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Malaria-related anaemia: a Latin American perspective

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Malaria is the most important parasitic disease worldwide, responsible for an estimated 225 million clinical cases each year. It mainly affects children, pregnant women and non-immune adults who frequently die victims of cerebral manifestations and anaemia. Although the contribution of the American continent to the global malaria burden is only around 1.2 million clinical cases annually, there are 170 million inhabitants living at risk of malaria transmission in this region. On the African continent, where Plasmodium falciparum *is the most prevalent human malaria parasite, anaemia is responsible for about half of the malaria-related deaths. Conversely, in Latin America (LA), malaria-related anaemia appears to be uncommon, though there is a limited knowledge about its real prevalence. This may be partially explained by several factors, including that the overall malaria burden in LA is significantly lower than that of Africa, that* Plasmodium vivax, *the predominant* Plasmodium *species in the region, appears to display a different clinical spectrus and most likely because better health services in LA prevent the development of severe malaria cases. With the aim of contributing to the understanding of the real importance of malaria-related anaemia in LA, we discuss here a revision of the available literature on the subject and the usefulness of experimental animal models, including New World monkeys, particularly for the study of the mechanisms involved in the pathogenesis of malaria.*

Key words: *Plasmodium falciparum* - *Plasmodium vivax* - malaria - anaemia - haemoglobin - Latin America

Malaria is the most important parasitic disease worldwide, causing 225 million clinical cases and an estimated 781,000 fatalities annually, and represents a major global public health problem (WHO 2010). Although four *Plasmodium* species have been classically responsible for human malaria, *Plasmodium falciparum* has been the most prevalent overall, particularly in Africa (86%). On this continent, the greatest mortality is associated with cerebral malaria and severe anaemia, mostly in children less than five years of age, in malaria holoendemic areas (Guerra et al. 2010). In sub-Saharan Africa, pregnant women are also at higher risk of cerebral malaria and anaemia, which are consequently the major causes of perinatal morbidity and mortality. Both of these malarial complications are responsible for a great number of spontaneous abortions, stillbirths, premature deliveries and low birth weight (Dicko et al. 2003).

Because of the higher *P. falciparum* global prevalence, morbidity and mortality, most research efforts on malaria pathogenesis have been focused on this species (Akhwale et al. 2004). However, *Plasmodium vivax* represents the second most prevalent species, responsible for an estimated 25-40% of the reported malaria clinical cases (Westenberger et al. 2010). Until recently, there was a mistaken belief that *P. vivax* was always a benign disease, however, there is growing body of evidence of the high prevalence of severe and complicated *P. vivax* malaria cases, including severe anaemia (Genton et al. 2008, Tjitra et al. 2008, Kochar et al. 2009, Alexandre et al. 2010, Andrade et al. 2010). Additionally, it is likely that the incidence of anaemia may be higher than what is currently diagnosed. Multiple factors indicate that the public health relevance of *P. vivax* may be more significant than was traditionally thought: (i) *P. vivax* has a wider geographical range - potentially exposing more people to the risk of infection, (ii) it is less amenable to control and (iii) most importantly, infections with *P. vivax* can cause severe clinical syndromes (Tjitra et al. 2008).

Approximately 170 million people live at risk of *P. vivax* and *P. falciparum* transmission in 21 countries in Latin America (LA) and the Caribbean (Guerra et al. 2008, 2010). Nearly 60% of the malaria cases in the Americas are reported from Brazil and the other 40% are reported from Colombia (14.2%), Peru (8.8%), Venezuela (5.4%), Bolivia (1.9%) and Ecuador (1.1%). Caribbean cases include those reported in Haiti (2.8%). Central American countries report the occurrence of malaria cases as Guatemala (3.8%), Panama (0.4%) and Honduras (1.5%). In terms of malaria species distribution, 74% of infections are caused by *P. vivax*, 25% by *P.*

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falciparum and < 0.01% by *Plasmodium malarie*. All the species together contributes a mortality estimated in less than 0.1% to the overall mortality caused by malarial infections (WHO 2009).

The benefits of a detailed knowledge of *P. vivax* transmission and its clinical burden are identical to those of *P. falciparum*. The development of the Malaria Atlas Project has shown that the global mapping of malaria is a fundamental step to (i) understand the epidemiology of the disease on a global scale, (ii) appraise the equity of global financing for malaria control and (iii) set the basis for disease burden estimation. Although significant progress has been made in *P. falciparum* mapping, in the case of *P. vivax*, such maps have been developed only recently, making any strategic planning in LA more difficult (Guerra et al. 2010).

To elucidate the molecular mechanisms involved in the pathogenesis of malaria-induced anaemia, this review address the malarial anaemia immune pathogenesis process and the relevance of currently available experimental animal models, particularly New World monkeys, which are susceptible to human malaria parasites (Alexandre et al. 2010, Andrade et al. 2010).

