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Investigating the Pathogenesis of Severe Malaria: A Multidisciplinary and Cross-Geographical Approach

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Abstract. More than a century after the discovery of Plasmodium spp. parasites, the pathogenesis of severe malaria is still not well understood. The majority of malaria cases are caused by Plasmodium falciparum and Plasmodium vivax, which differ in virulence, red blood cell tropism, cytoadhesion of infected erythrocytes, and dormant liver hypnozoite stages. Cerebral malaria coma is one of the most severe manifestations of P. falciparum infection. Insights into its complex pathophysiology are emerging through a combination of autopsy, neuroimaging, parasite binding, and endothelial characterizations. Nevertheless, important questions remain regarding why some patients develop life-threatening conditions while the majority of P. falciparum-infected individuals do not, and why clinical presentations differ between children and adults. For P. vivax, there is renewed recognition of severe malaria, but an understanding of the factors influencing disease severity is limited and remains an important research topic. Shedding light on the underlying disease mechanisms will be necessary to implement effective diagnostic tools for identifying and classifying severe malaria syndromes and developing new therapeutic approaches for severe disease. This review highlights progress and outstanding questions in severe malaria pathophysiology and summarizes key areas of pathogenesis research within the International Centers for Excellence for Malaria Research program.

INTRODUCTION

Malaria is a major global infectious disease caused by parasitic protozoans of the genus Plasmodium. Of the five Plasmodium species that infect humans, Plasmodium falciparum and Plasmodium vivax cause the majority of cases, and P. falciparum is the most virulent and responsible for the majority of deaths. Despite recent reductions in the overall malaria case incidence, malaria remains a leading cause of morbidity and mortality in the developing world. In 2012, there were an estimated 207 million cases of malaria and over 600,000 deaths. The majority of malaria deaths (90%) occur in children in Africa, where falciparum malaria accounts for as many as one in six childhood deaths and is the biggest killer of African children between the ages of 1 and 4 years. Outside Africa, there are a variety of transmission settings where P. falciparum, P. vivax, or both are present. In lower transmission settings in South America, India, and southeast Asia, adult populations are at higher risk for severe malaria.

Malaria is a complex disease, and the spectrum of disease manifestations differs between children and adults. Symptoms can range from none, in individuals with asymptomatic parasitemia, to mild, in patients with undifferentiated fever, to severe, in patients with life-threatening anemia, metabolic acidosis, cerebral malaria (CM), and multiorgan system involvement. Only a small minority of infections, less than 1–2%, leads to severe malaria. Because pathogenetic mechanisms are complex and poorly understood, current treatment primarily relies on antimalarial drugs and supportive care. Here we focus on recent advancements in understanding the molecular pathogenesis of CM and the variable presentations between children and adults.

Several pathogenetic mechanisms have been proposed for CM including mechanical microvascular obstruction by sequestered infected erythrocytes (IEs), activation of immune cells and release of pro-inflammatory cytokines, endothelial dysfunction, dysregulation of coagulation pathways, blood-brain barrier (BBB) permeability, and brain swelling. Furthermore, autopsy studies have subdivided pediatric cases into two different groups based on histopathological patterns. The CM1 group has sequestration only, while CM2 group has sequestration plus vascular pathology (ring hemorrhages, fibrin-platelet thrombi, and monocytes). Ring hemorrhages and cerebral thrombosis are also described in a proportion of adult cases, but whether there is an equivalent CM1/CM2 dichotomy in adults is less clear. Recent findings implicate a specific subset of parasites that adhere to endothelial protein C receptor (EPCR) in severe childhood malaria. As EPCR plays a key role in regulating coagulation and endothelial cytotoxic protective and barrier properties, this raises the possibility there may be linkages between IE cytoadhesion and microvascular complications in CM. However, the precise molecular processes that account for the pathophysiological differences between CM1, CM2, and adult CM are poorly understood. Elucidating key pathogenetic mechanisms in CM and severe malaria may suggest new treatment options to improve patient outcomes.

Unlike P. falciparum, P. vivax rarely causes severe disease in healthy travelers and is a less deadly parasite. Factors that may contribute to the lower virulence are that P. vivax only infects reticulocytes and the absence of the cytoadhesion protein family responsible for sequestration in P. falciparum infections. These differences limit the blood-stage parasite
burden and spectrum of cytoadhesion-based complications. Another distinction is that *P. vivax* has dormant liver hypnozoite stages, which can reactivate and lead to blood-stage relapses. Relapses contribute to vivax morbidity, but the mechanisms leading to severe vivax disease remain to be elucidated. This review covers recent findings on the pathological pathways in pediatric and adult CM, as well as severe malaria cases in low-transmission settings in South America and India because of *P. vivax* infections, highlighting progress and outstanding questions in severe malaria pathophysiology in the context of the pathogenesis research activities within the International Centers of Excellence for Malaria Research (ICEMR) program.

### SEVERE FALCIPARUM MALARIA IN CHILDREN AND ADULTS

The clinical presentations of severe falciparum malaria differ between children and adults. In particular, adults have a higher mortality rate and more multiorgan system involvement than children. A recent large multicenter comparison of artesunate versus quinine in the treatment of severe malaria in adults and children reported adult and pediatric mortality rates of 18.5% and 9.7%, respectively. The major organs affected in adult severe malaria are brain (CM), lungs (acute respiratory distress syndrome [ARDS]), liver (jaundice), and kidneys (acute renal failure) (Figure 1). Although the overall mortality of adult CM is about 15–20%, the risk of death depends on associated vital organ dysfunction and is increased 3-fold in the presence of acidosis and renal failure. In children, the three major disease complications are CM, severe anemia, and acidosis, but ARDS and renal failure are rare (Figure 1). Although the three disease syndromes can occur singly or as overlapping syndromes, severe malaria anemia commonly affects younger children, and CM and metabolic acidosis are more commonly found in slightly older children. CM and metabolic acidosis are each associated with high mortality rates in children (12% and 14%, respectively), and the presence of both increases the risk of death. The severity of disease may be exacerbated by both higher parasite burdens and the tissue-specific patterns of IE sequestration. Thus, there is significant research effort to understand factors that contribute to parasite blood-stage multiplication potential and cerebral homing of IEs.

Although severe malaria predominantly affects African children in high-transmission settings and adults in lower transmission settings, the same differences in disease complications and mortality were observed between adults and children in Rourkela, India. Collectively, these findings suggest there are different pathophysiological disease mechanisms in children and adults, but the molecular mechanisms underlying these differences are not fully understood. The different clinical symptoms could result from differences in host malaria immune status, since malaria transmission intensity is much higher in Africa than other regions where adults experience severe malaria. Alternatively, they could potentially relate to different parasite binding types, human polymorphisms, or age-dependent changes in the vascular system response to falciparum-induced inflammation.

***P. falciparum*** and cerebral malaria: a histopathological and ultrastructural perspective. A major pathological feature of *P. falciparum* malaria is that the mature stage IEs sequester from blood circulation by binding to the endothelial lining of blood vessels. Histopathological studies of fatal malaria had largely focused on adults in hypoendemic areas and soldiers in military theatres until recently, when a group based in Malawi undertook a case-control study, comparing the gross and microscopic pathology in children dying with clinically defined CM to the pathology in malaria-infected children with non-malarial causes of death.

