PLASMODIUM FALCIPARUM CLINICAL MALARIA IN DIELMO, A HOLOENDEMIC AREA IN SENEGAL: NO INFLUENCE OF ACQUIRED IMMUNITY ON INITIAL SYMPTOMATOLOGY AND SEVERITY OF MALARIA ATTACKS

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Abstract. Six hundred eighty-nine Plasmodium falciparum malaria attacks were observed during a three-year period among 226 inhabitants of the village of Dielmo, Senegal, an area of high malaria transmission. Malaria attacks were defined as clinical episodes with fever (body temperature ≥ 38.0°C) or reporting of fever or headache or vomiting, associated with a parasite:leukocyte ratio above an age-dependent pyrogenic threshold identified in this population. The symptom frequencies were tested against age, gender, and parasite density using a random-effect logistic regression model and the study of distinguishable clinical presentations was carried out by multi-correspondence analysis. There was little difference between the severity of symptoms during the initial course of attacks in young children and adults, and this severity was not correlated with the duration of the pathologic episode. It was not possible to distinguish objectively different malaria attack types according to the severity of clinical manifestations. In contrast, the duration of fever, symptoms, and parasite clearance were significantly longer among the youngest children than among the oldest children and adults. These findings suggest that of the two components of protective immunity, anti-parasite immunity and anti-toxic immunity, only the first would play a major role as age increases. They suggest also that the initial clinical presentation of malaria attacks is not predictive of the level of protective immunity.

The main goal of the current malaria control strategies is to prevent malaria mortality by diagnosing and treating clinical manifestations of the disease as early as possible. This depends not only on the availability of efficient health services but also on the knowledge and the attitude of the patients or of their parents and on their perception of the initial symptoms that influence their therapeutic approach at home. Although a number of studies have been published on the signs and symptoms of clinical malaria in endemic populations, almost all of these studies were conducted in hospitals or outpatient clinics. Few detailed data are available on the range of clinical manifestations of malaria at the community level, their relative frequency, and their value in diagnosing the disease and initiating treatment. Since access to health facilities is difficult for most rural populations of tropical Africa, a better knowledge of clinical malaria at the community level could help to develop simple rules that would be necessary for reducing malaria mortality in these populations.

A better knowledge of the whole range of malaria morbidity would also determine whether different clinical forms of nonsevere malaria attacks exist. The distinction between forms of malaria attacks that differ in the severity of clinical expression could have implications for the evaluation of control methods. The assessment of the clinical efficiency of malaria drugs or vaccines could vary according to the clinical forms that have been chosen to evaluate them. Recently, it has been proposed to differentiate mild from very mild forms of malaria attacks and it was hypothesized that this difference could reflect heterogeneity in parasite virulence. Furthermore, some research aims to develop a vaccine against clinical manifestations of malaria rather than against the parasite itself. It is not known if the immunity acquired by persons living in highly endemic areas acts more by decreasing the frequency of malaria episodes or by decreasing the signs and symptoms to the point that clinical malaria episodes remain undetected.

The present study was undertaken among the population of Dielmo, Senegal that is exposed to high and perennial malaria transmission. In this population, the incidence of clinical malaria was highest in the children two years of age, with an average of six malaria attacks per year. This decreased rapidly to reach, between the age of 10 and 15 years, its minimum level of one attack every seven years observed among adults. The objectives of the present study were 1) to analyze the age-dependent variations of clinical manifestations during nonsevere malaria attacks, 2) to determine if clinical forms of malaria attacks may be distinguished by the severity of manifestations, and 3) to serve as a basis to the elaboration of improved rules for clinical diagnosis and treatment.

PATIENTS AND METHODS

Study population and case and data management. The study took place from May 28, 1990 to May 31, 1993. The entire population of Dielmo village was involved in a prospective study described elsewhere. Briefly, to identify all episodes of morbidity, the 247 villagers were put under daily medical surveillance. At least one physician, a technician or a nurse, and three medical field workers were present in the village 24 hr a day, seven days a week. Among the population (female: male ratio = 0.98), 20.4% were children less than five years of age and 26.8% were children 5–14 years of age.

Informed consent was obtained from all adult participants and from parents or legal guardians of minors. The project was approved by the Conseil de perfectionnement de l’Institut Pasteur de Dakar.

Active case detection was carried out and each villager was visited daily at home. For each clinical episode with a history of fever, headache, or vomiting, thick blood films and medical examinations were made. The clinical signs and symptoms were systematically recorded on an ad hoc ques-
tional questionnaire. All the questionnaires (in Wolof and in Serere, the native languages) were established with the assistance of linguists and physicians of the corresponding ethnic groups. They were validated by ethnonlinguistic studies using the focus group method. They concerned 16 categories of symptoms that were recognized by the patients or their parents and 38 clinical signs looked for by the physician or the nurse. The body temperature was measured rectally in children less than seven years of age. The axillary temperature was measured in persons more than six years of age and the result was corrected by an addition of +0.5°C. We used a clinical mercury thermometer (Coopérative Pharmaceutique Française, Melun, France). Asthenia when present was considered as very mild when it did not limit activity, as mild when it limited the usual activity (playing, school attendance, or agricultural work), or as intense when the person was unable to remain sitting and was confined to bed. Thirst was considered as pathologic when it was perceived as unusually intense or frequent by the patient or their parents. Diarrhea was defined by an increased number (usually > 3/ day) of liquid stools. The allegations of cold sensation, pain, or nausea were not considered in those less than six years of age. Thick blood films were made in triplicate during clinical episodes. One thick smear was stained with Giemsa without previous dehemoglobinization and examined immediately so that a decision regarding treatment could be made; the other two were dehemoglobinized, stained, and stored.