Epidemiology of malaria-related anaemia in LA - It has been estimated that nearly 50% of the population, distributed in 21 countries of the American continents, is exposed at some level to the risk of malaria transmission (Gusmao 1999). Of these countries, Brazil and Colombia experience greater than 60% of the malaria cases. In LA, malaria exhibits epidemiological characteristics that appear to be particular to the region. There is an extraordinary parasite genetic differentiation due to bio-geographic barriers such as the Andean ridge, which separates endemic areas on the Pacific coast of the region from those in the Amazon and Orinoco Basins. In the case of *P. falciparum*, there is substantial spatial and temporal heterogeneity in the proportion of infections caused by each parasite population (Cortese et al. 2002, McCollum et al. 2007). This has resulted in the isolation of parasite populations and limited dispersion of mutations, such as those associated with anti-malarial drug resistance in *P. falciparum* populations, which may have consequences for malaria control strategies. Drug resistance associated mutations that are common in the Amazon and Orinoco Basins have not been introduced to the Pacific coast region (Bacon et al. 2009, Corredor et al. 2010). Additionally, there is a dense forest, known as the Darien gap, which geographically separates Central America from South America and maintains parasite populations that harbour significantly different drug sensitivity profiles (Restrepo-Pineda et al. 2008). Interestingly, communities in these distinct geographical areas correspond to highly diverse ethnic groups (e.g., African descendants, native Indians and mestizos) with critical genetic differences in their Duffy (Fy) blood groups, which influence *P. vivax* malaria susceptibility (Hadley & Peiper 1997). As a consequence of this complexity, but also because of the limited resources traditionally allocated to malaria research in this region, the epidemiology of malaria, including the prevalence of anaemia in these areas, is still poorly understood.

An exhaustive search for studies addressing malaria-related anaemia in the 21 countries of LA known to have malaria transmission has indicated that only 56 studies have been published in this period. This represents the work spanning the more than 60 years since the initiation of the Malaria Eradication Campaign. Twenty-four of the studies reported anaemia and/or information on haemoglobin (Hb) levels in community-based malaria patients (Table I), 18 studies were based on reports of hospitalised malaria patients (Table II) and 14 corresponded to cross-sectional surveys in malaria-endemic communities (Table III). Additionally, 70% of the published studies were contributed by only three countries: Brazil, Venezuela and Colombia, significantly limiting the geographical distribution of the available information.

Unfortunately, not only are studies scarce but they are also not comparable because (i) the designs of the studies were variable, (ii) the populations studied were different, (iii) the definition of anaemia was not universal, (iv) it is difficult to thoroughly understand the history of the infection, as several of the studies were cross-sectional and not prospective, (v) co-infections were frequently present, such as bacteraemia or helminth infection and (vi) patients with haemoglobinopathies or glucose-6-phosphate dehydrogenase (G6PD) deficiencies presented at different prevalence rates. Therefore, although severe anaemia was reported in these studies, it is not possible to determine its prevalence, which appears to be low regardless. These findings indicate the need for better-designed studies of malaria-related anaemia that assess not only its overall prevalence, but also its association with malnutrition and with common co-infections and diseases, such as helminth infections, bacterial infections and hepatitis.

Clinical spectrum of anaemia in human subjects - The clinical manifestations of malaria depend on multiple factors from the parasite, parasite-host interactions and host factors, including socio-economic conditions. Some of the host factors that have been studied more in-depth include immunity, particularly the production of pro and anti-inflammatory cytokines, genetic traits, α or β-thalassemia, Fy phenotype, sickle cell traits and age. Parasite factors such as endemicity, drug resistance, *Plasmodium* species, parasite multiplication rates and antigenic polymorphism are of great relevance for anaemia development. Moreover, social-geographical factors, such as access to treatment, cultural and economic factors, health policies and transmission intensity greatly contribute to increased risk of anaemia.

Malarial anaemia is usually normocytic and normochromic (Phillips et al. 1986, Bashawri et al. 2002), without spherocytes or schistocytes. However, the anaemia associated with malaria can also be microcytic and hypochromic due to the high frequencies of haemoglobinopathies and iron deficiency in endemic countries (Bashawri et al. 2002).

