ANALYSIS OF REPEATED HEMOGLOBIN MEASURES IN FULL-TERM, NORMAL BIRTH WEIGHT KENYAN CHILDREN BETWEEN BIRTH AND FOUR YEARS OF AGE
III. THE ASEMBO BAY COHORT PROJECT

PETER D. McELROY, ALTAF A. LAL, WILLIAM A. HAWLEY, PETER B. BLOLAND, FEIKO O. TER KUILE, AGGREY J. OLOO, SIOBÁN D. HARLOW, XIHONG LIN, AND BERNARD L. NAHLEN
Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan; Vector Biology and Control Research Center, Kenya Medical Research Institute, Kisumu, Kenya; Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, Michigan

Abstract. Anemia is an important public health problem. During very early childhood numerous factors affect hemoglobin (Hb) concentration over time, making single cross-sectional measurements difficult to interpret when studying the natural history of anemia or evaluating anemia control strategies. We analyzed repeated Hb measures contributed by 942 Kenyan children between birth and 48 months of life using a mixed effects model, with a regression spline used to describe the population mean Hb profile, and random intercepts and slopes and first-order autoregressive correlation structure to accommodate the within-individual correlation among the repeated Hb measures. The approach facilitates the study of time-stationary and time-varying covariates that influence Hb in early life. The fitted mean Hb profile obtained from the analytic model is consistent with the observed mean Hb of the study population. Village of residence was associated with greatest difference in mean Hb at time of birth (16 versus 19 g/dL; P < 0.0001). Monthly weight-for-age was also associated with mean Hb after 3 months of age. This is the first description of an analysis strategy specifically for repeated Hb measures collected in a longitudinal field study in Africa. The strategy will facilitate improved study of time-varying covariates thought to influence pediatric anemia.

An estimated 30% of the world's population is anemic, or roughly 1.3 billion people. Infants and young children from industrialized and developing countries bear most of the anemia burden. Hemoglobin (Hb) concentration is an important clinical measurement used in decisions regarding clinical diagnosis, treatment, and public health interventions for anemia. For example, recent clinical trials of human recombinant erythropoietin (rhEPO) therapy for treating the anemia of prematurity included Hb as one endpoint for determining dosage and timing of rhEPO. Reductions in anemia prevalence following introduction of iron-fortified formula or cereals into populations have largely been determined with cross-sectional Hb measures.

General analysis of data from a cross-sectional study design is inadequate for addressing questions concerning natural history of disease, particularly in situations where the exposures of interest continuously change over time. Single cross Hb measurements offer a limited ability to discern the relative importance among different causes of anemia and do not capture the natural within-individual variation in Hb due to unmeasured environmental, nutritional, and genetic factors present in almost any field study of anemia.

Serial Hb measurements obtained over time from the same individuals, at appropriately spaced intervals, can help improve the evaluation of anemia risk factors. A repeated measures design, with appropriate analysis, permits direct study of Hb change over time within individuals, and thus provides a more efficient approach for examining effects of covariates.

In preparation for studying determinants of malarial anemia in a large longitudinal body of data, we used more than 14,000 Hb measurements collected from 942 Kenyan infants enrolled in the Asembo Bay Cohort Project (ABCP) to describe an observed and modeled process of Hb decline, recovery, and stabilization over the first 4 years of life. This model accommodates the autocorrelation present in the repeated Hb measures (dependent variable) obtained from each child, and permits inclusion of both time-stationary and time-varying covariates of interest. This paper introduces the study design and population and outlines the development of the analytic model for repeated Hb data. Finally, we consider several sociodemographic factors for purposes of illustrating the analytic model and for explaining which covariates require consideration in subsequent analyses of malarial anemia.

MATERIALS AND METHODS

The Asembo Bay Cohort Project. Asembo Bay is a rural community on the shore of Lake Victoria (1,100 meters above sea level) located 40 km west of Kisumu, Kenya. In 1992, the Centers for Disease Control and Kenya Medical Research Institute initiated the ABCP, a large community-based study of morbidity and mortality associated with Plasmodium falciparum infection in pregnant women and their children and the role of cellular and humoral immune responses during the development and maintenance of protection against clinical malaria. The 70 km² ABCP area includes 15 contiguous villages with approximately 25,000 residents. More than 98% of the community is comprised of the Luo tribe.

