of the known interaction between SP (an antifolate antimalarial) and folic acid supplementation (45).

Children received their first dose of six-weeks of iron supplementation on the day of enrollment. Treatment groups included: (1) daily-supervised iron (DS), (2) daily-unsupervised iron (DU), (3) twice-weekly-supervised iron (TS), and, (4) twice-weekly-unsupervised iron (TU). The target oral dose of iron (Ferrous Sulfate syrup 40 mg/ml, 27.5% elemental iron, Laboratory and Allied Ltd, Nairobi, Kenya) was 3-6 mg/kg per day in the daily iron groups and 6-12 mg/kg per week in the intermittent iron groups (divided into two doses of 3-6 mg/kg each, separated by 3-4 days). Iron was dosed according to body weight (<5kg: 1.25ml/day, 5-10kg: 2.5ml/day, >10kg: 5.0ml/day). Caretakers were given the complete six-week supply of iron supplementation for their child, and participants in both the unsupervised and supervised arms received identical instructions in the local language with regard to the method of administration, expected side effects, safety and correct dose of iron supplementation. Plastic screw-cap bottles were used, labeled with personal identifiers and dosing instructions.

Children in the two supervised arms of the study were visited daily (DS) or twice-weekly (TS) for a period of six weeks by a trained study staff member who administered the iron. Unsupervised participants took the iron syrup home and were not visited except for health concerns upon the caretaker’s request.

**Study design.** The study was an un-blinded randomized trial using a 2x2 factorial design. Compounds were randomized so that all children in one compound were assigned to either a daily or twice-weekly regimen that was either supervised or unsupervised. A total sample size of 1,040 children was estimated to yield at least 80% power at 5% significance in order to detect a 5 g/L difference in mean hemoglobin at the end of intervention period between any of the treatment groups, accounting for 20% loss to follow-up, and assuming an average of 2 children per compound, and a design effect of two. Secondary endpoints included hematological recovery, microcytosis, all cause morbidity, clinical malaria and malaria parasitemia.

**Recruitment and randomization.** Following a census survey in October 2000, caretakers from all compounds containing households with children <5 years of age were invited for
screening to a central location in their respective villages. A brief questionnaire was completed, and axillary body temperature and anthropometric indices were measured. Capillary blood samples (0.5 mL) were collected by finger or heel prick into EDTA microtainer tubes (Becton-Dickinson, UK). During screening, hemoglobin concentrations were measured using a portable Hemocue system (HemoCue, AB, Angelholm, Sweden). Thick and thin malaria blood smears were collected. All children were examined by a clinical officer prior to enrollment. Acutely ill children, children with hemoglobin <50 g/L, or children with higher hemoglobin values who were clinically unstable, were referred to the hospital.

Following laboratory assessment, children meeting all of the following criteria were eligible for randomization: (1) Age 2-59 mo, (2) Coulter Counter Hemoglobin 50 to 109 g/L, (3) asexual parasite count <20,000 per mm³, (4) no history of intake of iron, sulfadoxine-pyrimethamine, or amodiaquine use, or blood transfusion within the last 2 weeks, (5) no known sickle cell disease. Children 2-6 months of age were included in accordance with the guidelines for the integrated management of childhood illness (IMCI) (42). A computer-generated random number listing was used to sequentially assign eligible children to one of four treatment groups, using the housing compound as the randomization unit. Balanced block randomization (12 compounds per block) ensured equal distribution in time and space among the four treatment groups. The allocation sequence was computer generated by FTK before the start of the study. Assignment to the study groups was independently conducted by MD.

Children fulfilling the entry criteria were approached the following day for clinical examination and a structured questionnaire was completed to record details of socio-economic and educational status of the caretaker. Health passports were issued allowing free health care at local clinics and the hospital.

**Follow-up.** Follow-up samples, smears and clinical information were collected at the end of the 6-week intervention period (+/- 1 day). To determine differences in the duration of any treatment effect on hemoglobin levels children were seen again at 12-weeks (+/- 1 day) at which time the same information was collected. At the 6-week follow-up, caretakers of participants enrolled to the two unsupervised groups were asked open-ended and prompted questions (yes/no/don't know) regarding adherence, whereas all caretakers were also elicited for prompted questions regarding perceived side effects in their children during the intervention period.

