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Thrombocytopenia in malaria: who cares?

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Despite not being a criterion for severe malaria, thrombocytopenia is one of the most common complications of both Plasmodium vivax *and* Plasmodium falciparum *malaria. In a systematic review of the literature, platelet counts under 150,000/mm³ ranged from 24-94% in patients with acute malaria and this frequency was not different between the two major species that affected humans. Minor bleeding is mentioned in case reports of patients with* P. vivax *infection and may be explained by medullary compensation with the release of mega platelets in the peripheral circulation by megakaryocytes, thus maintaining a good primary haemostasis. The speculated mechanisms leading to thrombocytopenia are: coagulation disturbances, splenomegaly, bone marrow alterations, antibody-mediated platelet destruction, oxidative stress and the role of platelets as cofactors in triggering severe malaria. Data from experimental models are presented and, despite not being rare, there is no clear recommendation on the adequate management of this haematological complication. In most cases, a conservative approach is adopted and platelet counts usually revert to normal ranges a few days after efficacious antimalarial treatment. More studies are needed to specifically clarify if thrombocytopenia is the cause or consequence of the clinical disease spectrum.*

Key words: *Plasmodium falciparum* - *Plasmodium vivax* - malaria - thrombocytopenia - platelets

Malaria affects almost all blood components and is a true haematological infectious disease. Anaemia and thrombocytopenia are the most frequent malaria-associated haematological complications (Wickramasinghe & Abdalla 2000) and have received more attention in the scientific literature due to their associated mortality. On the other hand, thrombocytopenia is less studied, causes negligible mortality and is an isolated phenomenon; there is no report of a single patient in the literature who has died only because of malaria-associated thrombocytopenia.

In the current field of Travel Medicine, the rapid increase in the number of people travelling to tropical areas has added a great challenge for malaria diagnosis because the thick blood smear (the standard diagnosis in endemic areas) has high specificity but only when performed by experienced microscopists. The presence of thrombocytopenia in acute febrile travellers returning from tropical areas has become a highly sensitive clinical marker for malaria diagnosis (D'Acremont et al. 2002). Another study has reported 60% sensitivity and 88% specificity of thrombocytopenia for malaria diagnosis in acute febrile patients (Lathia & Joshi 2004). The sensitivity of thrombocytopenia together with the acute febrile syndrome was 100% for malaria diagnosis, with a specificity of 70%, a positive predictive value of 86% and a negative predictive value of 100% (Patel et al. 2004).

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Thrombocytopenia is a well-documented and frequent complication in *Plasmodium vivax* malaria. In one study, platelet count normalised after treatment and only one patient, concomitant with the lowest platelet count, exhibited "purpuric lesions" on the lower extremities (Hill et al. 1964).

Since the beginning of the 1970s, there have been reports proposing that malaria-associated thrombocytopenia is quite similar in *P. vivax* and *Plasmodium falciparum* infections (Beale et al. 1972). However, more recent data in India has shown how thrombocytopenia exhibited a heightened frequency and severity among patients with *P. vivax* infection (Kochar et al. 2010).

In 1903, the young physician Carlos Chagas (who become more famous afterwards for the discovery of American trypanosomiasis, which is named after him), published his MD thesis on the Hematological Studies on Paludism (Chagas 1903). Within it, he described anaemia and leukocyte abnormalities, but also normal megakaryocytes in the bone marrow were referred to in patients with acute and chronic malaria from Rio de Janeiro. He also drew our attention to evidence of bleeding in the 46 patients he followed.

In the city of Manaus, state of Amazonas, located in the Western Brazilian Amazon, Djalma Batista authored Paludism in the Amazon, a book in which he described observations about patients with malaria seen at his private clinics (Batista 1946). Similar to Carlos Chagas, there is no mention of platelet count in his study because it was not routinely performed. However, there is a vivid description of haemostasis disorders in some patients. Particularly noteworthy is the presence of huge spleen enlargement and prolonged bleeding time accompanied by recurrent gingival bleeding.