Another key feature of malarial anaemia is the presence of inadequate reticulocytosis, despite the degree of anaemia. In acute uncomplicated malaria due to *P. falciparum*, the haematocrit may be normal during the first 24 h after the onset of fever, but afterwards there can

TABLE I

Studies reporting anaemia and/or haemoglobin (Hb) status in malaria-infected patients in the field, in Latin America (1983-2010)

References	Locations	Patients (n)	Age range or average (years)	Species	Severe anaemia	Main findings
De Souza (1983)	Belém (Brazil)	99	> 18	P.f.	No	Rise in Hb levels within seven days
Sanchez Perovani et al. (1983)	Cuba	NA	NA	NA	NA	Haemolytic anaemia
Rodriguez-Morales et al. (1986)	Cuba	150	NA	NA	NA	25.3% of haemolytic anaemia
Menendez-Capote et al. (1997)	La Habana (Cuba)	15	NA	P.v.	No	Haemolytic anaemia due to G6PD deficiency
Gutierrez et al. (1998)	Caracas (Venezuela)	29	NA	P.v. and P.f.	Yes	Anaemia associated to severe malaria
Ventura et al. (1999)	Belém (Brazil)	100	0-14	P.v.	No	Anaemia in 82.7%
Torres et al. (2000)	Ocamo (Venezuela)	26	> 14	P. f., P.v. and P.m.	Yes	Haemolytic anaemia
Sempertegui et al. (2002)	Esmeraldas (Ecuador)	77	0.5 -5	P.f.	Yes	-
Amaral et al. (2003)	Belém (Brazil)	30	2-10	P.f. and P.v.	No	Anaemia in 86.7%
Hamer et al. (2003)	Esmeraldas (Ecuador)	71	0-5	P.f.	No	Lower rate of Hb recovery associated to chloroquine resistance
Echeverri et al. (2003)	Turbo (Colombia)	104	2-75	P.v.	No	Anaemia in 46%; young age and female sex associated with anaemia
Zamora et al. (2005)	Buenaventura (Colombia)	150	28.0	P.f. and P.v.	No	Anaemia in 50%
Moyano and Mendez (2005)	Buenaventura (Colombia)	242	27.8	P.f.	No	Anaemia in 14.9%
Marquino et al. (2005)	Sullana (Peru)	197	28.4	P.f.	No	-
Ferreira et al. (2007)	Manaus (Brazil)	247	> 18	P.f. and P.v.	No	Anaemia in 33%
Osorio et al. (2007)	Quibdo (Colombia)	85	3-62	P.f.	No	Anaemia in 29.4%
Rodríguez-Morales et al. (2007)	Carupano (Venezuela)	120	4-89	P.v.	No	-
Carmona-Fonseca (2008)	Turbo and El Bagre (Colombia)	93	4-10	P.f. and P.v.	No	Mild anaemia
Uscategui et al. (2009)	Turbo and El Bagre (Colombia)	93	4-10	P.f. and P.v.	No	Anaemia in 80%
Fernandes et al. (2008)	Belém and Paragominas (Brazil)	199	NA	P.f. and P.v.	Yes	Anaemia in 36%
Gomez et al. (2009)	Sifontes (Venezuela)	123	23.4	P.f. and P.v	No	Anaemia in 26.2% (pregnant women)
Caicedo et al. (2009)	Tumaco (Colombia), Manaus (Brazil)	246	18-45	P.v. and P.f.	Yes	Hb inversely related to parasitaemia in both sites
Caicedo et al. (2010)	Tumaco (Colombia)	96	15-44	P.v. and P.f.	No	Inverse correlation of folate and Hb levels
Melo et al. (2010)	Careiro (Brazil)	54	5-14	P.v.	No	Helminthes coinfection protect against anaemia

G6PD: glucose-6-phosphate dehydrogenase; NA: not available; P.f.: *Plasmodium falciparum*; P. m.: *Plasmodium malarie*; P.v.: *Plasmodium vivax*.

TABLE II

Series of cases which reported anaemia and/or haemoglobin status in hospitalized malaria-infected patients in Latin America (1950-2010)

