Parasite Burden and Severity of Malaria in Tanzanian Children

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ABSTRACT

BACKGROUND
Severe Plasmodium falciparum malaria is a major cause of death in children. The contribution of the parasite burden to the pathogenesis of severe malaria has been controversial.

METHODS
We documented P. falciparum infection and disease in Tanzanian children followed from birth for an average of 2 years and for as long as 4 years.

RESULTS
Of the 882 children in our study, 102 had severe malaria, but only 3 had more than two episodes. More than half of first episodes of severe malaria occurred after a second infection. Although parasite levels were higher on average when children had severe rather than mild disease, most children (67 of 102) had high-density infection (>2500 parasites per 200 white cells) with only mild symptoms before severe malaria, after severe malaria, or both. The incidence of severe malaria decreased considerably after infancy, whereas the incidence of high-density infection was similar among all age groups. Infections before and after episodes of severe malaria were associated with similar parasite densities. Nonuse of bed nets, placental malaria at the time of a woman’s second or subsequent delivery, high-transmission season, and absence of the sickle cell trait increased severe-malaria risk and parasite density during infections.

CONCLUSIONS
Resistance to severe malaria was not acquired after one or two mild infections. Although the parasite burden was higher on average during episodes of severe malaria, a high parasite burden was often insufficient to cause severe malaria even in children who later were susceptible. The diverging rates of severe disease and high-density infection after infancy, as well as the similar parasite burdens before and after severe malaria, indicate that naturally acquired resistance to severe malaria is not explained by improved control of parasite density. (Funded by the National Institute of Allergy and Infectious Diseases and others.)
ALTHOUGH ALMOST 600,000 AFRICAN
children die each year from malaria,¹
most infections in children are mild.²⁻³
Fundamental questions about the pathogenesis
of malaria remain unresolved, such as the rela-
tive contributions of parasite burden and host
inflammation to severe disease.⁴ In areas where
transmission is stable, severe malaria is unlikely
to occur after 5 years of age, presumably as a
result of immunity,⁵ and mathematical models sug-
gest that protection against noncerebral severe
malaria develops after one or two infections.⁶
The mechanism of protective immunity is un-
clear; it might, for example, involve the reduction
of parasite density or the blocking of parasite
virulence to prevent disease. IgG transferred
from immune adults clears blood-stage parasites
and symptoms in sick children,⁷ but the targets
of protective IgG remain undefined.

To better understand the pathogenesis of se-
vere malaria and acquired immunity, we under-
took an intensive birth-cohort study of 882 chil-
dren in northeastern Tanzania. We examined the
relationship between the Plasmodium falciparum
parasite burden and the severity of malaria within
individual children over time and the risk of se-
vere malaria during the first and subsequent
infections.

METHODS

STUDY POPULATION
The study population was part of a longitudi-
nal birth cohort in the Muheza district, an area of
intense malaria transmission with an entomo-
logic inoculation rate of approximately 400 infec-
tive mosquito bites annually.⁸ The incidence
of malaria declined sharply in this area after the
study closed in 2006.⁹ Newborns were enrolled
in the study between September 2002 and Novem-
ber 2005. Children were followed for an average
of 2 years and for as long as 4 years. Written in-
formed consent was obtained from all the chil-
dren’s mothers before enrollment.

STUDY PROCEDURES
Children were examined at birth, once every 2
weeks during infancy, once every month after infancy, and
during any illness. Blood smears were collected at
all visits, regardless of whether symptoms were
present. Parasitemia was defined as any P. falci-
parum detected in a Giemsa-stained blood smear,
and high-density infection requiring parenteral
treatment was defined as a parasite density of
more than 2500 parasites per 200 white cells, in
accordance with Tanzanian Ministry of Health and
Social Welfare guidelines. P. falciparum—specific
histidine-rich protein 2 (PfHRP-2) levels in plas-
ma were measured with the use of a sandwich
enzyme-linked immunosorbent assay (ELISA)¹⁰
(see the Supplementary Appendix, available with
the full text of this article at NEJM.org).

