EFFECT OF PLASMODIUM FALCIPARUM PARASITEMIA DENSITY ON HEMOGLOBIN CONCENTRATIONS AMONG FULL-TERM, NORMAL BIRTH WEIGHT CHILDREN IN WESTERN KENYA, IV. THE ASEMBO BAY COHORT PROJECT

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Abstract. The relative importance of acute high-density versus persistent low-density Plasmodium falciparum parasitemia in contributing to the public health problem of malarial anemia remains unclear. The Asembo Bay Cohort Project in western Kenya collected monthly hemoglobin (Hb) and parasitologic measurements and biweekly assessments of antimalarial drug use among 942 singleton live births between 1992 and 1996. A mixed-model analysis appropriate for repeated measures data was used to study how time-varying parasitemia and antimalarial drug exposures influence mean Hb profiles. Incidence of World Health Organization-defined severe malarial anemia was 28.1 per 1,000 person-years. Among children aged less than 24 months, concurrent parasitemia was significantly associated with lower mean Hb, especially when compared to children with no concurrent parasitemia. Increased densities of the 90-day history of parasitemia preceding Hb measurement was more strongly associated with mean Hb levels than concurrent parasitemia density. While the highest quartile of 90-day parasitemia history was associated with lowest mean Hb levels, children in the lowest 90-day exposure quartile still experienced significantly lower Hb levels when compared to children who remained parasitemia-free for the same 90-day period. The results highlight the importance of collecting and analyzing longitudinal Hb and parasitologic data when studying the natural history of malarial anemia.

INTRODUCTION

The prevalence of Plasmodium falciparum parasitemia in young children may exceed 70% in many African regions.1-5 In areas with intense P. falciparum transmission, only a small proportion of infections ultimately manifest as severe disease.6 In these areas, severe malarial anemia (SMA) is the primary presentation of serious malarial disease among children less than 2 years of age.7,8 However, parasitemia frequently remains asymptomatic and thus untreated for prolonged periods. The role that untreated parasitemia plays in the maintenance of clinical immunity, and in the pathogenesis of malarial anemia, remains unclear.9 Malarial anemia associated with both high- and low-density parasitemia has been described.10,11 While higher-density is associated with symptoms (fever) and acute anemia, asymptomatic lower-density parasitemia may also result in anemia, particularly if the parasitemia persists for prolonged periods upon lack of, or ineffective, treatment.12-14

Renewed interest in the relationship between parasitemia dynamics and anemia has evolved in response to control methods that alleviate parasitemia in high transmission areas, without necessarily preventing it. Lower parasitemia may ultimately be achieved through use of insecticide-impregnated bednets or, in the future, vaccines.15,16 However, the omnipresence of parasitemia in intense transmission areas has complicated the study of how this exposure generates anemia in young children. Community-based rates of SMA (parasitemia > 10,000/µl and Hb < 5 g/dl) are also lacking for most transmission settings.17 To explore how the dynamic nature of parasitemia and antimalarial drug exposures influence Hb in young children, we have analyzed over 14,000 repeated Hb observations from children under four years of age living in an area with intense, perennial P. falciparum transmission. This study was part of the Asembo Bay Cohort Project in western Kenya; a longitudinal study of the epidemiology and immunology of P. falciparum infection and associated morbidity and mortality in pregnant women and their children.18

METHODS

Data were collected between June 1992 and October 1996 as part of the Asembo Bay Cohort Project (ABCP). Institutional review boards from the Centers for Disease Control and Prevention and the Kenya Medical Research Institute approved the study protocol. Informed consent was obtained from all adult participants and from parents or legal guardians of minors. The procedures for enrollment of pregnant women and subsequent delivery and follow-up of their infants have been detailed.18,19 Each infant was visited by an ABCP village monitor (VM) every 2 weeks after birth. A questionnaire ascertained presence and duration of malaria symptoms, use of medications, and attendance at health facilities since the previous visit. Axillary body temperature was measured and recorded. A thick blood film was obtained from any child with axillary temperature ≥ 37.5°C. At monthly intervals the same clinical data were collected, plus height, body weight, and a capillary blood specimen for Hb measurement and thick blood film preparation. At any time a mother could notify the VM of a sick child and have his/her axillary temperature measured. If ≥ 37.5°C, a blood film was obtained. Follow-up continued until the end of the study, a child’s death, migration, or refusal to participate.