During the 12-week study period, a passive morbidity surveillance system was used to monitor the frequency of clinic and hospital attendance. Study staff members were assigned to each of 3 local clinics and the hospital. Caretakers were asked to report to the clinic with their child when suspecting illness where a morbidity questionnaire was filled and a medical examination performed. Children with symptomatic malaria (axillary temperature ≥37.5°C with any malaria parasitemia) detected at follow-up visits or through passive case detection, and those without fever but with
high-density parasitemia (> 5,000 /mm\(^3\)) were treated according to national guidelines. Children who developed severe anemia (hemoglobin <50 g/L) or presented with any other severe disease were referred to the hospital for further management. Their study drugs were discontinued as they all received daily iron from the hospital. They were, however, included in the analyses on an intention to treat basis. Children who were treated for other non-severe illnesses during the intervention period continued to receive study treatments. Children who were anemic at 12 weeks were given another dose of SP and 6 weeks of daily iron supplementation.

**Laboratory methods.** An ACT 10 Coulter Counter (Coulter Co., Florida, USA, Serial no. AD04108) was used to obtain hemoglobin and mean corpuscular volume (MCV). Thick blood smears were Giemsa stained and *Plasmodium* parasites were counted against 300 leukocytes/\(\mu\)L and densities presented per microliter assuming a leukocyte count of 8000/\(\mu\)L. Malaria parasitemia refers to the presence of any asexual *Plasmodium* species detected by microscopy. Serum samples were stored within 6 hours at -20°C. Hemoglobin genotype was determined by hemoglobin electrophoresis.

**Informed consent.** The study was approved by the institutional ethical review boards of the Kenya Medical Research Institute (KEMRI), Nairobi, Kenya by the Centers for Disease Control and Prevention (CDC), Atlanta, USA, and the Academic Medical Center, University of Amsterdam. Written informed consent was obtained from caretakers for each individual participant.

**Statistical analyses.** Z-scores for height-for-age (HAZ), weight-for-age (WAZ) and weight-for-height (WHZ) were calculated using Epi Info (v6, Atlanta, GA, USA). All analyses were conducted on an intention-to-treat basis. SUDAAN v8.0 was used for analysis of differences in proportions and medians (Research Triangle Institute), and all other analyses were conducted in SAS v8.0 (Cary, NC). The intra-class correlation coefficient (ICC) and related design effect were calculated from an unconditional means model.

Differences in mean hemoglobin (using the values obtained from the Coulter Counter®) after 6 and 12-weeks from enrollment were assessed by use of a linear regression model with repeated measures, adjusting the standard errors for clustering at the compound level. After assessment of the three-way interaction between supervision, iron regimen and time (\(P = 0.35\)), the final model had supervision, iron regimen, time and their two-way interaction terms as main effects, and continuous baseline hemoglobin and age as covariates. The analytical approach takes into account testing of two independent hypotheses: effect of supervision and dosing regimen. In studies using a factorial design, adjustment for testing two hypotheses is not recommended (46). However, because we made two comparisons for each treatment at two different time points (6 and 12 weeks) within each hypothesis, p-values have been adjusted
for multiple (four) testing, using a sequential Bonferroni method (47).

For the purpose of this study severe anemia is defined as a hemoglobin <50 g/L, moderate as a hemoglobin of 50-79 g/L and mild anemia as 80-109 g/L (40, 48-51). Microcytemia was defined as a MCV value below the age-specific cut-off: 0-5 months: 70fl, 6-11 months: 73fl, and greater than 12 months: 75fl (52). Hematological recovery (Hb ≥110 g/L for those with mild anemia at enrollment, and Hb ≥80 g/L for those with moderate anemia at enrollment), absence of microcytemia, and presence of malaria parasitemia at 6 and 12 weeks were compared using the chi-square test adjusting for clustering at the compound level. The incidences of all visits to the clinic, first or only episode of non-malaria morbidity (fever and negative malaria smear), or clinical malaria (fever and malaria parasitemia), respectively, were calculated based on time up to the episode, the end of the main intervention period (6-weeks), or loss to follow-up. Logistic regression was used to assess the impact of treatments on side effects with the presence or absence of the symptom on admission as a covariate.