Children were classified as having severe ma-
laria in accordance with World Health Organiza-
tion (WHO) criteria¹¹ (hemoglobin level <5 g per
deciliter, prostration, more than one convulsion
in the past 24 hours, respiratory distress, or hypo-
glycemia). Children were classified as having mod-
erate or severe malaria if they did not fulfill the
WHO criteria but had at least one of the follow-
ing signs: a hemoglobin level lower than 6 g per
deciliter, hyperthermia (temperature, >40°C), one
convulsion in the past 24 hours, or a respiratory
rate greater than 40 breaths per minute. Most
cases of severe and moderately severe malaria
(78.4%) were treated with quinine. The results of
analyses of moderately severe malaria are pre-
sented in the Supplementary Appendix. Children
were classified as having mild high-density in-
fec tion if they had a parasite density of more
than 2500 parasites per 200 white cells in the ab-
sence of severe or moderately severe symptoms.
Children were followed until the end of the study (May 2006) or until they dropped out of
the study.

The study protocols were approved by the
Division of Microbiology and Infectious Diseases
at the U.S. National Institutes of Health and by
the institutional review boards of Seattle BioMed
and the Medical Research Coordinating Com-
mittee in Tanzania.

STATISTICAL ANALYSIS
All cases of parasitemia occurring within 28 days
after a previous case were considered to be a sin-
gle infection. We estimated age-specific inci-
dence rates of infection, mild high-density infec-
tion, and severe malaria using a Nelson–Aalen
estimator¹² with confidence intervals calculated
by means of a nonparametric bootstrap method.
A generalized-estimating-equation approach for
Poisson regression was used to compare age-
specific rates of severe malaria and high-density
infection. The risk of severe malaria during a
first infection was estimated by dividing the number of children with severe malaria during a first infection by the total number infected at least once; similar risk calculations were performed for subsequent infections, after children with previous episodes of severe malaria were excluded. Log-transformed parasite density before and after a first episode of severe malaria was compared with the use of a paired t-test.

Cox proportional-hazards models were fit to determine the influence of study variables (village of residence, use or nonuse of bed nets, presence or absence of sickle cell trait, α-thalassemia genotype, transmission season [low or high], birth season, sex, and a single variable combining status with respect to placental malaria and maternal parity) on time to first episode of severe malaria. A multivariate generalized-estimating-equation model with log-transformed parasite density as the dependent variable was developed to assess the effect of all study variables on the parasite burden. All reported P values are two-sided; P values lower than 0.05 were considered to indicate statistical significance. Details of the survival analysis and generalized-estimating-equation methods are provided in the Supplementary Appendix.

### RESULTS

#### STUDY POPULATION

During the recruitment period, 1045 mothers (of 1075 children) gave consent to participate and gave birth at the Muheza Designated District Hospital. After exclusions (see the Supplementary Appendix), 882 singleton infants were included in the analyses (Table 1), of whom 688 (78.0%) were followed for at least 1 year. Baseline characteristics were similar in the overall population and among the children followed for more than 1 year. Most of the children (62.7%) slept with bed nets, and among these children, 29.7% slept with bed nets that were insecticide-treated. The prevalences of sickle cell trait (16.5%) and α-thalassemia (11.8% homozygous and 40.9% heterozygous) were similar to those in other East African populations.13,14

