CHANGES IN PLATELET COUNT IN UNCOMPLICATED AND SEVERE FALCIPARUM MALARIA

Wattana Leowattana[1], Noppadon Tangpukdee[1], Sai Kaung Thar[2], Souwanit Nakasiri[1], Siripun Srivilairit[3], Shigeyuki Kano[4], Polrat Wilairatana[1] and Srivicha Krudsood[5]

[1]Department of Clinical Tropical Medicine, [2]Bangkok School of Tropical Medicine, [3]Hospital for Tropical Diseases, [5]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [4]Department of Tropical Medicine and Malaria, Research Institute, National Center for Global Health and Medicine (NCGM), Tokyo, Japan

Abstract. This study investigated alterations in platelet counts pre- and post-treatment with artemisinin derivatives in uncomplicated and severe falciparum malaria. Serial platelet counts were taken over 4 weeks for 110 uncomplicated and 110 severe falciparum malaria patients admitted to the Hospital for Tropical Diseases during 2005-2008. On admission, prior to treatment, thrombocytopenia was found in 73.6% of uncomplicated falciparum malaria patients and 90.9% of severe falciparum malaria cases. Platelet levels significantly lower in severe malaria cases. Although initial platelet counts were lower than normal in both study groups, they slowly increased significantly over time, and approached normal levels by several weeks post-treatment. No bleeding was evident during treatment, and none of the patients required a platelet transfusion. Platelet transfusions are not required for malaria patients with thrombocytopenia who have no bleeding.

Key words: uncomplicated malaria, severe malaria, platelet counts, thrombocytopenia

INTRODUCTION

Malaria is an important infectious disease in tropical and subtropical countries, having high morbidity, mortality, and resulting in economic loss (WHO SEARO, 2006a). The most serious form of disease is caused by *Plasmodium falciparum* (WHO, 2006). The manifestations of malaria are extremely variable. In malaria patients, the clinical manifestations may vary depending on infecting malaria species, geographical region, individual immunity, treatment received, and other factors. The most common presentations in uncomplicated *P. falciparum* malaria are fever, headache, fatigue, malaise, muscle pain, abdominal pain, and diarrhea. Complicated malaria cases may have all the signs and symptoms found in uncomplicated malaria with the addition of loss of consciousness, convulsions, abnormal bleeding, circulatory collapse, pulmonary edema, respiratory distress and death. Hematological changes may also occur due to several factors (Wickramasinghe and Abdalla, 2000).

Correspondence: Dr Noppadon Tangpukdee, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand.
Tel: +66 (0) 2354 9159; Fax: +66 (0) 2354 9158
E-mail: tmntp@mahidol.ac.th

Platelets play a critical role in the pathogenesis of malaria infection (McMorran *et al*, 2009). Thrombocytopenia is a common clinical finding in *P. falciparum* malaria infection (Jeremiah and Uko, 2007). Occasionally, profound thrombocytopenia may occur in severe falciparum malaria, but in general, a reduced platelet count is unrelated to clinical severity of infection (Looareesuwan *et al*, 1992; WHO, 2000). A decreasing platelet count corresponding to increasing in parasitemia has been found with *P. falciparum* infection (Rojanasthien *et al*, 1992). Many studies have evaluated platelets in malaria infection, but few studies have followed platelet counts for up to 28 days post-treatment in uncomplicated and severe falciparum malaria cases. This study evaluated platelet counts before and during treatment with artemisinin derivatives, and for 28 days after treatment in uncomplicated and severe falciparum malaria cases.

MATERIALS AND METHODS

Study site and recruitment procedures

The study was conducted at the Hospital for Tropical Diseases, in Bangkok, Thailand. An independent statistician generated a randomized list of patients, in blocks of 10 patients, before recruitment into the study. Subjects were then randomly enrolled into the study. A total of 220 in-patients with *P. falciparum* malaria were recruited; 110 had uncomplicated malaria and 110 had severe malaria, using World Health Organization (2000) criteria. Pre-inclusion criteria were: a patient (male or female) (1) admitted to the hospital for treatment of *P. falciparum* malaria during January 2005-December 2008, with a body weight ≥40 kg, age ≥18 years, (2) with a positive blood film for asexual *P. falciparum* parasites on admission, (3) enrolled in an artesunate-mefloquine drug protocol, (4) with no history of treatment with antimalarial drugs, (5) who remained in hospital for 4 weeks after starting antimalarial therapy, and (6) who provided blood samples for examination. Inclusion criteria were: (1) having clearance of asexual-forms of *P. falciparum* from the peripheral blood with therapy, (2) having no evidence of concomitant non-falciparum malaria infection, co-infection, or superimposed infection on admission or during follow-up, (3) having no reappearance of asexual-forms of parasitemia during the 4-week study period. A standard case-record form was used for recording demographic information, details of clinical signs and symptoms, and all examinations. The study was reviewed and approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Antimalarial chemotherapy

For uncomplicated malaria, the antimalarial chemotherapy used was a single dose of oral artesunate (200 mg/day), with mefloquine (8 mg/kg/day) daily for 3 days.

