Brief Report

The etiology of severe anemia in a village and a periurban area in Mali

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Running Title: THE ETIOLOGY OF SEVERE ANEMIA IN TWO AREAS IN MALI

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Severe anemia is one of the major complications of malaria in Africa. We studied two populations, one in a village and the second in a periurban area in Mali to understand the preventable factors in the disease. The two correlates of disease were parasitemia above 100,000 parasitized red blood cells per µL and low baseline hemoglobin. All cases of moderate to severe anemia occurred in children under 3.2 years of age. Raising baseline hemoglobin level and lowering peak parasitemia in infants and young children may reduce the incidence of severe anemia resulting from malarial infection.
Introduction

Severe anemia (hemoglobin [Hb] < 5 g/dL) in malaria causes increased mortality in African children, especially when associated with dyspnea (respiratory distress), sepsis, and cerebral malaria. If severe anemia presents with respiratory distress, chemotherapy alone is not adequate; transfusion lessens mortality but has the problem of availability and exposure to blood-borne pathogens, including HIV and hepatitis B and C. Although the pathogenesis of severe malarial anemia is multifactorial, the major preventable factors have not been adequately identified. The clearest part of the equation is bone marrow suppression (dyserythropoiesis), which was carefully characterized in 1980; however, transient suppression of bone marrow without associated hemolytic anemia cannot explain the sudden drop in Hb that is characteristically seen in malarial infections. Thus, an important unanswered question is whether the hemolytic anemia that accompanies the bone marrow suppression in malarial infection is caused by high parasitemia, or is related to another mechanism, such as immune-mediated red cell destruction. In a study in Kenya, severe anemia was associated with a drop in red blood cell CR1 and CD55 and a rise in immune complexes on the red blood cell surface. No difference in parasitemia was observed between the children with a drop in Hb below 5 g/dL and the control group with symptomatic malaria (fever and parasitemia) in the absence of severe anemia. In the present report, factors associated with moderate to severe anemia in malaria-infected children were determined in a longitudinal village-based study.
Study design

A longitudinal study of malaria in 397 (1999) and 398 (2000) subjects aged 6 months to 20 years in the village of Donéguébougou and the periurban setting of Sotuba in Mali presented the opportunity to study factors that may contribute to moderate to severe anemia (Hb below 6 g/dL) in the setting of malarial infection. The transmission in both study sites is seasonal (from June to November). The intensity of transmission (number of infectious bites per person during the transmission season) determined by spray catch/human landing catch was 21.1/167 and 19.2/137.3 in Donéguébougou and 0.74/12.2 and 0.28/3.64 in Sotuba for 1999 and 2000, respectively. Hb was determined by hemoglobin analyzer (Hemaque®, Lake Forest, CA) and the parasitemia (parasites/µL) was determined on a Giemsa-stained thick film (parasites per 300 leukocytes, assuming 7,500 leukocytes/µL). The children were seen weekly from July to December 31 for evaluation of symptoms and axillary temperature. Any child who was sick either during the visit or between visits was evaluated clinically, including a malarial blood film. Baseline hemoglobin and parasitemia were determined at the beginning of study and monthly. The study was approved by IRBs in Mali and at the NIH, and informed consent was obtained for all subjects.
Results and discussion

Moderate to severe anemia (defined as less than 6 g/dL) occurred in 6 children in Donéguébougou and in 2 children in Sotuba (Table 1A). All cases occurred in children less than 3.2 years of age out of a total study population of 63 and 48 children in this age group in Donéguébougou and Sotuba, respectively. This is the same age distribution of anemia seen in cases from The Gambia,\(^6\) where the intensity of transmission is similar to that of Donéguébougou. Of the 9 episodes of severe anemia (2 in the same child), 6 were associated with high parasitemia (>100,000 infected red blood cells per µL). The Hb level in children with high parasitemia continued to fall after treatment, reaching a nadir within 7 days in 5 of 6 episodes, similar to the observations in a study performed in Thailand\(^7\). The relative risk of moderate to severe anemia (Hb <6 g/dL) in children less than 3 years of age who had fever and parasitemia >100,000 per µL compared to those who had fever and parasitemia >2,500 to <100,000 per µL was 9.5 (95% CI 2.4 to 37.3; p = 0.001). In a multivariate analysis, hyperparasitemia in children below 3 years at the beginning of the study year, when adjusted for baseline hemoglobin, remained significantly associated with Hb <6 g/dL (RR = 6.3, 95% CI = 1.49 to 26.7; p = 0.012). A similar correlation between admission parasitemia and the level of anemia has been described in Thailand\(^7\) and Tanzania\(^8\). Previous studies have demonstrated a reduction in the incidence of severe and moderate to severe anemia by 50% or more in the setting of intermittent treatment with antimalarial drugs\(^9,10\), although rebound of high parasitemia occurred after discontinuation of therapy\(^11\).