![Figure 1](image-url). The major clinical complications associated with adult and pediatric severe malaria. Clinically severe malaria is a multisystem disorder that can affect different organs and differs in presentation between children and adults. The major clinical complications in children are cerebral malaria, severe malaria anemia, and metabolic acidosis. In adults, cerebral malaria is frequently accompanied by multiorgan system complications, including metabolic acidosis, acute kidney failure, jaundice, and acute respiratory distress (ARDS).
FIGURE 2. Schematic representation of the pathological differences between cerebral malaria CM1 and CM2. Autopsy studies in children have divided CM cases into two groups based on histological features.16 CM1 cases have infected erythrocyte sequestration in the cerebral microvasculature and no associated vascular pathology. CM2 cases are defined by cerebral sequestration plus intra- and perivascular pathology, including ring hemorrhages, fibrin-platelet thrombi, and intravascular monocytes. In the CM2 group, infected erythrocyte (IE) sequestration is frequently associated with fibrin-platelet thrombi in both capillaries and postcapillary venules. Insets provide examples of pathological features observed in CM2 cases described in Dorovini-Zis and others.15 Inset (A) shows a small branching capillary in which the upstream region is filled with sequestered IEs and one of the branches is occluded by a thrombus. This event is associated with a ring hemorrhage in which the microvessel is partially denuded of endothelial cells and is surrounded by a zone of necrosis and a ring of uninfected red blood cells in the white matter. Inset (B) shows a small vessel packed with sequestered IEs and surrounded by extravasated fibrinogen indicating increased permeability of the blood–brain barrier. Inset (C) shows a microvessel filled with monocytes containing phagocytosed hemozoin pigment. Intravascular pigmented monocytes are found adherent to the microvessel wall, but do not transverse across the blood–brain barrier. The molecular mechanisms driving the CM1 and CM2 pathophysiology are incompletely understood. Intercellular adhesion molecule 1 (ICAM-1) and endothelial protein C receptor (EPCR) are candidate brain endothelial receptors,18,31 but it is not known if the same parasite adhesion types are associated with CM1, CM2, and adult CM (not pictured). Recent studies reported that binding of IE to EPCR was associated with the development of severe malaria18 and that decreased EPCR staining on endothelial cells and increased fibrin deposition occurred at the site of IE adhesion in cerebral microvessels during fatal pediatric CM.20 This association suggests there may be causal links between cytoadhesion and microvascular pathophysiology. However, fibrin deposition is not found in CM1 and is less prominent in adult CM, highlighting gaps in our understanding of CM pathophysiology.
A striking finding was that ~25% of children who met the standard clinical case definition of CM during life (P. falciparum parasitemia, Blantyre Coma Score ≤ 2, no other obvious cause of coma) had no evidence of the pathological hallmark of CM, the cerebral sequestration of IE. All of these children had a non-malarial cause of death identified at autopsy. This finding highlights the difficulty of assigning the true cause of coma in children in geographic regions with high rates of apparently asymptomatic malaria infections and emphasizes the need for better CM diagnostics to guide treatment decisions. Among those who did have evidence of cerebral sequestration of IEs (“true CM”), two distinct pathological patterns were noted, CM1 and CM2 (Figure 2). Approximately one-third of the true CM patients had histologic evidence of sequestration only (CM1); the other two-thirds had evidence of intra- and perivascular pathology (fibrin thrombi, “ring” hemorrhages involving uninfected red cells, and intravascular accumulation of white blood cells). Although fibrin and intravascular monocytes are less prominent features in adult CM autopsy studies, ring hemorrhages are found in approximately 30–50% of adult cases (Table 1). Furthermore, in the classic histopathological study of Spitz on World War II U.S. military soldiers who died of acute falciparum malaria, thromboses and ring hemorrhages were commonly observed together, leading Spitz to speculate that ring hemorrhages were caused by thrombosis. Thus, although the CM2 pattern is not commonly described in adult cases (Table 1), it is possible that thrombotic lesions may play a role in some adult CM cases. Overall, the variability of pathological findings indicates that CM is not a histologically uniform syndrome and suggests there may be different pathophysiological mechanisms in CM1 and CM2, and potentially between children and adults.

From histopathological studies, activation of endothelial cells and breakdown of the BBB are evident. Parasites can stimulate intracellular signaling events in endothelial cells through direct adhesion to receptors such as CD36 or intercellular adhesion molecule 1 (ICAM-1) or release of soluble factors. This affects cerebral endothelial cell structure and function, which in turn may mediate changes in the BBB function in CM, but the parenchyma of the brain is rarely involved. Nevertheless, in both children and adults, neuropathology has been associated with ring hemorrhages and increased brain volume in response to fever, anemia, and seizures. Understanding the relative contribution of these potential mechanisms to brain swelling may suggest treatment strategies.

**Imaging approaches to investigate disease pathogenic mechanisms.** As illuminating as autopsy studies have been, they are inherently limited by the necessity of only studying patients who have died, and by only studying them at one point in the process, the time of death. Imaging modalities that could be used during life, which could be repeated to capture a process, would be helpful in studies of malaria pathogenesis.

**Orthogonal polarization spectral imaging.** Clear images of microcirculatory blood flow in mucosal surfaces (sublingual, rectal) obtained via orthogonal polarization spectral imaging allow for “real-time” visualization of microvascular obstruction related to sequestered IEs. This approach has revealed significant disturbances in microvascular blood flow that were variable between adjacent microvessels and increased in proportion to disease severity. These abnormalities disappeared after patient recovery, highlighting an important role for reduced microcirculatory blood flow in severe malaria.

### Table 1

| Characteristic | Pediatric | Adult |
|---------------|-----------|-------|
| **Intravascular pathology** | | |
| Infected erythrocytes in microvessels of gray and white matter | Yes15,16,33 | Yes15,16,35 |
| RHs | | |
| Increased BBB permeability to plasma factors associated with RH | No15,16,33 | Yes15,16,35 |
| Increased BBB permeability to plasma factors associated with sequestered IEs | NA15 | Yes15 |
| Microvascular thrombosis associated with necrosis of endothelial lining and perivascular hemorrhages | Yes15 | Yes15 |
| Fibrin thrombi | No15,16,33 | Yes15,16,35 |
| Pigment-containing monocytes | No15,16,33 | Yes15,16,35 |
| **Perivascular pathology** | | |
| Reactive astrocytes | No15 | Extremely rare33 |
| Durck’s granuloma (reactive microglia, astrocytes, and lymphocytes) | Yes15 | Yes15 |
| Axonal injury associated with RH or vascular thrombosis | NA | Yes15 |
| Myelin loss associated with RH | NA | Yes15 |
| Diffuse myelin damage associated with sequestered IEs | Limited15 | Yes15 |
| Axonal injury associated with sequestered IEs | Yes15 | Yes15 |

BBB = blood–brain barrier; CM = cerebral malaria; IEs = infected erythrocytes; NA = not applicable; RHs = ring hemorrhages; ? = not reported.
However, because the expression of surface receptors varies between organs, what is seen in accessible areas may not reflect what is happening in the brain.

**Ocular funduscopy.** The eye and the brain have similar embryologic origins, and the microvasculatures of the two organ systems share important features. In addition, the optic fundus can be readily observed and studied during life in patients with severe malaria. In conjunction with the Malawi autopsy study, ophthalmologists described a unique malarial retinopathy consisting of white-centered hemorrhages, vessel color changes, and peri- and extramacular whitening. At least one of these findings was present in all cases of true CM (i.e., patients with evidence of cerebral sequestration of IEIs at autopsy), and although recognition of the retinopathy requires a trained observer with relatively expensive equipment (direct and indirect ophthalmoscopes), it has created the opportunity, exploited by the ICEMR program, to use a more specific clinical case definition of CM. Retinal hemorrhages correlate, numerically, with the ring hemorrhages seen in fatal cases of pediatric CM. Vessel color changes reflect the presence of sequestered, parasitized, and de-hemoglobinized red cells, while the whitening represents areas of impaired perfusion.

Ophthalmologic observations on adults with severe malaria are relatively sparse, but they are consistent with the reported pediatric findings in that approximately one-third of adults meeting the standard clinical case definition of CM have no evidence of malarial retinopathy. Retinal hemorrhages are commonly observed, but vessel color changes, seen in ~32% of children with CM, are only rarely seen in adults. The severity of malaria retinopathy is strongly associated with malaria mortality in both adults and children.

**Neuroimaging.** Neuroimaging neatly addresses the two primary deficiencies of the autopsy approach: survivors can be imaged and serial studies can be carried out throughout the course of the acute illness. However, the worldwide distribution of sophisticated radiological capacity does not include malaria-endemic areas, so most descriptions of neuroimaging findings in malaria patients have been single case reports from patients hospitalized in more developed countries.