For severe malaria, the regimen was artesunate 2.4 mg/kg intravenously, followed by 2.4 mg/kg intravenously at 12 and 24 hours, followed by a once daily dose of artesunate for a total of 5 days. Once the patients could tolerate oral therapy, artesunate at a dose of 100 mg/day was given. Two doses of oral mefloquine were given (25 mg/kg in divided doses) 8 hours apart, 12 hours after the last dose of artesunate.

Baseline and follow-up studies

Age, gender, bodyweight, initial temperature, duration of fever prior to admission, history of malaria, and initial parasite count, were recorded. Baseline labo-

ratory data, determined were red-blood-cell count, hemoglobin, hematocrit, white-blood-cell count, and platelet count using an automated cell counter (Advia 120 Hematology System, Siemens Medical Solutions Diagnostics; commercial reagents by Roche Diagnostics). Severe thrombocytopenia was defined as a platelet count of $<10 \times 10^3/\mu l$, and thrombocytopenia as $<150 \times 10^3/\mu l$ (Diz-kugukkaya *et al*, 2006). Thick and thin blood films were prepared from fingerprick blood samples and stained with Giemsa. Peripheral blood concentrations of asexual forms of *P. falciparum* were estimated by counting the number of parasites per 200 white blood cells on thick smears, multiplied by the white blood cell count, or by counting the number of asexual forms per 1,000 erythrocytes on thin smears, multiplied by the red blood cell count. Hyperparasitemia was defined as an asexual-malaria parasite density ≥5% (WHO, 2006).

Statistical analysis

Quantitative and qualitative data were expressed as means with standard deviation (SD) and number of observations with percentage (%), respectively. All *p*-values reported were from 2-tailed testing; statistical significance was set at 0.05. Descriptive statistics were used to summarize baseline values and demographic data. The chi-square test was used to compare proportions and the *t*-test was used to analyze continuous data. Pearson's correlation and Spearman's rank correlation were used to analyze the relationship between initial platelet count, age, gender, history of malaria in the past year, days of fever preceding admission, splenomegaly, hepatomegaly, severity, and hyperparasitemia, as appropriate. Repeated measures of ANOVA were used to test differences in platelet level at each follow-up, compared with baseline.

Sample size was calculated, based on a previous report (Tangpukdee *et al*, 2008), with the assumption of 80% power, 25% effect size, and an alpha of 0.05; at least 108 cases per group were required to determine a statistically significant difference between groups. To round out the numbers, 110 cases of uncomplicated *P. falciparum* malaria and 110 cases of severe *P. falciparum* malaria were recruited into the study.

RESULTS

The sample totaled 220 in-patients, composed of 110 with uncomplicated, and 110 with severe, falciparum malaria. Most had contracted infection along the Thai-Myanmar border. The subjects' demographic data and pretreatment characteristics are shown in Table 1. Most baseline data were significantly different, except for age, body mass index (BMI) and presentation with splenomegaly. All patients completed the course of antimalarial drug therapy and were asymptomatic and negative for asexual-form parasites before discharge from the hospital.

Different baseline variables were analyzed for possible correlations. The values for Pearson's correlation coefficient, Spearman's rank correlation coefficient, and *p*-values for uncomplicated, severe, and total falciparum malaria patients, are shown in Table 2. In uncomplicated falciparum malaria patients, there was a weak, but significant, correlation between gender difference and initial platelet count. The initial platelet counts in males tended to be lower than those of females. For both groups, we found a significant negative correlation between duration of fever pre-admission and initial platelet count, but a weak correlation in the severe group. To investigate correlations between initial

Table 1
Pre-treatment baseline characteristics of the patients.

