

## SHORT REPORT: THROMBOCYTOPENIA IN FALCIPARUM MALARIA IS ASSOCIATED WITH HIGH CONCENTRATIONS OF IL-10

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**Abstract.** Over the last decade, a number of observations have suggested that platelets may play a role in the pathophysiology of severe malaria. However, somewhat paradoxically, thrombocytopenia is not associated clearly with outcome. We studied the relationship between thrombocytopenia and cytokines in Kenyan children with severe malaria and showed that thrombocytopenia (platelet count  $< 150 \times 10^9/L$ ) strongly correlates with high levels of interleukin (IL)-10. Several studies have shown that high levels of IL-10 are associated with a favorable outcome in severe malaria. Taken together, these data suggest why thrombocytopenia has a complex relationship with severe disease and suggest one mechanism whereby IL-10 may modify the outcome of severe disease.

### INTRODUCTION

Acute malaria is often associated with mild or moderate thrombocytopenia in non-immune adults and in children from malaria-endemic areas and is a sensitive but non-specific indicator of infection with malaria parasites. Profound thrombocytopenia is unusual, and thrombocytopenia is rarely associated with hemorrhagic manifestations or a component of disseminated intravascular coagulation either in non-immune adults or children in endemic areas.<sup>1–3</sup> Moreover, in most clinical studies, thrombocytopenia is associated neither with the severity of disease or death in malaria.

Nevertheless, platelets have been implicated in animal, clinical and experimental studies of malaria pathogenesis. Platelets mediate a syndrome of cerebral malaria in an animal model of malaria infection.<sup>4</sup> Histopathological studies of children who have died of severe malaria showed that platelet clumps with and without infected erythrocytes are frequently found in the vasculature.<sup>5</sup> Finally, infected erythrocytes may adhere to platelets, and the clumps of infected erythrocytes and platelets have been associated with severe disease.<sup>6</sup>

Therefore, there remains a paradox that, while thrombocytopenia is associated with infection and platelets have been implicated in the pathogenesis of severe disease, most studies suggest that low counts of platelets are not associated with an adverse outcome.<sup>2,7</sup> Without understanding this paradox, it is not possible to evaluate the true contribution of platelet to the pathogenesis of severe malaria.

The causes of thrombocytopenia in acute malaria are poorly understood. Certainly, increased platelet destruction is significant during malaria infection. The platelet lifespan is reduced during malaria,<sup>8</sup> which seems to be associated with a diffuse pattern of platelet sequestration rather than a predominant splenic or hepatic clearance.<sup>9</sup> Thrombocytopenia is often associated with palpable splenomegaly<sup>10</sup> and circulating immune complexes.<sup>1</sup>

The contribution of abnormalities of platelet production to thrombocytopenia during malaria infection is less clear. Early studies in malaria patients described dysmorphic megakaryocytes in the bone marrow of patients with acute falciparum

malaria.<sup>11</sup> Other investigators showed less lobulated and immature megakaryocytes in Gambian children with acute malaria.<sup>12</sup> Moreover, the biosynthesis and regulation of thrombopoietin (TPO), the main growth factor for megakaryocytes and thrombocytopoiesis, seems to be normal in patients with malaria.<sup>8</sup>

The cytokines released during an acute inflammatory response contribute to the pathogenesis of thrombocytopenia. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) has been associated with platelet consumption in mice,<sup>13</sup> and the administration of interleukin (IL)-10 to healthy volunteers has been shown to cause thrombocytopenia.<sup>14</sup> TNF- $\alpha$  and IL-10 have been reported to play an important role in the development of *Plasmodium falciparum* malarial anemia,<sup>15</sup> but the role of these cytokines has not been studied in the development of thrombocytopenia in patients with acute malaria. We therefore examined the association of these cytokines with thrombocytopenia to elucidate the relationship between thrombocytopenia and severe disease.

### MATERIALS AND METHODS

**Patients.** This study was conducted at Kilifi District Hospital (KDH), Kilifi, Kenya. The epidemiology of malaria in Kilifi District has been described elsewhere.<sup>16</sup> We studied 120 consecutive children (6 months to 10 years of age) admitted with a primary diagnosis of malaria with any asexual *P. falciparum* parasitemia to KDH or seen at the outpatient clinic as part of a larger clinical study of malarial anemia (C. Casals-Pascual and others, *Blood*, in press). Severe malaria was defined as children with hemoglobin (Hb)  $< 5$  g/dL and evidence of cardiovascular compromise/respiratory distress (deep breathing, intercostal muscle recession, prostration, or lethargy) or if they presented with hyperparasitemia ( $> 50$  parasites/500 red blood cells [RBCs]) or with oxygen saturation  $< 90\%$ . All children were treated according to the KDH clinical protocols as previously described. The parents/guardians of children were invited to participate in the study. Consent was obtained in the local language (Kiswahili or Kigiriyama). The National Ethical Committee in Kenya gave ethical approval for the study.