References	Locations	Patients (n)	Age range or average (years)	Species	Severe anaemia	Main findings
Chiriboga et al. (1950)	Cuzco (Peru)	67	NA	NA	No	
Ruiz-Gil et al. (1994)	Lima (Peru)	20	1-57	P.v.	No	85% of anaemia
Sarabia et al. (1996)	Guayaquil (Ecuador)	NA	NA	NA	Yes	Anaemia was the major complication in pregnant women
Navarro et al. (1998)	Caracas (Venezuela)	45	Children	P.v. and P.f.	No	84% of anaemia
Gonzalez et al. (2000)	Medellín (Colombia)	291	Adults	P.v. and P.f.	Yes	Mild anaemia in 44.6% and severe anaemia in 5.1%
Noronha et al. (2000)	Manaus (Brazil)	61	0-14	P.f.	No	54.5% of anaemia
Bardales-Tuesta and Puente-Olortegui (2000)	Iquitos (Peru)	186	15-70	P.f.	Yes	Severe anaemia in 41.9% in ICU patients
Fernandez et al. (2001)	La Ceiba and Tegucigalpa (Honduras)	53	15-29	P.f. and P.v.	Yes	26.7 vs. 67.5% of anaemia
Navarro et al. (2003)	Sucre (Venezuela)	6	4-44	P.v.	Yes	100% of anaemia
Jarude et al. (2003)	Rio Branco (Brazil)	445	12-49	P.f. and P.v.	No	39.3% vs. 14.8% of anaemia in pregnant women
Espinoza et al. (2005)	Guayaquil (Ecuador)	80	25.2	P.f. and P.v.	Yes	60% of anaemia in pregnant women
Rodríguez-Morales et al. (2006a)	Sucre (Venezuela)	78	3-97	P.v.	Yes	94.9% of anaemia; 10.3% of severe anaemia
Rodríguez-Morales et al. (2006b)	Sucre (Venezuela)	35	24.0	P.f. and P.v.	No	96% vs. 71% of anaemia in women
Rodríguez-Morales et al. (2006c)	Sucre (Venezuela)	12	16-40	P.v.	Yes	100% of anaemia and association to miscarriage and preterm delivery
Castano et al. (2006)	Tumaco and Turbo (Colombia)	199	1-82	P.f.	Yes	Anaemia associated to severe malaria
Rodríguez-Morales et al. (2009)	Margarita Island (Venezuela)	28	16-40	P.f. and P.v.	Yes	100% of anaemia
Ramos Júnior et al. (2010)	Manaus (Brazil)	18	8-39	P.v.	Yes	Haemolytic anaemia in G6PD deficient patients
Alexandre et al. (2010)	Manaus (Brazil)	17	0-80	P.v.	Yes	29.4% of severe anaemia

G6PD: glucose-6-phosphate dehydrogenase; ICU: intensive care unit; NA: not available; P.f.: *Plasmodium falciparum*; P.v.: *Plasmodium vivax*.

TABLE III

Cross-sectional surveys on haemoglobin (Hb) and/or haematocrit status in population living in malaria-endemic areas in Latin America (1956-2009)

References	Locations	Patients (n)	Age range (years)	Main findings
Ronnefeldt (1956)	Colombia	2,219	All	Lower Hb levels in more endemic areas
Lisker et al. (1965)	Cajinicilapa, Ometepec and San Pedro Mixtepec (Mexico)	1,505	0-51	No association of Hbs and G6PD deficiency with malaria endemicity
Faich and Mason (1975)	Distrito 13 and Los Planes de las Delicias (El Salvador)	853	6-16	Recent malaria associated to anaemia
Barraviera et al. (1988)	Humaitá (Brazil)	32	12-44	Association of previous malaria and splenomegaly with anaemia
Cardoso et al. (1992)	Porto Velho (Brazil)	1,068	> 0.5	Anaemia associated to younger age, pregnancy and recent malaria infection
Cardoso et al. (1994)	Urupá (Brazil)	133	All	Malaria and iron deficiency associated to anaemia
Blair et al. (1997)	El Bagre (Colombia)	NA	All	Anaemia in 30% of < 15 years and in 25% of > 15 years
Pérez Mato (1998)	Mavaca (Venezuela)	103	0.5-60	91% of anaemia; anaemia and malaria prevalence associated to younger age and malnutrition
Roshanravan et al. (2003)	Iquitos (Peru)	1,023	0-50	Anaemia was more frequent in P.f. vs. P.v.
Ferreira et al. (2007)	Acrelândia (Brazil)	389	5-90	37.5% of anaemia; younger age, female sex, pregnancy and recent malaria were identified as risk factors for anaemia
Magris et al. (2007)	Alto Orinoco (Venezuela)	924	All	Low levels of anaemia
García-Casal et al. (2008)	Betania del Topocho (Venezuela)	184	1-94	89.6% of anaemia
Grenfell et al. (2008)	Orinoco (Venezuela)	183	0-15	70.5% of anaemia; anaemia was associated to non-constant access to healthcare
Vitor-Silva et al. (2009)	Careiro (Brazil)	198	5-14	25.9% of anaemia

G6PD: glucose-6-phosphate dehydrogenase; NA: not available; P.f.: *Plasmodium falciparum*; P.v.: *Plasmodium vivax*.

be a progressive fall in the haematocrit level (Wickramasinghe & Abdalla 2000) despite the initiation of antimalarial treatment (Phillips et al. 1986) and even in the absence of parasites in the blood smear (English et al. 2002) or the administration of blood transfusions.

During malaria infection, there are soluble derivatives released by the parasite that induce bone marrow (BM) dysfunction. These derivatives are therefore implicated in the pathogenesis of malarial anaemia (Silverman et al. 1987, Miller et al. 1989, Jootar et al. 1993). This is reinforced by the observation that, as a consequence of repeated infections or suboptimal treatment, children may be partially immunocompromised and so asymptomatic during chronic *P. falciparum* infections. Despite having very low Hb levels (Kurtzhals et al. 1999), these children display absolute reticulocyte counts lower than expected for the degree of the anaemia (Wickramasinghe & Abdalla 2000). These low counts might indicate some degree of BM dysfunction (Kurtzhals et al. 1997). For *P. vivax* infection, it has been observed that the decrease in Hb concentrations can be attributed to the activity of the parasites. The destruction of erythrocytes is marked at the beginning of the infection and erythrocytes do not return to pre-infection numbers in a short period of time, despite the elimination of the infection. This can be primarily explained by *P. vivax* invasion to reticulocytes, which prevents the establishment of the normal erythrocyte population (Collins et al. 2003).