	Uncomplicated falciparum malaria (*N*=110)	Severe falciparum malaria (*N*=110)	*p*-value
Gender (%male/%female)	58.2/41.8	72.7/27.3	0.02
Age (years)			
Mean (SD)	29.5 (11.9)	27.7 (11.5)	0.25
Body Mass Index (kg/m^2)			
Mean (SD)	19.7 (2.7)	20.8 (3.0)	0.07
Highest fever pre-admission (ºC)			
Mean (SD)	37.9 (0.9)	39.1 (0.5)	<0.01
Days of fever pre-admission			
Mean (SD)	4.4 (2.3)	6.7 (4.3)	<0.01
Geometric mean of parasite density (/μl)	9,540	47,754	<0.01
Min-Max	(119-98,781)	(207-845,214)	
Percentage of patients with:			
Splenomegaly	8.2	7.3	0.80
Hepatomegaly	13.6	31.8	<0.01
History of malaria in past year	44.5	66.4	<0.01
Laboratory data [Mean (SD)]			
Hemoglobin (g%)	12.5 (2.0)	11.5 (2.4)	<0.01
Hematocrit (%)	37.0 (6.0)	34.9 (6.7)	<0.01
White blood cell count (x10^3/μl)	6.1 (2.8)	5.4 (1.8)	0.05
Platelet count (x10^3/μl)	120.3 (80.1)	65.0 (33.1)	<0.01

Table 2
Correlation coefficient between platelet counts on Day 0 (Plt 0) with age, gender, history of malaria in past year, days of fever pre-admission, presence of splenomegaly, severity, and hyperparasitemia, in uncomplicated and severe falciparum malaria patients.

	Plt 0: Uncomplicated falciparum malaria (*N*=110)	Plt 0: Severe falciparum malaria (*N*=110)	Plt 0: All falciparum malaria (*N*=220)
Age (years)	(-0.12)	0.08	0.01
Gender (male/female)	0.36[a]	(-0.40)	0.20[a]
History of malaria in past year (yes/no)	0.12	-0.13	(-0.13)[b]
Days of fever pre-admission	(-0.61)[a]	(-0.37)[a]	(-0.53)[a]
Splenomegaly (yes/no)	(-0.18)	0.08	(-0.30)
Hepatomegaly (yes/no)	0.02	0.12	0.17[b]
Severity (uncomplicated/severe)	NA	NA	(-0.46)[a]
Hyperparasitemia (yes/no)	NA	0.02	0.30[a]

[a]$p < 0.01$; [b]$p < 0.05$; NA; not applicable

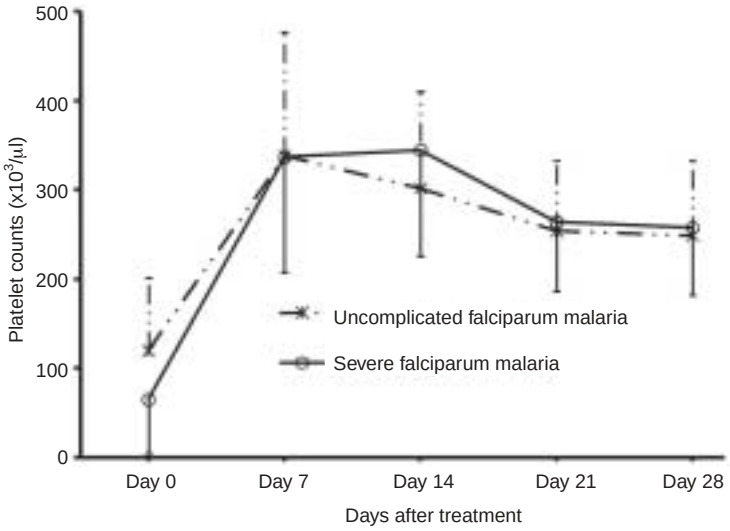

Fig 1–Mean platelet counts on Days 0, 7, 14, 21, and 28 for uncomplicated and severe falciparum malaria patients (*p*-values: within group <0.01, between groups <0.01).

platelet count and age, gender, history of malaria in past year, days of fever pre-admission, presentation with splenomegaly, presentation with hepatomegaly, severity, and hyperparasitemia, the two patient groups were combined into one; the correlation coefficient values and *p*-values were then investigated (Table 2). There were weak but significant ($p<0.01$) correlations between initial platelet count and gender difference, hyperparasitemia, days of fever pre-admission, and severity. History of malaria during the previous year and presentation with hepatomegaly were significantly correlated with initial platelet count ($p<0.05$).