Enrollment of pregnant women. An initial census of the entire ABCP area was conducted in February 1992. Data concerning family composition (number of persons per household and birth dates) were recorded, and pregnant women were invited to participate in the study. All subsequent pregnancies were identified during a census conducted each month by 2 traditional birth attendants per village. A village monitor employed by the Project approached each pregnant woman, explained the purposes and details of the study, and obtained informed written consent before her enrollment. The study protocol was reviewed and approved by
the institutional review boards at the Centers for Disease Control and Prevention (CDC) and the Kenya Medical Research Institute (KEMRI). Two questionnaires were administered in the local language to obtain sociodemographic data (education level, literacy, type of housing, antimosquito measures used) and reproductive history data (gravidity, history of miscarriage or stillbirth, number of children currently alive).

**Delivery of infants.** Deliveries were monitored by a traditional birth attendant who completed a data form that described details of each birth event. Presence/absence of a delivery complication, location of birth (home/hospital/clinic), number of infants born, birth status (alive or stillbirth), and sex were recorded. A maternal capillary blood specimen was obtained, along with umbilical cord and placental blood films (maternal side) for parasitemia detection and measurement of Hb. Birth weight was obtained within 24 hr.特別 trained staff recorded gestational age within 2 days using a standardized Dubowitz scoring system. Delivers missed by a traditional birth attendant were recorded on special forms the next day.

**Longitudinal follow-up of children.** Once every two week visits. Following birth, the village monitors visited each child at home every 2 weeks and assessed the presence and duration of 13 different illness symptoms, health care facility attendance, and use of antimalarial or traditional therapies. Length/height measurement of Hb and for immunologic studies. Length/height and body temperature was measured with a digital thermometer and recorded. In instances of an axillary temperature ≥37.5°C, a thick and thin blood film was obtained and specially labeled for priority microscopic examination. Priority blood films were delivered to the CDC/KEMRI laboratory the same day and examined within hours. If *P. falciparum* parasitemia was detected in a feverish child, he or she was visited the following day and treated with a single dose of sulfadoxine/pyrimethamine (SP).

**Monthly visits.** The same biweekly data collection procedures described above were also conducted at monthly intervals. Monthly visits also included collection of a finger-stick capillary blood specimen for thick film preparation, regardless of a child’s clinical status. A heparinized capillary blood specimen (200–750 μl) was also obtained for measurement of Hb and for immunologic studies. Length/height and body weight were also measured and recorded. In instances of an axillary temperature ≥37.5°C, the same biweekly protocol for priority blood film examination and treatment with SP was followed.

**Laboratory procedures. Hemoglobin.** Blood specimens were delivered to 1 of 4 central collection points and transported daily to the CDC/KEMRI laboratory near Kisumu. Hemoglobin (g/dL) was measured using the HemoCue® method (HemoCue, Angelholm, Sweden). The HemoCue® system uses the principle of Hb oxidation to hemoglobin by sodium nitrite, and subsequent conversion of hemoglobin to hemiglobinazide with sodium azide. The reagents for these reactions are contained within a small disposable microcuvette of approximately 10 μl. Hemoglobin standards and control specimens were included with each batch of 100 samples.

**Microscopy.** Blood films were stained with Giemsa and examined for the presence of plasmodium with a 100× oil-immersion objective and 10× ocular (total magnification = 1,000×). The number of asexual parasites per 300 leukocytes was used to estimate parasitemia density (with an assumed leukocyte density of 8,000/μl). Two hundred thick film fields were examined before identifying a slide as negative. The lower limit of parasite detection was approximately 4 asexual forms/μl.

**Analysis considerations.** To help reduce potential confounding by several variables, this analysis is limited to the data contributed by singleton, full-term, normal birth weight infants. Low birth weight (LBW) (<2,500 g) and preterm delivery (<37 weeks gestation) are well-documented risk factors for anemia of prematurity in infancy. The higher rate of growth relative to iron endowment at birth in premature infants is the second leading cause of iron deficiency and thus an important factor in the pathogenesis of anemia. Low birth weight and preterm infants were thus excluded to ensure more valid estimates of typical Hb among normal children in this study population. Estimates of a correlation matrix for repeated Hb measures would be biased if observations contributed by siblings were included. Thus, data from a second (n = 255) and third (n = 13) child born into the cohort by the same mother were excluded.