The study encompassed 1762.8 child-years and 38,261 blood smears, including 6319 P. falciparum–positive blood smears representing 3933 independent infections; 65.4% of the positive blood smears were documented during scheduled visits. Of the 882 children, 715 (81.1%) had at least one P. falciparum infection. A total of 102 children (11.6%) had severe malaria, with 122 episodes of severe malaria overall. Most of the children who had severe malaria (99 of 102) had only one or two episodes. Of the 15 children who had a second episode, 7 presented with the same syndrome on both occasions, and 7 of 15 second episodes occurred during infancy (Fig. S6 in the Supplementary Appendix), when the incidence of severe malaria peaks. Of the 688 children followed for at least 1 year, 624 (90.7%) became infected, and 98 (14.2%) had severe malaria.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (N = 882)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age — yr</td>
<td>26.0±6.3</td>
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<tr>
<td>Maternal parity — no. (%)</td>
<td></td>
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<tr>
<td>First delivery</td>
<td>254 (28.8)</td>
</tr>
<tr>
<td>Second delivery</td>
<td>201 (22.8)</td>
</tr>
<tr>
<td>Third or subsequent delivery</td>
<td>427 (48.4)</td>
</tr>
<tr>
<td>Maternal residence area — no. (%)</td>
<td></td>
</tr>
<tr>
<td>Magila</td>
<td>144 (16.3)</td>
</tr>
<tr>
<td>Muheza township</td>
<td>400 (45.4)</td>
</tr>
<tr>
<td>Mkanyageni</td>
<td>188 (21.3)</td>
</tr>
<tr>
<td>Bwembwera</td>
<td>150 (17.0)</td>
</tr>
<tr>
<td>Bed-net use — no. /total no. (%)</td>
<td>455/726 (62.7)</td>
</tr>
<tr>
<td>Birth weight — kg</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Duration of follow-up — wk</td>
<td>104.2±53.5</td>
</tr>
<tr>
<td>Sex — no. (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>457 (51.8)</td>
</tr>
<tr>
<td>Female</td>
<td>425 (48.2)</td>
</tr>
<tr>
<td>Transmission season at birth — no. (%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>432 (49.0)</td>
</tr>
<tr>
<td>Low</td>
<td>450 (51.0)</td>
</tr>
<tr>
<td>Sickle cell trait — no. /total no. (%)</td>
<td>142/861 (16.5)</td>
</tr>
<tr>
<td>Parasitemia during follow-up — no. (%)</td>
<td>715 (81.1)</td>
</tr>
<tr>
<td>Postneonatal death — no. (%)</td>
<td>35 (4.0)</td>
</tr>
<tr>
<td>Parasite-positive blood smears — no. /total no. (%)</td>
<td>6319/38,261 (16.5)</td>
</tr>
<tr>
<td>Treated at home before presentation — %</td>
<td></td>
</tr>
<tr>
<td>Severe malaria</td>
<td>4.1</td>
</tr>
<tr>
<td>Mild malaria with high parasite density</td>
<td>2.7</td>
</tr>
<tr>
<td>Mild malaria with any parasite density</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ±SD.
Thirty-five children (4.0%) died; 11 deaths were attributed to malaria. The overall mortality rate in our cohort was lower than that previously reported in this area\textsuperscript{15}; the difference may be related to the intensive follow-up in our study (see the Supplementary Appendix). The proportion of childhood deaths attributable to malaria (31%) was similar to that in other African communities.\textsuperscript{16-18}

**PARASITE BURDEN AND SEVERE MALARIA**

The ranges of parasite densities during severe malaria and during mild or asymptomatic malaria overlapped considerably, both in the overall cohort (Fig. S1A in the Supplementary Appendix) and in the subset of children who had severe malaria during follow-up (Fig. S1B in the Supplementary Appendix). Assessment of PfHRP-2 levels, another measure of parasite biomass, had similar results (Fig. S1C in the Supplementary Appendix). The mean parasite density was substantially higher during episodes of severe malaria than during mild or asymptomatic infections (P<0.001), largely because severe malaria was rare during low-density infections (Fig. S2 in the Supplementary Appendix). However, most high-density infections were not associated with severe malaria: 253 of 882 children had a total of 444 high-density infections with only mild symptoms, including 36 completely asymptomatic episodes.

Even among the 102 children who had severe malaria, most (67) had high-density infection with only mild symptoms before the first episode of severe malaria (21 children) (Fig. 1A), after the first severe episode (55 children) (Fig. 1B), or both. Both parasite density and PfHRP-2 levels were significantly higher during a mild high-density infection than during an episode of severe malaria; the mean ratio of parasite level during mild infection to parasite level during severe episodes was 6.3 (95% confidence interval [CI], 3.8 to 10.5; P<0.001; 67 children), and the geometric mean PfHRP-2 level was 4826 ng per milliliter (95% CI, 3378 to 6894) during a mild high-density infection versus 1305 ng per milliliter (95% CI, 516 to 3298) during a severe episode (P=0.01; 39 children).

**RISK OF FIRST EPISODE OF SEVERE MALARIA**

The incidence of infection increased during the first 6 months of life, then remained stable (Fig. 2A). The change in timing of the analysis of blood smears from every 2 weeks to every month might have resulted in an underestimation of the incidence of infection after infancy, but it would not have affected the persistent plateau thereafter. The overall cumulative incidence rates of infection, severe malaria, and mild high-density infection were, respectively, 2.41 (95% CI, 2.21 to 2.62), 0.05 (95% CI, 0.04 to 0.06), and 0.26 (95% CI, 0.22 to 0.30) episodes per child-year. The incidence of severe malaria — but not the incidence of mild high-density infection — diminished after infancy (Fig. 2B). The incidence rates of infection and severe malaria are similar to those that have been reported in other areas of high transmission\textsuperscript{19,20} (see the Supplementary Appendix).