Data on the real burden of thrombocytopenia associated with malaria is contradictory in the literature and it is not usually considered when conducting patient selection. Table I shows the major publications estimating the frequency of thrombocytopenia. Most of these data were published in the late 1990s, probably in time with the surge in the availability of affordable automated machines capable of performing full blood counts (FBC). Manual platelet counting is time-consuming and usually needs to be requested by the physician with the routine blood count in most of the endemic areas for malaria. In only three publications is there an adequate randomised enrollment of patients with appropriate sample size calculation to estimate the frequency of bleeding and its association with the respective platelet count (Lacerda 2007, Silva 2009, Kochar et al. 2010). Only one study has ruled out other common causes of thrombocytopenia that are also endemic in the studied area (Lacerda 2007). There is a wide range of thrombocytopenia occurrence in these reports, which may be explained by distinct selection criteria of the enrolled patients. There are also differences in the selection of outpatients or inpatients from tertiary care centres that tend to present with severe thrombocytopenia. Furthermore, clinical manifestations of thrombocytopenia are usually described as case reports and most of these are due to *P. vivax* (Table II).

In 2005, 138 of 684 (20.1%) malarial cases hospitalised in a tertiary care centre in Manaus had thrombocytopenia as the cause of admission, which corresponded to 6.8% of hospitalisations due to all causes in this reference institution (unpublished observations). Hospitalisation, however, does not add any benefit to the patient and because there is no evidence for any intervention, this simply increases public health costs in underdeveloped and under-resourced areas.

Pathogenesis of malarial thrombocytopenia - Coagulation disturbances - A study based on 31 American soldiers in Vietnam with chloroquine-resistant falciparum malaria noted the following changes in the acute phase of the disease using the same patients as their own controls during convalescence: decrease in the platelet count and prothrombim activation time, increase in the activated thromboplastin time, and reduction in factors V, VII and VIII with normal fibrinogen (Dennis et al. 1967). This report suggested that thrombocytopenia was simply a consequence of the coagulation disorders presented by these patients, an idea that persisted for many decades in the literature. In another series of 21 patients with falciparum malaria, six had developed disseminated intravascular coagulation (DIC). The authors noted that the patients with more severe thrombocytopenia also had DIC and that there was correlation between platelet count and C3 protein levels. However, the reduction in C3 was proportional to that in parasitaemia, suggesting that thrombocytopenia was not independently associated with C3 (Srichaikul et al. 1975). In Manaus, 2004, a study with falciparum and vivax patients demonstrated a negative correlation between platelet counts, thrombin-anti-thrombin complex and D-dimers, suggesting that the activation of coagulation could be partially responsible for thrombocytopenia (Marques et al. 2005).

Splenomegaly - The spleen in malaria has played a crucial role in the immune response against the parasite, as well as controlling parasitaemia due to the phagocytosis of parasitised red blood cells (RBCs) (Engwerda et al. 2005). Some data suggested that platelets were sequestered in the spleen during the acute infection (Skudowitz et al. 1973). In the experimental model with *Plasmodium chabaudi*, thrombocytopenia was absent in splenectomised mice, showing that the spleen was essential for thrombocytopenia (Watier et al. 1992). The term hypersplenism was proposed to describe the clinical picture of the enlarged spleen followed by the decrease in one or more peripheral blood lineages (usually reverted after splenectomy), probably due to sequestration or destruction of cells inside the spleen, in liver diseases, which lead to increased portal system pressure. However, it is recently believed that not only mechanical alterations take place, but also compromise of haematopoietic growth factors produced in the liver (Peck-Radosavljevic 2001). On the other hand, the isolated spleen enlargement does not explain *per se* the destruction of cells as formerly believed. This organ represents outstanding architectural organisation and controls, with great sophistication, the exposure of cells screened by it. In patients with malaria, the increase in the macrophage-colony stimulating factor is associated to thrombocytopenia, suggesting that macrophages play a role in the destruction of these particles (Lee et al. 1997). In the comparison of spleens from patients with severe falciparum malaria vs. those of control and septic patients, it was shown that splenic dendritic cells are increased in malaria and there is a reduction in B lymphocytes and macrophages in the splenic cords (Urban et al. 2005). The mechanisms related to the formation of splenic hematomas are mostly associated with *P. vivax* infection and the interface with thrombocytopenia is noted to be imprecise (Lacerda et al. 2007). In vivax malaria, the role of the spleen in the expression of *vir* genes is still unrecognised. *P. vivax* passing through the spleen would activate the transcription of polymorphic Vir proteins to escape from macrophage destruction in this organ. On the other hand, these same proteins would permit the binding of parasitised RBCs to barrier cells, creating an isolated microenvironment in the spleen that would be rich in reticulocytes (del Portillo et al. 2004). More recent studies with the murine model of *Plasmodium yoelii* evidenced that there was higher parasite accumulation, reduced motility, loss of directionality, increased residence time and altered magnetic resonance only in the spleens of mice infected with the non-lethal 17X strain (Martin-Jaular et al. 2011). This same model has never been used to study the role of the spleen in thrombocytopenia, but opens new avenues for functional and structural studies of this lymphoid organ.

