Clinical Manifestations of Plasmodium falciparum Malaria Experimentally Induced by Mosquito Challenge


A system for experimental challenge with malaria that is reliable, predictable, and safe in human volunteers is imperative in the development of a successful malaria vaccine. A system utilizing the bites of membrane-fed anopheline mosquitoes carrying Plasmodium falciparum to infect volunteers was first reported by Chulay et al. [1] in 1985. Since then, this model has been used as the method of challenge in numerous malaria vaccine and chemoprophylaxis studies, with consistent results and few complications [2–13]. The present report summarizes the clinical and laboratory manifestations of P. falciparum malaria induced by challenge with membrane-fed anopheline mosquitoes in 118 volunteers infected between 1985 and 1992.

Methods

This study was conducted as a retrospective review of the medical records and investigator records from 18 separately conducted challenge trials [2–13]. Permission to review these records was obtained from a principal investigator for each trial, or the principal investigator conducted the review.

Each record was reviewed for predefined symptoms (fever, chills, fatigue, malaise, myalgias, arthralgias, headache, nausea, vomiting, diarrhea, abdominal pain), signs (maximum daily temperature, pulse, systolic blood pressure), and laboratory data (levels or absence of white blood cells [WBC] with differential, platelets, blood urea nitrogen [BUN], creatinine, aspartate aminotransferase [AST], alanine aminotransferase [ALT], and total bilirubin) that we established for this analysis. Not all symptoms, signs, and laboratory data were recorded for all subjects. Furthermore, although several studies used symptom scales in an effort to quantitate the severity of symptoms, these scales were not uniform among the studies using them; symptoms were therefore recorded as either present or absent on each study day. Symptom data were recorded only if a positive or negative response was noted in the and International Environmental and Scientific Affairs, US Department of the Navy or Army.

Reprints or correspondence: Dr. Stephen L. Hoffman, Malaria Program, Naval Medical Research Institute, 12300 Washington Ave., Rockville, MD 20852.

* Present affiliations: Ochsner Clinic of Baton Rouge, Louisiana (L.W.P.C.); US Army Medical Research Unit, Nairobi, Kenya (D.M.G.); NABI, Rockville, Maryland (L.F.); Emerging Infectious Diseases Program, Bureau of Oceans and International Environmental and Scientific Affairs, US Department of State, Washington, DC (J.R.D.); Division of Infectious Diseases, Bowman-Gray School of Medicine, Winston-Salem, North Carolina (D.A.H.); Dermatology Service, Fitzsimons Army Medical Center, Aurora, Colorado (T.W.M.); Glaxo Wellcome, Inc., Research Triangle Park, North Carolina (J.D.C.).

The Journal of Infectious Diseases 1997;175:915–20


Written informed consent was obtained from all volunteers, and all studies were conducted in accordance with guidelines established by and with the approval of the institutional review board at each of the study locations.

Financial support: US Army Medical Research Command; US Naval Medical Research and Development Command (work unit 63002A.810.00101.

HFX.1433).

The opinions and assertions contained herein are those of the authors and are not to be construed as reflecting the views of the Department of the Navy or Army.
(r₉) was calculated using Minitab release 9 (Minitab, State College, PA).

**Results**

**Patient population.** Records for 118 volunteers who had been infected while participating as vaccinees (58), infectivity controls (56), or recipients of doxycycline prophylaxis (4) were reviewed. The median age of volunteers was 25 years (range, 18–49); 105 participants were men and 13 were women. Seventy-eight volunteers were white, 26 were black, and 2 were nonblack Hispanic; racial and ethnic data were not recorded for 12 volunteers. All volunteers were healthy by history and physical examination, with no evidence of hepatitis B or human immunodeficiency virus infection, hemoglobinopathy, or other chronic disease by laboratory analysis. One patient had glucose-6-phosphate dehydrogenase deficiency.

Volunteers were infected by exposure to the bites of 1–5 *Anopheles stephensi* or *Anopheles freeborni* mosquitoes subsequently verified to carry sporozoites. The chloroquine-sensitive NF54 strain (n = 78) [15], the 3D7 (n = 16) [16] and CVD1 (n = 8) [17] clones of NF54, or the chloroquine-resistant 7G8 clone (n = 16) [18] of *P. falciparum* were used in all challenges. Volunteers were evaluated daily by history, physical examination, and blood smear starting 5–7 days after challenge and continuing until day 30; evaluations were then done weekly for an additional 4–8 weeks.

