Comparative Hematologic Analysis of Uncomplicated Malaria in Uniquely Different Regions of Unstable Transmission in Brazil and Colombia

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Abstract. Information on malaria-associated anemia in adult patients is scarce in South American populations. From 2004 to 2006, malaria patients 18 to 45 years of age were recruited in a descriptive cross-sectional study from two different towns: Manaus, in the Brazilian Amazon (120 patients) where Plasmodium falciparum incidence is lower (≈20%), and in Tumaco on the Colombian Pacific Coast (126 patients) where P. falciparum incidence is higher (≈90%). Relationships between hematologic parameters and independent variables were explored using cross-tabulations and multiple linear regression analyses. We found an inverse relationship of hemoglobin (Hb) levels with days of illness in both sites. In Manaus but not in Tumaco, red cell distribution width (RDW) was related to asexual parasitemia. Reticulocytes were higher in Plasmodium vivax infection in Tumaco. Only in Tumaco, two patients with P. falciparum infection presented with severe anemia (Hb < 7 g/dL). Etiologic factors associated with hematologic changes in malaria seem to be multifactorial. More studies are needed to clarify the anemia determinants in uncomplicated malaria in South America, where malaria transmission is mostly unstable.

INTRODUCTION

Malaria in humans is produced by five different Plasmodium species, of which Plasmodium falciparum and Plasmodium vivax are the most abundant and coexist in most endemic regions worldwide, particularly in Asia and Latin America. Malaria-related anemia is caused by diverse pathophysiologic mechanisms that are still poorly understood. However, it is a common complication in both severe and non-severe clinical malaria cases and is responsible for much of the disease burden, with notable morbidity and great mortality, particularly in children. In addition, anti-malarial drugs such as primaquine, are able to trigger life-threatening acute intravascular hemolysis with severe anemia and acute renal failure in subjects with serious glucose-6-phosphate-dehydrogenase (G6PD) deficiency, and contribute to anemia in endemic populations. Moreover, in these areas co-infections with intestinal parasitic infestations, as well as iron, folate, and vitamin B12 deficiency, contribute significantly to anemia.

Although parasite-induced hemolysis during schizont burst certainly contributes to the development of anemia, a more general hemolytic process with destruction of non-parasitized erythrocytes and also bone marrow impairment are considered major anemia inducing factors. Anemia is well-documented in malaria-endemic regions of Africa and Asia but limited information is available about its prevalence in Latin America, where the predominant parasite species is P. vivax and both severe anemia and cerebral malaria are reported less frequently than in other endemic continents. This might be explained by its unstable transmission profile (<0.1 autochthonous cases per 1,000 people/year), the primary parasite species present, ethnic differences, or even by poor recording practices.

Furthermore, the clinical determinants of malaria-associated anemia have not been reported in the South American populations living in endemic areas. According to the Pan American Health Organization (PAHO), in 2006 Brazil reported most of the cases of malaria in the Americas (548,597 cases), followed by Colombia (120,107 cases). We aimed to study the relationships between selected hematologic parameters and time of disease, race, gender, previous exposure to malaria, and parasite species in patients with malaria from two distinct malaria-endemic sites in Brazil and Colombia considered to be among the most endemic regions of both countries.

PATIENTS AND METHODS

Study sites and population. Between December 2006 and August 2007, we conducted a cross-sectional study in all 126 adult patients (between 18 and 45 years of age) who presented to the outpatient clinics of the Vector Control Program and to San Andres Hospital, in Tumaco, Colombia with signs and symptoms suggestive of malaria (fever, chills, arthralgia, myalgia, headache and/or fatigue). Tumaco is a sea level municipality located in the Department of Nariño on the Colombian Pacific Coast (106,000 people) (Figure 1). Malaria transmission occurs throughout the year, with two seasonal transmission peaks (from April to May and from September to October).

The predominant species is P. falciparum (4,127 cases in 2006), followed by P. vivax (1,032 cases in 2006). Communities in this region are racially mixed, with ~70% Afro-Colombians and 30% Spanish-Amerindians. A high prevalence of Duffy-negative individuals in this region explains the higher prevalence of P. falciparum.