Bone marrow alterations - The finding of a *P. vivax* trophozoite inside a human platelet suggested that thrombocytopenia could be the result of invasion of these particles by the parasites themselves, similar to what was classically proposed for RBCs. As these same authors did not find parasites inside megakaryocytes, they proposed that the penetration took place in the peripheral circulation (Fajardo & Tallent 1974). However, this observa-

TABLE I

Systematic review of studies, estimating thrombocytopenia in malarial patients (1997-2011)

References	Study site	Type of patients	Age range	Species	n	Thrombocytopenia % [criterion (mm^3)]
Mohanty et al. (1997)	India	Inpatients and outpatients	All ages	*P.v.*	24	29 (< 150,000)
				P.f.	76	39 (< 150,000)
Noronha (1998)	Brazil	Inpatients and outpatients	< 14 y	*P.f.*	54	51.8 (< 150,000)
Kortepeter and Brown (1998)	USA	Inpatients and outpatients	> 18 y	*P.f./P.v.*	79	74 (< 150,000)
Murthy et al. (2000)	India	Inpatients	10-80 y	*P.f.*	158	40.5 (< 150,000)
Gonzalez et al. (2000)	Colombia	Inpatients	All ages	*P.f.*	113	33.6 (< 150,000)
				P.v.	128	39 (< 150.000)
Alecrim (2000)	Brazil	Inpatients	> 12 y	*P.v.*	73	91.8 (< 150,000)
		Outpatients			319	60.8 (< 150,000)
Silva et al. (2000)	Brazil	Inpatients	All ages	*P.v.*	429	46.6 (< 140,000)
Oh et al. (2001)	South Korea	Inpatients and outpatients	> 17 y	*P.v.*	101	85.1 (< 150,000)
Robinson et al. (2001)	Australia	Inpatients	NA	*P.f./P.v./P.o.*	246	71 (< 150,000)
Mourão et al. (2001)	Brazil	Inpatients	< 12 y	*P.f.*	255	73.7 (< 150,000)
Lacerda et al. (2001)	Brazil	Inpatients	> 12 y	*P.f.*	218	87.6 (< 150,000)
Ladhani et al. (2002)	Kenya	Inpatients	Children	*P.f.*	1,369	56.7 (< 150,000)
Park et al. (2002)	Brazil	Inpatients	All ages	*P.v.*	237	61.6 (NA)
Mohapatra et al. (2002)	India	Inpatients and outpatients	15-60 y	*P.v.*	110	3.6 (< 100,000)
Bashawri et al. (2002)	Saudi Arabia	Inpatients and outpatients	2 m-74 y	*P.v./P.f.*	727	55.6 (< 150,000)
Araújo Filho et al. (2003)	Brazil	Inpatients and outpatients	4-64 y	*P.v.*	68	20.6 (< 50,000)
Echeverri et al. (2003)	Colombia	Outpatients	All ages	*P.v.*	104	8 (< 130,000)
Jadhav et al. (2004)	India	Inpatients and outpatients	All ages	*P.v.*	973	65 (50,000-150,000)
				P.f.	590	50 (50,000-150,000)
Marques (2004)	Brazil	Inpatients and outpatients	> 15 y	*P.f.*	44	79 (< 150,000)
				P.v.	106	94 (< 150,000)
Rodriguez-Morales et al. (2005)	Venezuela	NA	NA	*P.v.*	116	87.6 (< 150,000)
Rodriguez-Morales et al. (2006)	Venezuela	Inpatients	< 12 y	*P.v.*	78	58.9 (< 150,000)
Casals-Pascual et al. (2006)	Kenya	Inpatients and outpatients	6 m-10 y	*P.f.*	120	34.4 (< 150,000)
Kumar and Shashirekha (2006)	India	Inpatients and outpatients	All ages	*P.v.*	27	88.8 (< 150,000)
Lacerda (2007)	Brazil	Outpatients	> 18 y	*P.v.*	142	71.8 (< 150,000)
				P.f.	26	65.4 (< 150,000)
Koltas et al. (2007)	Turkey	Outpatients	All ages	*P.v.*	90	NA
Taylor et al. (2008)	Indonesia	Outpatients	All ages	*P.v./P.f.*	151	78.8 (< 150,000)
Tan et al. (2008)	Thailand	Inpatients and outpatients	Pregnant women	*P.v.*	523	22 (< 75,000)
				P.f.	694	34 (< 75,000)
Silva (2009)	Brazil	Outpatients	All ages	*P.v.*	397	77.1 (< 150,000)
Rasheed et al. (2009)	Pakistan	Inpatients	All ages	*P.v./P.f*	502	80 (< 150,000)
Shaikh et al. (2009)	Pakistan	Outpatients	All ages	*P.v./P.f.*	124	82.5 (< 150,000)
Prasad et al. (2009)	India	Inpatients	< 5 y	*P.f.*	40	85 (< 150,000)
Gonzalez et al. (2009)	Venezuela	Outpatients	3-67	*P.v.*	59	55.9 (< 150,000)
Poespoprodjo et al. (2009)	Indonesia	Inpatients	0-3 m	*P.v./P.f. and mixed*	179	61.3 (< 100,000)
Khan et al. (2009)	Qatar	Outpatients	All ages	*P.v.*	81	63 (< 150,000)
Maina et al. (2010)	Kenya	Outpatients	< 5 y	*P.f.*	523	49 (< 150,000)
Kochar et al. (2010)	India	Inpatients and outpatients	All ages	*P.v./P.f. and mixed*	1,064	24.6 (< 150,000)
George and Alexander (2010)	India	Inpatients	18-66 y	*P.v.*	30	93.3 (< 150,000)
Srivastava et al. (2011)	India	Inpatients	All ages	*P.v.*	50	82 (< 150,000)