Since previous studies had indicated that symptoms occurred as a function of an age-dependent pyrogenic threshold and high transmission induced protective immunity in older children and adults, treatment of malaria cases with quinine was restricted to acute attacks according to the following criteria: 1) fever with a parasite:leukocyte ratio ≥ 2 in children less than 10 years of age, 2) fever with a parasite:leukocyte ratio ≥ 0.5 in pregnant women, or 3) fever with a positive thick blood smear in individuals with symptoms compatible with severe malaria or in individuals returning from an area of low endemicity where they had lived for more than one year during the three years prior to the study. For the remaining patients, appropriate symptomatic or specific treatment of nonmalarial diseases was provided. When fever persisted to the next day, another thick blood smear was made. Criteria for antimalarial treatment remained unchanged. However, in the absence of clinical improvement associated with 1) a parasite density close to the treatment threshold, 2) a positive thick blood smear in infants and pregnant women, or 3) a parasite:leukocyte ratio ≥ 0.5 in adults, the requirement for antimalarial treatment was decided considering all the patient’s clinical, biological, and epidemiologic data. The antimalarial treatment of uncomplicated malaria attacks consisted of daily oral doses of 25 mg/kg of Quinimax® (Sanofi-Winthrop, Gentilly, France) (Quinimax® contains 71.4% quinine-resorcin bichlorhydrate, 18.6% quinidine-resorcin bichlorhydrate, and 5% cinchonine-resorcin bichlorhydrate) administered every 8 hr by a medical field worker or a physician. The duration of the Quinimax® course was three or seven days as described elsewhere,11 which in this population proved equally efficient.

Clinical signs and symptoms (hot sweats, shivering, asthenia, vomiting, diarrhea, and headache) and axillary or rectal temperature were recorded every 8 hr for seven days and recorded on an ad hoc form. Thick blood smears were prepared between day 3 and day 10 after the beginning of the treatment to check for parasitologic efficacy. These blood films were also dehemoglobinized, stained, and stored.

All thick blood film readings were standardized.12 Two hundred microscopic oil-immersion fields were examined on each slide (about 0.5 μl of blood) by the same experienced technician at the end of the study. The parasite counts considered for the analysis were those obtained by this independent examination.

The hemoglobin level and hematocrit rates were measured at the moment of the inclusion of villagers in the study and on the occasion of several later blood sampling sessions destined for immunologic studies during asymptomatic periods.

The population was asked not to use any drugs without informing the team, and urine tests were carried out regularly (3,798 tests during the study period) to detect the presence of antimalarial drugs, using the modified Saker-Solomons test.13 Less than 1.4% of the results of these tests were compatible with unknown self-treatment during the study period and were possibly related to traditional treatment that may produce positive urine test results without any antimalarial activity (Rogier C, unpublished data).

Statistical analysis. Malaria attacks were defined as clinical episodes with fever (body temperature ≥ 38.0°C), alleged fever, headache, or vomiting associated with a parasite:leukocyte ratio above an age-dependent pyrogenic threshold identified in this population.10 The level of this threshold varied by 2.45 trophozoites per leukocyte, maximum at one year of age, to 0.5 trophozoites per leukocyte, minimum after 60 years of age, matching the description of a decreasing parasite tolerance. We only considered the persons who continued to live in Dielmo during the follow-up period and met the following criteria at the moment of the malaria attack: 1) at least 50% of their life since birth spent in Dielmo or an area of high malaria endemicity, 2) continued presence in the village or a maximum absence of 30 days during the six months preceding the study period, and 3) continued presence in the village or a maximum absence of one year during the three years preceding the study period. Pregnant women are the object of a separate analysis.14

The clinical recovery time after treatment was calculated as the time elapsed between the moment of the first dose of Quinimax® and the time when all the symptoms disappeared, estimated by the time midway between the last symptomatic visit and the first visit free of any symptom. The time of disappearance of fever (< 38.0°C) after treatment was calculated using the same rules. The total duration of fever or the total duration of the symptoms were calculated between the moment of the first report of symptoms and time when fever or all the symptoms disappeared. The probability of clinical recovery as a function of time was graphically represented using the Kaplan-Meier method. Differences in recovery time between the oldest age groups (10–19 years versus ≥ 20 years) were tested by the Mann-Whitney test. The parasitologic efficacy was assessed by the percentage of thick blood smears free of asexual parasites and the parasite density during the five-day period after the beginning of the quinine treatment or following the maximum parasite density...
registered during the clinical attacks treated by antipyretic drugs.

In the study of clinical manifestations recorded during malaria attacks, we tested the differences of frequency of each sign and symptom according to three explanatory variables: age, gender, and parasite density. The explanatory variables were always included together in both the logistic and linear models and their interaction terms were also tested. Due to the diminution of mean parasite density with age, we used a ratio to test the effect of parasitemia, irrespective of the effect of age. This ratio, the maximum parasite density ratio (MPDR), was calculated by dividing the maximum parasite density observed during the clinical attack by the pyrogenic threshold level corresponding to the patient’s age.

We used a random-effect logistic regression model to test proportions taking into account the interdependence of successive clinical episodes in the same person (EGRET: Statistical and Epidemiology Research Corp., Seattle, WA). With this model, the estimated odds ratio can be considered as estimations of individual relative risk. The effect of explanatory variables was tested by the likelihood ratio test. The correlation between observations, representing the effect of between-person variation, was also tested by a likelihood ratio test.