Role of the funding source. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Based on census data from October 2000, 2,315 children aged 2-59 months were identified in 1,069 compounds. Consent to participate was obtained from 87% (2,052). Eighty five percent (1,748 of 2,052) were available for screening between 29th of November 2000 and 23rd of January 2001. Among those screened, the overall prevalence of severe, moderate and mild anemia was 0.69%, 14.7% and 58.9%, respectively, and children between 12 to 18 months of age had the lowest mean hemoglobin concentration (Figure 1). Of those screened, 1,065 children were initially determined to satisfy the enrollment criteria and were randomized to one of the four treatment groups; 16 of these were dropped before they received the first dose of iron because subsequent information revealed that they did not fulfill the entry criteria. The trial profile for the 1,049 study children (681 compounds) fulfilling entry criteria is shown in Figure 2 and their baseline characteristics in Table 1. The study groups were not different for any of the clinical, laboratory and socio-economic variables. The prevalence of stunting, wasting and underweight on enrollment was 31.0%, 7.1% and 23.2%, respectively. The mean weight at enrollment was 10.9 kg (range 3.2-20.0 kg) and the mean dose of elemental iron received was 4.04 (range of 2.75-5.47) mg/kg/day.

Loss to follow-up by 6 weeks was 8.9% (n=93) and equally divided among the four study groups (P = 0.51). This included three deaths: two due to severe malaria (hospital diagnosis, one DS and one TU), and one with unknown cause (TS). No other children were admitted to
Figure 1. Prevalence of anemia and mean hemoglobin concentrations by age category among 1748 children 2-59 months of age screened for enrollment into the anemia treatment study. n = 161 (2-5m), 198 (6-11m), 162 (12-17m), 192 (18-23m), 192 (24-29m), 166 (30-35m), 143 (36-41m), 187 (42-47m), 174 (48-53m), 173 (54-59m).

