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Major Article

Platelet profile is associated with clinical complications in patients with vivax and falciparum malaria in Colombia

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ABSTRACT

Introduction: Thrombocytopenia is a common complication in malaria patients. The relationship between abnormal platelet profile and clinical status in malaria patients is unclear. In low and unstable endemic regions where vivax malaria predominates, the hematologic profiles of malaria patients and their clinical utility are poorly understood. The aim of this study was to characterize the thrombograms of malaria patients from Colombia, where *Plasmodium vivax* infection is common, and to explore the relationship between thrombograms and clinical status. **Methods:** Eight hundred sixty-two malaria patients were enrolled, including 533 (61.8%) patients infected with *Plasmodium falciparum*, 311 (36.1%) patients infected with *Plasmodium vivax* and 18 (2.1%) patients with mixed infections. **Results:** The most frequently observed changes were low platelet count (PC) and high platelet distribution width (PDW), which were observed in 65% of patients; thrombocytopenia with <50,000 platelets/μL was identified in 11% of patients. Patients with complications had lower PC and plateletcrit (PT) and higher PDW values. A higher risk of thrombocytopenia was identified in patients with severe anemia, neurologic complications, pulmonary complications, liver dysfunction, renal impairment and severe hypoglycemia. The presence of thrombocytopenia (<150,000 platelets/μL) was associated with a higher probability of liver dysfunction. **Conclusions:** Young age, longer duration of illness and higher parasitemia are associated with severe thrombocytopenia. Our study showed that thrombocytopenia is related to malaria complications, especially liver dysfunction. High PDW in patients with severe malaria may explain the mechanisms of thrombocytopenia that is common in this group of patients.

Keywords: Thrombogram. Thrombocytopenia. Platelet. *Plasmodium.* Severe malaria. Colombia.

INTRODUCTION

Malaria is a disease caused by infection with *Plasmodium* parasites[1] and is a major health problem worldwide. According to the World Malaria Report, 219 million malaria cases and 660,000 malaria-related deaths occurred worldwide in 2010[2].

Plasmodium falciparum is the main cause of malaria-related death worldwide; however, other species can also cause serious illness[3,4]. Clinical complications in malaria patients include cerebral malaria, severe anemia (SA), acute kidney failure (AKF), pulmonary edema (PE), severe hypoglycemia, shock, disseminated intravascular coagulation (DIC), acidosis and massive hemolysis. A patient who presents with one or more of these conditions is diagnosed with severe malaria (SM) and has an increased risk of mortality[5].

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Different organs can be affected during a malaria episode, which results in localized or systemic injury. Hematological changes, especially anemia and thrombocytopenia, are common[6,7]. Thrombocytopenia has been associated with *Plasmodium knowlesi*[8], *Plasmodium falciparum*[9], *Plasmodium vivax, Plasmodium ovale*[10] and *Plasmodium malariae* infections[11]. The evidence is mixed regarding the existence of a correlation between platelet count (PC) and parasitemia[8-10,12-14]. In a systematic review, Lacerda *et al.* found no differences in the frequency of thrombocytopenia across species of *Plasmodium;* the frequency of thrombocytopenia ranged from 24-94% in all studies[6]. The frequency of thrombocytopenia ranged from 8.0-64.2% in Colombian patients[15-17].

Various mechanisms have been proposed to explain thrombocytopenia during malaria episodes, including platelet destruction by immune mechanisms[14,18-20]; low medullar platelet production[14,21,22]; low thrombopoietin (TPO) synthesis[23]; platelet sequestration in organs, such as the spleen[24,25] or brain[26-28]; and systemic sequestration[29-31]. These changes are transient, and in general, patients recover completely after malaria treatment[32].

The 2013 World Health Organization (WHO) criteria that define complicated malaria do not include severe thrombocytopenia[33]. Studies have postulated that malaria should be suspected in travelers who present with fever and thrombocytopenia[34]. In malaria patients, thrombocytopenia is generally mild or moderate[13,17], and bleeding is rare[35,36]. One study found that severe thrombocytopenia in malaria

was an independent predictor of death (odds ratio (OR)=13.3, 95%CI=3.2-55.1)[37], but this finding has been contested[9]. Thrombocytopenia is more frequent in pregnant women with malaria[38] and is especially pronounced in primigravidae. Additionally, there is an inverse relationship between parasite density and PC[39].