Subjects were treated within the first 24 h of parasitemia (detected by thick blood film) in 110 of 117 infections; in 7 volunteers participating in a doxycycline prophylaxis protocol [11], treatment was initiated 24–72 h after the detection of parasitemia. In 1 volunteer, parasites were never detected on blood smear, despite the presence of typical symptoms on days 14–16; blood obtained on day 19 was culture-positive, and this individual was treated on day 30. Treatment regimens consisted of oral chloroquine (600 mg initially, followed by 300 mg 6, 24, and 48 h later) with (n = 22) or without (n = 80) primaquine (26.3 mg base daily for 5 days) for the chloroquine-sensitive strains or single-dose mefloquine (1000 or 1250 mg orally, n = 16) for infection with the 7G8 clone.

Symptoms and signs. All but 4 patients developed symptoms or signs during the follow-up period after challenge that could be attributed to malaria (table 1). Symptoms were first recorded from 6 to 23 days after exposure to sporozoites. The median duration of clinical illness was 3 days (range, 1–9). The most frequent symptoms included myalgias or arthralgias, malaise or fatigue, headache, and chills. Symptom frequency increased significantly after the identification of parasites in peripheral blood and the initiation of therapy (table 2).

Fever (oral temperature ≥38.0°C) was documented in 62 volunteers, with a median duration of 2 days (range, 1–5; table 1). Four volunteers experienced oral temperatures of ≥40.0°C. Four patients received intravenous fluids for dehydration; 3 additional patients were noted to have significant orthostatic blood pressure drops (>10 mm Hg decline in systolic pressure with accompanying symptoms) that responded to oral rehydration. One volunteer developed shortness of breath, beginning 2 days prior to the detection of parasitemia and persisting for 3 days, for which an etiology could not be determined. No patient was found to have altered mental status or signs of cerebral malaria, and in no cases was hepatomegaly or splenomegaly detected.

**Laboratory results.** Changes in WBC count, hemoglobin level, and platelet count were assessed at baseline, the day of initial parasitemia, 36–72 h after the first detection of parasitemia, and ~3 weeks after the detection of parasitemia (table 3). Significant declines in total WBC and platelet counts were noted, reaching a nadir 2 days after initiation of therapy (P < .0001, 2-tailed t test, paired samples). In 10 of 83 volunteers, the WBC count was <3000/µL (minimum, 1400/µL; normal range, 4000–11,000/µL). The absolute neutrophil count was <1000/µL in 7 of 79 subjects (minimum, 658/µL). Thrombocytopenia (platelet count <100,000/µL) occurred in 10 of 83 volunteers; the minimum recorded platelet count was 73,000/

---

**Table 1.** Frequency and duration of symptoms and fever associated with experimentally induced *P. falciparum* malaria.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Frequency (%)</th>
<th>Median (range)</th>
<th>Mean ± SD (geometric mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any symptom</td>
<td>114/118 (97)</td>
<td>3 (1–9)</td>
<td>3.78 ± 1.93 (3.28)</td>
</tr>
<tr>
<td>Arthralgia/myalgia</td>
<td>90/114 (79)</td>
<td>3 (1–8)</td>
<td>2.77 ± 1.53 (2.39)</td>
</tr>
<tr>
<td>Malaise/fatigue</td>
<td>84/106 (79)</td>
<td>2 (1–8)</td>
<td>2.81 ± 1.61 (2.38)</td>
</tr>
<tr>
<td>Headache</td>
<td>90/117 (77)</td>
<td>3 (1–7)</td>
<td>2.99 ± 1.52 (2.58)</td>
</tr>
<tr>
<td>Chills</td>
<td>80/118 (68)</td>
<td>2 (1–4)</td>
<td>1.89 ± 0.84 (1.71)</td>
</tr>
<tr>
<td>Nausea with or without vomiting</td>
<td>48/117 (41)</td>
<td>1 (1–8)</td>
<td>2.10 ± 1.52 (1.71)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>18/111 (16)</td>
<td>2 (1–7)</td>
<td>2.22 ± 1.62 (1.80)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16/111 (14)</td>
<td>1 (1–5)</td>
<td>1.50 ± 0.71 (1.36)</td>
</tr>
<tr>
<td>Oral temperature &gt;38°C</td>
<td>62/101 (61)</td>
<td>2 (1–5)</td>
<td>2.08 ± 1.06 (1.83)</td>
</tr>
</tbody>
</table>
Table 2. Frequency of symptoms before and after initiation of malaria chemotherapy.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. positive (%)</th>
<th>Before treatment (%)</th>
<th>After treatment (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthralgias/myalgias (n = 114)</td>
<td></td>
<td>51 (45)</td>
<td>77 (68)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Malaise/fatigue (n = 106)</td>
<td></td>
<td>52 (49)</td>
<td>76 (72)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Headache (n = 117)</td>
<td></td>
<td>51 (44)</td>
<td>81 (69)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Chills (n = 118)</td>
<td></td>
<td>39 (33)</td>
<td>62 (53)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Nausea with or without vomiting (n = 117)</td>
<td></td>
<td>13 (11)</td>
<td>46 (39)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Abdominal pain (n = 111)</td>
<td></td>
<td>9 (8)</td>
<td>14 (13)</td>
<td>NS</td>
</tr>
<tr>
<td>Diarrhea (n = 111)</td>
<td></td>
<td>3 (3)</td>
<td>15 (14)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Oral temperature &gt;38°C (n = 97)</td>
<td></td>
<td>23 (23)</td>
<td>53 (53)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* Determined using McNemar’s 2-sample test for binomial proportions for matched-pair data, according to Rosner [14]. NS, not significant.