m: months; NA: non-available; *P.f.*: *Plasmodium falciparum*; *P.o.*: *Plasmodium ovale*; *P.v.*: *Plasmodium vivax*; y: years.

TABLE II

Collated case reports of *Plasmodium vivax*-associated thrombocytopenia (1964-2011)

References	Study site	Platelet count (x 1,000/mm^3)	Bleeding	Platelet transfusion	Observation
Hill et al. (1964)	United States of America	20-49	Petecchiae	No	Experimental infection
Takaki et al. (1991)	Solomon Islands	NA	NA	NA	DIC
Anstey et al. (1992)	Bali	22	No	No	-
Ohtaka et al. (1993)	NA	NA	NA	NA	PAIgG increase
Yamaguchi et al. (1997)	Thailand and Sri Lanka	22-53	No	No	PAIgG increase
Victoria et al. (1998)	Brazil	1	Several	Yes	ITP
Kakar et al. (1999)	India	5	No	No	-
Makkar et al. (2002)	India	8	Gingival bleeding	Yes	-
Holland et al. (2004)	Mexico	19	Epistaxis	Yes	-
Lacerda et al. (2004)	Brazil	1	Gingival bleeding	Yes	ITP
Aggarwal et al. (2005)	India	6	Petecchiae	Yes	-
Katira and Shah (2006)	India	14-92	No	Yes	-
Komoda et al. (2006)	South America	15	NA	NA	-
Kaur et al. (2007)	India	30	No	No	Acute renal failure
Song et al. (2007)	South Korea	25-20	No	No	DIC, lung edema, acute renal failure and shock
Kaur et al. (2007)	India	30	Petecchiae	No	Acute renal failure
Lacerda et al. (2008)	Brazil	6	No	No	Chronic splenomegaly
Vij et al. (2008)	India	NA	Gingival bleeding	No	NA
Rifakis et al. (2008)	Venezuela	57	No	No	Hydronephrosis and shock
Parakh et al. (2009)	India	5-42	Petecchiae	No	Cerebral malaria, shock and acute renal failure
Thapa et al. (2009)	India	11	Petecchiae and mucosal bleeding	Yes	Hepatitis and shock
Harish and Gupta (2009)	India	1	Intracranial bleed	No	Seizures
Bhatia and Bhatia (2010)	India	NA	Yes	NA	NA

DIC: disseminated intravascular coagulation; ITP: immune thrombocytopenic purpura; NA: non-available; PAIgG: platelet-associated IgG.

tion was never seen again in the literature. Likewise, a "dysmegakaryopoiesis" was proposed, similar to what happened in the human malarial anaemia model, where dyserythropoiesis was triggered by cytokines (Menendez et al. 2000). In the few studies that examined the bone marrow for this purpose, megakaryocytic lineage was apparently preserved (Naveira 1970, Beale et al. 1972). Thrombopoietin indeed seems to rise during the acute disease even in the presence of liver compromise, suggesting that no bone marrow inhibition is seen (Kreil et al. 2000). Additional data from FBC samples in vivax patients showed that there is a significant negative correlation between platelet count and mean platelet volume (Lacerda 2007), suggesting that megakaryocytes are able to release mega platelets in the circulation to compensate for the low absolute number of platelets in the periphery. Similar results were shown in children with falciparum malaria (Maina et al. 2010). These mega platelets are probably able to sustain a good primary haemostasis that could explain the low frequency of severe bleeding in malarial patients, as shown in Table II. Non-human primates, on the other hand, are an unexplored model to study megakaryopoiesis alterations and its implication on thrombocytopenia (Llanos et al. 2006).