The median age at the first episode of severe malaria was 38.7 weeks (interquartile range, 28.6 to 63.8). The risk of severe malaria did not decrease after the first infection; approximately 50% of children had at least two mild or asymptomatic infections before their first severe episode (Fig. 2C).

**SEVERE MALARIAL ANEMIA**

As in other African communities,\textsuperscript{21,22} most children with severe malaria typically presented with a single condition (Fig. 3), such as respiratory distress, convulsions, or severe anemia. Children had more than one condition in only 12.3% of severe episodes (15 of 122), which is similar to the proportions reported in previous studies.\textsuperscript{21,22} Children with convulsions were older and had lower parasite densities than children with severe anemia, although the differences were not significant (P=0.09 and P=0.21, respectively) (Table S1 in the Supplementary Appendix). Of the 102 children who had severe illness, 6 (5.9%) died during an episode of severe malaria. Three of the 15 children (20%) who presented with overlapping severe malaria conditions died.

Most children with severe malarial anemia (26 of 40, 65%) had their hemoglobin level measured in the previous 2 months. These children had a median reduction of 47% (interquartile range, 28 to 65) in the hemoglobin level at the time of severe malarial anemia. In 15 of the 26 children (58%), hemoglobin levels were higher than 8 g per deciliter in the 2 months before the episode of severe malarial anemia, which suggests that there was a decrease of more than 3 g per deciliter during the acute infection. Fewer children (8 of 26, 31%) had a hemoglobin level lower than 7 g per deciliter in the 2 months before an episode of severe malarial anemia.
Placental Malaria and Other Risk Factors

In Cox regression analyses of the time to the first episode of severe malaria, placental malaria and maternal parity were combined as one variable, because these factors interact to influence the incidence of infection. Bed-net use (P<0.001) and residence in Muheza or Mkanyageni (P<0.001 and P = 0.02, respectively) reduced the risk of severe malaria, whereas the high-transmission season (P = 0.05) and placental malaria at the time of second or subsequent delivery (P = 0.04) increased the risk. In a multivariate Cox model (Table 2), bed-net use and residency in Muheza or Mkanyageni reduced the risk of severe malaria by 52%, 61%, and 53%, respectively (P = 0.001, P<0.001, and P = 0.02, respectively); sickle cell trait reduced the risk by 41% (P = 0.11), a finding that was similar to the results of previous studies. The high-transmission season increased the risk of severe malaria (P = 0.05).

We fit a generalized-estimating-equation model with log-transformed parasite density as the dependent variable (Table 2). Sickle cell trait and bed-net use significantly reduced parasite density (P<0.001), whereas placental malaria at the time of second or subsequent delivery (P = 0.04) and the high-transmission season (P<0.001) significantly increased parasite density. The effects of these factors on parasite density parallel their effects on the risk of severe malaria, which suggests
that interventions that reduce parasite density might prevent life-threatening disease by a mechanism that differs from naturally acquired resistance.
Most of the children in our study had severe malaria after one or more previous infections, rather than during their first infection, and most such children had much higher parasite densities (six times as high) with only mild symptoms in previous or subsequent infections. Taken together, the data suggest that severe malaria cannot be explained by parasite burden alone and that factors such as parasite virulence and host inflammation might play key roles in immunopathogenesis. Furthermore, acquired immunity to severe malaria is not explained by improved control of parasite density and therefore might target distinct antigens or processes involved in severe syndromes.

On the basis of rates of infection and hospitalization due to malaria, the risk of noncerebral severe malaria was thought to be limited to the first infection or two. This prospective study provides evidence that the risk of severe malaria is stable over several infections. As transmission decreases in many parts of Africa, the risk of severe malaria shifts to older age groups, and the idea that only minimal exposure is required to generate immunity may lead to complacency regarding this danger. Conversely, the relatively poor efficacy of the antimalarial agents recommended at the time of our study (treatment with artemisinins was implemented as policy after our study ended) may have contributed to high transmission, as well as to the early peak incidence and rapid acquisition of immunity to severe malaria.

The low risk of recurrent severe malaria and the rapid decrease in the incidence of severe malaria after infancy are consistent with earlier evidence that immunity to severe malaria is acquired rapidly. Recurrences of severe malaria are equally likely to be the same syndrome as the first episode or a different syndrome (Table S6 in the Supplementary Appendix). This fact and the observation that a child’s single episode of severe malaria is manifested as a single defining syndrome suggest that immunity to severe malaria is not syndrome-specific and therefore that targets of protective immunity may be conserved among syndromes.