Computed tomography scan technology is relatively uncomplicated and affordable, and the process itself is quick. This approach was the first used to illuminate disease pathogenesis in malaria patients, and highlighted the importance of increased brain volume. Most of these studies were done before the importance of malarial retinopathy was recognized, though the possibility of classification errors complicates interpretation of these findings.

Individual case reports of magnetic resonance imaging (MRI) findings in patients with CM (as reviewed in reference 59) have described a variety of findings, all of which have been corroborated by larger, systematic studies in Thai adults and Malawian children. Increased brain volume is strongly associated with a fatal outcome in children. Cortical involvement (often restricted to specific lobes), and changes in the periventricular white matter, the corpus callosum, and the thalami are common in children with retinopathy-positive CM.

Both of the larger studies were limited by the strength of the magnet (0.2 tesla [T] in Thailand, 0.35 T in Malawi). A collaborative effort between two independent ICEMR projects (Table 2) will address this problem while simultaneously addressing disparities between the clinical manifestations of severe disease in adults and children. The joint effort is currently being carried out between two hospitals, one located in Malawi and one in India, both of which have MRI facilities. Adults and pediatric patients with severe malaria in India (retinopathy-positive CM, with and without other organ system involvement) will undergo MRI on a 1.5 T machine, and their findings will be compared with those in retinopathy-positive CM pediatric patients in Malawi. The clinical protocol has been standardized between the two field sites, and four MRI sequences will be common to both projects, as their magnet strengths are different. To ensure the accurate interpretation and comparison of MRI findings in

| ICEMR       | Research activities related to pathogenesis of malaria                                                                 |
|-------------|------------------------------------------------------------------------------------------------------------------------|
| Southeast Asia | Collecting descriptive data on malaria patients attending local hospitals at sentinel sites, including data on disease manifestation |
| South Asia (India) | Investigating the molecular and cellular basis of severe Plasmodium falciparum and severe Plasmodium vivax infections in hospital patients recruited at multiple locations in India |
| India        | Assessing the role of interindividual variations in endothelial responsiveness to TNF in the development of cerebral malaria |
| East Africa (Uganda) | Investigating the role of prompt and effective therapy for minimizing the risk of severe malaria in cohorts of children living in high-endemic settings |
| Southern African (Zambia/Zimbabwe) | Collecting descriptive data on clinical diagnoses for persons seeking care at rural health centers |
| Southern African (Malawi) | Collecting hospital-based data on febrile illnesses (malarial and non-malarial) |
| Amazonia     | Collaborating with south Asia ICEMR (India) on MRI findings in adults and child development                                    |
| Latin America (outside Amazonia) | Observational, hospital-based observations of severe F. vivax malaria; 16S rRNA molecular and blood culture analysis of severe malaria cases |
| Southwest Pacific | Clinical profile of malaria in different epidemiological settings in Colombia, and their association with parasite and host immunological status |
|              | Determine the effects of immune status, nutritional factors, and helminth coinfection on complicated malaria cases in Colombia |
|              | Collecting data on childhood severe malaria admissions to major hospital serving Madang Province |

ICEMR = International Centers of Excellence for Malaria Research; MRI = magnetic resonance imaging; TNF = tumor necrosis factor.
these sequences, all the images will be scored and shared between the radiologists, via a web-based platform to enhance standardization. This study will permit, for the first time, the clinical characterization of pediatric and adult CM by neuroimaging and a precise comparison of carefully clinically defined cohorts of CM patients of different ages and from different continents. Such extensive MRI techniques have never been applied systematically to patients with acute malaria and represent a promising approach to investigating the relationship between brain swelling and the onset of CM.

Vascular activation/dysfunction and coagulation pathways in severe malaria. The brain swelling observed during CM both in Indian adults and Malawian children might be the consequence of disruption of the BBB associated with the pathogenic processes of CM, resulting in vasogenic edema. This hypothesis is currently being investigated as part of a Malawi–India inter-ICEMR initiative (Table 2) and is in line with the emergence of the endothelial cell as a central player in the pathophysiology of the neurologic syndrome. Although its involvement as a substrate for IE sequestration in the brain was identified very early on, results published over the past decade have highlighted the complex role of cerebral endothelial cells in the development of CM. One of the main goals of the India ICEMR is to investigate parameters inherent in the host endothelium that may result in an increased susceptibility to severe malaria in Indian adults infected with P. falciparum, an axis of research that is divided into three main approaches.

Variations and heritability of the host endothelial responsiveness to tumor necrosis factor alpha. A central component of CM pathophysiology is the activation of microvascular endothelial cells, resulting from both the cytoadherence of IE to their surface and the pro-inflammatory effects of local and systemically released cytokines. The consequences of this endothelial inflammation are numerous and include the upregulation of endothelial receptors for enhancing IE and platelet sequestration; the further release of cytokines and chemokines and the trigger of a tumor necrosis factor (TNF)-dependent pro-apoptotic pathway (as reviewed in reference 68). We hypothesized that variation in the responsiveness of endothelial cells to TNF in different individuals could be a factor affecting the severity of disease in patients infected with P. falciparum (Figure 3A). Indeed, endothelial cells derived from CM and uncomplicated (UM) children patients from Malawi were shown to display significantly different ex vivo responsiveness to TNF. When compared with UM, CM-derived endothelial cells express significantly higher levels of parasite and platelet receptors, produce more endothelial microparticles, release more pro-inflammatory cytokines, and are more prone to undergo apoptosis on stimulation with TNF. On the basis of these results, it was hypothesized that genetic variations within promoter, intron, or exon sequences of endothelial inflammatory genes may, in part, determine the clinical course in CM patients, as has been described in sepsis.

Using a large number of freshly isolated microvascular endothelial cells from adult patients admitted to Ispat General Hospital in Rourkela, we are planning to compare the response to TNF between CM and UM patients from India and investigate the different factors, extrinsic or intrinsic, leading to the

![Figure 3](https://example.com/image3.png)

**Figure 3.** Proposed influence of the host endothelial responsiveness to tumor necrosis factor (TNF) on the severity of malaria infection. Low endothelial TNF responders are less prone to upregulate receptors involved in the sequestration of infected erythrocyte (IE) and platelets than high responders. This leads to a minimal adhesion of IE and host cells and a lower pro-apoptotic signal for the endothelial cells, which might account for the absence of pathology. High responders, however, are over-activated in the presence of TNF, leading to high adhesion of IE and a strong pro-apoptotic signal, possibly resulting in the breakdown of the blood–brain barrier and, ultimately, to vasogenic edema (A). Potential clinical benefits offered by angiopoietin (Ang)-1 as a quiescence agent for high TNF-responding endothelial cells during cerebral malaria (CM) (B).
interindividual differential activation of the endothelium between
the two patient categories. The comparative analysis of the
variation in transcripts between the two high and low TNF-
responding groups of endothelial cells will give us insights into
the pathways involved in the acute activation observed in CM
patients, and will be compared with the results obtained in
Malawian children. This project is carried out not only with a
view to understanding the molecular basis of disease but also
to identifying patients at risk by analyzing specific single nucle-
otide polymorphisms associated with high and low responders.
It will also assess if there are age-specific differences in endo-
thelial responsiveness. Understanding the mechanistic basis
of vascular dysfunction in severe malaria may suggest new
treatment options.

Reversibility of the systematic endothelial activation in CM
patients. The presence of TNF as a trigger of inflammation in
malaria led to the assessment of a TNF-blocking approach in
CM. Although in vitro treatments produced favorable results,
anti-TNF clinical trials failed to reduce mortality in these patients.28,29 The use of a targeted compound block-
ing the downstream endothelial activation signaling cascade
resulted in a reduction of endothelial inflammation in vitro.30
However, this effect was only observed when the compound
was administered simultaneously with the cytokine, which
would be effectively impossible in vivo. Since most of the
patients admitted to the ward have already high levels of
TNF, an acute therapy might work by dampening the existing
endothelial inflammatory response in CM patients. Angio-
poietin (Ang)-1 has recently become a topic of increasing inter-
est in endothelial cell quiescence and survival,31 and plasma
Ang-2/Ang-1 ratio has been shown not only to be crucial for the
endothelial activation but also to discriminate UM and CM.
Indeed, high levels of Ang-2 are associated with mortality in
patients with CM, whereas high levels of Ang-1 are associated
with UM (as reviewed in reference 75). Since the use of Ang-1
offers clinical benefits as a quiescence agent for endothelial cells
in an elegant model of sepsis,32 it is conceivable that restoring
the Ang-2/Ang-1 balance in favor of Ang-1 would block and
potentially reverse the ongoing inflammatory processes in CM
patients at the time of admission (Figure 3B).