Antibody-mediated platelet destruction - There is evidence that platelet-associated IgG (PAIgG) is increased in malaria and is associated with thrombocytopenia. However, this is a generic definition for all types of IgGs that may be found on the platelet surface, including antibodies stored inside platelet α-granules. Therefore, increased PAIgG could also be interpreted as platelet activation and exposition of IgGs on the surface, and not necessarily auto-immunity, as suggested in anecdotal case reports where antibodies against glycoproteins were detected in malaria (Panasiuk 2001, Conte et al. 2003). The detection of auto-antibodies against platelets by flow cytometry (Rios-Orrego et al. 2005) should not be seen as specific for malaria, as natural auto-antibody formation is a common defence of the infected organism

and is frequently seen in most viral, bacterial and parasitic diseases without any repercussion (Daniel-Ribeiro & Zanini 2000). Molecular mimicry, however, provides evolutionary advantage for microorganisms that escape immune aggression (Daniel-Ribeiro 2000). The relationship between malaria and auto-immunity has been discussed in the literature and the first epidemiological association was made based on the presence of fewer auto-immune diseases in malarigenous areas (Greenwood 1968). The formation of circulating immune complexes (CIC) in vivo in malaria, as well as in other infectious diseases, is a continuous process from antigens and antibodies and/or complement elements. CIC seems to modulate the immune response to several antigens that remain sequestered in B lymphocyte or dendritic cell-rich follicles for a longer time, which contributes to the formation of B-cell immunological memory, as seen in vaccine studies (Davidson 1985). During acute malaria, thrombocytopenia is most probably associated with the binding of parasite antigens to the surface of platelets to which antimalarial antibodies also bind, leading to the *in situ* formation of immune complexes (ICs) (Kelton et al. 1983). In an experimental model with *Plasmodium berghei*, the same correlation between platelet count and IC's was evidenced (Grau et al. 1988). No association was found with IgM (Beale et al. 1972). It is clear that CICs are elevated in vivax and falciparum malaria, but their role in the development of thrombocytopenia is still obscure (Touze et al. 1990, Tyagi & Biswas 1999) as well as its immune suppressing effect (Brown & Kreier 1982, Shear 1984). Because the generation of IC's is proportional to the amount of available antigen, the negative correlation between platelet count and peripheral parasitaemia reported in many studies (Lacerda 2007, Silva 2009) corroborates ICs as a potential mechanism of platelet destruction. The presence of amino acid residues tyrosine 193 [9Y(193)] and serine 210 [S(210)] on apical membrane antigen-1 (AMA-1) was significantly associated with normal platelet counts in *P. vivax* malaria independent of the level of parasitaemia that also provides supporting evidence for this (Grynberg et al. 2007). In only one study, circulating monocytes were found to phagocytose platelets, but this mechanism still needs to be associated to thrombocytopenia more closely (Jaff et al. 1985). The finding of immune thrombocytopenic purpura (ITP) secondary to malarial infection is rare and may be due to idiosyncratic auto-immune mechanisms not well understood (Lacerda et al. 2004).

Oxidative stress - Free radicals may play an important role in the platelet destruction in malarial infection. There is evidence that the decrease in total cholesterol in vivax malaria is due to lipidic peroxidation (Erel et al. 1998). Also, in vivax malaria, there is a negative correlation between platelet count and platelet lipid peroxidation in addition to the positive correlation between platelet count and the activity of gluthatione peroxidase and superoxide dismutase, which are considered anti-oxidant enzymes (Erel et al. 2001). In a study of 103 patients with acute falciparum malaria, there was a negative correlation between platelet count and nitrogen reactive intermediates (Santos 2000). There is also a strong association between platelet count and intra-platelet gluthatione peroxidase, suggesting that a compensatory mechanism is presented by platelets to face the oxidative burst found in malaria (Araujo et al. 2008).