Data concerning hookworm were not collected during the 1992–1996 study period, but a 1999 survey of the same study area found prevalence of hookworm infection to range from 2.3% in infants to 15.1% among children 12 to 35 months of age (ter Kuile FO, unpublished data).
Laboratory procedures. Microscopy. Thick blood films were Giemsa-stained and examined for the presence of plasmodia with a 100× oil immersion objective and 10× ocular. The number of parasites per 300 leukocytes was used to estimate asexual peripheral parasitemia density (assumed leukocyte density of 8,000/μL).28 Two hundred fields were examined before identifying a slide as “negative.” The lower detection limit was approximately four asexual forms/μL.

Hemoglobin. Hb (g/dl) was measured using the Hemocue system.21 (Use of trade names is for identification purposes only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.) This method uses the principle of hemoglobin oxidation to hemiglobin by sodium nitrite, and subsequent conversion of hemiglobin to hemiglobinazide with sodium azide. Standards of known Hb concentration and control specimens were included with each batch of 100 samples.

Assessment of exposure to antimalarials. Children potentially received several different antimalarial treatments from either ABCP staff or household members. A single dose of sulfadoxine/pyrimethamine (SP) was administered by the VM for each episode of axillary temperature elevation to hemiglobinazide with sodium azide. Standards of known Hb concentration and control specimens were included with each batch of 100 samples. SP treatment failures were treated with halofantrine (8 mg/kg).

Chloroquine (CQ), quinine, and traditional therapy were the primary household-administered treatments. Four different brands of CQ (150 mg CQ base) retailcd in Asembo Bay: Homaquine, Nivaquine, Dawaquine, Malaraquine. Oral quinine was available from local health dispensaries and clinics. Approximately 12 different locally-derived materials were identified as common traditional therapies. Traditional therapies consisted of roots and barks readily available at shops and open-air markets.

Two approaches were used to increase the validity of reported exposure to household-administered therapies. First, the VM asked if any medications had been administered to the child since the last visit. A “yes” response prompted further questioning to specifically identify the product(s). Specific drug names were recorded on the clinical data collection form. Directed questions regarding use of CQ, quinine, and traditional therapy were also asked. Mothers were asked, brand by brand, if a CQ product had been given since the previous visit, with yes or no responses recorded. No attempt was made to establish exact dosage or timing of household-administered treatments. Biochemical verification of reported antimalarial use was not employed.

Covariates: parasitemia, antimalarial treatment, maternal and child factors. To evaluate the effect of concurrent parasitemia exposures on mean Hb, continuous (log_{10} transformed) and categorical variables for parasitemia density were used. Categorical levels of concurrent parasitemia were determined from quartiles of the cumulative distribution of positive parasitemias observed in this study population (Figure 1). History of parasitemia exposure preceding each Hb measurement was evaluated by creating various exposure windows starting with the concurrent observation and increasing the width of time included (30, 60, 90, and 120 days). The geometric mean density of the blood film results was then calculated for each period.

Similarly, the effect of each antimalarial therapy on mean Hb was determined by creating various windows of duration since each child’s last drug exposure (<15, 15–29, 30–45, 46–60, 60–90, and 90–120 days). A variable was also derived for each child’s cumulative number of antimalarial exposures (drug-specific and combined) between birth and each successive monthly Hb.

Based upon analyses presented in an earlier paper,19 additional covariates were controlled for in all multivariate models. This included four time-stationary covariates: maternal peripheral parasitemia at birth, child’s sex, birth quarter, village of residence; and a time-varying covariate: growth status at each monthly visit (<10th percentile, normal, >90th percentile weight-for-age).