\( \mu L \). No concurrent infections were reported in any of the volunteers, and no bleeding episodes were reported. The mean hemoglobin level was slightly lower at all time points than baseline values (table 3). The greatest decline recorded for a volunteer at any one time from baseline was 3.6 mg/dL; the lowest hemoglobin value recorded after challenge was 11.8 mg/dL.

Two volunteers developed significant aminotransferase elevations (AST, 162 and 224 U/L; ALT, 314 and 353 U/L) at the time parasitemia was detected; these abnormalities returned to baseline. No increase in alkaline phosphatase or serum bilirubin was recorded in these 2 volunteers. Serum electrolytes, BUN, and creatinine remained normal in all volunteers. Trace proteinuria was detected by dipstick in 10 of 72 volunteers at the time of the first positive smear.

Parasitemia. The mean prepatent period (time from exposure until first detected parasitemia) for all volunteers was 11.5 days (range, 7–23); all 4 subjects with a prepatent period \( \geq 20 \) days were from 1 challenge group in one study, suggesting a difference in the infectivity of \( P. falciparum \) or a difference in the efficiency of transmission by \( A. stephensi \) in this group. There was a negative correlation between the length of the prepatent period and the number of infective bites (\( r_s = -0.343, P < .001 \)) but not the mean gland grade (defined in [3]) of the biting mosquitoes (\( r_s = 0.05, P > .50 \)). Incubation periods (time from exposure until first symptoms) could not be determined for the group, as 42 of 118 patients were asymptomatic at the time parasitemia was detected and therapy started.

The maximum geometric mean parasitemia was 46 parasites/\( \mu L \) (range, 4–1848). The maximum level of parasitemia did not correlate with the duration of symptoms (\( r_s = 0.049, P > .50 \)) or the height of the maximum temperature (\( r_s = 0.029, P > .50 \)) but correlated significantly with the total duration of parasitemia (\( r_s = 0.325, P < .01 \)). The median duration of parasitemia was 2 days (range, 1–7) once antimalarials were initiated, although parasites were noted discontinuously for as long as 7 days in 1 volunteer [19]. No recrudescence occurred in any of the volunteers in these studies.

Subgroup comparisons. Comparisons of the laboratory and symptom data between male and female volunteers yielded similar results, except that women had significantly lower levels of parasitemia (34 vs. 156/\( \mu L, P = .0009 \), 2-tailed \( t \) test assuming unequal variances) and a slightly shorter mean duration of parasitemia (1.6 vs. 2.2 days, \( P < .025 \)). No differences were noted in the duration of symptoms, maximum temperature, changes in hemoglobin level or white blood cell and platelet counts, maximum parasitemia, or prepatent period when volunteers were grouped according to race (data not shown). There were no differences in symptoms, signs, or labo-

Table 3. Mean values for white blood cell (WBC) count, platelet count, and hemoglobin level during the course of experimentally induced malaria.

<table>
<thead>
<tr>
<th></th>
<th>Before challenge</th>
<th>Day of first positive smear</th>
<th>After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (( \times 10^9/L ))</td>
<td>6.0 ± 1.5</td>
<td>5.5 ± 1.5</td>
<td>4.2 ± 1.5</td>
</tr>
<tr>
<td>ANC (( \times 10^9/L ))</td>
<td>3.4 ± 1.1</td>
<td>3.5 ± 1.1</td>
<td>2.5 ± 1.6</td>
</tr>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>14.8 ± 1.1</td>
<td>14.6 ± 1.1</td>
<td>14.4 ± 1.9</td>
</tr>
<tr>
<td>Platelets (( \times 10^9/L ))</td>
<td>266 ± 58</td>
<td>238 ± 66</td>
<td>177 ± 68</td>
</tr>
</tbody>
</table>

NOTE. ANC, absolute neutrophil count.
ratory data among patients challenged with NF54 (n = 78), 3D7 (n = 16), CVDL (n = 8), or 7G8 (n = 16) parasites.