The mean weekly platelet counts for both groups are shown in Fig 1. On admission, thrombocytopenia was found in 73.6% of uncomplicated falciparum malaria patients, and 90.9% of severe cases. Twenty-five point five percent of the uncomplicated group, and 60.6% of the severe group, had initial platelet counts < 50 x 10^3/μl. However, no severe thrombocytopenia was found. The lowest platelet count in uncomplicated cases was 11 x 10^3/μl and in severe cases was 12 x 10^3/μl. No hemorrhagic manifestations were observed or platelet transfusions required for any of the study patients. The initial mean platelet count among severe falciparum malaria patients was significantly lower than among uncomplicated falciparum malaria patients. Platelet levels in both groups returned to normal, with a peak platelet count 7 days after receiving treatment, then slowly decreasing to normal on Days 21 and 28. Platelet counts for both patient groups at different weeks were also compared. Fig 1 shows relatively low platelet counts on admission for both study groups (uncomplicated falciparum malaria group mean platelet count = 120.34 x 10^3/μl; severe falciparum malaria group mean platelet count = 65.06 x 10^3/μl). This was significantly different from other days ($p<0.01$), with mean platelet counts of 249.98 x 10^3/μl to 345.98 x 10^3/μl, closer to the normal platelet level of 311 x 10^3/μl, reported by Ryan (2006).

DISCUSSION

Thrombocytopenia is commonly found in patients suffering from *P. falciparum* and *P. vivax* malaria (WHO, 2006). The risk for thrombocytopenia is increased in malarial patients with a low immune status. Our results are consistent with those of Boehlen (2006) who noted the immunity of malaria patients may affect the platelet count. We found platelet counts in both uncomplicated and severe

P. falciparum malaria patients were often low; but both uncomplicated and severe falciparum-malaria patients had a wide range in platelet counts, with considerable overlap. Platelet counts were reduced in acute malaria, then returned to normal following antimalarial chemotherapy. A higher than average platelet count (mean 250.82 x 10^3/μl, SD 34.84, n=40) was reported by Soogarun *et al* (2004), in healthy subjects from Bangkok, Thailand. Twenty-six point four percent of uncomplicated falciparum malaria cases and 9.1% of severe falciparum malaria cases had normal initial platelet counts. Therefore, malaria should be included in the differential diagnosis of acute febrile diseases, even with normal platelet counts.

In malaria, thrombocytopenia is normally asymptomatic. Few cases of spontaneous bleeding at the time of infection have been reported. Bleeding was found to not significantly be correlated with platelet counts (Tan *et al*, 2008). Our findings agree with a previous study (Kriel *et al*, 2000) that found no evidence of bleeding was evident among the study patients during treatment. Patients with platelet counts <50 x 10^3/μl may be at increased risk for hemorrhagic complications. In this study, although 25.5% of uncomplicated malaria patients and 60.6% of severe malaria patients had platelet counts < 50 x 10^3/μl, no platelet transfusions were required. Kreil *et al* (2000) showed the severity of malaria infection and antimalarial treatment affect the rate of platelet recovery. Our study results are consistent with those of Kreil *et al* (2000): the severity of malaria affected the platelet count on admission (p<0.01). A recent study suggested that prompt antimalarial-drug treatment of *P. vivax* patients may lead to platelet recovery within 2 weeks (Kim *et al*, 2008). The current study found platelet levels in both uncomplicated and severe falciparum malaria patients returned to normal within 7 days after receiving treatment.

The immunopathophysiological processes causing thrombocytopenia in acute *P. falciparum* malaria are complex, varied and incompletely understood. A recent study (Tan *et al*, 2008) suggests decreasing platelet counts during malaria infection may be caused by platelet activation, splenic pooling, and a reduction in platelet lifespan to 2-3 days (the normal platelet lifespan is 7-10 days). One study showed that thrombocytopenia during malaria infection, caused by platelet-bound immunoglobulin, was a recognition trigger for splenic removal, and the threshold for removal was lower in malaria. The more heavily coated platelets were more likely to be removed. The principal determinant of thrombocytopenia, therefore, resulted from the degree of splenic clearance increase, rather than the absolute level of platelet antibody (Looareesuwan *et al*, 1992). Thus, the role of antibody in the pathogenesis of thrombocytopenia remains unexplained.

In conclusion, our study results agreed with previous studies: all the falciparum malaria patients had full recovery of their platelet counts after antimalarial treatment and none needed platelet transfusions. Early treatment with appropriate antimalarial drugs, and appropriate management, may support platelet recovery. Platelet transfusions may not be required in patients with malaria and thrombocytopenia, but no bleeding.

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