Analysis. A World Health Organization (WHO)-defined case of severe malarial anemia (SMA) included episodes of Hb < 5.0 g/dl with concurrent P. falciparum parasitemia density > 10,000/μl (blood films were not identified as normocytic or otherwise).22 This restrictive definition of SMA excludes life-threatening Hb accompanied by lower P. falciparum parasitemia at time of presentation.23 A more inclusive definition has included Hb < 5.0 g/dl in the presence of any-level parasitemia.24 Incidence rates and cumulative incidence estimates (attack rates) were calculated for 6-month age intervals. Only a child’s first episode of severe anemia was included in each 6-month interval.

Our analytic model used to examine the effect of parasitemia and other covariates on mean Hb profiles has been recently presented.19 Briefly, a mixed model analysis (SAS procedure PROC MIXED) accommodates the within-subject correlation present among repeated Hb measures contributed by each child, and permits simultaneous analysis of both time-stationary and time-varying covariates.25 To control for the confounding effect of pre-term delivery on infant Hb levels, analyses were limited to full-term (≥ 37 weeks gestation), normal birth weight (≥ 2,500 g) children. A preliminary unadjusted analysis of each parasitemia and antima-
Parasitemia density‡

High-density parasitemia (detectable parasitemia/H11021 exceeded 100,000/H9262 were P. falciparum in months of age, respectively. Most blood

laria exposure on mean Hb was conducted by including each variable separately into the model. Multivariate models were then constructed by adding each significant covariate from the crude analysis. Interactions between age and important covariates were also tested.

RESULTS

Parasitemia exposures over time. A total of 16,554 blood films (excluding SP treatment follow-up) were obtained during the longitudinal follow-up of 942 full-term, normal birth weight children born between 1992 and 1996. Most blood films (86.4%) were collected at the routine monthly visits. The mean time between birth and first detectable P. falciparum parasitemia (n = 823) and first detectable parasitemia ≥ 5,000/μl (n = 696) was 3.4 months (95% CI, 3.2–3.7) and 5.0 months (95% CI, 4.7–5.3), respectively. Ninety-five percent of first detectable parasitemias, or parasitemias ≥ 5,000/μl, occurred within 7 and 10 months of age, respectively.

Table 1 summarizes the 10,550 parasitemia exposures across age. The cumulative number of P. falciparum-positive blood films per 100 person-months remained stable between seven and 48 months of age. However, the cumulative number of high-density exposures per 100 person-months was highest among children seven to 24 months. The highest geometric mean parasitemia occurred in the 7 to 12-month age group. Ninety-five percent of all positive blood films were < 32,000/μl, 99% < 60,000/μl, and only 0.07% exceeded 100,000/μl (maximum density = 237,680/μl).

Antimalarial exposures over time. Age-specific antimalarial treatments given during follow-up are summarized in Table 2. The ABCP treatment protocol resulted in 2,843 SP doses for clinical malaria episodes during the 4-year study, with at least one dose administered to 669 (71%) children. Mean age at first SP treatment (i.e., first clinical malaria episode) was 6 months (95% CI, 5.5–6.3). The rate of SP treatment per 100 person-months was highest in the 7 to 12-month age group, and declined by 34%, 55%, and 69% in the second, third, and fourth years of life, respectively (all significant at P < 0.001).

Household-administered antimalarials are also summarized in Table 2. CQ was the most commonly reported antimalarial exposure, with 4,007 treatments administered over the 4-year period. Most children (85%) received at least one CQ exposure during follow-up. CQ treatments per 100 person-months were highest in the 7 to 12-month age group (32.7 per 100 person-months), and declined by 24%, 43%, and 50% in the second, third, and fourth years of life, respectively (all significant at P < 0.001). Quinine exposures were too rare to determine valid trends across age, but the data suggest a slightly higher exposure in the 7 to 12-month age group. Traditional therapies were a relatively common household exposure. A total of 3,242 traditional therapy treatments were reported, with 85% of children having received at least one treatment. As with the other antimalarials, exposures to traditional therapies declined after infancy.