The relationship between thrombocytopenia and clinical presentation is not sufficiently understood, and platelet changes beyond the PC have been either entirely unstudied or not studied in relation to other aspects of the clinical presentation. Moreover, in low and unstable endemic regions such as Colombia, the hematologic profiles of malaria patients and their clinical utility remain poorly understood. The aim of this descriptive study was to characterize the thrombograms of malaria patients from different regions of Colombia and to explore the relationship between clinical status and platelet alterations in ambulatory and hospitalized patients.

METHODS

Patients and sociodemographic characteristics

A retrospective analysis was conducted using clinical and laboratory data obtained from patients from malaria-endemic regions in Colombia. Patients were treated on an outpatient basis for *P. falciparum* and *P. vivax* and were enrolled in clinical and epidemiological studies conducted by the Grupo Malaria (Universidad de Antioquia) between 1997 and 2007[40-42]. A second study group consisted of malaria patients hospitalized between 2005 and 2010 (A. Tobón: unpublished data). The reference population consisted of patients from all age groups who presented at first- or second-tier health-care facilities in the municipalities of Turbo and Necoclí (Urabá Region, Antioquia State), El Bagre (Bajo Cauca Region) and Tumaco, Guapi and Timbiquí (Pacific coast), as well as individuals who were treated on an inpatient basis at third-tier hospitals in Medellin with malaria diagnosed microscopically by thick smear (**Table 1**). For all patients with a complete blood count (CBC), a convenience sample was taken. Eight hundred sixty-two patients were sampled.

Clinical and laboratory procedures

The thick smear was performed according to the WHO recommendations[43]. Following a medical assessment, a sample of blood was collected in ethylenediaminetetraacetic acid (EDTA) for cellular and biochemical studies, which included a CBC within 2 hours after sampling. The analysis was conducted in third- and fourth-generation automated analyzers (Cell-Dyn 3200, from Abbott Diagnostics®, Canada; Coulter HmX from Beckman Coulter®, United States of America; Celltac F MEK 8222, from Nihon Kohden®, United States of America and Sysmex KX-21N®, from Sysmex America, Inc. United States of America). Reference values for the platelet variables for the Colombian population were as follows: PC 150-400 x 10^3 platelet/µL, mean platelet volume (MPV) 6.5-13.5 fl, platelet distribution width (PDW) 15.4-16.8% and plateletcrit (PT) 0.085-0.287%[44].

The complications were classified according to the major criteria of the WHO[5], along with the additional minor criteria proposed for Colombian endemic regions[42]. The complications were classified as follows: 1) Liver failure: severe (total bilirubin >3 mg/dl; AST >120IU/L) or mild (total bilirubin >1.5-3mg/dl; AST >80-120IU/L); 2) Thrombocytopenia: profound (<25,000 platelets/µl) or severe (25-50,000 platelets/µl); 3) Renal impairment: severe (blood urea nitrogen (BUN) >60mg/dl or creatinine >3mg/dl) or mild (BUN 41-60 mg/dl or creatinine 1,5-3mg/dl). 4) Anemia: severe (hemoglobin <5g/dl) or moderate (hemoglobin 5-6.9g/dl); 5) Neurologic complication: cerebral malaria (seizures/coma) or extreme weakness; 6) Pulmonary injury: acute respiratory distress syndrome (ARDS)/

TABLE 1 - Platelet variables: descriptive statistics and categorical classification.

	PC[a]		PT[b]		MPV[c]		PDW[d]	
Number	862		720		720		654	
Mean	134,905		0.112		8.3		17.7	
Median	121,000		0.102		8.4		17.9	
SD	82,249		0.065		1.93		2.2	
Minimum	6,000		0.007		2.6		0.6	
Maximum	727,000		0.662		14.6		37.9	
Classification by category*	n	%	n	%	n	%	n	%
low	564	65.4	258	35.8	120	16.7	23	3.5
normal	293	34.0	450	62.5	598	83.1	207	31.7
high	5	0.6	12	1.7	2	0.3	424	64.8

PC: platelet count; **PT:** plateletcrit; **MPV**: mean platelet volume; **PDW**: platelet distribution width; **SD:** standard deviation *Reference values: [a]PC (platelets/µL): low <150,000; normal 150,000-450,000; high >450-000. [b]PT (%): low <0.085; normal 0.085-0.287; high >0.287; [c]MPV (fL): low <6.5[b]; normal 6.5-13.5; high >13.5; [d]PDW: low <15.4; normal: 15.4-16.8; high >16.8.

pulmonary edema or pleural effusion; 7) Acid-base disturbance: severe acidosis (pH <7.35 and HCO3 <15mEq/L) or acidosis (pH<7.35 and HCO_3 15-18mEq/L); and 8) Hypoglycemia: severe (<40mg/dl) or moderate (40-49mg/dl).