Immunopathology - Although several mechanisms appear to participate in the generation of anaemia in individuals acutely or chronically infected with malaria, the mechanisms can nevertheless be grouped into two main categories: (i) destruction of cells in the peripheral circulation and (ii) a reduction in or alteration of the production of erythroid precursors. Although host genetics may exert some influence for these two mechanisms, its role in anaemia pathogenesis is still poorly understood. For instance, host genetic diversity may explain, to some extent, variations in the frequency of anaemia when distinct areas are compared worldwide. The Fy-negative genotype, which prevails on the Colombian Pacific coast, for example, defines the higher prevalence of *P. falciparum* in that region, as compared to other regions where *P. vivax* is more prevalent (Caicedo et al. 2009). Likewise, there is evidence that FY*B/FY*X and FY*A/FY*X genotypes are associated with low levels of *P. vivax* parasitism, which may favourably impact Hb levels (Albuquerque et al. 2010). Recently, G6PD deficiency (Mediterranean type) was shown to protect against *P. vivax* infection (Leslie et al. 2010); however, protection by the African type, which is more prevalent in LA (Chagas et al. 2009), has never been shown.

Peripheral destruction of red blood cells (RBC) - An important part of the *Plasmodium* life cycle occurs as an obligate intraerythrocytic parasite, where it differentiates and multiplies at the expense of the host cell's nutrients up to the induction of its burst. Each merozoite released either from the liver or from an erythrocyte invades a new erythrocyte; in the case of *P. vivax*, its merozoites have a preference for immature red cells (reticulocytes), whereas in the case *of P. falciparum*, its merozoites invade erythrocytes of any age (Simpson et al. 1999, Rayner et al. 2005). This parasite-specific invasion preference entails the expression of receptors on the erythrocyte surfaces that are required for an invasion. These include the Fy group antigens and reticulocyte-binding ligands in the case of *P. vivax* (Galinski et al. 1992) or glycophorins A, B and C, sialoglycoproteins expressed on human erythrocytes, in the case of *P. falciparum* (Jaśkiewicz 2007). An obvious consequence of the parasite multiplication and the periodic burst of schizonts is the rupture of infected erythrocytes. Although this clearly contributes to the development of anaemia, it does not appear to be sufficient to explain the levels of anaemia attained in individuals exposed to the infection. It has been established that levels of parasitaemia of ≥ 50,000 parasites/μL are indicative of severe *falciparum* malaria (WHO 2000). An infection level that corresponds to the infection and destruction of approximately 1% of the total erythrocyte mass could be easily replaced by erythropoiesis under normal conditions. However, it seems that simultaneous to this mechanism, depuration of the parasitized erythrocytes also occurs as a consequence of the phenomenon of erythrocyte rigidity. This rigidity is induced by the transport of parasite antigens to the infected erythrocyte membrane and is followed by the deformation of the membrane, opsonisation by antibodies and complement and by macrophage activation (Wickramasinghe & Abdalla 2000). However, in many cases, the severity of malaria anaemia does not directly correlate with the degree of circulating parasitaemia (e.g. ≤ 1%), though detected parasitaemia does not reflect the total parasite load, as it does not take into account parasites sequestered in the microvasculature (Nakazawa et al. 1995). Additionally, erythrocytes of malaria patients have a decreased half-life compared to those of healthy individuals (Looareesuwan et al. 1991). Moreover, epidemiological and mathematical models have shown that between 8-12 non-parasitized erythrocytes may be destroyed for each *Pf*-RBC parasitized (Jakeman et al. 1999, Price et al. 2001).

Although the precise cause of the destruction of non-parasitized erythrocytes is unknown, several mechanisms have been postulated: (i) the production of auto-antibodies against the proteins that modify RBC membranes (Jakobsen et al. 1995), (ii) antibody-independent phagocytosis of phosphatidyl-serine exposure, secondary to damage mediated by reactive oxygen species (Bratosin et al. 1998, Serghides et al. 2003), (iii) recognition of malarial antigens on infected erythrocytes by immunoglobulins (Igs) and their further clearance by macrophages (Waitumbi et al. 2000), (iv) complement-mediated phagocytosis and/or haemolysis (Ritter et al. 1993) and (v) loss of complement regulatory proteins (CD35, CD55 and CD59) (Waitumbi et al. 2000, Stoute et al. 2003). Furthermore, it has been documented that the activities and absolute numbers of macrophages are increased during infection; together with erythrocyte changes (decreased deformability and deposition of Igs), this could assist in the depuration of non-parasitized erythrocytes during infection (Mohan et al. 1995).