Incidence of SMA. For purposes of international comparison, all 17,002 monthly Hb observations contributed by the first 1,166 singleton delivery contributions were considered (942 normal births, 76 full-term low birth weight, 20 pre-term low-birth weight, 19 pre-term normal birth weight, and 109 children with missing data for either variable). One-hundred fifty episodes of Hb < 5.0 g/dl were contributed by 127 children, and 86% had concurrent parasitemia. Table 3 presents crude estimates of SMA incidence for two case-

### Table 1

Asexual peripheral *Plasmodium falciparum* parasitemia exposures during longitudinal follow-up of 942 study participants, June 1992–August 1996, Asembo Bay Cohort Project, western Kenya

<table>
<thead>
<tr>
<th>Age (mo.)</th>
<th>No. children present</th>
<th>Person-months contributed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–6</td>
<td>942†</td>
<td>4.2431</td>
</tr>
<tr>
<td>7–12</td>
<td>671</td>
<td>3.074</td>
</tr>
<tr>
<td>13–24</td>
<td>519</td>
<td>4.642</td>
</tr>
<tr>
<td>25–36</td>
<td>323</td>
<td>2.744</td>
</tr>
<tr>
<td>37–48</td>
<td>167</td>
<td>1.112</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total blood film observations</th>
<th>4,665</th>
<th>3,603</th>
<th>4,527</th>
<th>2,728</th>
<th>1,031</th>
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</thead>
<tbody>
<tr>
<td>Any-density parasitemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total no. of events</td>
<td>1,902</td>
<td>2,380</td>
<td>3,278</td>
<td>2,195</td>
<td>795</td>
</tr>
<tr>
<td>events/100 person-mo.</td>
<td>44.8</td>
<td>77.4</td>
<td>70.6</td>
<td>79.9</td>
<td>71.5</td>
</tr>
<tr>
<td>maximum events per child</td>
<td>7</td>
<td>7</td>
<td>15</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>median events per child</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>interquartile range</td>
<td>1–3</td>
<td>2–4</td>
<td>3–9</td>
<td>3–10</td>
<td>2–7</td>
</tr>
<tr>
<td>% of blood films with event</td>
<td>40.8</td>
<td>66.1</td>
<td>72.4</td>
<td>80.5</td>
<td>77.1</td>
</tr>
<tr>
<td>High-density parasitemia (&gt;5000/μl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total no. of events</td>
<td>793</td>
<td>1,154</td>
<td>1,569</td>
<td>851</td>
<td>220</td>
</tr>
<tr>
<td>events/100 person-mo.</td>
<td>18.7</td>
<td>37.5</td>
<td>33.8</td>
<td>31.0</td>
<td>19.8</td>
</tr>
<tr>
<td>maximum events per child</td>
<td>4</td>
<td>7</td>
<td>12</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>median events per child</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>interquartile range</td>
<td>0–1</td>
<td>1–3</td>
<td>1–4</td>
<td>1–4</td>
<td>0–2</td>
</tr>
<tr>
<td>% of blood films with event</td>
<td>17.0</td>
<td>32.0</td>
<td>34.7</td>
<td>31.2</td>
<td>21.3</td>
</tr>
<tr>
<td>Parasitemia density‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>geometric mean</td>
<td>2,489</td>
<td>3,656</td>
<td>3,548</td>
<td>2,818</td>
<td>1,950</td>
</tr>
<tr>
<td>maximum</td>
<td>168,993</td>
<td>118,133</td>
<td>237,680</td>
<td>86,133</td>
<td>79,893</td>
</tr>
<tr>
<td>median</td>
<td>3,360</td>
<td>4,693</td>
<td>4,640</td>
<td>3,333</td>
<td>2,187</td>
</tr>
<tr>
<td>interquartile range</td>
<td>640–10,587</td>
<td>1,280–13,147</td>
<td>1,413–11,307</td>
<td>1,307–8,107</td>
<td>720–5,467</td>
</tr>
</tbody>
</table>