**Covariates.** Covariates of interest for describing the Hb curve in this phase of our investigation included maternal characteristics, child characteristics, environmental exposures, and *P. falciparum* infection status at birth. Categorical variables were created for several maternal characteristics that did not change during the course of the study (time-stationary covariates). These included maternal age at delivery (15–19, 20–29, and ≥30 years old), education level (≤3, 4–7, and ≥8 years), literacy (able to read a newspaper and write a letter), history of miscarriages and stillbirths (yes or no), gravidity (1, 2, and ≥3), number of live births (0–2, 3–6, and ≥7), and number of living offspring (0–2, 3–6, and ≥7). Environmental exposures included quality of house (high, medium, or low quality based on a summary score of type of door, floor, windows, walls, and roof), use of various vector-control measure at least 4 times per week (yes or no), and distance from the lake shore (<1.0, 1.0–1.9, 2.0–2.9, and ≥3 km). Child characteristics modeled as time-stationary covariates include birth year, birth quarter, sex, presence of a delivery problem, term intrauterine growth retardation (IUGR), and village of residence. Term IUGR status was defined as a birth weight less than the 10th percentile weight-for-gestational age by sex of an international reference group. Presence or absence of maternal peripheral, placental, and cord parasitemia were also examined individually.

Two time-varying covariates were also examined. Children with high weight-for-age may have experienced better nutrition over time and thus been at lower risk for iron deficiency. Likewise, children with growth faltering may have experienced inadequate nutritional requirements and have an increased risk of iron deficiency anemia. The reduced iron availability associated with poor nutrition may cause iron deficiency anemia in children who receive no iron supplementation. We thus classified children at each monthly visit as above the National Center for Health Statistics (NCHS)
HYATTsville, MD) 90th percentile weight-for-age (high growth), below the NCHS 10th percentile weight-for-age (low growth), or between the 10th and 90th percentile (normal growth). Persistent diarrhea in very young children may result in decreased absorption of nutrients necessary to maintain normal hematologic parameters. In an attempt to control for the effect of recent diarrheal episodes, cumulative number of reported episodes, and cumulative duration of episodes were determined for each month of observation.

**Modeling approach for repeated Hb measures.** Special statistical methods are necessary to accommodate the within-individual correlation present among the repeated Hb measures on each child. Ignoring this correlation may result in incorrect estimates of regression coefficients and invalid inferences regarding the scientific question. A mixed model analysis (Proc Mixed; SAS Institute, Cary, NC) was used to model the age-dependent process of Hb decrease and stabilization over the first 4 years of life. The mixed model approach permits inclusion of both fixed and random effects. Fixed effects model the influence of covariates on mean Hb, while random effects model the Hb correlation structure. This model does not require equally spaced nor equal numbers of Hb observations per child.\(^{16}\) The fitted model predicts the average Hb for the population at a given age, while controlling for statistically relevant covariates.

The physiologic decrease in Hb immediately after birth is well recognized.\(^{19,20}\) We used a cubic regression spline to capture the nonlinear trend of Hb over months since birth (note, months since birth is the equivalent of age): \[ \text{Hb} = \beta_0 + \beta_1 \text{month} + \beta_2 \text{month}^2 + \beta_3 \text{month}^3 + \epsilon, \] when month \(\leq 5.0\) months since birth, and \[ \text{Hb} = \beta_0 + \beta_1 \text{month} + \beta_2 \text{month}^2 + \beta_3 \text{month}^3 + \beta_4 (\text{month} - 5)^2 + \epsilon, \] when month \(> 5.0\) months since birth. A cubic regression spline is a smoothly joined piece-wise polynomial approach to modeling nonlinear relationships.\(^{21}\) The polynomial pieces of the 2 regression models are joined along the abscissa at points referred to as knots. The number and location of knots accommodate non-linear trends in data. A single knot at 5 months was used in this cubic regression spline to capture the observed relationship between age and Hb in this study population. Four other knots (at \(t = 2\), \(6\), \(7\), and \(8\) months) were individually examined before selecting the final knot position at 5 months.