The potential clinical benefits of Ang-1 are currently being
evaluated as part of the ongoing project on primary endothe-
rial cells at Ispat General Hospital. Using the endothelial cell
banks isolated from CM patients, the effects of Ang-1 on
TNF-stimulated endothelium will be measured, with a view to
develop new adjunct therapies and improve disease outcome
in CM.

The role of EPCR in adult CM. Recent studies reported
that binding of IE to EPCR was associated with the develop-
ment of severe malaria33 and that decreased EPCR staining
on endothelial cells and increased fibrin deposition occurred
at the site of IE adhesion in cerebral microvessels during fatal
pediatric CM.20 A causal relationship between cytoadhesion
and coagulopathy was therefore suggested for the first time,
and the pivotal role of EPCR in the organ specificity of the
syndrome was proposed.77,78 One of the major aims of the
India ICEMRs is to further investigate the role of EPCR in
the development of CM in Indian adults, as fibrin deposition is
a far less prominent pathological feature in southeast Asian
adults than African children who succumb to CM (Table 1).29,30
Since endothelial cell cultured from subcutaneous fat resemble
cerebral vascular endothelial cell and represent a useful ex vivo
model for examining brain endothelial alteration in the con-
text of CM,69 this approach is being carried out by performing
phenotypical analyses of primary subcutaneous endothelial
cells isolated from patients admitted at Ispat General Hospital,
followed by targeted gene expression profiling (RNA and
miRNA) and genetic analyses of genes selected for their rele-
vance in the protein C pathway. The results will 1) contribute
to a better understanding of the pathogenic mechanisms for
childhood and adult disease, 2) assess the overall importance
of EPCR in mediating the cytoprotective effects of activated
protein C (APC) in the brain, and 3) evaluate new avenues of
translational research. A collaborative protocol is currently
being developed between the India ICEMR and the clinical
team to extend these analyses to endothelial cells isolated
from postmortem brain biopsies samples of fatal CM.

Parasite biomass and severe malaria. It is difficult to mea-
sure the total parasite biomass of *P. falciparum* (circulating
and sequestered) from blood sampling because of the “hidden
sequestered” component. To overcome this challenge, a new
approach has been introduced by Dondorp and others34 in which
the plasma concentration of a soluble parasite molecule serves
as a surrogate for the total parasite biomass. *Plasmodium falci-
parum* histidine-rich protein-2 (HRP-2) is a water-soluble
protein produced throughout the parasite life cycle and released
largely (but not exclusively) at the time of schizont rupture.79,80
It has a long half-life and persists in the plasma for up to 21 days,
even after successful treatment81; HRP-2 detection (present/
absent) is the basis of many rapid diagnostic tests, but quantita-
tive measures of HRP-2 can discriminate between retinopathy-
positive and retinopathy-negative CM.82 can predict which
children with uncomplicated malaria are more likely to deteri-
orate,83 and can distinguish between patients with complicated
malaria, mild malaria, asymptomatic parasitemia, and non-
malarial fevers.84 A model, based on plasma half-life of HRP-2
in vivo, production rates of HRP-2 in vitro, and parasite multi-
plication rates suggests that HRP-2 concentrations reflect total
body parasite burden (sequestered and circulating parasites).70
In general, the associations between HRP-2 concentration and
disease severity support the hypothesis that parasite biomass
is a major determinant of malaria pathogenesis. However, a
recent longitudinal birth cohort study of Tanzanian children
followed from birth to 2–4 years of age indicated that while
parasite burden was higher on average in severe malaria epi-
isodes, high parasite burden was insufficient to cause severe
disease.85 Thus, high parasite burden appears to be an impor-
tant determinant in severe malaria, but other factors may act in
congest to precipitate severe malaria episodes.

Parasite invasion pathways and malaria severity. Higher
parasite biomass is a risk factor for severe malaria and may be
driving increased systemic inflammation, endothelial activation
markers, and metabolic acidosis by microvascular obstruction.
The circumstances leading to higher parasite burdens in severe
malaria are likely multifactorial and incompletely understood.
However, potential parasite factors are red blood cell (RBC)
invasion efficiency and the cytoadhesion efficiency of infected
RBCs. Mathematical modeling approaches suggest that inva-
sion efficiency can be a significant driver of peak parasite den-
sity during an infection and concomitant pathogenesis.86 Rodent
malaria parasites can shift from a nonlethal to a lethal form
following a change in preference from reticulocytes to older
normocytes resulting in huge increases in parasite biomass and
pathology.87 In humans, there is evidence that the efficiency
of the invasion process can be a virulence determinant in *P. falciparum* parasites. Clearly, this can be influenced by genetic polymorphisms within both the host and the parasite, as well as acquired immunity. In addition, the ability of parasites to invade RBCs using alternative receptors, known as invasion pathways, can facilitate immune evasion and persistence of malaria infections and ultimately contribute to malaria pathogenesis. Anemia may result from chronic low-burden infections.

Invasion potential has been measured in two ways: by parasite multiplication rate and by selectivity of RBCs. Both have been shown to be strongly associated with the severity of *P. falciparum* malaria in one population in southeast Asia, suggesting the existence of parasite molecular factors that mediate pathogenesis through increased proliferation. However, a similar study was carried out with parasite isolates from Africa and no association was found between invasion efficiency, selectivity, and disease severity. It is not clear whether this is due to regional differences in parasites or in host factors, such as the level of acquired antimalarial immunity.

Previous work carried out in several varied geographical areas have shown that natural *P. falciparum* isolates are capable of using multiple ligand–receptor invasion pathways, and exhibit variation in pathway usage, suggesting mechanisms by which invasion efficiency could be altered via parasite-based mechanisms. These studies have shown that both sialic acid–dependent and sialic acid–independent invasion pathways are commonly used by parasites collected directly from infected humans, and a few isolates have been shown to be able to switch between the use of sialic acid–dependent and sialic acid–independent pathways. Switching of one isolate was associated with reduced invasion efficiency.

With the genome sequenced, *Plasmodium* parasites have been found to possess a diverse number of ligands for invasion. Two superfamilies of invasion ligands, the reticulocyte-binding-protein-like (RBL) and the erythrocyte-binding-protein-like (EBL) have been identified. Much data from studies with *P. falciparum* suggests that each parasite ligand has a single cognate receptor, defining alternative invasion pathways and that there is a hierarchy of different ligand–receptor interactions. Further, variation can exist at the level of sequence and expression changes for these invasion ligands, suggesting a molecular basis for switching between the use of different invasion pathways, either for immune evasion, to change the parasite multiplication rate, and/or RBC selectivity. To better understand the molecular mechanisms driving higher parasite burdens in severe malaria, an ICMR group in India is addressing the interplay between parasite invasion efficiency and IE cytoadhesion phenotypes in disease severity.

**Parasite adhesion and severe malaria.** As described above, cytoadhesion of IEs is a major virulence determinant for CM complications. Furthermore, high parasite burdens and the massive sequestration of IEs in different tissue beds and resulting microvascular obstruction may lead to metabolic acidosis. The majority of falciparum infections are not severe, which suggests that the parasite is relatively well adapted to sequester in microvessels without killing the host. Cytoadhesion of IEs is predominantly mediated through the *var* gene/ *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family of adhesion proteins. PfEMP1 proteins are anchored at parasite-induced, knob-like protrusions on the erythrocyte membrane, exposing them to host antibodies. Clonal antigenic variation of *var* genes enables *P. falciparum* to evade anti-body destruction and to bind to different host receptors. Each parasite encodes approximately 60 different *var* copies with limited overlap of *var* gene repertoires between parasite haplotypes. The vast intra- and interstrain diversity in PfEMP1 repertoires enables parasites to establish chronic infections and repeatedly infect hosts with different parasite genotypes. A fundamental question for pathogenesis is whether specific PfEMP1 and host-receptor interactions have a causal role in severe malaria.