Table 1: Baseline characteristics of treatment groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DS (n=261)</th>
<th>DU (n=251)</th>
<th>TS (n=266)</th>
<th>TU (n=271)</th>
<th>F-test p-value&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
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<tr>
<td>Age (month); mean (SE)</td>
<td>27.8 (1.03)</td>
<td>28.7 (1.05)</td>
<td>28.2 (1.02)</td>
<td>26.9 (1.01)</td>
<td>0.66</td>
</tr>
<tr>
<td>Boys; No (%)</td>
<td>128 (49)</td>
<td>135 (54)</td>
<td>135 (51)</td>
<td>132 (49)</td>
<td>0.64&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caretaker’s education status&lt;sup&gt;5&lt;/sup&gt;; median years (interquartile range)</td>
<td>6.71 (5.66, 7.70)</td>
<td>6.88 (6.08, 7.70)</td>
<td>6.86 (5.89, 7.78)</td>
<td>6.76 (5.63, 7.85)</td>
<td>0.81&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wealth above median&lt;sup&gt;6&lt;/sup&gt;; No (%)</td>
<td>96/199 (48.2)</td>
<td>90/201 (44.8)</td>
<td>104/204 (51.0)</td>
<td>102/203 (50.3)</td>
<td>0.62&lt;sup&gt;5&lt;/sup&gt;</td>
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<tr>
<td><strong>Clinical</strong></td>
<td></td>
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</tr>
<tr>
<td>Weight for Age Z-score; mean (SE)</td>
<td>-1.20 (0.08)</td>
<td>-1.02 (0.08)</td>
<td>-1.10 (0.08)</td>
<td>-1.06 (0.08)</td>
<td>0.48</td>
</tr>
<tr>
<td>Height for Age Z-score; mean (SE)</td>
<td>-1.41 (0.09)</td>
<td>-1.35 (0.09)</td>
<td>-1.50 (0.09)</td>
<td>-1.33 (0.09)</td>
<td>0.57</td>
</tr>
<tr>
<td>Weight for Height Z-score; mean (SE)</td>
<td>-0.43 (0.07)</td>
<td>-0.21 (0.07)</td>
<td>-0.21 (0.07)</td>
<td>-0.32 (0.07)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
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<tr>
<td>Hemoglobin (g/L); mean (SE)</td>
<td>91.3 (0.79)</td>
<td>92.5 (0.80)</td>
<td>92.5 (0.78)</td>
<td>92.6 (0.77)</td>
<td>0.62</td>
</tr>
<tr>
<td>HbAS genotype; No (%)</td>
<td>74 (28)</td>
<td>74 (30)</td>
<td>57 (22)</td>
<td>61 (22)</td>
<td>0.23&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prevalence of malaria parasitemia; No (%)</td>
<td>157 (60)</td>
<td>143 (57)</td>
<td>158 (59)</td>
<td>169 (62)</td>
<td>0.74&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Parasite density (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>1948.0</td>
<td>2006.3</td>
<td>1504.2</td>
<td>2160.7</td>
<td>0.21</td>
</tr>
<tr>
<td>geometric mean [95% CI]</td>
<td>[1508.7, 2515.4]</td>
<td>[1537.4, 2618.8]</td>
<td>[1166.5, 1940.0]</td>
<td>[1687.3, 2766.3]</td>
<td></td>
</tr>
<tr>
<td>MCV &lt; cut-off for age&lt;sup&gt;7&lt;/sup&gt;; No (%)</td>
<td>142 (57)</td>
<td>142 (61)</td>
<td>162 (63)</td>
<td>159 (61)</td>
<td>0.63&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>DS = Daily Supervised; TS = Twice-a-week Supervised; DU = Daily Unsupervised; TU = Twice-a-week Unsupervised.
<sup>2</sup>adjusted for cluster design; <sup>3</sup>chi square test; <sup>4</sup>data based on number of households enrolled (n=808), not number of children; <sup>5</sup>Wilcoxon rank sum; <sup>6</sup>geometric mean parasite density in parasites per mm<sup>3</sup> for 627 parasitemic children; <sup>7</sup>data not available for <5% of children in each treatment group.
the hospital. Children lost to follow-up had significantly \((P = 0.01)\) lower hemoglobin concentrations at enrollment than those who were successfully followed for 6 weeks, but were not different for any other characteristics. None of the characteristics between treatment groups were significantly different after excluding children lost to follow-up (data not shown). Six children (4 compounds) were excluded from the analyses at the 6-week follow-up due to missing hemoglobin values. The average cluster size was 1.5 children per compound, and the design effect was small (1.035) indicating that the cluster design was 3.5% less efficient than a simple random sample.
Hematological response The interaction term between supervision (supervised versus unsupervised) and iron regimen (daily versus twice-weekly) was statistically significant ($P = 0.04$) and therefore all subsequent results are presented by the four treatment groups only. The interaction of time was not significant with either supervision ($P = 0.87$) or iron regimen ($P = 0.12$) but these terms were retained in the final model to allow determination of the duration of the treatment effect.

DS was associated with a significantly greater increase in hemoglobin at 6-weeks than TS and DU (Figure 3, and Table 2) indicating that both the frequency of iron dosing as well as supervision of the daily dose were significant determinants of treatment efficacy. In the unsupervised groups, the hemoglobin concentrations at 6-weeks were not different with daily and twice-weekly iron, but were maintained for longer in the daily group resulting in marginally higher hemoglobin concentrations at 12-weeks compared to the twice-weekly group. However this difference was not statistically significant ($P = 0.06$) after adjustment for multiple testing. Post-hoc assessment of the interaction between treatment effect and hemoglobin or age at enrollment indicated that the four-way interaction terms between supervision, iron regimen, time and hemoglobin at enrollment (< or $\geq$80 g/L) ($P = 0.48$), or age at enrollment (< or $\geq$18 months) (32) ($P = 0.20$), were not significant.