Statistical analysis

The Kolmogorov-Smirnov test was used to test quantitative variables for normality; the median values were compared using the Mann-Whitney U test when the variables were not normally distributed. Associations between the qualitative variables were analyzed with the Chi-square test. Spearman's correlation coefficient was applied to analyze specific relationships. The level of statistical significance adopted was 5% (p<0.05). All analyses were conducted using the IBM® SPSS® statistics program, version 19 (licensed for Universidad de Antioquia).

Ethical considerations

All patients provided their informed consent to enroll in this study as required by the ethics committee of the Medical Research Institute of the Medical School of the Universidad de Antioquia (Acta 31, July 2002 and Acta 05, June 2005). The review of patient medical records was approved by the hospital ethics committees from Pablo Tobón Uribe Hospital and San Vicente Foundation Hospital (Letter 5282, Oct. 2009).

RESULTS

We reviewed the clinical records of 862 patients with malaria, including 144 (16.7%) inpatients at the San Vicente Foundation Hospital and Pablo Tobón Uribe Hospital in Medellin. All patients were classified in 4 age groups: less than 1 year (n=8, 0.9%), 1-5 years (n=49, 5.7%), 5.1-15 years (n=187, 21.7%) and >15 years (n=618; 77.7%); the precise age was known in 610 patients (mean 25.9 years; standard deviation (SD) 16.6; median 22.8). In total, 62.9% of the patients were male.

In 592 patients, the mean duration of illness prior to seeking treatment was 6.2 days (SD 6.4; median 5.0; range 1-99). The *Plasmodium* species diagnosed were *P. falciparum*, 533 (61.8%) patients; *P. vivax*, 311 (36.1%) patients; and mixed infection, 18 (2.1%) patients. The clinical presentation was classified as acute malaria in 613 (71.1%) patients and complicated malaria in 248 (28.8%) patients.

Platelet profile

The values of PC, PT, MPV and PDW were not normally distributed (Kolmogorov-Smirnov, p=<0.001). Categorical sorting indicated that the most significant alterations were the low PC and high PDW, which were present in approximately 65% of patients **(Table 1)**.

In the 564 (65.4%) patients with PC values below 150,000 platelets/µL, thrombocytopenia was classified in 4 categories **(Table 2)**. Significant thrombocytopenia (<50,000 platelets/µL) was observed in 93 (11%) patients, which included 2% with <25,000 platelets/µL. Other frequent abnormalities included low values for PT and MPV.

The thrombogram and other variables

We did not identify differences by gender in the median values of PC (p=0.299), PT (p=0.833), MPV (p=0.660) or PDW (p=0.115) or by age (children-adults) in the median values of PC (p=0.914), PT (p=0.972), MPV (p=0.328) or PDW (p=0.640) (Mann-Whitney U test).

The analysis by species is presented in **Table 2**. The PT, MPV and PDW were significantly lower in the *P. vivax* cases compared with the *P. falciparum* cases (Mann-Whitney U test, p<0.05). PC was lower in the *P. vivax* cases, but this result was not significantly different from that in the *P. falciparum* cases (p=0.832). The frequency of thrombocytopenia (<150,000 platelets) in the patients with *P. falciparum* was 66.6% compared with 63.7% in the *P. vivax* cases. Severe or profound thrombocytopenia (<50,000 platelets) was observed in 55 (10.3%) of the 533 *P. falciparum* cases, 34 (10.9%) of the 311 *P. vivax* cases and 4 (22.2%) of the 18 mixed infections (Chi^2, p>0.05).

The plateletcrit was higher in mixed infections compared with *P. vivax* infections (p=0.041), and the PDW was lower in mixed infections compared with *P. falciparum* infections (p=0.011); additional comparisons by species were not significantly different.