Quantitative variables (body temperature and the hemoglobin level) were analyzed using a generalized estimating equation approach that allows the analysis of repeated measures, can be implemented for normal responses, and is available in the SPIDA statistical package (SPIDA Version 6; Statistical Computing Laboratory, Eastwood, New South Wales, Australia). We used an exchangeable correlation structure where the correlation between observations made in the same person at different times is assumed to be the same. This correlation structure was used instead of an autoregressive structure because the lengths of time between malaria episodes are variable. The effect of explanatory variables was tested by the Wald test.

The study of distinguishable clinical presentations was carried out by multi-correspondence analysis (MCA) available in the BMDP statistical software (BMDP Statistical Software, Inc., Los Angeles, CA). Multiple-correspondence analysis is an exploratory multivariate technique that converts frequency table data into graphic displays in which rows, i.e., individual cases, and columns, i.e., categories of variables, are depicted as labeled points on graphics. It is an extension of simple correspondence analysis. Instead of trying to compare them using proportions in several analyses, we decompose the measure of association between variables into a number of components. These components are represented by axis. The coordinates of each individual case and category of variables on these axes are calculated. These coordinates are computed so that each successive axis accounts for a decreasing portion of the total association between variables as represented by the familiar Pearson chi-square statistics or, in other words, for a decreasing portion of the total inertia, i.e., the diversity of clinical presentations. This permits most of the information included in the set of data to be graphically presented at the same time. In the plots, the proximity of points representing categories from two distinct variables may be interpreted as an association between these categories, e.g., the proximity of the points body temperature $\geq 40^\circ C$ and intense asthenia suggests an association of these clinical manifestations. The point’s proximity of categories from the same variable may be interpreted as the similarity of the mean clinical presentation between the groups of cases owning each of these categories, e.g., the proximity of the points male and female indicates similarity of the mean clinical presentation irrespective of gender. A scatterplot of cases was provided to study distinguishable clusters of clinical presentations: a multimodal distribution shows the existence of different clusters of clinical profiles. The categories of additional variables, e.g., age or parasite density, may be inserted as supplementary points without contributing to the principal inertia, i.e., the diversity of clinical presentations. The position of the categories of the additional variables in relation to the positions of the categories of the original variables could be interpreted in the sense of a visual version of regression on the principal components. Instead of studying the various regression coefficients for different additional variables, one plots the variables in the space of the original variables, then explores dependencies by examining the corresponding positions of the points for the additional variables.

RESULTS

Description of the sample. Over three years, from 28 May 1990 to 31 May 1993, 2,242 episodes of fever (body temperature $\geq 38^\circ C$ or a report of fever), headache, or vomiting were recorded among 228 persons (120 males and 108 females) in the population. Among these 2,242 episodes of fever, headache, or vomiting, the clinical file was incomplete in 94 episodes (4.2%) and the result of the thick blood smear was not available in 14 episodes (0.6%). These 108 episodes have been excluded from the analysis.

The analysis concerns 2,134 episodes of fever, headache, or vomiting observed in 226 residents (120 males and 106 females). At their inclusion into the study, 29 children among these 226 persons were newborn (included at their day of birth), 25 were less than two years old, 35 were 2–5 years old, 28 were 6–9 years old, 41 were 10–19 years old, and 68 were 20 or more years of age.

The recorded maximum parasite density was above the diagnostic threshold in 689 (32.3%) of these 2,134 pathologic episodes. Only one of these malaria attacks (0.15%) was severe, and entailed the death of a one-year-old child due to cerebral malaria despite immediate treatment with quinine and attempts at resuscitation.

Children born during the study had an average of 5.1 malaria attacks (range = 0–24) during the follow-up. Children less than two years of age at the beginning of the study had an average of 8.6 (0–24) malaria attacks, those 2–5 years of age had 6.3 malaria attacks (0–28), and those 6–9 years of age had 2.3 (0–8) malaria attacks. Individuals 10–19 years of age had 0.4 (0–2) malaria attacks and adults 20 or more years of age had 0.4 (0–2) malaria attacks. There was a significantly lower incidence rate of malaria attacks among carriers of the AS phenotype of hemoglobin than among carriers of the AA phenotype, and there was no difference in the incidence rate based on gender.

Signs and symptoms recorded during malaria attacks. The symptoms recorded during the 689 malaria attacks are
shown according to age in Table 1. In 680 (98.7%) of 689 malaria attacks, a body temperature ≥ 38°C (650 cases) or a report of fever (hot body feeling) was recorded (671 cases). The recorded maximal temperature was found to depend on age, gender, and parasite density (Tables 2 and 3). The value of the correlation between any pair of attacks for each patient (0.038) indicated that maximal temperatures recorded during several malaria episodes in the same person could be considered independent of each other. There was no significant interaction between effects of parasite density, age, and gender. The average temperature was 38.9°C (95% confidence interval [CI] = 38.7–39.1) among children 6–9 years of age, 38.8°C (38.5–39.0) among those 10–19 years old, and 38.4°C (38.1–38.8) among adults. It was higher among males (+0.17°C; \( P < 0.002 \)) than among females and was higher in the highest class of parasite density (MPDR ≥ 2.5, +0.22°C; \( P < 0.001 \)) than in the lowest class (MPDR < 2.5). There was no significant difference between means of maximal temperatures recorded at different moments of the day and their recording time did not differ significantly according to gender or age. Among 192 attacks in children less than two years of age, hypothermia (< 36°C) was recorded twice (1%): 35.9°C in a 12-month-old child with cerebral malaria and 35.8°C in a 19-month-old child. Except for case of cerebral malaria, no convulsions were observed in Dielmo during the study, either in the course of
Table 2
Frequency (%) of signs and symptoms that significantly differed as a function of gender or parasite density