* Number of children contributing follow-up time within each age interval.
† Total number of person-months contributed by enrollees within each age interval.
‡ Peripheral parasitemia density (asexual forms/μl). Figures include positive blood film results only.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>1–6</th>
<th>7–12</th>
<th>13–24</th>
<th>25–36</th>
<th>37–48</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. children present</td>
<td>942*</td>
<td>671</td>
<td>519</td>
<td>323</td>
<td>165</td>
</tr>
<tr>
<td>Person-months contributed</td>
<td>4,243</td>
<td>3,074</td>
<td>4,642</td>
<td>2,744</td>
<td>1,112</td>
</tr>
</tbody>
</table>

**Antimalarial drug exposures**

**Project-administered total treatments**

- Sulphadoxine/pyrethamethine: 615 tx/100 person-mo., 884 maximum per child, 886 median per child, 357 interquartile range
- Chloroquine: 1,147 tx/100 person-mo., 1,005 maximum per child, 1,159 median per child, 515 interquartile range
- Quinine: 31 tx/100 person-mo., 1.1 maximum per child, 5 median per child, 3 interquartile range
- Traditional remedies: 1,086 tx/100 person-mo., 853 maximum per child, 904 median per child, 326 interquartile range

**Household-administered total treatments**

- Chloroquine: 27.0 tx/100 person-mo., 32.7 maximum per child, 25.0 median per child, 18.8 interquartile range
- Quinine: 0.7 tx/100 person-mo., 1.1 maximum per child, 1.6 median per child, 0.5 interquartile range

* Number of children contributing follow-up time within each age interval
† Total number of person-months contributed by enrollees within each age interval

**EFFECT OF P. FALCIPARUM ON HEMOGLOBIN**

The 942 children contributed 14,317 Hb observations between birth and cessation of follow-up. Details regarding the overall mean follow-up time and mean Hb profile for this population have been described. A negative concurrent blood film was consistently associated with higher mean Hb (P < 0.0001) when compared to even the lowest parasitemia levels. Concurrent parasitemia, included as a categorical time-varying (monthly) variable and adjusted for other covariates, was strongly associated with lower mean Hb (P < 0.0001) between birth and 24 months of age (Figure 2). However, in children under 18 months of age the mean Hb profiles in the three highest parasitemia quartiles were significantly lower than for children in the 1–999/µL category, yet no significant differences in mean Hb were detected among the three highest categories of concurrent parasitemia presented in Figure 2.

The effect of low versus high concurrent parasitemia density on mean Hb was still difficult to discern when the log-transformed density values were analyzed as a continuous time-varying (monthly) covariate (Figure 3). All levels concurrent parasitemia were again associated with decreased mean Hb levels (P < 0.0001) when compared to mean Hb in the presence of a concurrently negative blood film. However, significant differences in the effect of concurrent parasitemia levels were only evident for the very lowest (10th percentile) and very highest (90th percentile) exposures.
TABLE 3.
Incidence of two different definitions of severe malarial anemia (SMA) among all children (including low birth weight and pre-term deliveries) enrolled in the Asembo Bay Cohort Project in western Kenya, June 1992 to August 1996