Thrombocytopenia severity and others variables

To explore the relationships between the severity of thrombocytopenia and age, duration of illness and hematological variables, the variables were compared among the patients with no alteration in PC (group A) and the patients with mild (B), severe (C) or profound (D) thrombocytopenia **(Table 3)**. Patient age was not different between the patients with normal or low PCs (p>0.05) but was significantly different between the patients with mild and severe thrombocytopenia (p<0.05), which indicates that younger age could be a risk factor for severe platelet reduction. The median duration of illness was similar between the non-thrombocytopenic patients and the patients with mild changes; however, the duration was significantly higher in the patients with PCs below 50,000/µL (p<0.05). Parasitemia was significantly higher and the erythrocyte count was significantly lower in the patients with PCs below 50,000/µL compared to the other patients groups **(Table 3)**.

The comparison between the patients with normal PC and the patients with any degree of thrombocytopenia showed significant differences in the leukocyte count, with the latter group characterized by significantly lower counts of lymphocytes (p<0.001), monocytes (p=0.003), neutrophils (p=0.010) and eosinophils (p=0.029).

In the cases of severe or profound thrombocytopenia, all variables with the exception of age were significantly different compared with the patients with normal counts. Lower leukocyte and erythrocyte counts, higher coagulation times [prothrombin time (PTi) and partial thromboplastin time (PTT)] and higher parasitemia were observed in the patients with severe or profound thrombocytopenia.

Finally, the comparison between the patients with mild, severe and profound thrombocytopenia revealed no leukocyte count differences (p>0.05). Young age, a longer duration

TABLE 2 -Thrombocytopenia level and platelet profile by species of *Plasmodium*.

		P. falciparum		*P. vivax*		Mixed infection		Total		Cumulative
Thrombocytopenia level	Platelets/µl	n	%	n	%	n	%	n	%	%
Profound	< 25,000	15	2.2	5	1.3	—	—	20	2.3	2.3
Severe	25,000-49,999	40	5.8	29	7.6	4	21.1	73	8.5	10.8
Moderate	50,000-74,999	59	8.6	48	12.6	3	15.8	110	12.8	23.5
Mild	75,000-149,999	241	34.9	116	30.5	4	21.1	361	41.9	65.4
Normal count	150,000-449,000	175	25.4	111	29.2	7	36.8	293	34.0	99.4
High count	> 450,000	3	0.4	2	0.5	—	—	5	0.6	100.0
Platelet count		n=533		n=311		n=18				
mean		136,710		132,002		131,611				
median		119,000		124,000		99,000				
SD		84,909		75,938		107,626				
Plateletcrit		n=467		n=241		n=12				
mean		0.118		0.102		0.109				
median		0.110[a]		0.100[a]		0.129[d]				
SD		0.068		0.057		0.041				
Mean platelet volume		n=467		n=241		n=12				
mean		8.5		7.8		8.7				
median		8.5[b]		8.3[b]		8.8				
SD		1.7		2.2		1.9				
Platelet distribution width		n=407		n=237		n=10				
mean		18.2		17.5		17.2				
median		18.5[c]		17.2[c]		16.7[e]				
SD		2.2		2.2		0.8				

P.: *Plasmodium;* **SD:** standard deviation. Mann-Whitney U test, **a**p=0.007; **b**p=0.003; **c**p=0.000; **d**p=0.041; **e**p=0.011.

TABLE 3 - Thrombocytopenia level stratified by quantitative variables (median values).

Group	A	B	C	D	C+D[b]	A vs B	A vs C+D
Thrombocytopenia (platelet count)	No (>150, 000)	Mild-moderate (50,000-150,000)	Severe (25,000-49,999)	Profound (<25,000)	Severe or profound (<50,000)	p= (Mann-Whitney U test)	
Age	23.3	23.0	19.0	17.0	19.0	0.112	0.129
Duration of disease (days)	4.0	4.0	7.0	7.0	7.0	0.683	0.009
Parasitemia (parasites/µL)	3,720	5,000	8,960	30,556	10,800	0.115	0.000
Hemoglobin (g/dL)	12.2	11.9	11.4	11.5	11.4	0.155	0.029
Erythrocyte count (#/µL)	4.46	4.4	4.2	4.2	4.2	0.461	0.002
Leucocytes (#/µL)	7,000	5,700	5,800	5,450	5,700	0.217	0.000
Lymphocytes (#/µL)	1,955	1,421	1,425	1,073	1,372	0.187	0.000
Monocytes (#/µL)	400	360	318	216	273	0.086	0.012
Neutrophils (#/µL)	4,379	3,732	3,949	3,397	3,700	0.483	0.001
PTi[a]	12.1	11.9	14.1	13.9	14.1	0.195	0.001
PTT[a]	33.0	33.0	35.5	39.0	36.7	0.871	0.002