The within-individual correlation of Hb observations over time was modeled using a random intercept, a random slope, and a first-order autoregressive, AR(1) process. The random intercepts and slopes model the between-individual variation, while the AR(1) process models the within-individual correlation. The AR(1) correlation structure assumes that the correlation between two measurements is modeled by an autoregressive process of order 1. A scatterplot of all Hb measures contributed between birth and 48 months, with accompanying reference range, shows the magnitude of anemia experienced by this population (Figure 1). After 2 months of age, approximately 70% of all Hb observations were below the \(-2\) SD cutoff for normal.

The mean Hb levels obtained during the first 8 weeks of life were remarkably consistent with published reference values from normal Caucasian American, Nigerian, and Jamaican\(^{24}\) populations (Figure 2). However, in comparison with these reference populations, the standard deviations of mean Hb for the ABCP children were generally 0.8–1.3 g/dL higher at each time point. The observed mean Hb level at 2 months of age (age of Hb nadir for a healthy population) was 10.9 g/dL (95% confidence interval [CI] = 10.7–11.1), well within normal limits (9.0–14 g/dL).\(^{25}\)

In contrast to Hb profiles observed in healthy children, the mean Hb level in the ABCP population continued to gradually decrease beyond 2 months of age. The mean Hb nadir was 9.2 g/dL (95% CI = 9.0–9.4), and was reached at 9 months of age, 7 months later than normal. This low mean concentration persisted into the 12th month. The observed monthly mean Hb values between 9 and 12 months were nearly 3.0 g/dL below a normal mean Hb for this age.\(^{25}\) Table 4 shows age-specific frequencies of mild, moderate, and severe anemia episodes.

**Fitted Hb over time since birth.** A comparison among the observed and fitted mean Hb profiles and a reference population is presented in Figure 3. The use of a cubic regression spline, with a single knot at 5 months, produced...
the best fitting model to accommodate the study population’s steep decrease in Hb over the first 6 months of life. The large sample size with multiple Hb observations per child resulted in a predicted population mean Hb profile with extremely narrow 95% confidence limits. For example, the predicted mean Hb nadir from the fitted model was 9.2 g/dL (95% CI = 9.1–9.3), reached at 10 months of age. Model coefficients and standard errors are shown in Table 5. The residual variance estimate was 4.408.

**Time-stationary covariates: maternal and household characteristics.** The Hb levels at delivery were similar for the 3 levels of maternal education, but a modest difference was detected between 3 and 7 months of age. Between these months, infants born to mothers with ≤3 years of education had mean Hb levels <1.0 g/dL lower than children born to mothers with ≥8 years of education \( (P < 0.001) \). No effect of education was apparent after age 12 months. No differences in mean Hb over time were detected for maternal age, literacy, gravid status, history of miscarriage or stillbirth, number of live births, number of living children, time since last delivery, housing construction, or distance from the lake shore. Children born to mothers free of parasitemia at delivery \( (P < 0.0001) \). However, no association was found between malaria infection of the placenta and mean Hb at birth, or at any age thereafter. Infrequent cord parasitemia (Table 3) precluded analysis of this variable.

**Time-stationary covariates: child characteristics.** As observed in many other populations throughout the world, male infants 6–24 months old had mean Hb values <0.5 g/dL below the female means \( (P = 0.010) \). Differences by sex disappeared after 24 months. The mean Hb level was also associated with village of residence. Data for the 2 villages with the highest and lowest Hb levels at birth are shown in Figure 4. In comparison with Ndwar/Nyagoma village, the mean Hb level at birth was substantially lower
in Nguka village (19 g/dL versus 16 g/dL, respectively; \( P < 0.001 \)). Between 12 and 18 months, significant differences in the mean Hb level were again apparent (\( P < 0.0001 \)) in these 2 villages. Birth quarter was associated with mean Hb level at time of delivery only, with the largest difference for these 2 villages. Birth quarter was associated with mean Hb level at time of delivery only, with the largest difference in Nguka village (19 g/dL versus 16 g/dL, respectively; \( P < 0.001 \)). Between 12 and 18 months, significant differences in the mean Hb level were again apparent (\( P < 0.0001 \)) in these 2 villages. Birth quarter was associated with mean Hb level at time of delivery only, with the largest difference detected between the second and fourth quarter (17.4 g/dL and 18.5 g/dL, respectively; \( P < 0.001 \)). Year of birth, IUGR status, and delivery location were not associated with differences in Hb at any age.