The frequency of gastrointestinal complaints (nausea, vomiting, diarrhea, or abdominal pain) did not differ between the three drug treatment regimens. The duration of nausea or vomiting in symptomatic volunteers infected with the 7G8 clone and receiving mefloquine was significantly longer (n = 9; duration, 3.00 ± 1.50 days) than in volunteers infected with NF54 or its clones who received chloroquine plus primaquine (n = 9; duration, 1.67 ± 1.66 days, P = .0007) or chloroquine alone (n = 29; duration, 1.46 ± 0.69 days, P = .0017, 2-tailed t test assuming unequal variance).

Discussion

To better define the clinical course of experimentally induced P. falciparum malaria, we reviewed data collected from 18 independently conducted studies. Inherent in such a review are confounding factors that commonly plague metaanalysis: missing data and differences in study design, methodology, and data collection [20]. These differences may result in bias. Although interpretation of these data must, therefore, be viewed with caution, the findings are generally consistent with previous observations regarding clinical malaria.

Previous understanding of the clinical course of induced P. falciparum malaria has been derived from observations made during the use of malaria therapy for the treatment of neurosyphilis, from antimalarial trials, and from studies of antimalarial resistance. Malaria therapy with Plasmodium vivax was first used systematically by Wagner-Jauregg [21] and was widely used in the United States and Europe in the prepenicillin era. In an effort to induce more sustained and severe hyperthermia, some investigators substituted P. falciparum for P. vivax. Early efforts were marred by high mortality, but successive work lowered mortality to an “acceptable” level of 4% [22]. Unlike the current challenge studies, the purpose of malaria therapy was to induce clinical illness, particularly fever >40°C, and maintain illness as long as the condition of the patient permitted or until the malaria resolved spontaneously. Under these circumstances, recrudescences were common (as a result of treatment with subcurative doses of quinine), in contrast to current methods, with which recrudescence has not been observed. The next generation of observations on sporozoite-induced P. falciparum malaria were made during studies of the clinical course of malaria and drug treatment studies [23, 24]. In these studies, it was common practice to allow volunteers to remain symptomatic and parasitemic for several days before treatment was initiated. In one review of challenge studies, this translated into a mean parasite density of 88,713/µL [25], a result >1000-fold higher than the mean parasite density observed in the present analysis. Despite these higher parasitemias and the greater severity of clinical manifestations, none of the >2000 volunteers studied in this manner died as a direct result of infection by P. falciparum.

In the studies reviewed, onset of symptoms was as early as 6 days after challenge and as late as 23 days. Because not all patients were symptomatic at the time of detection of parasitemia, an accurate incubation period could not be calculated. Incubation periods in other studies of experimentally induced malaria have closely approximated the prepatent period, with a mean of 12 days (range, 6–17) observed by James et al. [22] and also 12 days (range, 10–15) reported by Coatney et al. [24]. The mean prepatent period of 12 days (range, 6–23) observed in the present series corresponds closely to those reported by Coatney et al. (11 days; range, 9–13) [24] and Powell and McNamara (11 days; range, 9–13, in volunteers bitten by 1–5 infected mosquitoes) [26].

In this series, we were able to confirm an inverse correlation between the number of infectious bites and the prepatent period. In the series reported by Powell and McNamara [26], a significant difference was detected in the mean prepatent period in volunteers exposed to 1–5 bites compared with >10 bites (mean prepatent period, 11 vs. 7.6 days, respectively).

The spectrum of symptoms exhibited by volunteers in the present series is similar to those seen in nonimmune persons who are naturally infected (table 1). However, the volunteers did not become as ill as do many patients who contract malaria under natural conditions of exposure. We believe that this was attributable to the fact that they were diagnosed and treated early in the course of illness, before the parasite burden had increased significantly. The increase in frequency of symptoms and elevated temperatures after the initiation of chemotherapy noted in this review (table 2) has not been a feature of previous reports, although most patients in other studies appear to have been more symptomatic at the time treatment was initiated than our volunteers were at any time.