A. Severe malarial anemia (Hb <5.0 g/dl and parasitemia >10,000/μl)

<table>
<thead>
<tr>
<th>Age (mo.)</th>
<th>No.</th>
<th>SMA cases*</th>
<th>Person-months</th>
<th>Incidence rate†</th>
<th>(95% CI)</th>
<th>Attack rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6</td>
<td>1,166</td>
<td>13</td>
<td>5,111</td>
<td>30.5</td>
<td>(14.0–47.1)</td>
<td>1.1</td>
</tr>
<tr>
<td>7–12</td>
<td>799</td>
<td>14</td>
<td>3,570</td>
<td>47.0</td>
<td>(22.5–71.7)</td>
<td>1.8</td>
</tr>
<tr>
<td>13–18</td>
<td>619</td>
<td>7</td>
<td>2,756</td>
<td>30.4</td>
<td>(7.9–53.0)</td>
<td>1.1</td>
</tr>
<tr>
<td>19–24</td>
<td>467</td>
<td>4</td>
<td>2,130</td>
<td>22.5</td>
<td>(0.5–44.6)</td>
<td>0.9</td>
</tr>
<tr>
<td>25–30</td>
<td>387</td>
<td>2</td>
<td>1,730</td>
<td>13.9</td>
<td>(0.0–33.0)</td>
<td>0.5</td>
</tr>
<tr>
<td>31–36</td>
<td>271</td>
<td>1</td>
<td>1,161</td>
<td>10.3</td>
<td>(0.0–30.6)</td>
<td>0.4</td>
</tr>
<tr>
<td>37–42</td>
<td>187</td>
<td>0</td>
<td>687</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>43–48</td>
<td>109</td>
<td>0</td>
<td>364</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

* Figures do not include more than one episode per child per 6-month interval. Four children experienced two episodes of SMA.
† WHO-defined cases of SMA per 1,000 person-years observation. CI = confidence interval.

B. Severe malarial anemia (Hb <5.0 g/dl and any-density parasitemia)

<table>
<thead>
<tr>
<th>Age (mo.)</th>
<th>No.</th>
<th>SMA cases*</th>
<th>Person-months</th>
<th>Incidence rate†</th>
<th>(95% CI)</th>
<th>Attack rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤6</td>
<td>1,166</td>
<td>40</td>
<td>5,030</td>
<td>95.5</td>
<td>(66.0–124.9)</td>
<td>3.4</td>
</tr>
<tr>
<td>7–12</td>
<td>799</td>
<td>31</td>
<td>3,519</td>
<td>105.8</td>
<td>(68.6–142.8)</td>
<td>3.9</td>
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<td>13–18</td>
<td>619</td>
<td>17</td>
<td>2,726</td>
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<td>(39.4–110.3)</td>
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<td>19–24</td>
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<td>10</td>
<td>2,112</td>
<td>56.8</td>
<td>(21.7–92.0)</td>
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<td>387</td>
<td>15</td>
<td>1,691</td>
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<td>(52.8–160.1)</td>
<td>3.9</td>
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<tr>
<td>31–36</td>
<td>271</td>
<td>7</td>
<td>1,143</td>
<td>73.7</td>
<td>(25.4–145.4)</td>
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<tr>
<td>37–42</td>
<td>187</td>
<td>0</td>
<td>687</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>43–48</td>
<td>109</td>
<td>0</td>
<td>364</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

* Figures do not include more than one episode per child per 6-month interval. Six children experienced two SMA episodes and three children experienced three SMA episodes during their entire duration of follow-up.
† SMA cases per 1,000 person-years observation. CI = confidence interval. na = not applicable.

The current force of *P. falciparum* infection in western Kenya is formidable. Despite being born to mothers with life-long exposure to *P. falciparum*, half of the ABCP children became parasitemic by 3 months of age (75% within 4 months). Data collected from the same area nearly a decade earlier reflect a similar force of infection among children 6 to 60 months of age.34,35 Successful control strategies will need to take into account this early exposure to parasitemia and high rate of reinfection following parasite clearance with antimalarials. Even in this study, where children received SP therapy upon diagnosis of parasitemia and fever, it was rare (<5%) for a child to remain parasitemia-free for more than 3 months.