Our data suggest that the total parasite burden contributes to the pathogenesis of severe malaria, although this association does not prove causality: parasite levels are higher during severe episodes, and several risk factors for severe malaria also affect parasite density. Because these factors might modify the risk of severe malaria through their effect on parasite density, interventions designed to reduce parasite density, such as vaccines targeting merozoites, might provide at least partial protection against severe disease. Possibly because responses to multiple variants are required to block erythrocyte infection, early merozoite vaccine candidates did not reduce levels of blood-stage parasites, and their efficacy against severe malaria has therefore not been assessed.

Our findings are in agreement with those of earlier studies showing that sickle cell trait, the low-transmission season, and bed-net use are associated with reduced parasite density. A new finding is that placental malaria at the time of second or subsequent delivery increases the risk of severe malaria in a woman’s offspring, which is similar to the reported effect of placental malaria in such women on the overall risk of infection and on the risk of
The mechanism behind this observation is unknown, but it suggests that in utero exposure might influence immune responses to malaria parasites in early childhood.

Despite its association with disease, parasite burden alone does not completely explain severe malaria. Most children had high-density infection with only mild symptoms before the episode of severe malaria (21 of 102 children), after the episode (55 of 102), or both, and most children (67 of 102), regardless of whether they ever had a high-density infection, did not have their severe malaria episode at the time of their highest-density infection. Parasite sequestration complicates the quantification of parasite density in blood smears; however, analysis of PfHRP-2 levels, which are believed to be a better measure of total parasite mass, yielded the same conclusions as our blood-smear data.

We found that naturally acquired resistance to severe malaria was not accompanied by improved control of parasite density. Although the incidence of severe malaria decreases significantly after the first year of life, children continue to have high-density infections. Mean parasite densities do not differ significantly during infections occurring before and those occurring after severe malaria episodes. An understanding of parasite and host factors other than parasite burden that are associated with severe malaria might lead to identification of targets for new vaccines. For example, PfEMP1, a family of highly variant proteins found on the surface of infected erythrocytes, has been linked to severe disease and might be targeted by vaccines.

Clinical malaria in their offspring. We observed that severe malarial anemia was usually caused by an abrupt decrease in the hemoglobin concentration, often exceeding 3 g per

<table>
<thead>
<tr>
<th>Table 2. Factors That Influenced the Risk of Severe Malaria and Parasite Density during Infection.</th>
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<tbody>
<tr>
<td><strong>Factor</strong></td>
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<tr>
<td>Sickle cell genotype hemoglobin AS vs. AA</td>
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<tr>
<td>Bed-net use vs. no bed-net use</td>
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<td>Male sex</td>
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<td>Village of residence</td>
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<tr>
<td>Magila</td>
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<td>Bwembwera</td>
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<td>Mkanyageni</td>
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<td>Muheza township</td>
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<tr>
<td>High-transmission season vs. low-transmission season</td>
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<tr>
<td>Maternal parity and placental malaria status</td>
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<tr>
<td>First delivery, no placental malaria</td>
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<tr>
<td>Second or subsequent delivery, no placental malaria</td>
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<tr>
<td>First delivery, placental malaria</td>
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<tr>
<td>Second or subsequent delivery, placental malaria</td>
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</table>

* A Cox multivariate model was used to assess the time to the first episode of severe malaria.
† A multivariate generalized-estimating-equation model was used to assess parasite density (log₁₀ number of parasites per 200 white cells) during infection.
deciliter. Severe malarial anemia in children is commonly believed to develop as a chronic process, but the pattern we observed indicated a substantial contribution of the acute infection to hemoglobin loss. These two distinct dynamics of hemoglobin decrease could represent different pathologic processes.

Our study provides direct evidence that severe malaria is unlikely to recur but that it commonly occurs after previous infections, including high-density infections. The immunity to severe malaria is not related to improved control of parasite density. The epidemiologic evidence suggests that naturally acquired immunity targets a conserved feature of the various severe-malaria syndromes, such as parasite virulence or host inflammation. An understanding of the natural history of P. falciparum infection and disease can guide mechanistic studies of the pathogenesis of and immunity to severe malaria, which in turn can lead to the development of new therapeutics.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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REFERENCES

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