**Time-varying covariates: effect of growth and diarrheal episodes.** At each point in time after 3 months of age, children with elevated weight-for-age (90th percentile) had a higher mean Hb level than children with normal or low weight-for-age at the same age (Figure 5). The effect of high weight-for-age on Hb appeared to be strongest after 24 months. Neither cumulative frequency of diarrheal episodes nor cumulative duration of diarrheal episodes had a detectable effect on Hb at anytime over the first 3 years of life.

**Multivariate model including all covariates of interest.** A final multivariate model for predicted mean Hb over time since birth is shown in Figure 6 and includes the presence of maternal parasitemia at birth, child’s sex, village, and weight-for-age classification. Maternal education and birth quarter did not remain in this final model. Two scenarios (favorable and unfavorable) depict the mean Hb over time since birth. The favorable mean Hb profile represents female children from Ndwar/Nyagoma village born to mothers

This page contains tables, figures, and text related to malaria and its impact on hemoglobin levels in children. The tables provide data on parasitemia and anemia, while the figures illustrate the distribution of malaria-related time-stationary covariates at time of birth and the mean hemoglobin concentrations among different populations.
ANALYSIS OF REPEATED HEMOGLOBIN MEASURES

FIGURE 3. Analytic model for hemoglobin (Hb) concentration (g/dL) with time since birth as independent variable. Shown is a comparison between observed and fitted mean Hb values over first 48 months of life. Reference values for normal mean Hb are also presented.

FIGURE 4. Predicted hemoglobin (Hb) (g/dL) concentration over time for a time-stationary covariate (Nguka village versus Ndwara/Nyagoma village).

FIGURE 5. Predicted hemoglobin (Hb) (g/dL) concentration for a time-varying covariate: low, normal, and high weight-for-age children over time.

TABLE 5
Estimated coefficients and standard errors (SEs) obtained from mixed model analysis of the relationship between hemoglobin concentration and months since birth; months was included as a continuous variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>18.072</td>
<td>0.1181</td>
</tr>
<tr>
<td>Months</td>
<td>-5.182</td>
<td>0.0903</td>
</tr>
<tr>
<td>Months²</td>
<td>1.026</td>
<td>0.0198</td>
</tr>
<tr>
<td>Months³</td>
<td>-0.006826308*</td>
<td>0.00135211</td>
</tr>
<tr>
<td>(Months - 5)³</td>
<td>0.006822802†</td>
<td>0.00135953</td>
</tr>
</tbody>
</table>

* Coefficients for both cubic terms must be carried out to 9 significant digits.
† Coefficient = 0 when months ≤ 5 months since birth.

DISCUSSION

New approaches are needed to study factors that influence the natural history of sub-optimal Hb levels. Published data describing serial Hb measures obtained from African children over extended periods are scarce, and no analytic methods for such data have been proposed heretofore. Before meaningful and efficient analyses could be pursued with the ABCP longitudinal Hb data, it was essential to first describe the observed Hb pattern for this particular study population. The observed Hb data could then be used to formulate a statistical model capable of simultaneous evaluation of multiple covariates thought to influence Hb across age.

This report summarizes our analytical model to most efficiently address repeated Hb measures data obtained in early life. The model provides a useful approach for studying the role of a single factor or a multivariate array of genetic, nutritional, environmental, immunologic, and infectious disease risk factors for anemia. This strategy for conceptualizing causal pathways and testing hypotheses concerning pediatric anemia is particularly attractive for assessing the influence of exposure variables that naturally change as a child ages or as environmental or experimental conditions are modified over time.