PTi: Prothrombin time; **PTT:** Partial thromboplastin time. **a**PTi and PTT in seconds; **b**Mann-Whitney U test: between A and D, p<0.05 for all comparisons.

TABLE 4 - Median contrast of platelet features by clinical complications (major or minor).

	Platelet count			Plateletcrit			Mean platelet volume			Platelet distribution width		
Group	n	median	p	n	median	p	n	median	p	n	median	p
CM												
yes	248	72,000	0.000	183	0.070	0.000	149	8.6	0.537	154	18.9	0.000
no	614	137,000		537	0.111		571	8.4		500	17.5	
CM except thrombocytopenia												
yes	155	105,000	0.000	110	0.086	0.000	110	8.2	0.466	83	19.1	0.000
no	614	137,000		537	0.111		537	8.4		500	17.5	
Liver complication												
yes	170	90,500	0.000	123	0.073	0.000	123	8.4	0.126	99	19.1	0.289
no	264	129,000		184	0.100		184	8.0		144	18.7	
Renal complication												
yes	31	80,000	0.033	25	0.120	0.850	25	8.8	0.212	22	16.7	0.007
no	401	114,000		280	0.090		280	8.1		219	18.9	
Neurologic complication												
yes	15	41,000	0.000	11	0.13	0.876	11	9.7	0.024	11	16.7	0.003
no	847	122,000		709	0.10		709	8.4		643	17.7	
Pulmonary complication												
yes	17	55,000	0.001	11	0.13	0.649	11	9.3	0.278	10	16.7	0.525
no	845	122,000		709	0.10		709	8.4		644	17.7	
Hypoglycemia (<49g/dl)												
yes	4	40,500	0.004	3	0.04	0.010	3	9.8	0.649	2	18.3	0.751
no	333	119,000		242	0.09		242	8.0		182	19.2	
Severe anemia												
yes	26	105,500	0.307	20	0.11	0.956	20	8.8	0.776	18	17.6	0.336
no	836	121,500		700	0.10		700	8.4		636	17.7	
Acidosis (severe or moderate)												
yes	11	32,000	0.276	11	0.13	0.134	11	9.8	0.119	10	16.7	0.392
no	18	56,500		13	0.13		13	8.3		14	16.7	

CM: complicated malaria.

of illness and higher parasitemia were related to severe thrombocytopenia, a lower erythrocyte count and a longer coagulation time.

Clinical aspects

Clinical complications were diagnosed in 248 (28.8%) patients, and 206 (23.9%) patients exhibited complications other than severe thrombocytopenia. The complications were classified using major, 105 (12.2%) and minor, 143 (16.6%) criteria in accordance with the guidelines established by the WHO[5] and the Colombian endemic regions, respectively[42]. The complications were distributed as follows: 1) Liver failure: mild in 96 (11.1%) patients or severe in 74 (8.6%) patients; 2) Thrombocytopenia: severe in 73 (8.5%) patients or profound in 20 (2.3%) patients; 3) Renal impairment: mild in 21 (2.4%) patients or severe in 10 (1.2%) patients; 4) Anemia: moderate in 22 (2.6%) patients or severe in 4 (0.5%) patients; 5) Neurological complications: extreme weakness in 4 (0.5%) patients or seizures/coma in 11 (1.3%) patients; 6) Pulmonary injury: pleural effusion in 5 (0.6%) patients or ARDS/pulmonary edema in 12 (1.4%) patients; 7) Acid-base disturbances: acidosis in 5 (0.6%) patients or severe acidosis in 4 (0.5%) patients; and 8) Hypoglycemia: moderate in 3 (0.4%) patients or severe hypoglycemia in 1 (0.1%).patient.

TABLE 5 - Median values of hematological laboratory tests in relation to severe thrombocytopenia.