![Figure 3. Mean [95% CI] hemoglobin at enrollment (Day 0), 6 and 12 weeks from enrollment among preschool children receiving different iron supplementation regimens, western Kenya. Values at 6 and 12 weeks are modeled using linear regression adjusting for clustering at the compound level, continuous age and hemoglobin at enrollment; Comparisons between treatment groups at 6 and 12 weeks (shown in Table 2) were based on Wald chi-square statistic and are adjusted for multiple testing (47); DS= Daily Supervised; DU= Daily Unsupervised; TS= Twice-a-week Supervised; TU= Twice-a-week Unsupervised; n at 6 and 12 weeks, respectively: DS (238, 223), DU (226, 213), TS (250, 232), TU (237, 231); The P-values of the interaction terms in this model were: supervision by dose (0.04), supervision by time (0.87), dose by time (0.12). The initial model showed the 3-way interaction not to be significant (time by dose by supervision, $P=0.35$).}
Table 2: Difference in mean hemoglobin (g/L) at 6 and 12-weeks between treatment groups

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>At 6-weeks</th>
<th></th>
<th></th>
<th>At 12-weeks</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference [95% CI]</td>
<td>P-value</td>
<td>Difference [95% CI]</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS versus DU</td>
<td>3.0 [0.92, 5.1]</td>
<td>0.017</td>
<td>3.2 [0.92, 5.4]</td>
<td>0.017</td>
<td></td>
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<tr>
<td>DS versus TS</td>
<td>4.0 [2.0, 6.0]</td>
<td>0.0003</td>
<td>5.3 [3.1, 7.6]</td>
<td>0.0003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DU versus TU</td>
<td>1.1 [-0.95, 3.2]</td>
<td>0.29</td>
<td>2.5 [0.24, 4.7]</td>
<td>0.06</td>
<td></td>
<td></td>
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<tr>
<td>TU versus TS</td>
<td>-0.18 [-2.2, 1.8]</td>
<td>0.86</td>
<td>-0.32 [2.6, 1.9]</td>
<td>0.86</td>
<td></td>
<td></td>
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</tbody>
</table>

1DS= Daily Supervised; DU= Daily Unsupervised; TS= Twice-a-week Supervised; TU= Twice-a-week Unsupervised; n at 6 and 12 weeks, respectively: DS (238, 223), DU (226, 213), TS (250, 232), TU (237, 231); In each comparison, the first treatment group is compared in reference to the second treatment group. Difference in mean Hb (g/L) and corresponding 95% confidence intervals were obtained from linear regression models using repeated measures (n=1850), adjusting for clustering at the compound level, continuous age and hemoglobin at enrollment; P-values were obtained based on a Wald chi-square statistic and are adjusted for multiple testing (47); The P-values of the interaction terms in this model were: supervision by dose (0.04), supervision by time (0.87), dose by time (0.12). The initial model showed the 3-way interaction not to be significant (time by dose by supervision, P=0.35).

Overall, anemia had resolved (Hb ≥110 g/L) in 29.2% and 34.5% of the study children by 6 and 12 weeks respectively. Almost all children with moderate anemia (50-79 g/L) on enrollment had hemoglobin levels above 80 g/L by 6 weeks and 12 weeks (6 weeks: 98%, 86%, 79%, 90% and 12 weeks: 92%, 91%, 86%, 81% for DS, DU, TS, TU respectively). In those with mild anemia on enrollment, however, anemia had resolved in only a small proportion of children (6 weeks: 43%, 34%, 28%, 28% and 12 weeks: 46%, 41%, 34%, 29% for DS, DU, TS, TU respectively). The proportion of children experiencing hematological recovery was 54%, 42%,

Table 3: Hematological recovery and Mean Cell Volume ≥cut-off for age at 6 and 12-weeks by treatment group