For example, the method could be particularly advantageous in the design and analysis of a controlled trial of rhEPO therapy for treating the anemia of prematurity, a significant public health problem among marginalized populations. Investigators could substantially improve their ability to determine ideal dose and timing of rhEPO therapy by controlling for particular hemoglobinopathies (HbS) and presence of infection with human immunodeficiency virus at birth, while simultaneously considering time-varying factors such as ferritin level and growth rate. A model with a random intercept and slope for repeated Hb measures contributed by each child will capture within-individual variation in Hb, and thereby more precisely reflect the clinical influence of various rhEPO doses than earlier studies.2,3
Our description of the observed mean Hb profile, the Hb nadir, and prevalence of anemia in this large population of African children provides useful baseline information for investigators designing anemia control strategies in similar environments. Such data are necessary for the calculation of appropriate sample sizes and determination of the frequency and spacing of Hb measures and other exposures that influence Hb. For example, consider a future randomized controlled trial of a malaria vaccine among children less than 5 years of age in an area of holoendemic malaria. The primary objective of such a trial might be to estimate the vaccine’s efficacy for reducing all-cause mortality over a 2-year period. However, it would also be interesting to partially understand the mechanism through which a vaccine reduces mortality. It would thus be desirable to also monitor a smaller subsample of the vaccinated and placebo groups to determine the vaccine’s efficacy at improving Hb profiles during the first 24 months of life. These data, and analysis strategy, will greatly facilitate such an objective.

The mean Hb level in the first 2 months of life among children in this study was consistent with several reference values in normal healthy infants from industrialized and developing countries. Overall, the normal birth weight children included in this analysis began life and proceeded through the second month of life with a healthy mean Hb profile. Our objective was to describe mean Hb patterns following birth in children who began life without the disadvantage of LBW or prematurity. Approximately 12% of the ABCP children had birth weights < 2,500 g and 4% had a gestational age < 37 weeks. While exclusion of these children has no doubt resulted in an underestimate of the complete burden of anemia in this population, including them would have been inconsistent with most other published studies that describe hematologic indices in young children. Analysis of possible interactions between anemia risk factors and LBW are indeed important within the context of anemia control for African populations, but were beyond the scope of this presentation.

Maternal education level and birth quarter were associated with Hb in the crude analyses, but not in the final multivariate model. An explanation may be that education level was measuring a similar component of nutritional status as weight-for-age. If weight-for-age is a more proximal estimate of current nutritional status than maternal education, the significance of weight-for-age in the model would likely supersede that of education. Likewise, birth quarter is associated with *P. falciparum* transmission intensity immediately preceding birth, an exposure likely to influence presence of maternal parasitemia. In western Kenya, the second quarter (April–June) is generally a high transmission period likely to result in more maternal parasitemia, while the fourth quarter (October–December) corresponds to the lowest transmission period and least likelihood of maternal parasitemia at time of delivery (McElroy PD, unpublished data). Consequently, birth quarter was no longer significant after introduction of maternal parasitemia to the multivariate model.

Placental parasitemia at time of delivery is a recognized risk factor for LBW.27 One report also found placental parasitemia at delivery to be associated with anemia around 2 months of age.28 Two months of age is the exact time when anemia of prematurity is most evident, even among LBW infants born in the United States. The LBW associated with placental parasitemia in earlier studies may have accounted for the detection of reduced Hb values in children born to mothers with placental parasitemia. The lack of an association detected between placental parasitemia and Hb in this study may have been due to our exclusion of LBW infants. In full-term, normal birth weight children of Asembo Bay, placental parasitemia is not an independent risk factor for anemia.

Neither a recent diarrheal episode, cumulative number of episodes, nor duration of diarrheal episodes were associated with Hb over time since birth. This lack of association may be a consequence of the poor specificity of the mother-reported diarrheal data. No case definition for diarrhea was used in this study. The result may have been nondifferential misclassification of the diarrhea episodes, thus resulting in a biased effect of this exposure toward the null value (i.e., little or no effect on mean Hb).