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Males</th>
<th>Females</th>
<th>Parasite density &lt;2.5 times the diagnostic threshold</th>
<th>Parasite density ≥2.5 times the diagnostic threshold</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;38°C</td>
<td>4.6</td>
<td>7.0</td>
<td>8.2</td>
<td>2.8</td>
<td>5.7</td>
</tr>
<tr>
<td>38–38.9°C</td>
<td>34.8</td>
<td>42.3</td>
<td>39.3</td>
<td>36.6</td>
<td>38.0</td>
</tr>
<tr>
<td>39–39.9°C</td>
<td>46.0</td>
<td>40.3</td>
<td>44.2</td>
<td>42.8</td>
<td>43.5</td>
</tr>
<tr>
<td>≥40°C</td>
<td>14.6</td>
<td>10.4</td>
<td>8.2</td>
<td>17.8</td>
<td>12.8</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot body feeling</td>
<td>95.9</td>
<td>99.1</td>
<td>&lt;0.01</td>
<td></td>
<td>97.4</td>
</tr>
<tr>
<td>Asthenia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>33.8</td>
<td>24.9</td>
<td>&lt;0.01‡</td>
<td></td>
<td>29.6</td>
</tr>
<tr>
<td>Very Mild</td>
<td>8.0</td>
<td>9.8</td>
<td>&lt;0.01‡</td>
<td></td>
<td>8.8</td>
</tr>
<tr>
<td>Mild</td>
<td>9.9</td>
<td>10.5</td>
<td>&lt;0.03§</td>
<td></td>
<td>10.2</td>
</tr>
<tr>
<td>Intense</td>
<td>48.4</td>
<td>54.8</td>
<td>0.06§</td>
<td></td>
<td>51.4</td>
</tr>
<tr>
<td>Digestive signs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>43.7</td>
<td>56.0</td>
<td>&lt;0.005</td>
<td></td>
<td>49.5</td>
</tr>
<tr>
<td>Pathological thirst</td>
<td>39.6</td>
<td>52.0</td>
<td>&lt;0.02</td>
<td></td>
<td>45.4</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pallor</td>
<td>5.9</td>
<td>12.4</td>
<td>&lt;0.01</td>
<td></td>
<td>8.7</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleeping troubles</td>
<td>9.1</td>
<td>16.3</td>
<td>&lt;0.01</td>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>No. of cases (100%)</td>
<td>391</td>
<td>298</td>
<td>364</td>
<td>325</td>
<td>689</td>
</tr>
</tbody>
</table>

*P values are based on random effect logistic regression models including age, gender, and parasite density as explanatory variables.
† No asthenia versus asthenia.
‡ No asthenia or very mild asthenia versus higher levels of asthenia.
§ Intense asthenia versus lower levels of asthenia.

Table 3
Mean and confidence intervals (CIs) of maximum body temperature (°C) recorded during 689 malaria attacks as a function of parasite density, age, and gender

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
<th>Parasite density &lt;2.5 times the diagnostic threshold</th>
<th>Parasite density ≥2.5 times the diagnostic threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>0±1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
<td>39.0</td>
<td>38.8–39.2</td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>39.0</td>
<td>38.8–39.1</td>
</tr>
<tr>
<td>2±5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>102</td>
<td>39.1</td>
<td>39.0–39.3</td>
</tr>
<tr>
<td>Female</td>
<td>80</td>
<td>39.0</td>
<td>38.8–39.1</td>
</tr>
<tr>
<td>≥6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>61</td>
<td>38.8</td>
<td>38.6–39.0</td>
</tr>
<tr>
<td>Female</td>
<td>37</td>
<td>38.7</td>
<td>38.4–39.0</td>
</tr>
</tbody>
</table>

Malaria attacks or during the other episodes of fever. Vomiting was recorded in 39.9% of attacks, with an equivalent frequency regardless of the class of parasite density and gender. The diminution of frequency observed with age, from 40.4% among children 0–9 years of age to 33.9% among persons ≥ 10 years of age, was not significant. There was a significant between-person variation in the frequency of vomiting (P < 0.001). The frequency of diarrhea was significantly higher among children 0–1 years of age (27.6%) than among the oldest persons (9.2%; P < 0.0001). The frequency and the severity of asthenia were equivalent in all age groups and entailed confinement to bed in 51.4% of the attacks. The frequency and the severity of the asthenia were significantly increased in the highest parasite density class (P < 0.05). There was a significant between-person variation in its frequency and its severity (P < 0.02), but there was no effect of the gender. The abnormal thirst frequency increased significantly with the recorded maximal body temperature (P < 0.01). A one degree increase in body temperature corresponded to an increase by an odds ratio of 1.36 (95% CI = 1.08–1.71) of the abnormal thirst frequency.