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>At 6-weeks</th>
<th></th>
<th></th>
<th>At 12-weeks</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR [95% CI]</td>
<td>P-value</td>
<td>RR [95% CI]</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematological recovery*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS versus DU</td>
<td>1.25 [1.04, 1.51]</td>
<td>P = 0.04</td>
<td>1.09 [0.91, 1.30]</td>
<td>P = 0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS versus TS</td>
<td>1.36 [1.13, 1.64]</td>
<td>P = 0.0004</td>
<td>1.26 [1.04, 1.53]</td>
<td>P = 0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DU versus TU</td>
<td>1.11 [0.90, 1.38]</td>
<td>P = 0.19</td>
<td>1.26 [1.02, 1.56]</td>
<td>P = 0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TU versus TS</td>
<td>0.98 [0.79, 1.21]</td>
<td>P = 0.97</td>
<td>0.92 [0.74, 1.15]</td>
<td>P = 0.48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean cell volume ≥cut-off for age

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>At 6-weeks</th>
<th></th>
<th></th>
<th>At 12-weeks</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR [95% CI]</td>
<td>P-value</td>
<td>RR [95% CI]</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS versus DU</td>
<td>1.29 [1.05, 1.58]</td>
<td>P = 0.058</td>
<td>1.19 [0.94, 1.50]</td>
<td>P = 0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS versus TS</td>
<td>1.28 [1.04, 1.58]</td>
<td>P = 0.049</td>
<td>1.36 [1.05, 1.75]</td>
<td>P = 0.049</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DU versus TU</td>
<td>1.03 [0.82, 1.30]</td>
<td>P = 0.81</td>
<td>1.05 [0.79, 1.40]</td>
<td>P = 0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TU versus TS</td>
<td>0.97 [0.77, 1.22]</td>
<td>P = 0.78</td>
<td>1.08 [0.80, 1.47]</td>
<td>P = 0.78</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n at 6 and 12 weeks, respectively: DS (238, 223), DU (226, 213), TS (250, 232), TU (237, 231); In each comparison, the first treatment group is compared in reference to the second treatment group. RR [95% CI]: Relative Risk and 95% confidence intervals; P-values obtained from chi-square test were adjusted for clustering at the compound level and multiple testing (47); * defined as Hb ≥80 g/L for those with Hb 50-79 g/L at enrollment, and Hb ≥ 110 for those with Hb 80-109 g/L at enrollment.
Table 4: Malaria and all-cause morbidity from passive and active surveillance

<table>
<thead>
<tr>
<th></th>
<th>DS</th>
<th>DU</th>
<th>TS</th>
<th>TU</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause sick child visits&lt;sup&gt;1&lt;/sup&gt;</td>
<td>170 (6.46)</td>
<td>128 (5.02)</td>
<td>157 (5.91)</td>
<td>135 (5.07)</td>
</tr>
<tr>
<td>Non-malaria morbidity&lt;sup&gt;2&lt;/sup&gt;</td>
<td>118 (4.48)</td>
<td>101 (3.96)</td>
<td>121 (4.55)</td>
<td>97 (3.64)</td>
</tr>
<tr>
<td>Clinical malaria&lt;sup&gt;2&lt;/sup&gt;</td>
<td>14 (0.53)</td>
<td>22 (0.86)</td>
<td>19 (0.72)</td>
<td>27 (1.01)</td>
</tr>
<tr>
<td>Malaria parasitemia&lt;sup&gt;3&lt;/sup&gt;</td>
<td>88/239 (36.8)</td>
<td>94/228 (41.2)</td>
<td>89/249 (35.7)</td>
<td>109/240 (45.4)</td>
</tr>
<tr>
<td>6-12-weeks</td>
<td>DS</td>
<td>DU</td>
<td>TS</td>
<td>TU</td>
</tr>
<tr>
<td>All-cause sick child visits&lt;sup&gt;1&lt;/sup&gt;</td>
<td>136 (5.21)</td>
<td>128 (5.20)</td>
<td>132 (4.94)</td>
<td>149 (5.53)</td>
</tr>
<tr>
<td>Non-malaria morbidity&lt;sup&gt;2&lt;/sup&gt;</td>
<td>85 (3.21)</td>
<td>79 (3.16)</td>
<td>85 (3.01)</td>
<td>91 (3.28)</td>
</tr>
<tr>
<td>Clinical malaria&lt;sup&gt;2&lt;/sup&gt;</td>
<td>17 (0.66)</td>
<td>16 (0.65)</td>
<td>21 (0.76)</td>
<td>20 (0.73)</td>
</tr>
<tr>
<td>Malaria parasitemia&lt;sup&gt;3&lt;/sup&gt;</td>
<td>82/219 (37.4)</td>
<td>82/207 (39.6)</td>
<td>86/221 (38.9)</td>
<td>86/215 (40.0)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Number of visits (incidence reported as visits per child year); <sup>2</sup>Number of first time episodes (incidence reported as episodes per child year); <sup>3</sup>n/N (%) at 6 and 12 weeks; None of the differences between treatment groups were statistically significant (P > 0.05) after adjustment for multiple comparisons.