Despite extensive sequence diversity, the majority of *var* genes can be classified into three main subfamilies (A, B, and C) on the basis of upstream gene sequence and chromosomal location. Interstrain sequence comparisons have also identified three unusual strain-transcendent *var* genes (*var1csa*, *var2csa*, type 3 *var*). Each PfEMP1 protein encodes multiple adhesion domains called Duffy binding-like (DBL) and cysteine-rich interdomain region domains. PfEMP1 adhesion domains are classified into different types (α, β, γ, δ, etc.) and subtypes based on sequence similarity. Using adhesion domain classification, interstrain sequence comparisons have revealed a small number of tandem domain arrangements of 2–4 domains, called domain cassettes (DC), which are unusually conserved between parasite genotypes.

The prototypical example of a specific PfEMP1 and disease is malaria in pregnancy. In this case, the strain-transcendent VAR2CSA mediates placental binding. It has been more challenging to determine if a specific PfEMP1 subset is associated with CM because of the difficulty of studying the brain. Analysis of *var* gene expression in patients has suggested that most infections contain a heterogeneous population of parasites expressing a mixture of A, B, or C *var* genes. In hosts with limited malaria immunity and severe pediatric malaria, the ratio of PfEMP1 variants appears to be skewed toward higher group A expression. These findings suggest that group A PfEMP1 encode adhesion traits that facilitate parasite multiplication in malaria naive hosts and may include binding properties that predispose to severe malaria. As individuals acquire anti-PfEMP1 antibodies through repeated infections, the proportion of group B and C variants appear to increase. However, even in pregnant African women who have acquired considerable antimalarial immunity, there was high *var2csa* expression from parasites recovered from placenta, but mixed *var2csa* and A, B, C *var* expression from parasites circulating in the blood. Thus, the parasite strategy of having a heterogeneous population appears to persist even after individuals have acquired substantial antimalarial immunity.

More recently, it was shown that parasites expressing PfEMP1 proteins encoding DC8 or DC13 are strongly selected on human brain microvascular endothelial cells in vitro and are highly expressed in children with severe malaria or CM. The DC8 is found in an unusual chimeric gene between groups B and A and the DC13 is restricted to group A variants. Both DC8 and DC13 proteins, as well as a subset of other group A variants, were found to encode a novel binding property for EPCR, the receptor for APC. As the APC–EPCR pathway plays a key role in regulating blood coagulation and endothelial barrier properties, this has raised the possibility that there may be a linkage between IE binding and CM pathogenesis. However, given the different clinical presentation and autopsy findings in children and adults (Table 1), an important question is whether different PfEMP1 variants are associated with CM1, CM2, and adult CM.
As discussed above, one possibility is that host polymorphisms or age-specific differences in endothelial responses may contribute to pathophysiological differences. Alternatively, different parasite binding variants may be associated with CM1, CM2, and adult CM. For instance, ICAM1 has also been proposed to be a cerebral sequestration receptor.31 Therefore, one possibility is that ICAM1, EPCR binding variants play a more predominant role in CM1 where fibrin-platelet clots and ring hemorrhages are absent, whereas EPCR binding variants are predominant in CM2 (Figure 2). To evaluate if parasite binding phenotype influences disease pathogenesis, more information is needed on the binding specificity of DC8, DC13, and other group A—expressing parasites for ICAM1 and EPCR.111,112 In addition, multiple domains in DC8 PfEMP1 bind to brain endothelial cells.115 Therefore, this analysis should include defining the other host receptors that act in concert with EPCR to mediate firm endothelial binding, as these adhesion traits may also influence microvascular pathology.

Although considerable work has been done on var gene expression in severe pediatric malaria,16,107,108,113,116 almost no information exists on DC8 or DC13 var gene expression in adult severe malaria. One of the aims of the India ICEMR is to investigate the expression of var genes in Indian adults. This question is also being evaluated as part of a collaborative effort between multiple independent ICEMRs using carefully clinically defined cohorts, in which patients in India have undergone MRI, fundoscopic examinations and have been evaluated for endothelial responsiveness to TNF. By having a precise comparison between MRI and fundoscopic findings, PfEMP1 expression, and host endothelial phenotypes, it may be possible to distinguish if host or parasite factors contribute to different pathological manifestations.

SEVERE VIVAX MALARIA IN CHILDREN AND ADULTS

The other major Plasmodium species infecting humans is P. vivax. Although P. vivax infections are rare in most of Africa because of the high percentage of the human population with the Duffy blood group antigen—negative phenotype that is highly resistant to RBC infection,117,118 it is estimated that over 2.5 billion people are at risk of P. vivax transmission. Approximately 91% of the populations at risk of transmission are in central and southeast Asia.117 Furthermore, in Brazil, P. falciparum cases are declining, and P. vivax has become the dominant parasite species in many endemic areas.119

Historically, P. vivax has been considered a relatively benign parasite, but recently there has been a renewed appreciation that it carries a significant morbidity and mortality burden in endemic regions.21,120,121 Furthermore, a 5–15% mortality rate was reported in the early neurosyphilis therapies of patients with P. vivax.21 Part of the explanation for the “benign” reputation, despite the evidence for mortality, is that vivax parasites are highly restricted to reticulocytes and therefore cannot achieve the same high parasite biomass as P. falciparum.22 A second difference is that P. vivax possesses relatively poor IE adhesive capacity compared with P. falciparum.123 Major questions for vivax pathogenesis include how does a parasite that is limited to lower grade parasitemias cause severe malaria? And is severe disease a consequence of vivax infection alone, the relapsing nature of P. vivax, or do other comorbidities influence disease severity? Within the ICEMRs, work is being done to better understand the prevalence and severity of P. vivax infections in Latin America and Asia and to characterize factors that may contribute to disease severity.

Clinically, vivax infections are associated with a chronic debilitating febrile illness that can be accompanied by chills, vomiting, malaise, and headache.21 On a per parasite basis, P. vivax is highly potent at inducing pro-inflammatory cytokines, such as TNF21,120,121,123,124 and has a much lower pyrogenic threshold than P. falciparum (180 vivax parasites/μL compared with 1,000 falciparum parasites/μL).124,125 The most frequent severe complications of vivax infection are severe anemia and acute respiratory distress.121 Cerebral malaria is a rare complication of P. vivax mono-infection, although it has been reported in India.126 In general, even less is known about the pathogenetic mechanisms in vivax malaria than P. falciparum, and it is not known if P. vivax CM cases reflect a particular strain of P. vivax, and/or a region-specific host susceptibility.

Parasite adhesion and severe vivax malaria. Unlike P. falciparum, P. vivax IEs become more deformable as they mature,127 and all parasite stages are visible in peripheral blood smears.21 However, late-stage schizont forms are underrepresented in peripheral blood,119 suggesting sequestration may occur. The lack of a continuous culture system has hindered research into P. vivax cytoadhesion, but the mechanism is distinct from P. falciparum because P. vivax IEs lack knob-like protrusions and do not encode var genes.22 Ex vivo studies have shown that P. vivax IEs adhere to placental cryosections as well as human lung—albeit at 10–15 times lower binding levels than P. falciparum.128 A strong candidate for P. vivax cytoadhesion and rosetting functions is a family of variant sub-telomeric genes named vir.129 On the basis of the sequence analysis, VIR proteins are classified into different groups, which have been found to have different subcellular localizations and functions.130 To study the cellular trafficking and adhesive functions of VIR proteins, they have been transfected into a poorly cytoadhesive P. falciparum strain (3D7), permitting gain of function studies. Two of three transfected VIR proteins were transported to the IE surface and one conferred ICAM1 binding activity.131 Whether cytoadhesion has a role in organ-specific disease complications is currently being investigated. There are few autopsy findings from polymerase chain reaction–confirmed P. vivax mono-infections. In one postmortem series from Brazil, ARDS and pulmonary edema was associated with accumulation of neutrophils in the interalveolar space, and scattered P. vivax IEs were present inside the pulmonary capillaries.132 A single autopsy performed in India showed monocyte infiltrates in alveolar capillaries.133 It has been postulated that P. vivax sequestration in pulmonary microvessels may trigger the inflammatory influx,134 but more work is needed to prove this hypothesis.