Likewise, between September 2004 and September 2006, a sample of 120 adult patients (between 18 and 45 years of age) who presented to the outpatient clinics of the Tropical Medicine Foundation of Amazonas, in Manaus, Brazil was randomly enrolled. Manaus is the capital of the Amazonas State, in the Brazilian Amazon, 30 meters above the sea level, with a population of ~1,500,000 people (Figure 1). Malaria transmission occurs throughout the year, with one seasonal transmission
Transmission is clearly related to the new areas of unplanned growth on the outskirts of the city. The predominant species is *P. vivax* (40,698 cases in 2006), followed by *P. falciparum* (9,849 cases in 2006). The population in this region is racially mixed.

**Selection criteria and ethical clearance.** Patients diagnosed with malaria by a thick blood smear and residents in the endemic area under study were asked to participate in the study if they were between 18 and 45 years of age and able to understand and sign an informed written consent. Volunteers were not included if they reported any chronic disease likely to create anemia (e.g., sickle cell anemia, thalassemia, neoplasia, autoimmune diseases), or if they were pregnant. Ethical clearance to draw blood from human volunteers was obtained from the Institutional Review Boards of the University del Valle, the San Andres Hospital, and the Tropical Medicine Foundation of Amazonas. Written informed consent was obtained from all patients before blood was drawn or any additional information about them was collected. All the patients were treated according to the malarial treatment guidelines of the World Health Organization (WHO) recommendations. Slides were then independently examined by two experienced microscopists and parasitemia reported as the number of asexual parasites/µL after counting the number of asexual parasites/100 leukocytes in high-magnification fields. Five mL of blood were taken in EDTA-Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for the automated complete blood count (hemoglobin [Hb], red cell distribution width [RDW], leukocytes, and reticulocytes) using a Celta Alfa Counter (NYHON-KOHON) in Tumaco and a Pentra 120-Retic (ABX Diagnostics) in Manaus.

**Statistical analyses.** Descriptive statistics were obtained for all variables. For continuous variables normality was assessed. If a particular variable did not follow a reasonable near-normal distribution, mathematical transformations were made. Cross tabulations were used to compare mean Hb levels with days of illness, quartiles of parasitemia levels, quartiles of previous malarial episodes, and quartiles of day of illness. Analysis of variance (ANOVA) was used to test the null hypothesis of no differences among means of the different variables. All reported *P* values were two-tailed. The associations between Hb levels and days of illness, parasitemia, and previous malarial episodes as continuous variables were adjusted using linear regression. Regression models were constructed to evaluate the effect of different groups of potential confounders on the relationship between Hb levels and relevant independent factors. The variables included in each model are described in more detail in each table. Generally, the assumptions for linear regression held for each of the models. Residual and influential analyses were performed. In the analysis of Hb levels one extreme observation was detected, and analyses were done with and without this particular data, with similar results. All statistical analyses were performed using the statistical software package Stata 7.0 (Stata Corporation, College Station, TX).

**RESULTS**

**Population characteristics.** A description of the participants is summarized in Table 1. Male gender predominated in both populations, showing a higher representation in Manaus than in Tumaco (70.4% versus 56.3%, *P* value = 0.02). Participants’ mean age was 31.9 years for Tumaco, and 30.7 years for Manaus. Most participants (69.8%) were Afro-descendents in Tumaco compared with only 6.7% in Manaus (*P* value < 0.001). In Tumaco 59.5% of the patients reported living in urban areas, as compared with 52.5% in Manaus (*P* value = 0.29). The distribution of infections by *Plasmodium* species was very different, with 88.9% and 16.7% of patients infected with *P. falciparum* in Tumaco and Manaus, respectively (*P* value < 0.001). Self-reported smoking status was also different, with 17.5% participants in Tumaco classified as current smokers and 34.5% in Manaus (*P* value = 0.003). The number of days of acute illness and the number of previous malaria episodes was higher in Manaus than in Tumaco.

**Hemoglobin levels.** Mean Hb levels in Tumaco were lower than in Manaus (11.6 ± 2.0 versus 13.4 ± 1.8, Student’s *t* test, *P* value < 0.001). As expected, female gender showed lower mean levels of Hb compared with males, both in Tumaco and Manaus. Mean Hb in smokers was 12.3 ± 1.8 g/dL compared with non-smokers 11.4 ± 2.01 g/dL in Tumaco (Student’s *t* test, *P* value = 0.05). This difference was much smaller in the
Manaus population. No difference in Hb levels was seen for age, or between African descendants and other ethnic origins. No difference in the mean hemoglobin levels was seen between rural and urban residence in Manaus or Tumaco. In Tumaco, two patients with *P. falciparum* infection were diagnosed with severe anemia according to the WHO criterion of severity (Hb < 7 g/dL). No study patient with severe anemia was seen in Manaus.