**Laboratory investigations.** A 3-mL venous blood sample was collected into EDTA and processed immediately. A full blood count was obtained by a hematology analyzer (Coulter MD II; Coulter Corp., Miami, FL). Peripheral blood films were stained with 3% May-Grünwald-Giemsa. Absolute

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TABLE 1  
Population description

	Platelets on admission > 150 × 10 <sup>9</sup> /L (N = 78)	Platelets on admission < 150 × 10 <sup>9</sup> /L (N = 41)	P value*
Age (median, IQR) (years)	2.25 (1.58–3.33)	3.0 (1.58–4.75)	NS
Male/female (ratio)	0.78	0.75	NS
Hb (median, IQR) (g/dL)	7.5 (5.4–10.4)	6.6 (5.3–9.6)	NS
WAZ-score (median, IQR)	-1.97 (-2.8 to -0.83)	-1.72 (-2.4 to -0.9)	NS
Parasites/μL (median, IQR)	10,306 (2,465–133,200)	17,688 (2,938–118,140)	NS
IL-10 (median, IQR) (pg/mL)	54.6 (31.8–162.3)	127.4 (68.8–261.3)	0.002
TNF-α (median, IQR) (pg/mL)	15.1 (7.6–27.3)	18.9 (8.9–28.3)	NS
IFN-γ (median, IQR) (pg/mL)	2.7 (1.8–4.2)	3.1 (1.9–4.7)	NS
IL-12 (median, IQR) (pg/mL)	288.8 (213.7–378.7)	239.6 (142.9–283.4)	0.012
Mean platelet volume (median, IQR) (fL)	8.3 (7.5–9.3)	9.1 (8.1–10.2)	0.03

\* Mann-Whitney test.  
NS, not significant.

parasite counts were calculated for each child using either thin films (counting the number of parasitized RBCs/500 RBCs) or from thick films (counting the number of parasitized RBCs/200 white blood cells [WBCs]) stained with 10% Giemsa and with reference to the data from the full blood count. Plasma concentrations of TNF-α, IL-10, interferon (IFN)-γ, IL-12, and erythropoietin (Epo) were measured by ELISA (R&D Systems, Abingdon, United Kingdom).

**Statistical analyses.** The values of parasitemia, TNF-α, IL-10, IL-12, IFN-γ, and platelet counts were normally distributed when log-transformed. We used Pearson's correlation to measure the association between the variables studied. Groups were compared using the non-parametric Mann-Whitney test. We used logistic regression to study the association of independent variables with thrombocytopenia (dependent variable). For binary logistic regression, thrombocytopenia was treated as a dichotomized variable using a cut-off for platelet counts of 150 × 10<sup>9</sup>/L (SPSS 11.0; SPSS, Chicago, IL).

## RESULTS

More than one third (41/129 or 35%) of the children admitted with acute malaria had thrombocytopenia (platelets < 150 × 10<sup>9</sup>/L). All children, independently of the degree of anemia on admission, had platelet counts > 300 × 10<sup>9</sup>/L a week after treatment. These levels were maintained 1 month after treatment. Children with thrombocytopenia on admission had a significantly higher mean platelet volume (MPV) on admission of 9.1 fL (IQR, 8.1–9.2 fL) than children with normal platelet counts (8.3 fL [7.5–9.3 fL]; Mann-Whitney, *P* = 0.03). The MPV a week after treatment was similar for both groups.

The subgroup of children with thrombocytopenia (*N* = 41) was compared with children with platelet counts > 150 × 10<sup>9</sup>/L (*N* = 78; Table 1). No differences in age, sex ratio, or nutritional status (weight-for-age Z-score) were found when children with thrombocytopenia were compared with children with normal platelet counts. Children with thrombocytopenia had lower Hb values and higher *P. falciparum* parasitemias. However, these differences did not reach statistical significance. Children with acute malaria and thrombocytopenia had significantly higher levels of plasma IL-10 (*P* = 0.002) and lower levels of IL-12 (*P* = 0.012).

However, when thrombocytopenia was used as the depen-

dent variable in a logistic regression model including parasitemia, TNF-α, IL-10, IFN-γ, IL-12, nutritional status, and age as independent variables, the only variable significantly associated with thrombocytopenia was IL-10 (Exp [B], -3.1; 95%CI, -1.7 to -5.7; *P* < 0.01). The same association was confirmed when platelet counts on admission were used as a continuous dependent variable in a regression model (slope [B], -39.6; 95%CI, -59 to -20; *P* < 0.01; Figure 1).