	Plasmodium falciparum				*Plasmodium vivax*				All patients[a]			
		Severe thrombocytopenia[b]				Severe thrombocytopenia[b]				Severe thrombocytopenia[b]		
Variable	n	yes	no	P value[c]	n	yes	no	P value[c]	n	yes	no	P value[c]
Parasites/µL	391	10,492	4,360	0.000	135	9,760	5,640	0.339	537	10,800	4,680	0.000
Total bilirubin	269	1.8	0.9	0.000	135	1.8	1.1	0.008	416	1.7	1.0	0.000
Direct bilirubin	268	0.9	0.3	0.000	135	0.6	0.3	0.006	416	0.7	0.3	0.000
Aspartate aminotransferase	266	64	33	0.001	100	54	36	0.013	375	57.0	34.0	0.000
Alanine aminotransferase	65	82	27	0.001	100	54	28	0.028	173	54.0	27,0	0.000
Blood urea nitrogen	269	18.0	12.7	0.002	117	16.9	11.1	0.005	379	16.9	12,0	0.000
Prothrombin time	139	14.3	11.8	0.000	15	12.4	14.0	0.111	159	14.1	12,0	0.000
Partial thromboplastin time	142	36.0	32.3	0.005	14	44.0	34.7	0.096	160	36.7	33,0	0.000
Hemoglobin	533	11.4	11.9	0.234	311	11.1	12.2	0.034	844	11.3	12,1	0.029
Erythrocyte count/µL	512	4,185	4,400	0.065	261	4,080	4,500	0,004	787	4,185	4,430	0,002
White blood cells/µL	531	6,100	6,000	0.784	310	4,850	6,500	0.001	860	5,700	6,000	0.054
Creatinine	278	1.0	1.0	0.599	138	0.90	0.94	0.528	412	1.0	0.9	0.425
Serum glycemia	230	94.5	100	0.261	103	183	109	0.028	337	100	103	0.454

[a]Including mixed infections; [b]<50,000 platelets/µL; [c]Mann-Whitney U test.

Patients with complicated malaria (with or without severe thrombocytopenia) exhibited significantly lower median values for PC and PT and higher median values for PDW (**Table 4**). Median PC values were significantly lower in the patients with complications other than severe anemia or acidosis. In the patients who experienced complications, the median PT was lower and the median PDW was higher compared with the patients who did not experience complications. Furthermore, the PDW was significantly lower in the patients with renal or neurologic complications. The MPV was similar across patients.

Severe and profound thrombocytopenia (<50,000 platelets/µL) were related to clinical complications (O.R. 1.6; 95% 95%CI=1.5-1.8; p≤0.001). A higher probability of thrombocytopenia was observed in patients with severe anemia (OR=3.2; 95% CI=1.3-7.9; p=0.007), neurologic complications (OR=12.0; 95%CI=4.0-35.0; p≤0.001), pulmonary complications (OR=8.0; 95%CI=3.0-21.0; p≤0.001), liver dysfunction (OR=3.9; 95%CI=2.2-6.9; p≤0.001), renal impairment (OR=3.7; 95%CI=1.7-8.1; p=0.001) and severe hypoglycemia (OR=38.6; 95%CI 3.9-385; Fisher, p=0.002). The presence of thrombocytopenia (<150,000 platelets/µL) was associated with a higher probability of liver dysfunction (OR=2.4; 95%CI=1.5-3.8; p≤0.001).

Finally, we compared the laboratory data among malaria patients with and without severe thrombocytopenia and stratified by *Plasmodium* specie (**Table 5**). The laboratory data were significantly different across categories, with the exception of the white blood cell count, serum creatinine and serum glycemia. In the patients infected with *P. falciparum*, severe thrombocytopenia was related to abnormal values on all liver-function tests, BUN, PTi and PTT; in the patients with *P. vivax*, a relationship was identified between severe thrombocytopenia and abnormal values on liver-function tests, BUN, serum glucose, erythrocyte and white cell counts.

DISCUSSION

In addition to anemia, a reduction in the number of platelets is one of the more well-known hematologic changes observed in patients with malaria. While platelet-count changes are particularly associated with *P. falciparum* malaria[9,12,45], this pathology has also been identified in malaria caused by other species, such as *P. vivax*[36,46]. Changes in other platelet parameters, such as PT, MPV or PDW, have not been well described in malaria patients.