Severe anemia was not observed during the study. Variations in hemoglobin levels according to age at the moment of inclusion into the study (most in 1990) and on the occasion of later blood sampling sessions (271 in 1991, 277 in 1992, 102 in 1993, 130 in 1994, and 57 in 1995) among asymptomatic persons are shown in Table 4. The difference in hemoglobin levels according to age was significant (P < 0.001). There was a significant improvement (P < 0.03) in the hemoglobin level of the youngest children (< 4 years old) after their inclusion into the study that was not observed among the oldest persons. Among 1,078 tested blood samples (initial samples and later sampling), no severe anemia (< 5 g/dL) was observed. The hemoglobin levels were less
Table 4
Mean (g/dL) and confidence intervals (CIs) of hemoglobin levels at the moment of the inclusion and on the occasion of later blood sampling sessions as a function of age (asymptomatic persons)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Inclusion</th>
<th>Later* blood samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Mean 95% CI</td>
<td>No. Mean 95% CI</td>
</tr>
<tr>
<td>0–1</td>
<td>20 9.9 9.4–10.6</td>
<td>39 10.3 9.3–11.3</td>
</tr>
<tr>
<td>2–3</td>
<td>18 9.6 8.9–10.3</td>
<td>46 11.4 10.3–12.6</td>
</tr>
<tr>
<td>4–5</td>
<td>19 10.3 9.3–11.3</td>
<td>46 10.9 10.4–11.3</td>
</tr>
<tr>
<td>6–7</td>
<td>15 11.9 11.4–12.4</td>
<td>56 11.2 10.9–11.6</td>
</tr>
<tr>
<td>8–9</td>
<td>13 12.5 11.7–13.3</td>
<td>48 11.8 11.4–12.1</td>
</tr>
<tr>
<td>10–19</td>
<td>50 12.3 11.9–12.8</td>
<td>198 12.5 12.3–12.7</td>
</tr>
<tr>
<td>≥20</td>
<td>106 13.2 12.8–13.7</td>
<td>404 13.1 12.9–13.3</td>
</tr>
</tbody>
</table>

* Median period between inclusion and later blood samples: 2 years, interquartile interval 25–75%: 1–3 years.

Figure 1. Representation of the dispersion of signs, symptoms and individual characteristics as a function of the two first factorial axes obtained in multi-correspondence analysis.

The abscissa and ordinate in Figure 1 represent the severity of clinical manifestations and, to some extent, the frequency of the signs and symptoms, respectively. The negative coordinates on the abscissa correspond to the lowest body temperatures (< 38°C), the absence of asthenia limiting common activity, and the absence of signs or symptoms. The points corresponding to these terms have been encircled in Figure 1A (Circle 1). Reciprocally, the positive coordinates on the horizontal axis correspond to the most intense clinical manifestations. Points corresponding to the categories temperature ≥ 40°C, sweats, anorexia, intense asthenia, and abnormal thirst were gathered on most of the factorial plans (Circle 2, Figure 1A).

The points corresponding to the categories of the supplementary variables, i.e., age, gender, phenotype of hemoglobin, and the MPDR are shown in Figure 1B. The points corresponding to the gender, the phenotypes of hemoglobin, and the youngest age groups (0–1 and 2–5 years) are close together in Figure 1B (*) and in the other factorial plans: there was no difference in average clinical presentation between males and females, between AA and AS hemoglobin.

Study of clinical forms distinguishable by the severity of manifestations. Figure 1 shows the plan representation of the dispersion of signs, symptoms, and individual characters as a function of the two first factorial axes obtained in multiple correspondence analysis of 689 malaria attacks. The abscissa and ordinate represent the severity of clinical manifestations and, to some extent, the frequency of the signs and symptoms, respectively. A, clustering of various clinical manifestations. The points corresponding to the absence of signs and symptoms are in italics and preceded by an N. The points corresponding to the maximum body temperature categories < 38°C, 38–38.8°C, 39–39.9°C, and ≥ 40°C are linked by a broken line. The points corresponding to the asthenia (Asth.) categories N. asthenia, Very mild asthenia, Mild asthenia (limiting usual activities), and Intense asthenia (with confinement to bed) are also linked by a broken line. For definitions of circles 1 and 2: see the Results. Tr. = trouble, B, clustering of groups of individuals according to gender, age, hemoglobin phenotype, and maximum parasite density ratio. The points corresponding to the categories of parasite density < 1.5T (maximum parasite density < 1.5 times the level of the diagnosis threshold), 1.5 to < 2.5T, 2.5 to < 4T, and ≥ 4T are linked by a thin line. The points corresponding to the age groups 0–1 y (children 0–1 years of age), 2–5 y, 6–9 y, and ≥ 10 y are linked by a thick line. The points corresponding to the categories males, females, and AA and AS phenotypes of hemoglobin are superposed (*).
carriers, and between infants (0–1 years) and young children (2–5 years). The points corresponding to the categories 0–1 years and ≥10 years on the one hand and an MPDR < 1.5T (maximum parasite density ≤ 1.5 times the level of the age-dependent diagnosis threshold) and an MPDR ≥ 4T on the other hand were never very distant from each other. This indicates that there were no great differences in average clinical presentation between the different age groups and between the different levels of parasite density.

The coordinates on the first axis of the points corresponding to the different age and parasite density categories were always close together. This suggests that the average clinical presentations of the different age groups and the different parasite density categories had equivalent average clinical severities. The difference between the average clinical severity of cases having a low parasite density (MPDR < 1.5) and cases having a high parasitemia (MPDR ≥ 4) was more important than the difference between the average clinical severity of cases less than two years of age and cases 10 or more years of age.

To study the existence of forms naturally distinguishable by the severity of clinical manifestations, we have considered the distribution of 689 malaria attacks according to their coordinates on the abscissa. This distribution was unimodal and slightly asymmetrical (shifted to the most severe clinical forms). In absence of multimodal distribution, it was not possible to distinguish groups of malaria attacks differing in the severity of clinical manifestations or in other clinical factors.