Cross-tabulations of crude and adjusted mean Hb levels by days of illness, parasitemia, and previous malarial episodes are shown in Table 2 for Manaus and in Table 3 for Tumaco. In both populations mean Hb levels decreased as days of illness increased, and this trend persisted after adjusting for potential confounders, including age, gender, place of residence, smoking status, parasite species, parasitemia, and previous malarial episodes. An independent association was seen between Hb levels and parasitemia and previous malaria episodes in Manaus, but not in Tumaco.

**Red cell distribution width.** The median values of RDW was 13.6% (interquartile range [IQR] 12.7, 15.0) for Tumaco and 13.0% (IQR 12.8, 13.3) for Manaus.

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tumaco (Colombia)</th>
<th>Manaus (Brazil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean 95% CI n</td>
<td>Mean 95% CI n</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>31.9 (30.4, 33.4)</td>
<td>30.7 (29.3, 32.1)</td>
</tr>
<tr>
<td>Asexual parasites/µL†</td>
<td>2775 (2230, 3453)</td>
<td>1373 (1004, 1878)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)*</td>
<td>11.6 (11.2, 11.9)</td>
<td>13.3 (13.0, 13.6)</td>
</tr>
<tr>
<td>Reticulocytes (%)†</td>
<td>1.29 (1.16, 1.43)</td>
<td>2.69 (2.36, 3.07)</td>
</tr>
<tr>
<td>RDW</td>
<td>13.9 (13.5, 14.2)</td>
<td>13.0 (12.8, 13.3)</td>
</tr>
</tbody>
</table>

### Table 2

Mean crude and adjusted hemoglobin (Hb) count by quartile s of parasitemia, previous malaria episodes, and days of illness in Manaus, Brazil

<table>
<thead>
<tr>
<th>Variables</th>
<th>Crude</th>
<th>Model 1*</th>
<th>Model 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean‡</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Parasitemia§ (quartiles)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23–820</td>
<td>23</td>
<td>13.1</td>
<td>22</td>
</tr>
<tr>
<td>850–2800</td>
<td>20</td>
<td>13.6</td>
<td>19</td>
</tr>
<tr>
<td>2801–5280</td>
<td>15</td>
<td>12.5</td>
<td>15</td>
</tr>
<tr>
<td>5281–46800</td>
<td>0.028</td>
<td>0.028</td>
<td>0.028</td>
</tr>
<tr>
<td><strong>Previous malaria episodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25</td>
<td>13.4</td>
<td>37</td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>13.6</td>
<td>35</td>
</tr>
<tr>
<td>2–3</td>
<td>16</td>
<td>12.7</td>
<td>15</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>29</td>
<td>13.0</td>
<td>28</td>
</tr>
<tr>
<td><strong>Days of illness¶</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>39</td>
<td>13.6</td>
<td>25</td>
</tr>
<tr>
<td>3–4</td>
<td>36</td>
<td>13.4</td>
<td>46</td>
</tr>
<tr>
<td>5–6</td>
<td>15</td>
<td>13.1</td>
<td>15</td>
</tr>
<tr>
<td>&gt; 6</td>
<td>28</td>
<td>12.8</td>
<td>29</td>
</tr>
</tbody>
</table>

**P value:** analysis of variance (ANOVA) test.

* Model 1; age, African descent, gender, current smoker.
† Model 2; age, African descent, gender, current smoker, parasite species, parasitemia, previous malaria episodes, and days of illness.
‡ Mean; arithmetic mean.
§ Number of asexual parasites/µL of blood.
¶ Fever, chills, arthralgia, myalgia, headache and/or fatigue.
Most studies of malaria-related anemia from the African Continent, which is highly endemic for *P. falciparum* malaria, focus on vulnerable groups, such as children and pregnant women. In South America, where most of the malaria endemic areas have unstable transmission, adults are the most affected population. It seems, therefore, relevant to explore possible relationships between demographic and clinical characteristics of the malarial infection with hematologic parameters in populations from the Brazilian Amazon and from the Colombian Pacific Coast. These areas represent two typical scenarios of malaria transmission in South America. The two populations have different profiles, mainly in their demographic and malaria exposure backgrounds, as shown in our results. The unstable transmission is evidenced by only 2.4% of patients from Tumaco and 24.2% of patients from Manaus who have reported more than three episodes of malaria throughout their lives.