Some studies have found that patients infected with *P. vivax* or *P. falciparum* develop anemia, monocytosis, eosinopenia and lymphopenia[45]. This study found a progressive reduction in hemoglobin levels and white blood cell counts with reduced PCs, which resulted in anemia, monocytopenia, neutropenia and lymphopenia. Thrombocytopenia presented frequently in the patients infected with *P. falciparum* (67%) and *P. vivax* (64%). The PC did not differ by species, which suggests that a similar mechanism governs this interaction in *P. vivax* and *P. falciparum* infections. Significant differences in PT and MPV were observed among species, but the values remained

within the normal range. The PDW was significantly higher in the patients infected with *P. falciparum* compared with those infected with *P. vivax*; however, both median values were above the normal range. These findings and the results of various studies that have demonstrated a rapid recovery of PCs following the initiation of antimalarial treatment suggest that thrombocytopenia is related to a systemic endothelial and platelet activation rather than medullary suppression. Systemic endothelial activation likely mediates platelet sequestration and does not depend exclusively on the formation of platelet aggregates and parasitized erythrocytes, as observed in *P. falciparum* infections[47].

Interestingly, we found that the patients with *P. vivax* infection and severe thrombocytopenia had more significant changes in leukocyte and erythrocyte counts compared with the patients without thrombocytopenia. These changes, which were not identified in the patients infected with *P. falciparum,* favor a stronger inflammatory response in severe *P. vivax* infections that can affect bone marrow via the production of tumor necrosis factor alpha (TNF-α). This hypothesis is supported by a study that identified increased production of TNF-α in *P. vivax* malaria and a stronger host immune response in *P. vivax* malaria compared with *P. falciparum* malaria[48].

Various mechanisms have been proposed to explain thrombocytopenia in malaria; however, its clinical significance remains uncertain. Thrombocytopenia can be interpreted as evidence of damage and has been associated with clinical complications and high parasitemia[9,12,37]. However, some studies have suggested that thrombocytopenia has a protective effect, which reduces the probability of red blood cell aggregation[28] or mediates parasite destruction. The latter effect has been observed *in vitro* in the early stages of erythrocytic infection with *P. falciparum* and in a mouse model with *Plasmodium chabaudi*[49]. In this study, we identified a significant relationship between severe thrombocytopenia and other clinical complications, but we were not able to establish causality.

Thrombocytopenia has been associated with disturbances in coagulation and has been implicated in cases of disseminated intravascular coagulation (DIC)[50]. In children with severe malaria, prolonged PTi, PTT and thrombin time (in 47.5, 35.0 and 62.5% of patients, respectively) may lead to platelet hypoaggregation[46]. Activation of coagulation may also be responsible, in part, for thrombocytopenia[6]. Our study showed that the patients infected with *P. falciparum*, but not *P. vivax,* had more prolonged PTi and PTT when the thrombocytopenia was more severe. This difference may be the result of increased procoagulant activity in *P. vivax* compared with *P. falciparum* infections, which develops in response to increased endothelial activity induced by TNF-α levels and the consequent formation of thrombin-antithrombin III[48].

In this study, patients with severe or profound thrombocytopenia showed higher parasitemia compared with patients without thrombocytopenia, which is in agreement with previous findings. Low PCs have been associated with increased parasite load. This association may result from sensitization induced by the parasitized red blood cells in the platelet, with consequent increased platelet sensitivity to adenosine diphosphate (ADP) and higher dense-granule secretion[45,46]. These changes could ultimately promote platelet aggregation on the endothelium, which has been demonstrated in cerebral malaria[27].

Plateletcrit is a measure of platelet mass, the clinical significance of which should be interpreted in terms of the number and size of the platelets. Whitfield et al.[51] demonstrated that in healthy subjects, there was an inverse relationship between the platelet size (MPV) and the platelet number (PC) and proposed that the total platelet mass is closely regulated by changes on MPV or PC. We found that the PT was low in 36% of patients with malaria. This finding may be explained by a combination of low PC with normal MPV (63%), low PC and MPV (35%) and normal PC with low MPV (2%). The reduction in circulating platelet mass is best explained by a reduction in platelet number rather than a reduction in platelet size. It is suspected that in malaria, sequestration leads to pseudothrombocytopenia[52].