**Treatment of malaria attacks.** Among 689 observed malaria attacks, 566 (82%) were treated with quinine and 123 were treated symptomatically, including the 56 malaria attacks in persons 10 or more years of age (except pregnant women) who did not require quinine. Among children less than 10 years of age, treatment with quinine was complete and the results of the clinical supervision were recorded for the total duration of the treatment in 514 (81.2%) of 633 malaria attacks. These 514 attacks were retained for the analysis of the parasitologic and clinical course. In 52 cases (8.2%), the clinical supervision file was incomplete. Finally, in 67 cases (10.6%), the patient recovered from the malaria attack with symptomatic treatment (antipyretic: paracetamol) and no dose of antimalarial drug. These 67 cases were not treated with quinine for the following reasons. 1) There was an underestimation of the parasite density (PD) by the emergency microscopic examination while the parasite density measured by the examination of reference was above the therapeutic decision threshold (PD = 2 trophozoites/leukocyte): 39 cases (58.2%). 2) There was a rapid disappearance of symptoms before the examination of emergency thick smear or the beginning of quinine treatment: 17 cases (25.4%). 3) An emergency thick smear was made during the increasing phase of the parasite density: two cases. 4) Diagnosis was carried out retrospectively on a systematic thick smear that was not read as an emergency case: three cases. 5) The PD was below the therapeutic decision threshold but above the level of the diagnosis threshold: three cases. 6) There was an error in interpretation of therapeutic rules: three cases.

**Clinical recovery time after the beginning of treatment with quinine.** Among children less than 10 years of age, the median duration between the first report of symptoms and the beginning of the treatment with quinine was 5 hr (percentiles = 25–75: 2–16). Among 514 attacks treated with quinine retained in the analysis, fever (≥ 38.0°C) was absent in 18 cases (3.5%). Fever had disappeared before the beginning of the treatment in a high proportion (24.8–42.8%) (Table 5) of the cases, increasing with age and ranging from 24.8% in children 0–1 years old to 42.8% in children 6–9 years old. The disappearance time of fever after the beginning of treatment decreased with age (Table 5 and Figure 2). The between-person variation in the probability of disappearance of fever before the 12th hour was significant (P < 0.001).

Symptoms associated with fever (hot body feeling, shivering, sweats), headache, asthenia, and vomiting also disappeared before the beginning of treatment in a high proportion (20.6–35.0%) of the cases, with similar age-dependent and between-person variation patterns (Table 6).

**Clinical recovery time after the first report of symptoms.** From the first report of symptoms, the average time of disappearance of fever was 26.7 hr (95% CI = 22.8–30.7) among children 0–1 years of age treated with quinine, 19.4 hr (17.2–21.7) among children 2–9 years of age treated with quinine, and 20.8 hr (14.3–27.2) among persons ≥10 years of age who were symptomatically treated. This time did not differ significantly between those 10–19 years of age and adults ≥20 years of age (P = 0.31, by Mann-Whitney test).

We found a clearly slower decrease of fever among children less than two years of age compared with the other subjects, regardless of the type of treatment (Figure 3).

From the first report of symptoms, the average time of symptoms clearance was 30.2 hr (95% CI = 26.1–34.2) among children 0–1 years of age treated with quinine, 21.7 hr (19.3–24.0) among children 2–9 years of age treated with quinine, and 34.6 hr (25.9–43.3) among persons ≥10 years of age who were symptomatically treated. In this last group, the time was significantly longer among adults ≥20 years.

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**Table 5**

Disappearance of fever during 496 malaria attacks treated with quinine: frequency of disappearance before the treatment, disappearance time after the beginning of treatment, and probability of disappearance before the 12th hour of treatment

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of cases with fever</th>
<th>Disappearance before treatment (%)</th>
<th>Persistence at the beginning of treatment</th>
<th>Disappearance time</th>
<th>Disappearance before the 12th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>161</td>
<td>40</td>
<td>24.8</td>
<td>121</td>
<td>15.0 13.0–17.1</td>
</tr>
<tr>
<td>2–5</td>
<td>279</td>
<td>93</td>
<td>33.3</td>
<td>186</td>
<td>14.0 12.1–15.9</td>
</tr>
<tr>
<td>6–9</td>
<td>56</td>
<td>24</td>
<td>42.8</td>
<td>32</td>
<td>11.1 7.1–15.0</td>
</tr>
</tbody>
</table>

* OR = odds ratio; 95% CI = 95% confidence interval; - = reference group.

---

**Formulas:**

- $P_{0.31}$
- $95\%$ CI
- $OR$
of age (46.3 hr, 95% CI = 32.8–59.8) than among those 10–19 years of age (23.8 hr, 95% CI = 13.9–33.6; \( P < 0.01 \), by Mann-Whitney test).

The durations of clearance of symptoms or fever showed no correlation with the severity of symptoms measured by the coordinates of the malaria attacks on the first axis obtained in the MCA (\( r^2 < 0.005 \)).

**Parasitologic evolution.** At least one control thick blood smear made after the beginning of treatment was available in 433 (84%) of 514 malaria attacks treated with quinine. At the third day of treatment (between 48 and 71 hr after the first dose of quinine), the average ratio of the parasite density estimated on control thick smear (CPD) to the maximal parasite density (MPD) observed during the attack was 2% (95% CI = 0.6–3.5, \( n = 258 \)). The proportion of patients with negative thick smears in children 0–1, 2–5, and 6–9 years of age was 38%, 54%, and 79%, respectively (\( P < 0.01 \)).

From the fourth to the sixth day after the beginning of treatment (between 72 and 119 hr after the first dose of quinine), the average CPD:MPD ratio was 0.1% (95% CI = 0.02–0.2, \( n = 220 \)). The between-person effect on the probability of detecting parasitemia at day 3 and between day 4 and day 6 after the beginning of treatment was significant (\( P < 0.05 \)).