The major findings of this study were 1) lower Hb levels in patients from Tumaco as compared with Manaus, 2) an inverse relationship between Hb and time of acute disease in both Tumaco and Manaus, 3) Hb levels were related to both parasitemia and previous malaria episodes, 4) RDW was related to parasitemia only in Manaus, 5) reticulocytes were higher in *P. vivax* infection in Tumaco only, and 6) the report of severe anemia in two adults with *P. falciparum* infection in Tumaco.

There are many possible explanations for the difference in the Hb levels during the acute malarial episode between the two localities. Because this difference was independent of the malaria species, other non-evaluated covariates such as the nutritional status of the populations and the prevalence of severity of erythrocyte defects, e.g., G6PD deficiency, could explain such a finding.

Iron deficiency has been identified as the major cause of anemia in populations living in malaria endemic areas in Brazil. Similar results were found in a study of Amerindians from Venezuela, where anemia was predominantly hypochromic and microcytic. Similarly, in Colombia microcytic anemia was found in 59.6% of adults with malaria.

The G6PD deficiency status was not assessed in this study, and it may have contributed to the observed differences in anemia between the two populations during an acute infection with malaria, which induces oxidative stress per se. In previous studies in Manaus, the prevalence of G6PD deficiency among patients with *P. vivax* malaria was estimated to be 8.7%, whereas in a population similar to that of Tumaco, G6PD deficiency was 12.0%.

Likewise, helminthic infections were not assessed. However, hookworm infections, which are more associated with anemia in the tropics, would probably be more relevant in school-aged children and the elderly, ages not enrolled in this study.

The relationship between days of illness and Hb levels both in Tumaco and Manaus is consistent with previous observations made in Buenaventura, also on the Colombian Pacific Coast, where an independent linear and inverse relationship between self-reported days of illness and Hb levels was found in uncomplicated malarial infection. In Tumaco no relation
could be demonstrated between parasitemia and previous exposure to malaria, in contrast to the findings from Manaus. The independent association of high parasitemia with low Hb levels in Manaus may partly be explained by the inclusion of patients from the countryside or surrounding areas from Manaus who seek medical assistance at the Tropical Medicine Foundation of Amazonas, with more frequent nutritional disturbances, including anemia. This is reflected in the delayed time for the diagnosis among patients seen in Manaus. In terms of the association between Hb and previous exposure to malaria in Manaus, one may assume that most of the previous infections referred by the patients were caused by *P. vivax*, because this is the most prevalent species in the Brazilian Amazon since 1990. On the Colombian Pacific Coast, however, *P. falciparum* is usually the major species among African descendants. This suggests therefore that cumulative infections by *P. vivax* could worsen the hematologic status during the acute infection in the long-term, as compared with areas endemic for *P. falciparum*. A cross-sectional study in a peri-urban locality in the Brazilian Amazon, also more endemic for *P. vivax*, found that the more recent the malaria episode, the higher the prevalence of anemia.

The similar levels of Hb among patients with different *Plasmodium* species in both Manaus and Tumaco mirror what was previously seen in other unstable malaria transmission areas in Brazil, suggesting that *P. vivax* and *P. falciparum* infections present similar characteristics in terms of the induction of anemia.

Because both sites have continuous malaria transmission throughout the year and the samples were collected homogeneously during all the months of the study, we believe that the seasonal peaks may have not biased the results from one or the other center. However, in terms of comparison with other studies from the literature, this information about the time of the year when the samples were collected constitutes a relevant variable, as already pointed out for severe malaria in places in Africa where malaria transmission is unstable and seasonal.

The RDW was used as a surrogate marker of the release of young erythrocytes and reticulocytes from the bone marrow. The data from Manaus suggest there was higher hemolysis in the bone marrow, however the studies do not associate these finding with disease or explore mechanisms of disease.

In conclusion, because of the scarcity of studies evaluating anemia in uncomplicated malaria in Latin America, little is known about the impact of this hematologic complication on the health of people living in Brazil and Colombia, where more than half of the cases of malaria reported in the Western Hemisphere are found. The data presented here suggest that the development of anemia in malaria is affected by distinct epidemiologic and individual variables. Further studies in these endemic areas are needed to clarify the mechanisms of the disease triggered by this parasite, and especially to understand the factors leading to the severe anemia cases observed.

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REFERENCES