Parasite invasion pathways and vivax malaria severity. In contrast to the deadly P. falciparum, which is able to invade RBCs of all age, it has been suggested that the lack of fatalities from P. vivax malaria is related to its unique restriction to invasion and growth in reticulocytes. The Duffy blood group antigen on RBCs has a key role in invasion.118 This protein is recognized by the P. vivax Duffy binding protein (DBP),135 a leading vivax vaccine candidate. Although the identification of Duffy-dependent and Duffy-independent strains in Madagascar136 indicates that P. vivax can use alternative invasion pathways, it is unknown how extensively Duffy-independent strains are distributed throughout the world. In addition, a single amino acid polymorphism in the Duffy antigen Fy(a)/Fy(b) affects P. vivax
invasion efficiency and the risk of clinical vivax in Brazil but the effect of this polymorphism has not been examined in other parts of the world.

Despite the strong preference of *P. vivax* for reticulocytes, there is still a relatively poor understanding of why *P. vivax* is unable to invade normocytes or of the potential role of alternative invasion pathways in disease severity. A reticulocyte-binding protein complex was identified (PvRBP-1 and PvRBP-2), which plays a key role in reticulocyte binding and invasion. A related protein family was subsequently discovered in *P. falciparum* and named reticulocyte homology or RBL proteins. *Plasmodium vivax* genome sequences indicate the presence of numerous RBL paralogs, and intriguingly an additional DBP paralog, which might contribute to different modes of invasion, immune evasion, and pathogenesis. Within the India ICEMR, *P. vivax* in vitro invasion assays are being conducted to characterize the role of invasion pathways in disease severity.

**CROSS-ICEMR COMPARISON OF RESEARCH ACTIVITIES RELATED TO SEVERE MALARIA**

The ICEMR program covers a wide range of malaria transmission intensities for *P. falciparum* and *P. vivax*. Within the ICEMR program, nine ICEMRs based in south Asia, India, east and southern Africa, Amazonia, and southwest Pacific are collecting descriptive data on the characteristics and outcomes of patients admitted with severe malaria (Table 2). This broad approach can provide a better understanding of the relationship between severe malaria outcomes across the endemicity spectrum and may lend itself to meta-analysis to understand risk factors for incidence of severe disease. In addition, individual ICEMRs are investigating the role of prompt and effective therapies on minimizing severe malaria outcomes in African children and assessing the clinical profile and their association with the parasite and host immunological status and the role of nutritional factors and helminth coinfections in complicated malaria cases in Colombia (Table 2).

**CONCLUSIONS**

Although the pathophysiology of CM is complex, pediatric autopsy studies have demonstrated two major patterns: cerebral microvessels with sequestered IEs alone (CM1) and cerebral microvessels with IE sequestration plus evidence of endothelial dysfunction and activation of coagulation (CM2). Ring hemorrhages and cerebral thrombosis are also described in a proportion of adult cases, but whether there is an equivalent CM1/CM2 dichotomy in adults is unclear. Neuroimaging studies have highlighted an important role for brain swelling in pediatric CM, which is less commonly observed in adult CM. A recent focus has been the microvascular interactions between *P. falciparum* IEs and cerebral endothelial cells, and how these binding interactions may contribute to disease presentation. Furthermore, because of the inaccessibility of cerebral microvessels, dermal biopsies provide a noninvasive approach to profile the endothelial reactivity of patients with severe or non-severe malaria complications. It has been postulated that EPCR-binding parasites associated with severe pediatric malaria may impair the protein C pathway in cerebral microvessels and thereby directly contribute to coagulopathy and endothelial barrier disruption. However, further work is needed to understand to what extent parasite adhesion or endothelial phenotypes may contribute to the pathophysiological differences between CM1, CM2, and adult CM.

By comparison to *P. falciparum*, the lower lethality of *P. vivax* may relate to invasion and growth in reticulocytes and lower cytoadhesive properties. Nevertheless, despite its benign reputation, there has been a surge in reports on severe vivax malaria and a growing appreciation that *P. vivax* is not harmless. Recent studies in Peru suggest that severe vivax can occur in monoendemic malaria regions. Although highly restricted to reticulocytes, genome projects have revealed a large expansion of invasion ligand gene families in *P. vivax*. Thus, it will be important to investigate if invasion pathways influence vivax disease severity. Within the ICEMR program, current research efforts are focused on understanding disease mechanisms, as an important prerequisite to developing new tools to diagnose and treat severe malaria.

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**REFERENCES**

1. World Health Organization, 2013. *World Malaria Report*. Available at: http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/w.
2. Murray CJ, Ortblad KF, Guinovart C, Lim SS, Wolock TM, Roberts DA, Dansereau EA, Graetz N, Barber RM, Brown JC, Wang H, Duber HC, Naghavi M, Dicker D, Dandona L, Salomon JA, Heuton KR, Foreman K, Phillips DE, Fleming TD, Flaxman AD, Phillips BK, Johnson EK, Coggeshall MS, Abd-Allah F, Abera SF, Abraham JP, Abubakar I, Aburaddad LJ, Abu-Rmeileh NM, Achoki T, Adeyemo AO, Adou AK, Adua JC, Agardh EE, Akena D, Al Kahbouri MJ, Alasoori D, Albritt MJ, Alcala-Cerra G, Alegretti MA, Alemu
2012. Global malaria mortality between 1980 and 2010: a systematic analysis. Nature 415: 673–679.

Beales PF, Brabin B, Dormian E, Gilles HM, Loutain L, Marsh K, Molyneux ME, Olliaro P, Schapira A, Touze JE, Hien TT, Warrell DA, White N. 2000. Severe falciparum malaria. Trans R Soc Trop Med Hyg 94 (Suppl 1): S1–S80.

Marsh K. 1992. Malaria—a neglected disease? Parasitology 104 (Suppl): S53–S69.

Marchiafava E, Bignami A, 1894. On summer-autumnal fevers. Charles TE, ed. Two Monographs on Malaria and the Parasites of Malarial Fevers. London, United Kingdom: The New Sydenham Society, 1–393.

Clark IA, rockett KA, 1994. The cytokine theory of human cerebral malaria. Parasitol Today 10: 410–412.

van der Heyde HC, Nolan J, Combes V, Gramaglia I, Grau GE, 2006. A unified hypothesis for the genesis of cerebral malaria sequestration, inflammation and hemostasis leading to microcirculatory dysfunction. Trends Parasitol 22: 503–508.

Wassmer SC, Combes V, Grau GE, 2011. Platelets and microelements in cerebral malaria: the unusual suspects. Drug Disc Today Dis Mech 8: e15–e23.

Francischetti IM, Seydel KB, Monteiro RQ, 2008. Blood coagulation, inflammation, and malaria. Microcirculation 15: 81–107.

Mohanthy S, Heyderman RS, Wassmer SC, 2009. Disregulation of coagulation in cerebral malaria. Mol Biochem Parasitol 166: 99–108.

Medana JM, Turner GD, 2006. Human cerebral malaria and the blood-brain barrier. Int J Parasitol 36: 555–568.

Mohanty S, Taylor TE, Kampendonk S, Potchen MJ, Panda P, Majhi M, Mishra SK, Wassmer SC, 2014. Magnetic resonance imaging during life: the key to unlock cerebral malaria pathogenesis? Malar J 13: 276.