When dealing with the poorly described *P. vivax*-induced anaemia phenomenon, another relevant issue is the increasing body of evidence of *P. vivax* chloroquine resistance in many endemic areas, including some regions of LA (Santana Filho et al. 2007), which tends to increase peripheral parasitaemia for a longer period of time. It has been demonstrated that in areas where chloroquine resistance has been identified, severe disease (especially severe anaemia) is also frequent, which raises the possibility of a causal effect (Price et al. 2009). Biomarkers of resistance are urgently needed to validate this ecological association.

BM alterations - The mechanisms of BM dysfunction induced by malaria appear to be multiple but are still only partially known. The first observations of BM dysfunction during malaria infection occurred over 60 years ago, when reticulocytopaenia was documented in humans during *P. falciparum* and *P. vivax* infection. Afterwards, it was shown that patients with acute *P. falciparum* infections had low reticulocytaemia (or inappropriate reticulocytosis), which was accompanied with suppression of erythropoiesis (erythroid hypoplasia) (Camacho et al. 1998). Despite having increased cellularity in BM aspirates, there were no significant differences in the total numbers of erythroblasts (Abdalla & Wickramasinghe 1998). These findings provided evidence of a decrease in the erythroid response at the BM level. Children with chronic malaria, low parasitaemias (< 1%) and severe anaemia display erythroid hyperplasia and dyserythropoiesis (Abdalla et al. 1980, Wickramasinghe et al. 1989, Abdalla & Wickramasinghe 1998) accompanied by ineffective erythropoiesis and decreases in circulating reticulocytes.

Inadequate production of reticulocytes suggests insufficient erythropoiesis, which may be the result of either hypoproliferative erythropoiesis or hyperproliferative but ineffective erythropoiesis. Ineffective erythropoiesis is generally associated with intramedullar destruction of erythroid precursors by erythrophagocytosis or dysplastic changes of these precursors, which can be recognised by cytoplasmic vacuolation, abnormal nucleus (bilobed), nuclear budding, formation of interchromatin and intracytoplasmatic bridges and nuclear fragmentation.

It appears that in acute and some chronic anaemia patients, there are two major forms of anaemia: (i) anaemia with erythroid hypoplasia with or without dyserythropoiesis and (ii) anaemia with erythroid hyperplasia and dyserythropoiesis. Acute malaria infection in adults may be accompanied by a reduction in total erythropoietic activity. In these cases, there may be normal BM or reduced cellularity with erythroid hypoplasia. In cases of high parasitaemia, there may even be ineffective erythropoiesis of the residual erythropoietic activity (hypoproliferative erythropoiesis).

Several studies have documented a loss of precursor cells in the BM (Dörmer et al. 1983), reduced use of iron by the erythrocytes and dysplasic changes in the BM (Knuttgen 1987). However, in other studies, no evidence of dyserythropoiesis was found in children with acute *P. falciparum* infection (Das et al. 1999). Therefore, it has been proposed that, in contrast to chronic malaria, dyserythropoiesis plays a minor role in the pathogenesis of anaemia during an acute malaria infection (Das et al. 1999, Jakeman et al. 1999). Some studies suggest that ineffective erythropoiesis and dyserythropoiesis play greater roles (Abdalla et al. 1980, Weatherall et al. 1983) in chronic malaria infection, which may be accompanied by a severe anaemia characterised by an erythroid hyperplasia and dysplasia, some degree of erythrophagocytosis and low levels of reticulocytes. In this case, the dyserythropoiesis is directly related to malaria and is not caused by deficiencies of folate, vitamin B12 or iron (Abdalla et al. 1984).

However, ineffective erythropoiesis during malaria could develop through different mechanisms, such as altered Hb synthesis in vitro and premature death of normoblasts (Srichaikul et al. 1973, 1976).

Susceptible populations - In malaria-endemic regions, the most susceptible populations to suffer severe and complicated disease, including anaemia, are children < five years of age and pregnant women. Although older children and adults still suffer repeated malaria infections, the disease frequency and severity is progressively reduced. This clinical immunity does not develop in areas of low endemicity or seasonal exposure to parasites; therefore, the disease affects all groups in these regions (Miller et al. 1994). Pregnant women, although previously clinically immune, become more susceptible to developing the pathogenic processes that affect both the mother and the foetus, and subsequently the newborn.