Why did severe anemia not develop in the 14 other children less than 3.2 years of age who had parasitemia ≥100,000 per µL (Table 1B)? The children without severe anemia had a
higher baseline hemoglobin (average of three highest hemoglobin determinations) than those who developed severe anemia \[11.3 \pm 0.19 \text{ (SE)} \text{ and } 9.7 \pm 0.25 \text{ (SE), respectively; } p<0.001\]. These children also had a significantly higher mean hemoglobin at the time of treatment \[10.2 \pm 0.17 \text{ (SE)}\] than the children who had severe anemia \[7.95 \pm 0.59 \text{ (SE); } p < 0.001\]. Furthermore, baseline hemoglobin was significantly associated with \(Hb < 6 \text{ g/dL} \) (RR = 0.60, 95% CI = 0.46 to 0.80, \(p = < 0.001\)). As in the severely anemic group, Hb levels fell in most children (11 of 14) following treatment and reached a nadir (between 7.6 and 9.7 g/dL) within 7 days of the initiation of therapy. Thus, the major differences between the two groups of children with high parasitemia were the baseline hemoglobin and the hemoglobin at the time of treatment. The lower hemoglobin at the time of treatment in the children who had severe anemia may reflect higher parasitemia during the time leading up to treatment. The lower baseline hemoglobin that predisposes these children to severe anemia may result from repeated malarial infections and iron deficiency.

To further evaluate the factors in severe anemia, we looked at baseline hemoglobin in children less than 10 years of age. The hemoglobin dropped after infancy and then rose after age three to a plateau at age five (Fig. 1A and 1B). In a placebo-controlled study comparing the effect of chloroquine prophylaxis for malaria from birth to age three, the hemoglobin in the control group fell below the treatment group to hemoglobin levels similar to the levels in present study\(^{12}\). This study\(^{12}\) and the present one indicate that baseline hemoglobin is affected by malaria. The rise in baseline hemoglobin at age three in the present study suggests that children below 3 are affected differently by malaria than older children, since high parasitemia (>100,000 per µL) continued to occur in Sotuba after the age of three without severe anemia.
Three children developed severe anemia without high parasitemia. One child had no parasitemia above 6,000 per µL on three blood films during the 2 weeks before treatment; another had a parasitemia of 15,000 per µL. The lowest Hb in both of these children was 5.3 g/dL. One child's Hb rose immediately after treatment. The failure to evaluate and treat the other child was an oversight, as the protocol dictated a complete evaluation for the presence of severe anemia even in an asymptomatic child. Fortunately, the child’s Hb returned to baseline without treatment. Anemia in these children may fit into the category of other causes of anemia such as immune hemolysis. Its course may be similar to the severe anemia that developed during *P. falciparum* infections in partially immune Aotus monkeys, some of whom had no parasitemia on blood films but were parasite positive on polymerase chain reaction of the blood. The third child had G-6-P D deficiency and was treated with Fansidar™, likely precipitating severe hemolytic anemia that reached its low point 14 days after treatment.

African children with severe anemia and malaria clearly fall into multiple categories, including those without high parasitemia. In our study, however, low baseline hemoglobin and high parasitemia were the most significant risk factors for severe anemia. Other factors such as dyserythropoiesis and Fansidar™ treatment in G-6-P D deficiency were probably also responsible for the anemia in a minority of cases. The difference between our data and those of Stoute et al., in which immune-mediated red cell destruction was thought to be the major cause of malaria-associated anemia, may be explained by differences in the intensity of transmission; the intensity in their study reached hundreds of infections per year and the infections occurred throughout the year, as compared with lower rates and seasonal transmission in our study. The major implications of our study are twofold. First, raising the baseline hemoglobin by
intermittent antimalarial therapy may decrease the incidence of severe anemia, particularly in
settings of seasonal malarial transmission. Second, effective vaccines against malaria that lower
peak parasitemia may raise baseline hemoglobin and reduce the frequency of severe anemia.

Acknowledgements

We thank Dr Richard Sakai for logistical support and the populations of the two villages for their
cooperation throughout the study.
References


### Table 1A. Moderate to severe anemia in malaria

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<th>Age (years)</th>
<th>Gender*</th>
<th>Site†</th>
<th>Hb‡ type</th>
<th>G-6-P D§</th>
<th>Per µL</th>
<th>%</th>
<th>Rx# failure*</th>
<th>Hb (g/dL)</th>
<th>Baseline</th>
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* M, male; F, female.
† D, Donéguébougou; S, Sotuba.
‡ Hb, hemoglobin.
§ G6PD, glucose-6-P dehydrogenase.
¶ Infected red blood cells at time of treatment, expressed as number per µL of blood and as % of red blood cells infected (estimated from Hb and number per µL.
# Rx, antimalaria treatment: F, fansidar; Q, quinine; C chloroquine.
© Same patient at A, 2 years; and B, 2 months later.
* ?, Unknown.
Table 1B. Children less than 3 years old who have parasitemia above 100,000/mL without developing Hb less than 6 g/dl

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<tr>
<th>Patient identifier</th>
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<th>Site†</th>
<th>Hb type</th>
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<th>Per µL</th>
<th>%</th>
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* M, male; F, female.
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‡ Hb, hemoglobin.
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Rx, antimalaria treatment: F, fansidar; Q, quinine; C chloroquine.
?
?, Unknown.
Figure 1A. The baseline hemoglobin (95% confidence interval) in Doneguebougou during 1999 and 2000
Figure 1B. The baseline hemoglobin (95% confidence interval) in Sotuba during 1999 and 2000
The etiology of severe anemia in a village and a periurban area in Mali

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