This increase in frequency of symptoms was not unique to any one treatment regimen; in particular, gastrointestinal symptoms occurred with equal frequency with the three treatment regimens, which is probably representative of the frequency of these symptoms in acute malaria. Investigators involved in challenge studies in which mefloquine was used noted that gastrointestinal side effects were more severe and vomiting more frequent than in studies relying on chloroquine. The increased duration of nausea and vomiting in volunteers treated with mefloquine is consistent with this observation, although differences in the infecting P. falciparum strains is an alternative explanation. There are several possible explanations for why clinical manifestations increased after initiation of treatment in some volunteers. One is that our screening procedure was so sensitive that, in a number of patients, we detected parasitemia before the parasites reached the threshold necessary to induce clinical manifestations and that subjects then developed symptoms and signs as they would have in the disease’s natural history. Another explanation is that initiation of treatment in these subjects induced rupture of parasites and release of parasite “toxins” [27], which led to the clinical manifestations.
In this series, platelet counts as low as 73,000/µL were observed without clinical manifestations. Thrombocytopenia, with counts as low as 20,000/µL, has been reported in up to 86% of naturally acquired *P. falciparum* infections [28] but has rarely been associated with evidence of bleeding diathesis [29]. Platelet counts in the present series reached their nadir 2 days after the initial detection of parasitemia, coinciding with the median time to parasite clearance. This finding is consistent with the hypothesis that hemolysis of parasitized red cells releases adenosine diphosphate, which leads to platelet activation [30]. Activated platelets are functional but have a shortened life span; platelet counts could thus be expected to continue to decline after the last infected erythrocytes rupture, before climbing back toward baseline.

Anemia was not a prominent feature in sporozoite-inoculated volunteers. Although hemoglobin values declined by as much as 3.6 mg/dL in 1 individual, no volunteer’s hemoglobin level fell below 11.8 mg/dL. The patterns of rise and fall of hemoglobin levels varied considerably among the volunteers, and no consistent pattern of decline in hemoglobin level was identified. Phlebotomy may be an important factor contributing to changes in hemoglobin level in these volunteers.

In acute malaria, with the exception of severe malaria, the initial leukocyte count is normal or decreased [31]. The fall in leukocyte count is usually attributed to a decline in the granulocyte count, which may be offset in part by a rise in circulating monocytes. Despite absolute neutrophil counts of <1000/µL in 7 of 79 volunteers studied, no complications attributable to granulocytopenia were noted. In human infections with *P. vivax*, declines in neutrophil counts (to as low as 360/µL) are a result of a decrease in circulating neutrophils, with a corresponding increase in the marginal pool [32], suggesting that the observed neutropenia in acute malaria is due to a redistribution of neutrophils and not to neutrophil deficiency.

Proteinuria was identified in 10 patients in this review and never exceeded trace amounts by dipstick testing. Transient proteinuria, which characteristically resolves within 1–2 weeks of treatment, has been reported in 20%–70% of *P. falciparum*-infected patients [33]. The exact cause is unknown, but both glomerular and tubular lesions appear to be involved. An association between the fever and proteinuria has been demonstrated. Most importantly, this early proteinuria due to *P. falciparum* infection resolves within 1–2 weeks after appropriate treatment. Severe renal insufficiency has been reported as complicating *P. falciparum* infection [34] but was not a feature of the volunteers in the studies we reviewed, all of whom maintained normal BUN and creatinine levels throughout the monitoring periods.

Pathologic changes in the liver are a uniform feature of *P. falciparum* infection [35], and aminotransferase elevations may occur commonly in clinical cases [36]. Two patients in the present review had elevations of liver enzymes. Neither had a high parasite density (80 and 104/µL), but both patients had maximum temperatures of ≥40°C and were febrile or otherwise symptomatic for 4 days, suggesting that they were among the most ill patients in these studies. Both patients also reported nausea after starting chloroquine therapy but denied other gastrointestinal symptoms. Such symptoms have been reported to be frequent in *P. falciparum* infection when the liver enzymes are elevated but are not predictive of abnormal liver enzymes [31].

Experimental infection of volunteers with *P. falciparum* by the bite of 5 infected anopheline mosquitoes has proven to be a safe and reliable method for challenge. Although nearly all volunteers will be symptomatic, the illness is frequently mild. Detection of parasitemia reliably occurred at parasite densities of <0.05%, a level 100-fold less than the parasitemia that defines severe malaria. No patient in this review met criteria for severe malaria and all recovered without sequelae. In addition, no recrudescence of infection occurred, eliminating the risk of delayed clinical illness or secondary transmission.

References