Some studies have identified a relationship between the decrease in PC and the appearance of clinical complications, such as anemia, respiratory distress syndrome and cerebral malaria[37]. We found that the patients with severe (25,000-49,999 platelets/μL) or profound thrombocytopenia (<25,000 platelets/μL) had more pathological changes in their clinical status compared with the patients without thrombocytopenia. Furthermore, parasitemia, the duration of disease, and TP and TPP increased with an increasing severity of thrombocytopenia (mild→moderate→severe→profound), whereas erythrocyte and leukocyte counts decreased.

In this study, the patients who experienced complications had lower PC and PT values and higher levels of PDW compared with the patients who did not experience such complications. Specifically, the median PCs were significantly lower in the patients with hepatic complications (90,500 vs. 129,000; $p \leq 0.001$), renal complications (80,000 vs. 114,000; $p=0.017$), neurologic complications (42,000 vs. 122,000; $p \leq 0.001$), pulmonary complications (55,000 vs. 122,000; $p=0.001$) and severe hypoglycemia (40,500 vs. 119,000; $p=0.004$). The low PC may result from the adhesion of platelets to the sites of vascular injuries and the mediation of malaria-parasitized red blood cells (pRBC) sequestration to the endothelium through von Willebrand-factor strings[53].

When platelets decrease in number, bone marrow megakaryocytes are stimulated by thrombopoietin, and their nucleus becomes hyperlobulated with a higher deoxyribonucleic acid (DNA) content. These stimulated megakaryocytes produce larger platelets. Thus, platelets with a higher MPV are expected to be present in autoimmune thrombocytopenia when megakaryocytic stimulation is present. Conversely, platelets with a lower MPV are expected in thrombocytopenic states associated with marrow hypoplasia or aplasia[54]. Interestingly, 83% of the patients studied had normal MPV, and it appears that neither of these two mechanisms associated with thrombocytopenia was significantly activated.

The examination of PDW with MPV provides information regarding thrombocyte volume disturbance. Thrombocyte volume heterogeneity occurs because of the production factors in the bone marrow. PDW is an index of thrombocyte volume heterogeneity similar to erythrocyte distribution, and both MPV and PDW are markers of platelet immaturity and platelet activation[55]. A high PDW predominated in the patients studied (65%), and we identified significantly higher PDW in the total sample of severe patients; however, the analysis for each complication indicated conflicting results, which could be a result of the differential patterns of inflammation in the spectrum of clinical complications or statistical effects based on the low number of cases when the complications were individually analyzed.

In healthy human subjects, no correlation was identified between PCT, MPV or PDW values and platelet aggregation results[54]; however, these relationships could be modified in the presence of inflammation.

It has been reported that PDW increases over storage time because of the formation of abnormally small and large platelets[54], and it is well known that pseudo-thrombocytopenia occurs as a result of exposure to EDTA when used in blood sampling[56]. There is a need for multi-center and prospective studies with large sample sizes to elucidate the utility and clinical importance of these parameters in malaria.

Our results indicate that thrombocytopenia accompanies malaria complications, especially liver dysfunction. Major changes in total bilirubin, direct bilirubin, aspartate aminotransferase, alanine aminotransferase, prothrombin time and partial thromboplastin time were observed in severe thrombocytopenia (<50,000 platelets/μL). The PCs were significantly lower in the patients who experienced liver, renal, neurologic, and pulmonary complications and severe hypoglycemia.

The liver is a key regulator of platelet production through the synthesis of TPO. Thus, hepatic dysfunction may explain, in part, the thrombocytopenia observed in these patients; however, more data are required because TPO appears to rise during infection by *P. falciparum,* even when liver function is compromised[23].

Differences between *P. falciparum* and *P. vivax* infections may arise from the different mechanisms by which the physiopathology of thrombocytopenia is mediated, i.e., via clumping in *P. falciparum* and via medullar suppression in *P. vivax*. A better understanding of these mechanisms is still needed.

Despite the inherent limitations of retrospective analyses, these results may contribute to the understanding of severe thrombocytopenia as a negative prognostic marker in patients with malaria. Our findings suggest a need to study hepatic function and to establish close clinical monitoring of malaria patients. Furthermore, there is a substantial need for prospective studies to elucidate the utility and clinical importance of these parameters in malaria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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