Platelet aggregation - Platelets from patients with acute malaria are highly sensitive to adenosine diphosphate (ADP) addition in vitro (Essien & Ebhota 1981), and it is believed that ADP release following haemolysis could contribute to higher platelet aggregation. Actually, the incubation of platelets with *P. falciparum*-parasitised RBCs also increases platelet aggregation *per se* in vitro, especially after ADP and thromboxane A_2 addition (Inyang et al. 1987). Even electron microscopic examination of non-stimulated, fresh platelets from malarial patients show centralisation of dense granules, glycogen depletion and microaggregates and phylopoids as a sign of in vivo activation, which could be responsible for a pseudo-thrombocytopenia due to sequestration of these activated particles in the interior of the vessels (Mohanty et al. 1988). Contradictory data were presented showing aggregation impairment in severe falciparum patients after ADP addition in vitro (Srichaikul et al. 1988). *P. falciparum* induces systemic acute endothelial cell activation and the release of activated von Willerbrand factor (vWF) immediately after the onset of the blood-stage infection (Mast et al. 2007). Even without consumptive coagulopathy, the increase in soluble glycoprotein-1b (GP1b) concentrations results from vWF-mediated GP1b shedding, a process that may prevent excessive adhesion of platelets and parasitised erythrocytes (Mast et al. 2010). Antimalarial drugs have also been shown as potential inhibitors of platelet aggregation in vivo and in vitro, what precludes careful inclusion and exclusion criteria of patients to be used in clinical research (Cummins et al. 1990).

The relationship between thrombocytopenia and severe malaria - Severe thrombocytopenia (platelet count under 50,000/mm^3), despite not being considered severe malaria according to World Health Organization criteria (WHO 2010) due to the inability to cause death *per se*, has been occasionally associated with severity (Gerardin et al. 2002, Rogier et al. 2004) or not (Moulin et al. 2003). But thrombocytopenia has also been described in severe vivax patients (Kochar et al. 2005, Andrade et al. 2010). In 17 patients from Manaus affected by any of the WHO malaria severity criteria with confirmed *P. vivax* monoinfection, 14 presented with thrombocytopenia, suggesting that this haematological complication can be explored as a marker of the severity for this species (Alexandre et al. 2010). From the case reports described in Table II, the association between severe cases with thrombocytopenia is evident. However, that can be due to bias publication, where prospective studies would be needed to validate this association. On the other hand, considering that many studies point to a clear negative correlation between platelet count and parasitaemia (Grynberg et al. 2007, Silva 2009), it should be investigated if thrombocytopenia could be used in the surveillance of drug resistance, where higher parasitaemias for prolonged periods are usually found. Interestingly, in areas where thrombocytopenia and other types of clinical severity are

frequently reported, resistant parasites are also being simultaneously detected (Santana Filho et al. 2007, Tjitra et al. 2008), possibly explaining why the prevalence of thrombocytopenia worldwide is not homogeneous.

On the other side of the clinical presentation of plasmodial infection, platelet counts were never performed in asymptomatic parasite carriers. However, due to the very low parasitaemia (sometimes submicroscopic) presented by these patients, it is possible that platelet counts are normal and parallel clinical symptoms (Suarez-Mutis et al. 2007).

Avoiding the consensual understanding that platelets are particles devoted to the maintenance of primary haemostasis, it has been shown that platelets participate in the pathogenesis of microvascular malaria, adhering to the endothelium when it is previously stimulated with tumor necrosis factor (TNF) (Lou et al. 1997). Even in the non-stimulated cerebral endothelium, platelets may adhere and facilitate the adhesion of *P. falciparum*-parasitised RBCs, through CD36 is ubiquitous in endothelial cells and, coincidentally, platelets (Wassmer et al. 2004). Platelets therefore act by stabilising and strengthening bridges between RBCs and endothelial cells, which is considered the cornerstone of severe falciparum malaria. Rosetting of parasitised RBCs is also mediated through CD36 in platelets in severe malaria (Pain et al. 2001, Chotivanich et al. 2004). In mice infected with *P. berghei* ANKA, mice deficient of tissue and uroquinase plasminogen activators demonstrated less capillary sequestration of platelets and less severe malaria (Piguet et al. 2000). Blocking GPIIb with anti-CD41 monoclonal antibodies in the first day of murine infection with *P. berghei* also led to higher production of interleukin (IL)-10, IL-1α, IL-6, interferon-α and TNF and less mortality among mice, suggesting that platelets may act as cofactors of severe malaria (Sun et al. 2003, van der Heyde et al. 2005). There was also an inverse correlation between platelet count and TNF in patients with vivax infection and no association between specific mutation G→A in the position 308 in the *TNF* gene (a polymorphism whose functional effect upon severe disease is hypothesised) and platelet count was observed. More severe patients presented more severe thrombocytopenia and higher TNF levels (Silva 2004). Platelets stimulated by parasitised RBCs may also trigger apoptosis in endothelial cells pre-treated with TNF in a pathway mediated by tumor growth factor (TGF)-β_1 from platelets (Wassmer et al. 2006a, b). Recent evidence showing *P. vivax*-infected RBCs adhering to lung endothelial cells and to the placental tissue ex vivo indicates that in vivax, mechanisms similar to those associated with falciparum severity may be involved (Carvalho et al. 2010). The contribution of platelets to this adhesion, however, requires further investigation.