Dorovini-Zis K, Schmidt K, Huyhn H, Fu W, Whitten RO, Milner D, Kamiza S, Molyneux M, Taylor TE, 2011. The neuropathology of fatal cerebral malaria in Malawian children. Am J Pathol 178: 2146–2158.

Taylor TE, Fu WJ, Carr RA, Whitten RO, Mueller JS, Fosiko NG, Lewallen S, Liomba NG, Molyneux ME, 2004. Differentiating the pathologies of cerebral malaria by postmortem parasite counts. Nat Med 10: 143–145.

Spitz S. 1946. The pathology of acute falicparum malaria. Mil Surg 99: 555–572.

Turner L, Lavstsen T, Berger SS, Wang CW, Petersen JE, Avril M, Bazzi AF, Freeth J, Jespersen JS, Nielsen MA, Magistrad P, Lusinj J, Smith JD, Higgins MK, Theander TG, 2013. Severe malaria is associated with parasite binding to endothelial protein C receptor. Nature 498: 502–505.

Bouwens EA, Stavenhuyf F, Mosnier LO, 2013. Mechanisms of anticoagulant and anti-inflammatory actions of the protein C pathway. J Thromb Haemost 11 (Suppl 1): 242–253.

Moxon CA, Wassmer SC, Milner DA JR, Chisala NV, Taylor TE, Seydel KB, Molyneux ME, Faragher B, Esmon CT, Downey C, Toh CH, Craig AG, Heyderman RS, 2013. Loss of endothelial protein C receptors links coagulation and inflammation to parasite sequestration in cerebral malaria in African children. Blood 122: 842–851.

Price RN, Tijjra E, Guerra CA, Yeung S, White NJ, Anstey NM, 2007. Vivax malaria: neglected and not benign. Am J Trop Med Hyg 77: 79–87.

Carlton JM, Adams JH, Silva JC, Bidwell SL, Lorenzo H, Caler E, Crabtree J, Angulo SV, Merino EF, Amedo P, Cheng Q, Coulson RM, Crabb BS, del Portillo HA, Essien K, Feldblum TV, Fernandez-Becerra C, Gilson PR, Gaye AH, Guo X, Kang's S, Kooij TW, Korsinczy M, Meyer EV, Nene V, Paulsen I, White O, Ralph SA, Ren Q, Sargeant TJ, Salzberg SL, Stoeckert CJ, Sullivan SA, Yamamoto MM, Hoffman SL, Wortman JR, Gardner MJ, Galinski MR, Barnwell JW, Fraser-Liggett CM, 2008. Comparative genomic of the neglected human malaria parasite Plasmodium vivax. Nature 457: 755–767.

Dondorp AM, Fanello CJ, Hendriksen RC, Gomes E, Seni A, Chhaganal KD, Bojang K, Olaosebikan R, Anunobi N,
Maitland K, Kivaya E, Agbeneyeza T, Ngah SB, Evans J, Gesase S, Khabakha C, Mtove G, Nadjm B, Deen J, Mwanga-Amumpaire J, Nansumba M, Karema C, Umulusi N, Uwimana A, Mokuolu OA, Adeoyin OT, Johnson WB, Tshefu AK, Onyamboko MA, Sakuthzew T, Ngum WP, Silamut K, Stepniowska K, Woodrow CJ, Bellotti D, Wills B, Onelko M, Peto TE, von Scidlein L, Day NP, White NJ, 2010. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. Lancer 376: 1647–1657.

Newton PN, Stepniowska K, Dondorp A, Silamut K, Chierakul W, Krishna S, Davis TM, Suputtamongkolkul S, Pfitzer B, Pukrittayakamee S, Ruangveeryuth R, Hanson J, Day NP, White NJ, 2013. Prognostic indicators in adults hospitalized with falciparum malaria in western Thailand. Malar J 12: 229.

White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM, 2014. Malaria. Lancet 383: 723–735.

Marsh K, Snow RW, 1997. Host-parasite interaction and morbidity in malaria endemic areas. Philos Trans R Soc Lond B Biol Sci 352: 1385–1394.

Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P, Peshu N, 1995. Indicators of life-threatening malaria in African children. N Engl J Med 332: 1399–1404.

Mohanty S, Mishra SK, Pati SS, Pattnaik J, Das BS, 2003. Compliations and mortality patterns due to Plasmodium falciparum malaria in hospitalized adults and children. Rourkela, Orissa, India. Trans R Soc Trop Med Hyg 97: 69–70.

MacPherson GG, Warrell MJ, White NJ, Looareesuwan S, Warrell DA, 1985. Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. Am J Pathol 119: 385–401.

Turner GD, Morrison H, Jones M, Davis TM, Looareesuwan S, Buley ID, Gatter KC, Newbold CI, Pukrittayakamee S, Nagachinta B, White NJ, Berendt AR, 1994. An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adherence molecule-1 in cerebral sequestration. Am J Pathol 145: 1057–1069.

World Health Organization, 2011. World Malaria Report 2011. Geneva, Switzerland: World Health Organization.

Milner DA Jr, Whitten RO, Kamiza S, Carr R, Liomba G, Dzamalala C, Seydel KB, Molyneux ME, Taylor TE, 2014. The systemic pathology of cerebral malaria in African children. Front Cell Infect Microbiol 4: 104.

Ngakata T, Hoang VT, Tegoshi T, Rabbege J, Ann TK, Aikawa M, 1992. Pathology of falciparum malaria in Vietnam. Am J Trop Med Hyg 47: 259–264.

Romiti M, Pongpomratn E, Tegoshi T, Looareesuwan S, Punpoowong B, Aikawa M, 1990. Human cerebral malaria in Thailand: a clinico-pathological correlation. Brain 113: 223–271.

Toro G, Roman G, 1978. Cerebral malaria. A disseminated pathology in cerebral malaria and CNS infections in Vietnam. Neurology 55: 104–111.

Jenkins N, Wu Y, Chakravorty S, Kai O, Marsh K, Craig A, 2007. Plasmodium falciparum intercellular adherence molecule-1-based cytoadherence-related signaling in human endothelial cells. J Infect Dis 196: 321–327.

Tripathi AK, Sullivan DJ, Stins MF, 2007. Plasmodium falciparum-infected erythrocytes decrease the integrity of human brain-blood barrier endothelial cell monolayers. J Infect Dis 195: 269–275.

Gillrie MR, Lee K, Gowda DC, Davis SP, Monestier M, Cui L, Hien TT, Day NP, Ho M, 2012. Plasmodium falciparum histones induce endothelial proinflammatory response and barrier dysfunction. Am J Pathol 180: 1028–1039.

Medana IM, Day NP, Sachanonta N, Mai NT, Dondorp AM, Pongponratn E, Hien TT, White NJ, Turner GD, 2011. Coma in fatal adult human malaria is not caused by cerebral oedema. Malar J 10: 267.

Ponsford MJ, Medana IM, Prapansip P, Hien TT, Lee SJ, Dondorp AM, Esiri MM, Day NP, White NJ, Turner GD, 2012. Sequestration and microvascular congestion are associated with coma in human cerebral malaria. J Infect Dis 205: 663–671.

Dondorp AM, Ince C, Charunwatthana P, Hanson J, van Kuijzen A, Faiz MA, Rahman MR, Hasan M, Bin YE, Ghose A, Ruangveeryat R, Limmathurotsakul D, Mathura K, White NJ, Day NP, 2008. Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. J Infect Dis 197: 79–84.

Aird WC, 2012. Endothelial cell heterogeneity. Cold Spring Harb Perspect Med 2: a006429.

Maccormick IJ, Beare NA, Taylor TE, Barrera V, White VA, Hiscott P, Molyneux ME, Dhillon B, Harding SP, 2014. Cerebral malaria in children: using the retina to study the brain. Brain 137: 2119–2142.

Beare NA, Lewallen S, Taylor TE, Molyneux ME, 2011. Redefining cerebral malaria by including malaria retinopathy. Future Microbiol 6: 349–355.

White VA, Lewallen S, Beare NA, Molyneux ME, Taylor TE, 2009. Retinal pathology of pediatric cerebral malaria in Malawi. PLoS One 4: e4317.