The current World Health Organization goal of a reduction in incidence of severe malaria morbidity and mortality, not *P. falciparum* infection, will guide future research objectives and allocation of resources.29 The clinical importance of reduced Hb, accompanied by the ease and rapidity with which it may now be measured under field conditions, make this hematologic parameter a potentially important outcome variable in malaria morbidity studies in young children. Efficacy trials of insecticide-impregnated bed nets, antimalarial drugs, and malarial vaccines may need to include Hb as an outcome if the true public health impact of the control measure is to be quantitated.30,31 Emerging evidence implicates reduced Hb in the multifactorial pathogenesis of cerebral malaria and respiratory distress, perhaps making serial Hb measures even more critical.32–34 The use of similar longitudinal data collection and analysis procedures may offer an improved understanding of how a prolonged state of reduced Hb interacts with the acute phase response and parasite sequestration to induce other severe malaria syndromes.35

With enrollment of considerably fewer study participants than in this study, and use of the described analytic approach, investigators may facilitate identification and eval-

---

**Figure 6.** Final multivariate model of predicted mean hemoglobin (Hb) (g/dL) concentration over time, controlling for maternal parasitemia at birth, child’s sex, village, and weight-for-age (time-varying).
uation of important time-varying immune factors that confer protection against malarial anemia. A more pertinent issue concerns the natural variability in Hb across individual children due to unmeasured characteristics such as genetic composition and household exposures that remain constant over time. In longitudinal studies accompanied by appropriate analysis, statistical power is increased since each person serves as his or her own control. When a repeated outcome measure is available, the influences of these unmeasured characteristics (third variables) that have an independent effect on the outcome are canceled. Cross-sectional data require an investigator to control for such variables, and thus collect data on those variables.

Finally, analysis of repeated measures data need not be limited to a continuous outcome variable as we have presented. Dichotomous and counted responses may be pursued using similar models. For example, a repeated measure for a dichotomous outcome obtained at monthly intervals might include "any parasitemia accompanied by fever in the past month." Alternatively, a counted response might include the "number of episodes of fever accompanied by parasitemia in the previous month." Random effects models for repeated outcomes like these assume each child has a different underlying risk for the event at each monthly observation, and that his or her risk will likely change with increasing age (parasitemia exposure duration). An individual's change in risk may not be determined from a single observation, since much of the risk may be attributable to factors for which no data are collected. Given repeated measures data and an appropriate analysis, we may ask how the available data for certain exposures affect a child's propensity to experience the morbid event each month. For a review of these methods, see Zeger and Liang. Through careful integration of data collection procedures, including the spacing and frequency of repeated outcome measures, followed by more efficient analytic procedures, challenging research objectives may be pursued with far fewer study participants than included in this study. This strategy will facilitate the study of how time-varying immunologic parameters, in the presence of certain host genotypes and cytokine profiles, are modulated by P. falciparum transmission intensity to ultimately influence the risk of malarial morbidity and mortality in the early years of life.

Acknowledgments: We thank the field, laboratory, and data management staff of the ABCP Study for dedicated service. In particular, we are grateful to Michael Onyango, Thomas Ondieki, and David Anyona. In addition, we thank Kathy Welch (University of Michigan) for technical assistance in data management.

Financial support: The ABCP Study was funded by the U.S. Agency for International Development (HRN 6001-A-00-4010-00). Peter D. McElroy received partial support through a Rackham Predoctoral Dissertation Fellowship from the University of Michigan.

Disclaimer: Use of trade names is for identification purposes only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

Authors' addresses: Peter D. McElroy, Division of TB Elimination, Centers for Disease Control and Prevention, Mailstop E-10, 1600 Clifton Road, Atlanta, GA 30333. Altay A. Lal and Peter B. Boland, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, Atlanta, GA 30341-3724. William A. Hawley, Feiko O. ter Kuile, and Bernard L. Nahlen, U.S. Embassy--Centers for Disease Control and Prevention, Unit 641 00, Box 421, APO, AE 09831-4100. Aggrey J. Oloo, Kenya Medical Research Institute, Box 1578, Kisumu, Kenya. Siobhán D. Harlow, Department of Epidemiology, School of Public Health, University of Michigan, 109 Observatory Street, Ann Arbor, MI 48109. Xihong Lin, Department of Biostatistics, School of Public Health University of Michigan, 109 Observatory Street Ann Arbor, MI 48109.

Reprint requests: Altaf A. Lal, Division of Parasitic Diseases, Centers for Disease Control and Prevention 4770 Buford Highway, Mailstop F-12, Atlanta, GA 30341-3724.

References