Two of the most feared malaria complications that are associated with an increased mortality, especially in children and pregnant women, are cerebral malaria and severe anaemia, with mortality rates of 5.6-16% in children (Marsh et al. 1995) and approximately 6% in pregnant women (Granja et al. 1998, Menendez et al. 2000, Weatherall et al. 2002). As mentioned, immunological factors and mechanisms appear to have great relevance in the anaemia pathogenesis during a malaria infection. Beside the role of the antibodies that are specific to malarial antigens and are exported to erythrocyte membranes, the potential role of the erythrocyte-targeted auto-antibodies and that of complement activation and cytokine imbalances are associated with an increased anaemia severity in children with malaria. Pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-6, are elevated during an acute malarial infection (Kern et al. 1989, Lyke et al. 2004) while anti-inflammatory cytokines, such as IL-10, are substantially decreased (Kurtzhals et al. 1998, 1999, Akanmori et al. 2000). Furthermore, elevated TNF-α levels are associated with an increased anaemia severity in children with malaria (Shaffer et al. 1991) and a low IL-10/TNF-α ratio is associated with an enhanced anaemia severity, suggesting that the relative expression of cytokines in the inflammatory milieu is an important determinant of severe malarial anaemia (Othoro et al. 1999, Perkins et al. 2000). It has also been observed that increased levels of TNF-α are associated with a decrease in erythroid progenitor cells, decreased iron uptake by erythrocytes, erythrophagocytosis of nucleated erythroblasts and dyserythropoiesis (Phillips et al. 1986, Silverman et al. 1987, Clark & Chaudhri 1988, Miller et al. 1989, Taverne et al. 1994). Moreover, recent studies show that hepcidin, a 25-amino-acid protein produced in the liver, is associated with the anaemia of inflammation in humans, where its production is increased 100-fold, resulting in both an impaired iron uptake in the gut and iron sequestration in macrophages (Ganz 2003, Means 2004).

Between 60-80% of Hb is degraded during the intraerythrocytic cycle, releasing haemozoin (Hz) and amino acids, which are used by the parasite to produce proteins. The presence of Hz in the cytoplasm of polymorphonuclear leukocytes and monocytes appears to be associated with the severity of the malarial infection, as it seems that cytoplasmic Hz is more frequently found in the complicated malaria cases than in the uncomplicated cases (Nguyen et al. 1995, Amodu et al. 1998, Lyke et al. 2003, López et al. 2004).

Experimental animal models of malarial anaemia - In-depth haematological studies in humans with malarial anaemia pose a number of ethical and technical challenges that preclude invasive procedures, particularly BM analyses over the course of the infection. The development of experimental animal models is therefore critical for understanding the mechanisms involved in the pathogenesis of severe anaemia. Although some molecular bases of malarial anaemia could be shared by several *Plasmodium* species, experimental evidence suggests that species-specific factors play significant roles. For example, a larger proportion of the non-infected RBCs are removed by erythrophagocytosis in *P. vivax*-infected individuals - it is estimated that ~32 non-infected RBCs are destroyed per every *P. vivax*-infected RBC (Collins et al. 2003), compared to approximately eight non-infected RBCs destroyed per every *P. falciparum*-infected RBC (Jakeman et al. 1999).

Four rodent malaria parasite species (*Plasmodium berghei*, *Plasmodium chabaudi*, *Plasmodium vinckei* and *Plasmodium yoelii*), have been extensively used to study malaria pathogenesis, including anaemia, due to their distinctive erythrocyte invasion profiles, which are similar to those observed with human parasites (Lamb et al. 2006). However, several features, such as anaemia in the presence of hyperparasitaemia and extramedullar erythropoiesis, which are frequently observed in rodents, are rare events in human malarial infections (Silverman et al. 1987, Yap & Stevenson 1992). Anaemia research in rodent models allows immunological studies and manipulations that are more difficult in other animal models, such as primates. Examples include in vivo depletion of macrophages and $CD4^+$ T cells, comparisons of resistant vs. susceptible strains, experiments involving cytokine knockout mice, such as IL-10 (Linke et al. 1996) and macrophage migration inhibitory factor-deficient mice (Stevenson et al. 2001, McDevitt et al. 2006). Non-human primate models appear to be more relevant in LA, due to their abundance in the region and the availability of several primate colonies.

New World monkeys (*Aotus* and *Saimiri*) have been used extensively for vaccine trials and drug testing using *P. falciparum* and *P. vivax*-adapted strains (Collins 1992, Obaldia 2001, Herrera et al. 2002). Malaria semi-immune *Aotus* monkeys immunised with merozoite vaccine candidates or exposed to *P. falciparum* were protected from hyperparasitaemia, but were more likely to develop severe anaemia after a second challenge (Egan et al. 2002, Jones et al. 2002). In this experimental model, low Hb levels were associated with low reticulocyte counts, suggesting that the ineffective erythropoiesis and removal of non-infected erythrocytes are at least part of the aetiological factors involved (Egan et al. 2002). *Aotus* monkeys that self-control parasite patency or that received anti-malaria treatment exhibited a robust reticulocytosis, indicating a direct effect of the parasite on erythroid progenitors. Interestingly, immunisation of *Aotus* monkeys with the *P. falciparum* CIDR1α domain of PfEMP1, a protein involved in sequestration, prevents the development of anaemia after re-infection (Makobongo et al. 2006). *Aotus* also appears to be a good model to study the role of hepcidin homeostasis. Although little is known about the relationship between hepcidin and malarial anaemia, one study developed in Colombia with the *Aotus* model showed that hepcidin levels decreased throughout the experiment in malaria and mock-infected animals (Llanos 2008). Regardless of this finding, it remains an area for further research.