Eighty-two control thick smears were collected during the five days following the observation of the MPD in 38 of 56 malaria attacks in persons \( \geq \) 10 years who did not receive quinine. The CPD:MPD ratio decreased rapidly: 33% (95% CI = 18–48) before the 24th hr, 22% (8–35) between the 24th and 47th hr, 3% (0–8) between the 48th and 71th hr, and 1% (0.3–3) between the 72th and 119th hr. This diminution was slightly more rapid among adults (\( \geq 20 \) years) than among younger individuals (\( < 20 \) years, no significant difference).

**Hemoglobin AS.** Only 3% of the malaria attacks occurred in AS individuals. These individuals had 10.3% of the pathologic episodes with a negative thick smear, 13.3% of the episodes with a parasitemia < 50% of the level of the pyrogenic threshold, and 3% of the episodes with a parasitemia \( \geq 50% \) of the level of the threshold but lower than the level of the threshold. This later category of pathologic episodes represented 7.7% (165 of 2,134) of all observations. The proportion of children, the mean of maximum body temperature, and the frequency of reported fever were slightly lower in these clinical episodes than in malaria attacks. The frequency of the other clinical manifestations did not differ significantly.

**DISCUSSION**

The criterion of reference for the diagnosis of malaria attacks was the observation of a parasitemia higher or equal to the level of the pyrogenic threshold that has been defined in this population. Only 0.13% of the thick blood smears of asymptomatic persons had a parasite density greater than

### Table 6

Disappearance of symptoms during 514 malaria attacks treated by quinine: frequency of disappearance before the treatment, disappearance time after the beginning of treatment, and probability of disappearance before the 12th hour of treatment\(^*\)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of cases</th>
<th>Disappearance before treatment (%)</th>
<th>Persistence at the beginning of treatment</th>
<th>Disappearance time before the 12th hour of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>165</td>
<td>34</td>
<td>20.6</td>
<td>131</td>
</tr>
<tr>
<td>2–5</td>
<td>289</td>
<td>80</td>
<td>27.7</td>
<td>209</td>
</tr>
<tr>
<td>6–9</td>
<td>60</td>
<td>21</td>
<td>35.0</td>
<td>39</td>
</tr>
</tbody>
</table>

\( OR = \) odds ratio; 95% CI = 95% confidence interval; - = reference group.
or equal to this threshold.\textsuperscript{10} It is therefore unlikely that this criterion led to a false-positive diagnosis of pathologic malaria episodes. On the other hand, calculation of the sensitivity of our diagnostic criteria needs a gold standard for the definition of malaria attacks. This gold standard does not exist. Defining malaria attacks by clinical episodes with a parasite density $> 50\%$ of the level of the threshold did not modify appreciably the clinical description of malaria attacks and the conclusions of our study.

The active case detection, the standardized procedures of clinical data collection, and the low proportion of cases excluded from the analysis limited selection and information bias. Prevarication bias and the tendency to provide an affirmative reply to questions were also limited, when it was possible, due to the clinical and visual verification (examination and temperature measurement) of the patient’s or parent’s reports and by the repetition of visits at the homes of patients (approximately three times per day). The preparation and use of questionnaires including ethnolinguistic input does not suppress interpersonal variations of the expression of symptoms or ensure that conclusions can be extrapolated to other communities. Thus, a difference in the perception and the reporting of symptoms could explain that the time of symptoms clearance, but not fever disappearance and parasite density decrease, was shorter among the youths 10–19 years of age than among adults $\geq 20$ years of age.

To our knowledge, the clinical descriptive studies of non-severe malaria attacks undertaken at the population level are rare. The study of Miller\textsuperscript{20} in Liberia involved 32 malaria attacks observed among 20 adults and 14 among 10 children. The frequencies of signs and symptoms were similar to those that we observed. Most earlier clinical studies were carried out in hospitals or clinics.\textsuperscript{21,31} In these studies, the case analysis was generally initiated only several days after clinical onset, and patients had frequently taken medications before their admission. Enrollment from health facilities unavoidably selects the most severe forms of malaria and those with the longest evolution. The high frequency of convulsions and complicated forms reported clearly illustrates the selection bias inherent in this mode of data collection and contrast with ours.

The absence of convulsions in our sample, the observation of only one case of severe malaria, and the absence of life-threatening anemia illustrate the efficiency of the rapid treatment of sick persons to avoid or limit complications of malaria. On the other hand, the only death due to malaria that we have observed was not caused by lack of or delay in treatment since the child had begun to receive quinine less than 12 hr after the beginning of symptoms. The treatment had been taken to the prescribed daily dose (25 mg/kg) and had not been vomited. The signs and symptoms of the disease were mild on the first day and it was 24 hr after the beginning of treatment that the child suffered convulsions and went into coma without ever resuming consciousness. This type of rapid development of cerebral malaria, despite correctly administered treatment, has already been described in Senegal.\textsuperscript{22} This suggests that the risk of development of severe forms cannot be totally prevented by the rapid treatment of cases with Quinimax\textsuperscript{8} and that some persons could be predisposed to this type of outcome.\textsuperscript{32}

Body temperature at the time of malaria attacks decreased with age. The temperature difference according to age could be partially explained by fixation of the hypothalamic thermostatic point\textsuperscript{33} at a lower level among older persons than among younger ones. However, the oldest persons controlled high parasitemia more rapidly and returned more rapidly to a normal body temperature than the youngest persons. Even if the maximal body temperature reached by children and adults during the malaria attacks has an equivalent level, the probability of recording a high temperature would be smaller among adults than among children. Furthermore, a correction of $+0.5^\circ\text{C}$ was systematically applied to the axillary temperature to be analyzable with the rectal temperature.\textsuperscript{34,35} This may also contribute to the observation of lower temperature in adults. Finally, the most striking observation was the very short duration of a high proportion