An important step in understanding the epidemiology of severe malaria morbidity is to quantify the incidence of specific clinical outcomes in various transmission settings throughout Africa.17 This task has not been frequently undertaken outside of hospital settings. We used the ABCP data to obtain the first community-based estimates of WHO-defined SMA incidence. These are likely minimal estimates of SMA incidence since active surveillance and prompt SP treatment upon diagnosis of fever with any-density parasitemia almost certainly reduced the likelihood of persistent mean of the log-transformed parasitemia observations during the preceding 90-day period had the strongest effect on mean Hb. Children with higher mean densities of parasitemia during the preceding 90-day period had significantly lower Hb concentrations, particularly in the first year of life (P < 0.0001). The effect of a 90-day parasitemia-free period and various levels (quartiles) of 90-day parasitemia exposure history is presented in Figure 4. While a higher 90-day history of parasitemia was associated with lower mean Hb, children with the lowest exposure still had significantly lower mean Hb values when compared to the 90-day parasitemia-free group.

**DISCUSSION**

The current force of *P. falciparum* infection in western Kenya is formidable. Despite being born to mothers with life-long exposure to *P. falciparum*, half of the ABCP children became parasitemic by 3 months of age (75% within 4 months). Data collected from the same area nearly a decade earlier reflect a similar force of infection among children 6 to 60 months of age.34,35 Successful control strategies will need to take into account this early exposure to parasitemia and high rate of reinfection following parasite clearance with antimalarials. Even in this study, where children received SP therapy upon diagnosis of parasitemia and fever, it was rare (<5%) for a child to remain parasitemia-free for more than 3 months.

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**FIGURE 2.** Effect of concurrent categorical levels of parasitemia density on mean hemoglobin (Hb) over time. Model adjusted for presence of maternal parasitemia at birth, sex, village of residence, and weight-for-age.
high-density parasitemia. Conversely, the repeated Hb observations on each child, over time, resulted in a greater likelihood of capturing SMA events. These data provide a more valid estimate of SMA than simple cross-sectional data. These estimates will assist future comparisons of rates of severe malaria morbidity in communities with varying \textit{P. falciparum} transmission intensity.\cite{24}

The pathogenesis of anemia is multifactorial, particularly among disadvantaged populations of children. Even the anemia associated with \textit{Plasmodium falciparum} alone is quite complex and poorly understood. Malarial anemia may result from direct destruction of parasitized erythrocytes.\cite{28,30} However, the degree of anemia that often develops in parasitemic individuals cannot be accounted for solely by hemolysis of parasitized erythrocytes.\cite{9} Immune-mediated destruction of non-parasitized erythrocytes (via binding of complement factor) and decreased deformability of these cells followed by splenic removal are other mechanisms, and may contribute more to malarial anemia than destruction of parasitized erythrocytes.\cite{29,30} A third etiology may be inadequate bone marrow response due to persistent low-grade parasitemia and cytokine production.\cite{31}

The lack of, or in some instances modest association between increased gradients of parasitemia density and reduced Hb in some previous hospital-based studies is possibly due to the simultaneous collection of these two parameters at time of admission.\cite{32,34} The ABCP data demonstrate that while presence of concurrent parasitemia in children under 24 months of age is associated with lower mean Hb, differential increases in level of concurrent parasitemia density

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Effect of parasitemia density on concurrent hemoglobin (Hb) concentration (log-transformed density included as continuous variable) while controlling for other covariates.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Effect of mean parasitemia density over previous 90-day period leading up to the hemoglobin (Hb) measure while controlling for other covariates and history of sulfadoxine/pyrimethamine treatment during the previous 30- to 45-day period. Thick, solid line represents a normal mean Hb concentrations for a healthy reference population.}
\end{figure}
appear less influential in reducing Hb (Figures 2 and 3). Consideration of the data in Figures 2 and 3 alone might lead to the erroneous conclusion that control methods employed to shift higher-density parasitemias to lower-densities will have only the slightest effect on improving mean Hb profiles of a population. Fortunately, these longitudinal data have permitted analysis beyond the effect of concurrent parasitemia density and provide a more meaningful examination of the temporal relationship between parasitemia history and anemia.