36%, and 36% at 6 weeks, and 55%, 49%, 42%, and 36% at 12 weeks for DS, DU, TS, TU respectively. Hematological recovery in the DS regimen was significantly higher than in the TS group (Table 3). Similarly, the probability of recovery was significantly higher in the DU compared to the TU group at 12-weeks, but not at 6-weeks (Table 3). Children in the DS group were less likely to be microcytemic at 6 and 12-weeks than the TS group.

**Morbidity and adverse effects** The incidence of all-cause sick child visits, clinical malaria, and non-malaria morbidity reported to the three clinics and the local hospital were not different between the daily and twice weekly treatment groups by 6 and 12 weeks (Table 4). Similarly the prevalence of malaria parasitemia was not different between daily and twice-weekly iron groups at 6 and 12-weeks.

None of the children were reported to have been overdosed (e.g. drunk the bottle). There were no statistically significant differences between any of the treatment groups in reported signs or symptoms at 6-weeks, when controlling for signs or symptoms at enrollment. Reports of dark stool increased in all four treatment groups, with the greatest increase in the DS and DU groups. Reports of body rash and vomiting decreased in all four groups, whereas that of respiratory tract infections, difficulty breathing, coughing, and diarrhea increased in all four groups. Reports of poor appetite decreased in the daily groups, but increased in the twice-weekly groups. The prevalence of constipation was not assessed on enrollment, but was commonly reported at 6 weeks (range DS=27% to TU=31%) although it was not significantly different between the treatment groups. Teeth staining, assessed by open-ended questions only, was reported once (DU regimen). In the unsupervised groups, 69 (15.1%) respondents admitted skipping doses or prematurely stopping supplementation of iron to their child (56.5% in the DU regimen, and 43.5% in the TU regimen). In the DU regimen, the most common
reasons were: vomiting (n=22), dark stool (n=6) and confusion with other drugs (n=2). In the 
TU group, vomiting (n=19), dark stool (n=5) and loss of appetite (n=3) were cited. None of the 
differences were statistically significant.

Discussion

Among children 2-59 months of age with mild to moderate anemia who received an initial 
antimalarial treatment (sulfadoxine-pyrimethamine), those receiving daily iron supplementation 
for 6 weeks experienced significantly greater hematological improvement over 12 weeks of 
follow-up than those receiving twice-weekly iron. A smaller difference was observed when 
iron was not given as directly observed therapy.

One of the main reasons that intermittent iron is considered in the control of anemia is the 
proposed advantage that less frequent dosing results in fewer side effects and may improve 
compliance. Of note is that Beaton et al. indicate that 'supervision' as judged by the reviewers 
on the subjective impression of “control” in each of the clinical trials was an important predictor 
of post-intervention anemia prevalence, and more so in the intermittent than in the daily group 
(32). It is likely that skipping of twice-weekly or weekly dosing will have a greater relative 
impact than skipping one of the daily doses. Our data indeed confirm that difference in 
adherence between the daily and twice-weekly regimens was an important determinant of 
treatment efficacy, as evidenced by the significant effect modification (P = 0.04) observed 
between the effects of dose regimens and the degree of supervision. It was in the daily iron 
regimen, however, and not in the twice-weekly group, that a substantial beneficial effect was 
achieved by administering the dose as directly observed therapy. This could not be explained 
by a difference in side effects as the prevalence of gastro-intestinal complaints as perceived by 
the care-taker did not differ between the four groups.