Methodologies have been implemented to study the BM compartment in *Aotus* monkeys experimentally infected with *P. falciparum* and *P. vivax* (Llanos et al. 2006). Interestingly, on-going studies have confirmed that high numbers of normoblasts are present in BM aspirates, suggesting that erythropoiesis is effective (unpublished data) (Llanos 2008). However, the molecular mechanisms involved in severe anaemia in semi-immune *Aotus* monkeys remain unknown (Egan et al. 2002).

Simian malaria parasites have been a critical resource for understanding the biology of *Plasmodium* (Brown & Brown 1965, Coatney 1968) for facilitating the development of anti-malarial drugs (Omar et al. 1973) and for characterising the mechanisms involved in the physiopathology of severe malaria (Davison et al. 1998). Unfortunately, comprehensive investigations on malaria pathogenesis have not been addressed using these experimental models (Galinski & Barnwell 2008). *Plasmodium coatneyi* and *Plasmodium fragile* share with *P. falciparum* the ultrastructural features that are involved in parasite sequestration and rosetting. Therefore, these parasite species mimic clinical complications of cerebral malaria associated with *P. falciparum* infections in humans (Kawai et al. 1993, Fujioka et al. 1994). *P. coatneyi* infection in macaques has also been associated with placental malaria, thrombocytopaenia, anaemia and disseminated intravascular coagulation (Kawai et al. 1993, Sein et al. 1993, Nakano et al. 1996, Smith et al. 1996, Davison et al. 1998, Collins et al. 2001, Moreno et al. 2007). *Plasmodium cynomolgi* is phylogenetically related to *P. vivax* and shares its capacity to produce hypnozoites (Krotoski et al. 1982). The patterns of relapses described in *P. vivax* are also present in rhesus macaques exposed to *P. cynomolgi* sporozoites, making this model ideal for studying the mechanisms of chronic anaemia (Schmidt 1986). Comparative experiments using *P. coatneyi* and *P. cynomolgi* in rhesus macaques are required to define whether the molecular mechanisms underlying the pathogenesis of malarial anaemia are shared between these two species. Unfortunately, such studies are restricted to primate centres outside LA.

Conclusions and perspectives - Beside the acknowledged importance of anaemia as a cause of morbidity and mortality in Africa and other endemic regions, little is known about its prevalence and burden in the malaria-endemic regions of LA. The few studies reported from this region appear to indicate that the incidence of severe anaemia is significantly lower than that reported from Africa or Asia. However, considering that specific haematological changes associated with malaria infection may vary with the level of malaria endemicity (Idro et al. 2006), nutritional status (Friedman et al. 2005), demographic factors (Barcus et al. 2007), malaria immunity (Langhorne et al. 2008) and the parasite species, it is essential to study and characterise the epidemiology and mechanisms involved in malarial anaemia in endemic regions of LA. The National Institutes of Health-National Institute of Allergy and Infectious Diseases is currently funding the establishment of the Centro Latino Americano de Investigación en Malaria (CLAIM) as a Centre of Excellence for Malaria Research (ICEMR). The Centre aims, as a priority, to determine the prevalence and severity of haematological manifestations attributable to malaria infection and their association with concomitant immune status, including nutritional factors and helminth coinfection. Although studies would initially cover only Colombia, Panama, Peru and Guatemala, further expansion of malaria research, including anaemia, would be extended to other countries of LA, to the Caribbean region and Brazil. CLAIM already interacts with the Brazilian Malaria Network, which aims to describe the determinants of *P. vivax*-related severe anaemia in hospitalised patients from a tertiary care institution, specifically focussing on the impacts of host genetics (G6PD deficiency and Fy genotypes) and chloroquine resistance.

These multi-country regional projects will allow the comparison of pathologies in settings with different malaria transmission intensities in communities with great ethnic, occupational and immune diversities. Moreover, the availability of the *Aotus* monkey animal model in several countries of the LA region represents a valuable resource to address important questions regarding malaria pathogenesis that cannot be studied in the human populations. Indeed, the accessibility to BM aspirates of non-human primates vaccinated with human malaria vaccine candidates opens an interesting new area of research to complement the analyses of the influences of specific anti-malarial immune responses in the generation of anaemia.

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