Higher mean parasitemia over the 90-day period preceding each Hb measure was associated with lower mean Hb among children < 24 months of age (Figure 4). While other windows of parasitemia exposure were statistically associated with lower mean Hb (e.g., 30, 45, 60, and 120 day exposure histories), these windows had a less dramatic effect than the 90-day exposure window. We do not propose that an exact 90-day period preceding each Hb measure is particularly critical in terms of anemia pathogenesis. The point is that parasitemia history, not concurrent parasitemia alone, is important to consider when studying malarial anemia or when evaluating the effectiveness of interventions to alleviate malarial anemia.3

Our analysis of how not only concurrent, but also preceding parasitemia levels affect mean Hb in this population supports the hypothesis that methods to alleviate higher-density parasitemia, such as impregnated bednets, will promote improved Hb status among young children in high transmission areas. This study also showed that higher density parasitemia was a more important contributor to reduced Hb levels in young children. While a complete absence of parasitemia during the preceding 90-day period was associated with the most favorable Hb profile, children with the lowest quartile of 90-day parasitemia had higher mean Hb profiles than their counterparts with the highest quartile of 90-day parasitemia, particularly in the first 18 months of life (the peak risk period for malarial anemia in many African regions).3 However, these data do not vindicate the role of lower-density parasitemia in generating malarial anemia. In comparison to the parasitemia-free children, a statistically lower mean Hb was observed in the youngest children with the lowest quartile of parasitemia exposure. While the predominant pathogenic mechanism of malarial anemia cannot be elucidated from this study, these data do not implicate persistent or repeated low-grade parasitemia as a high-risk exposure leading to unusually low Hb in this population.

Care should be taken in interpreting these findings. It is possible that lower-density parasitemias keep children in a sub-optimal hematologic state that prevents them from attaining the more favorable Hb concentrations of parasite-free children. Children with lower Hb due to persist lower-density parasitemia may be at increased risk for severe anemia due to their reduced capacity to buffer additional parasitologic insult. Likewise, even the lowest parasitemia levels were associated with reduced Hb in neonates (Figures 2 and 3).

The beneficial versus detrimental effects of CQ and SP on Hb status have not been adequately investigated beyond Day 28 in African children. Our analysis controlled for antimalarial remedies that could potentially confound the association between parasitemia and Hb. Given sufficient time for hematologic recovery and red blood cell replacement, it was important to determine whether Hb increased or decreased in children following these drug exposures. Many reports have implicated the lack of parasitemia clearance resulting from ineffective CQ therapy as a factor increasing the incidence of malarial anemia.18,35 The increase in anemia under such circumstances is thought to result from the persistent low-density infections that accompany ineffective CQ therapy. This study indicates that extended follow-up of patients beyond Day 28 following CQ and SP treatment provided additional information regarding hematologic response. The difference in mean Hb between children with a history of recent household CQ exposure was greatest 30–45 days following treatment. This is consistent with recent results from the Thai-Burmese border, a site of much less intense P. falciparum transmission.37 When compared to mean Hb levels following SP treatment, household use of CQ (likely an ineffective therapy for eliminating parasitemia) was associated with only a minimal increase (< 0.15 g/dL) in mean Hb.

Data are sparse concerning antimalarial effects of traditional remedies such as root and bark extractions in western Kenya. While many of these treatments may alleviate symptoms associated with P. falciparum infection (fever, vomiting, headache), there was no evidence to support a positive or negative effect on mean Hb. The fact that traditional remedies do not appear to have a detrimental influence on Hb is important given the popularity of these remedies in western Kenya. Our combined analyses have now examined dozens of factors for an effect on mean Hb over time.19 At this time, 90-day levels of parasitemia density appear to influence mean Hb in the first 18–24 months of life more than any other factor we have thus far studied. Socio-economic factors in this and other African populations appear far less important than parasitemia history.18,35 Lower-density parasitemia is associated with reduced Hb in younger (< 24 months), but not older children. Higher density parasitemia over time is associated with even lower Hb levels. While interventions that generate a parasitemia-free environment will certainly have the greatest impact on improving hematologic status of young African children, our observations offer hope for more successful control of malarial anemia with methods (bed nets or vaccines) that alleviate the frequency of the highest parasitemia densities.

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