In children in Kenya suffering from falciparum malaria, an inverse correlation between platelet count and plasmatic IL-10 was seen (Casals-Pascual et al. 2006). This interpretation is not straightforward, because IL-10 is generally associated with protection against severe disease. The authors hypothesise, though, that IL-10 could reduce platelet counts to avoid infected-RBC adhesion to the endothelium, as if thrombocytopenia was a mechanism of defence against severe disease and not the cause. Studies of vivax infection have shown thrombocytopenia to be associated with an increase in IL-1, IL-6, IL-10 and TGF-β (Park et al. 2003).

The role of platelet-derived microparticles (MPs) (submicron-sized vesicles released from cells upon activation or apoptosis) has yet to be determined in vivo. There is evidence that these MPs participate in the endothelial activation responsible for severe cerebral malaria in murine models (Combes et al. 2006). MPs were also associated with coma and thrombocytopenia in severe falciparum malaria patients (Pankoui Mfonkeu et al. 2010). Apparently, there is an increase in the amount of MPs in vivax malaria patients, which may play a role in the acute inflammatory symptoms of this disease (Campos et al. 2010); this role requires further investigation.

Clinical management of malarial thrombocytopenia - To date, there is no robust evidence on how to manage patients with malaria and thrombocytopenia. Platelet transfusion has been widely followed, but with no confirmed efficacy. The indication of prophylactic platelet transfusion when platelet counts are under 10,000/mm^3 probably applies only when the bone marrow is compromised and is not able to release efficacious platelets (Rebulla 2000). This does not seem to be the case in malaria. Keeping platelet counts between 50,000 and 100,000/mm^3 is a formal indication only in patients undergoing surgical procedures (Rebulla 2001). In a tertiary care centre in the Western Brazilian Amazon over a 12-month period, 10.4% (20/191) of patients who received platelet transfusion were diagnosed with vivax or falciparum malaria (Lacerda et al. 2006). The dosage was usually below that recommended in the literature (Schlossberg & Herman 2003). In 40% of patients, the only justifications for transfusion were maintaining a platelet count below 10,000/mm^3 and discrete bleeding. In a further 6% of patients, only a very low platelet count was described. In this group of 40% of patients, the alleged reason was minor bleeding despite having non-severe thrombocytopenia; in 33%, no indication was verified. These data point to the little existing evidence of the recommendations for platelet transfusion in these patients. The corrected count increment to evaluate transfusion efficacy was not calculated for any patient. The low efficacy of platelet transfusion in general is well described for several acute infectious diseases (de Paula et al. 1993), probably due to peripheral immune mechanisms of destruction that do not spare the transfused platelets. Indications for platelet transfusion in cases when DIC is suspected and diagnosed, the formal clinical indication persists, as recommended elsewhere (Franchini 2005). Due to the impossibility of using frozen platelets in routine clinical practice, other platelet substitutes and preparations are being investigated (Blajchman 2003). Except in atypical cases of ITP with severe bleeding, there is no evidence for the use of human intravenous immunoglobulin, even in cases of severe thrombocytopenia (Lacerda et al. 2004).

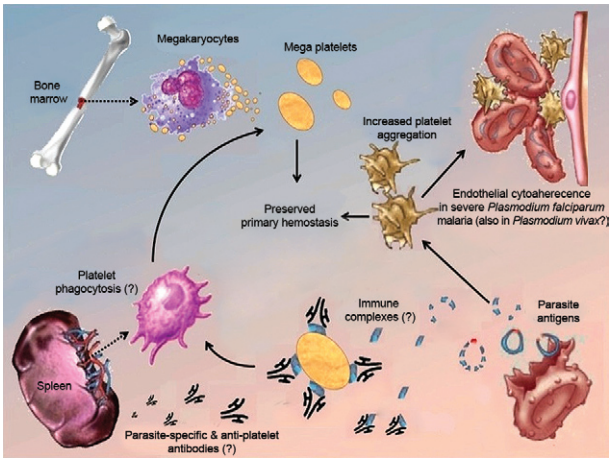
The use of corticoids has never been followed, probably due to the fact that the recovery of thrombocytopenia following antimalarial treatment is seen in almost all cases, with good prognosis for all species that infect humans (Lacerda 2007) and with the lack of robust evi-