The lack of difference in hematological response between the twice-weekly supervised and 
unsupervised groups suggests that low-compliance was not a major obstacle to intermittent 
dosing in this study. The relative short duration of the intervention may have contributed to 
the high compliance. A less likely explanation, which can not be excluded in the absence of a 
placebo control group, is that neither group benefited from twice-weekly iron supplementation 
and that all improvements in hemoglobin were due to the effects of antimalarial therapy 
received on enrollment, seasonal trends, or resulted from regression towards the mean. However, 
there is ample evidence suggesting that intermittent iron supplementation is efficacious under 
favorable conditions (15, 16, 19, 20, 28, 29, 32, 53).

The majority of the previous studies failed to show a substantial benefit from daily over 
intermittent iron in young children (32). The reason for this lack of empirical difference between 
regimens has been unclear. It may reflect a reduction in iron absorption in response to relatively
high intake of daily iron (54), but, the existence of a 'mucosal block' in humans has been strongly disputed (26). It has been suggested that the previous studies were unlikely to detect a difference since they evaluated treatment responses at the end of long intervention periods (≥ 8 weeks) in subjects with predominantly mild iron deficiency and low grade anemia, eventually resulting in the same optimal hemoglobin levels regardless of the dose schedule used (26). Our study provides further support for the lack of a functional mucosal block in humans. We may have had a greater chance of detecting a difference between daily and twice-weekly iron due to the inclusion of mild as well as moderately anemic children and by addressing malaria as the main other cause of anemia. We also evaluated a shorter course of iron than previous studies. Beaton et al, however, suggest that the relative difference between efficacy of weekly and daily iron appears to increase, rather than decrease, with duration of intervention (32).

Despite the relatively short duration of iron supplementation we observed marked increases in hemoglobin by six weeks in all four groups (Figure 3). Almost all children (88%) with moderate anemia (50-79 g/L) on enrollment had hemoglobin levels above 80 g/L by six weeks, including those randomized to the twice-weekly groups (84%). The majority of all children enrolled in the study, however, had failed to resolve their anemia (Hb <110 g/L; 62% in the supervised daily group), suggesting that longer supplementation is required to increase the overall efficacy. The short course of iron was chosen due to the uncertainty regarding the safety of long-term regimens in this area with intense malaria transmission (33). We found no difference between the four treatment groups in the risk of clinical malaria or malaria parasitemia during the intervention period, or the six weeks thereafter. In a recent randomized controlled companion study we also found no indication that a longer regimen (3-6 mg/kg/day for 12 weeks) was associated with a clinically relevant increase in the risk of malaria compared with iron-placebo (55). This, together with the limited efficacy of the 6-week regimen, suggests that the benefits of 12 weeks of iron supplementation in children with anemia are likely to outweigh any potential associated adverse effects caused by increased risk of malaria in this area. This is consistent with current recommendations from the International Nutritional Anemia Consultative group (INACG) (56, 57). Other causes of anemia should also be considered in order to maximize hematological recovery, including micronutrient deficiencies other than iron, and chronic inflammation unrelated to malaria, (51, 58-60). In addition, the high prevalence of HIV in this area of Kenya likely contributes to childhood anemia (61).

This study suggests that daily iron regimens when given as directly observed therapy, and possibly under unsupervised conditions, are superior to twice-weekly dosing in the treatment of anemia in children <5 years of age. It also suggests that unlike with daily iron, little additional hematological benefit can be expected from giving six weeks of twice-weekly iron as directly observed therapy. We conclude that similar to earlier recommendations for pregnant women (32), daily dosing should be the regimen of choice in the treatment of mild and moderate
anemia in pre-school children regardless of the level of compliance that can be ensured.

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