## DISCUSSION

Previous studies have not addressed the association of IL-10 and thrombocytopenia in acute malaria. Here, we showed that thrombocytopenia in children with acute malaria is strongly associated with plasma concentrations of IL-10 but not with *P. falciparum* parasitemia or other plasma cytokines.

There is evidence that IL-10 may directly induce thrombocytopenia. The administration of a low dose of recombinant human IL-10 (8 μg/kg/d) decreased platelet production in healthy adult volunteers.<sup>14</sup> In the same study, there was a corresponding reduction in splenic sequestration of platelets in the IL-10-treated group compared with the placebo-treated subjects. In the IL-10-treated group, there was a trend toward lower numbers of megakaryocyte colony-forming units (CFU-MKs) compared with volunteers who received placebo. This single study suggests that IL-10-induced reduc-

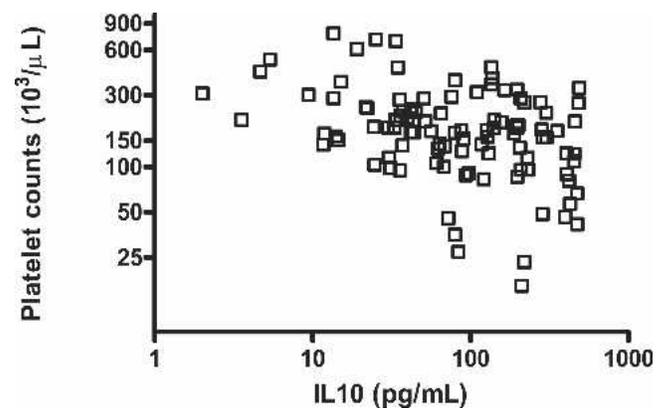


FIGURE 1. Regression scatter plot of platelet counts on admission with IL-10 levels.

tion in platelet count is caused, at least in part, by a reduction in platelet production.

Thrombocytopenia is a common finding in patients with HIV.<sup>17</sup> Therefore, the interpretation of thrombocytopenia as a marker of clinical outcome in malaria-endemic areas should take into account the HIV status of the population studied. In our study, only 2 of 120 children were positive for HIV, and it is therefore unlikely that HIV status confounded our results.

How do these findings explain the relationship between thrombocytopenia and severe disease? The clinical relevance of thrombocytopenia associated to *P. falciparum* malaria has been addressed in two large studies. In a clinical study in Kenyan children, platelet counts  $< 150 \times 10^9/L$  were found in 57% of children with acute malaria and were associated with age, prostration, and parasite density, but not with bleeding problems or mortality.<sup>2</sup> On the other hand, a recent study carried out in a hypoendemic area in Senegal identified thrombocytopenia as an independent predictor of death (odds ratio = 13.3).<sup>18</sup> However, other data from Southeast Asia do not support a relationship between thrombocytopenia and outcome.<sup>7</sup> These inconsistent relationships between disease severity or outcome and thrombocytopenia may reflect the differences in clinical epidemiology and the corresponding differences immune status and immunopathology in patients with severe malaria. Our study could not include death as a dependent variable because none of the 120 children studied died during the follow-up period. However, platelet counts in the subgroup of children with severe malarial anemia ( $N = 29$ ) were not significantly different from those with mild or moderate anemia. Therefore, our results do not support a relationship between thrombocytopenia and disease severity, in relation to severe malarial anemia, in *P. falciparum* malaria.

Indeed, the association between thrombocytopenia and IL-10 levels in malaria infection may explain, at least in part, the failure to find consistent associations of thrombocytopenia and poor outcome in malaria infection despite the evidence from clinical experimental and animal studies for a role of platelets in the pathophysiology of malaria. IL-10 itself is associated with less severe forms of clinical malaria,<sup>15</sup> and this relationship would confound any simple relationship between thrombocytopenia and outcome. It is possible that a reduction in platelet count mediated by IL-10 reduces the pathologic interaction between infected erythrocytes and platelets, associated with severe disease,<sup>5,6</sup> and is therefore one mechanism whereby IL-10 improves the outcome of severe malaria.

Our results suggest that the significance of platelets in the pathophysiology of falciparum malaria cannot be gauged by determining the simple relationship between thrombocytopenia and outcome. Further studies to define the role of platelets in the pathophysiology of severe malaria will require more detailed functional and histopathological approaches to measure the activation and fate of platelets during malaria infection.

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