dence of immune-mediated destruction of platelets as a major mechanism. It was also found that in patients with cerebral falciparum malaria, dexamethasone exacerbated the neurological symptoms and increased the frequency of gastrointestinal bleeding (Warrell et al. 1982, Hoffman et al. 1988). However, in none of these studies was platelet recovery analysed as a secondary endpoint.

Immune modulators are also candidates in the adjuvant antimalarial therapy (Muniz-Junqueira et al. 2005, Mohanty et al. 2006), based on the drug-induced inhibition of adhesion molecules in RBCs and platelets (Muniz-Junqueira 2007). The exploration of drugs known by their anti-inflammatory effect, modulating TNF, e.g., pentoxyfylline and thalidomide, upon severe malaria, could not only contribute to the understanding of the mechanisms of severity but also clarify the association between platelets and severe disease.

Thrombocytopenia in other infectious diseases - Many other acute and chronic infectious diseases share similar thrombocytopenia as part of the clinical picture and these mechanisms may be used by proxy to explain malarial disease.

Chronic thrombocytopenia is found in approximately 10% of patients with human immunodeficiency virus (HIV)-1 infection and in one-third of those with acquired immunodeficiency syndrome (Scaradavou 2002). The first cases of homosexuals with profound thrombocytopenia in New York were classified as ITP (Karpatkin 2002), involving the presence of serum IgG anti-GPIIIa (Karpatkin et al. 1995). Later on, this IgG was found to be directed against $GPIIIa_{49-66}$ (Nardi et al. 1997). More recently, molecular mimicry was proposed between *nef* HIV-1 protein and $GPIIIa_{49-66}$ (Li et al. 2005) Other chronic infectious diseases known to cause thrombocytopenia include chronic viral hepatitis, where CIC (Samuel et al. 1999) and PAIgG (Doi et al. 2002) are also implicated. In the case of hepatitis C virus infection, the blockage in the maturation of megakaryocytes is mediated by the viral RNA itself (Almeida 2003). Despite an associated medullary compromise in visceral leishmaniasis in the canine model of *Leishmania infantum* infection, antiplatelet IgG and IgM were also observed (Terrazzano et al. 2006). In acute infection with *Trypanosoma cruzi*, frequent thrombocytopenia is related to the presence of parasite trans-sialidase (Tribulatti et al. 2005). Furthermore, during infection with any of the four dengue viruses, thrombocytopenia is frequent and is supposed to be a criterion of dengue hemorrhagic fever (Mourão et al. 2007). Platelet phagocytosis ex vivo has already been shown as a potential mechanism in this acute viral disease (Honda et al. 2009). Thrombocytopenia is also observed in leptospirosis (Nicodemo 1993), typhoid fever (Huang & DuPont 2005), hantavirus infection (Santos et al. 2006), yellow fever (Monath 2001) and sepsis (Becchi et al. 2006), whose mechanisms are poorly understood. The high frequency of thrombocytopenia in other infectious diseases, as a rule, changes the paradigm that platelets are essential only to haemostasis, supporting their role as important contributors to modulate the immune response. In any case, studies focusing on the pathogenesis of thrombocytopenia in malarial patients should always rule out other concomitant infectious diseases, which is difficult in socio-economically deprived study populations suffering large burdens of multiple diseases.



Major mechanisms associated to malaria-triggered thrombocytopenia and the possible relationship with severe disease.

The frequency of thrombocytopenia (i.e., platelet count below 150,000/mm^3) in malarial infection ranges from 24-94% in the literature, despite the low occurrence of severe bleeding, even in the case of severe malaria. It is still unclear whether this haematological complication is more frequent in *P. vivax* or *P. falciparum* malaria. In Figure, the major mechanisms involved in the pathogenesis are highlighted, but further studies are still needed to clarify the impact of each mechanism and its clinical relevance. The clinical management of malarial thrombocytopenia is expectant and the level of evidence for platelet transfusion is insufficient to recommend this practice. It is not clear whether platelets are diminished during acute malarial infection as a consequence of the immune response to the parasite present or whether platelets are actually involved in the generation of severe disease.

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