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Kaplan-Meier estimate of the probability of continued fever ($\geq 38.0^\circ\text{C}$) after the first report of symptoms as a function of time, age, and type of treatment in 540 malaria attacks with fever.}
\end{figure}
of fever episodes during malaria attacks regardless of the age of the patients. In particular, 25% of young children (<2 years old) had fever episodes that resolved spontaneously within a few hours before treatment. This does not presume of the evolution of the disease in absence of treatment (some of them had further fever episodes despite treatment). However, this clearly illustrate the particularly erratic nature of the body temperature during malaria attacks. Although in our study most of these very short episodes of fever were probably detected, their detection required a very close surveillance system. For these reasons, the report of fever, and not only the observation of fever, is also an important criterion to take into account in the clinical definition of malaria attacks despite interpersonal and intercultural variations.

The temperature difference according to gender was not very important but it was significant in all age groups and was corroborated by the greatest frequency of reported fever among boys. We failed to find any substantial epidemiologic explanation for this phenomenon but it is possible that gender might have an effect upon tumor necrosis factor (Kwiatkowski D, unpublished data), immune responses, parasite density, and thus on the course of clinical attacks.

An important observation was the lack of any marked diminution of the severity of most presenting symptoms according to age. On one hand, fever was slightly more frequent among children than adults. However, we used an age-independent definition of fever while normal body temperature is higher among children than adults. Using an age-dependent definition of fever, the difference in its frequency between children and adults would be even less important. On the other hand, the level of asthenia as the average severity or frequency of other symptoms such as vomiting differed little between malaria attacks in the youngest children and those of adults. For example, the confinement to bed and the diminution in activity had a comparable frequency regardless of the age of the sick person. These levels of disability could be verified by field workers and their report was probably less subject to interpersonal variations than allegations of some other symptoms. This contrasts with the diminution of the incidence and duration of malaria attacks according to age, which reflects the progressive reinforcement of protective immunity in children and adults. Moreover, a similar age-independent symptomatology pattern was observed in Ndiop, a Senegalese village where the level of transmission is about ten times lower than in Dielmo and where the population is followed in the same way. However, these two populations differ markedly in terms of the pattern of age-dependent variations in malaria attack incidence rates: approximately 23% and 41% of the malaria attacks occur during adulthood in Dielmo and Ndiop, respectively. Our observations suggest that of the two components of protective immunity, anti-parasite immunity and anti-toxic immunity, only the first would play a major role as age increases. The control of high parasitemias was more efficient and more rapid among the oldest persons. In parallel, the duration of fever and clinical manifestations, with or without antimalarial treatment, decreased with age. This contrasts with the uncertain importance of anti-toxic immunity, whose decrease with age leads to an increase of the risk of fever at equal parasite density without limiting the severity of the symptoms when the parasitemia crosses the pyrogenic threshold.

Is it possible to distinguish various clinical forms of non-severe malaria attacks differing by their signs and symptoms? It is obvious that severity of symptoms may differ considerably between attacks, and these differences have been recently emphasized by several investigators. In a drug efficacy evaluation, Hogh and others used a classification of malaria attacks in four categories based on the general state, the allegation of fever, and the capacity for normal activity. This classification had been chosen a priori. Cox and others divided nonsevere malaria attacks into mild and very mild forms according to the perceived need for treatment. We used an exploratory method of analysis (MCA), and no sign or symptom had been chosen a priori as indicator of the severity of clinical manifestations. The first axis summarized this severity and quantified it. The proximity of the average clinical forms of malaria attacks of youngest children and those of adults is explicit on this axis. Furthermore, the distribution of the coordinates of the cases on the first axis, i.e., the clinical severity, was unimodal and it was not therefore possible to distinguish objectively different malaria attack types according to the severity of manifestations. We suggest that there would be a continuity between the mildest and the most severe clinical forms and that a distinction between mild forms and more severe forms would be arbitrary and would depend only on the criteria used to define these forms.

It is well established that in highly endemic areas only young children are at risk of dying of malaria while older children and adults are fully protected against the most severe forms of the disease. Without rapid treatment, several infants and young children in our study would probably have developed severe malaria. Surprisingly, there was no marked difference in the severity of symptoms during the initial course of attacks between young children and adults, and this severity was not correlated with the duration of the pathologic episode. This suggests that the initial clinical presentation of the malaria attacks is not predictive of the level of protective immunity.

Acknowledgments: We are grateful to the villagers of Dielmo for active participation and continuing collaboration in the project. We thank Dr. L. Ravinet (Sano®) for providing Quinimax®. We also thank Dr. G. Angel (Hôpital Principal de Dakar), Dr. P. Druilhe (Institut Pasteur, Paris), Dr. O. Garraud (Institut Pasteur de Dakar), Dr. P. Imbert (Hôpital Principal de Dakar), Dr. O. Puijalon (Institut Pasteur, Paris), and Pauline Roussilhon for reviewing the manuscript. We are also grateful to Dr. J. F. Etard (ORSTOM, Dakar) for help in the MCA analysis, Professor Kamini Mendis for useful discussion about the clinical questionnaires, Professor L. Pereira da Silva for constant support and encouragement, Abdoulaye Badiane, Assane Badji, Charles Bouganali, Hilaire Bouganali, Mamadou Camara, Joseph Faye, and Mamadou Senghor for technical support, and Dr. A. Spiegel (Institut Pasteur de Dakar) for further analysis of the Ndiop clinical data set.

Financial support: This work was supported by a grant from the Ministère de la Coopération et du Développement (Paris).

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