Lewallen S, White VA, Whitten RO, Gardiner J, Hoar B, Lindley J, Locman SP, McCormack A, Ward K, Tembo M, Mwenechenyana J, Molyneux ME, Taylor TE, 2000. Clinical-histopathological correlation of the abnormal retinal vessels in cerebral malaria. Arch Ophthalmol 118: 924–928.

Beare NA, Harding SP, Taylor TE, Lewallen S, Molyneux ME, 2009. Perfusion abnormalities in children with cerebral malaria and malarial retinopathy. J Infect Dis 199: 263–271.

Maude RJ, Beare NA, Abu Sayeed A, Chang CC, Charunwatthana P, Faiz MA, Hossain A, Yunus EB, Hoque MG, Hasan MU, White NJ, Day NP, Dondorp AM, 2009. The spectrum of retinopathy in adults with Plasmodium falciparum malaria. Trans R Soc Trop Med Hyg 103: 665–671.

Abu Sayeed A, Maude RJ, Hasan MU, Mohammed N, Hoque MG, Dondorp AM, Faiz MA, 2011. Malarial retinopathy in Bangladeshi adults. Am J Trop Med HYG 84: 141–147.

Beare NA, Taylor TE, Harding SP, Lewallen S, Molyneux ME, 2006. Malarial retinopathy: a newly established diagnostic sign in severe malaria. Am J Trop Med Hyg 75: 790–797.

Mohanty S, Mishra SK, Patnaik R, Dutt AK, Pradhan S, Das B, Patnaik J, Mohanty AK, Lee SJ, Dondorp AM, 2011. Brain swelling and mannitol therapy in adult cerebral malaria: a randomized trial. Clin Infect Dis 53: 349–355.

Newton CR, Peshu N, Kendall B, Kirkham FJ, Sowumni A, Waruiru C, Mwangi I, Murphy SA, Marsh K, 1994. Brain swelling and ischaemia in Kenyans with cerebral malaria. Arch Dis Child 70: 281–287.

Patankar TF, Karnad DR, Shetty PG, Desai AP, Prasad SR, 2002. Adult cerebral malaria: prognostic importance of imaging findings and correlation with postmortem findings. Radiology 224: 811–816.
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62. Looareesuwan S, Wilairatana P, Krishna S, Kendall B, Vannaphan S, Viravan C, White NJ, 1995. Magnetic resonance imaging of the brain in patients with cerebral malaria. *Clin Infect Dis* 21: 300–309.

63. Potchen MJ, Kampondeni SD, Seydel KB, Birbeck GL, Hammond CA, Bradley WG, DeMarco JK, Glover SJ, Ugorji JO, Latourette MT, Siebert JE, Molyneux ME, Taylor TE, 2012. Acute brain MRI findings in 120 Malawian children with cerebral malaria: new insights into an ancient disease. *AJNR 33*: 1740–1746.

64. Seydel KB, Kampondeni SD, Valim C, Potchen MJ, Milner DA, Muwali FW, Birbeck GL, Bradley WG, Fox LG, Glover SJ, Hammond CA, Heyderman RS, Chilingulo CA, Molyneux ME, Taylor TE, 2015. Brain swelling and death in pediatric cerebral malaria. *N Engl J Med* 372: 1126–1137.

65. Potchen MJ, Kampondeni SD, Ibrahim K, Bonner J, Seydel KB, Taylor TE, Birbeck GL, 2013. NeuroInterp: a method for facilitating neuroimaging research on cerebral malaria. *Neurology* 81: 585–588.

66. Ho M, White NJ, 1999. Molecular mechanisms of cytoadherence in malaria. *Am J Physiol* 276: C1231–C1242.

67. Wassmer SC, Combes V, Grau GE, 2003. Pathophysiology of cerebral malaria: role of host cells in the modulation of cerebral blood flow. *Transfusion* 43: 351–380.

68. Combes V, Costel N, Faure D, Wassmer SC, Grau GE, 2006. Cerebral malaria: role of microparticles and platelets in alterations of the blood-brain barrier. *Int J Parasitol* 36: 541–546.

69. Wassmer SC, Moxon CA, Taylor T, Grau GE, Molyneux ME, Craig AG, 2011. Vascular endothelial cells cultured from patients with cerebral or uncomplicated malaria exhibit differential reactivity to TNF. *Cell Microbiol* 13: 198–209.

70. Zehnbauer B, 2005. Population genetics in critical illness. *Crit Care Med* 33: 242–243.

71. Di Perri G, Di Perri IG, Monteiro GB, Bonora S, Henning C, Cassatella M, Miccoli R, Vento S, Dusi S, Bassetti D, Concì E, 1995. Pentoxifylline as a supportive agent in the treatment of malaria-induced histidine-rich protein (Pf HRP II) from *Plasmodium falciparum* in successfully treated acute falciparum malaria. *J Infect Dis* 171: 1091–1097.

72. van Hensbroek MB, Palmer A, Onyiorah A, Schneider G, Jaffar S, Dolan G, Mesnager H, Fanello CI, Day NP, White NJ, Dondorp AM, 2013. Diagnosing severe falciparum malaria in parasitaemic African children: a prospective evaluation of plasma PfHRP2 measurement. *PLoS Med* 9: e1001297.

73. Ho M, White NJ, 1999. Molecular mechanisms of cytoadherence in malaria. *Am J Physiol* 276: C1231–C1242.

74. Potchen MJ, Kampondeni SD, Valim C, Potchen MJ, Milner DA, Muwali FW, Birbeck GL, Hammond CA, Bradley WG, DeMarco JK, Glover SJ, Ugorji JO, Latourette MT, Siebert JE, Molyneux ME, Taylor TE, 2012. Acute brain MRI findings in 120 Malawian children with cerebral malaria: new insights into an ancient disease. *AJNR 33*: 1740–1746.

75. Seydel KB, Kampondeni SD, Valim C, Potchen MJ, Milner DA, Muwali FW, Birbeck GL, Hammond CA, Bradley WG, Fox LG, Glover SJ, Heyderman RS, Chilingulo CA, Molyneux ME, Taylor TE, 2015. Brain swelling and death in pediatric cerebral malaria. *N Engl J Med* 372: 1126–1137.

76. Potchen MJ, Kampondeni SD, Ibrahim K, Bonner J, Seydel KB, Taylor TE, Birbeck GL, 2013. NeuroInterp: a method for facilitating neuroimaging research on cerebral malaria. *Neurology* 81: 585–588.

77. Ho M, White NJ, 1999. Molecular mechanisms of cytoadherence in malaria. *Am J Physiol* 276: C1231–C1242.

78. Wassmer SC, Combes V, Grau GE, 2003. Pathophysiology of cerebral malaria: role of host cells in the modulation of cerebral blood flow. *Transfusion* 43: 351–380.

79. Combes V, Costel N, Faure D, Wassmer SC, Grau GE, 2006. Cerebral malaria: role of microparticles and platelets in alterations of the blood-brain barrier. *Int J Parasitol* 36: 541–546.

80. Wassmer SC, Moxon CA, Taylor T, Grau GE, Molyneux ME, Craig AG, 2011. Vascular endothelial cells cultured from patients with cerebral or uncomplicated malaria exhibit differential reactivity to TNF. *Cell Microbiol* 13: 198–209.

81. Zehnbauer B, 2005. Population genetics in critical illness. *Crit Care Med* 33: 242–243.

82. Di Perri G, Di Perri IG, Monteiro GB, Bonora S, Henning C, Cassatella M, Miccoli R, Vento S, Dusi S, Bassetti D, Concì E, 1995. Pentoxifylline as a supportive agent in the treatment of malaria-induced histidine-rich protein (Pf HRP II) from *Plasmodium falciparum* in successfully treated acute falciparum malaria. *J Infect Dis* 171: 1091–1097.

83. Ho M, White NJ, 1999. Molecular mechanisms of cytoadherence in malaria. *Am J Physiol* 276: C1231–C1242.

84. Potchen MJ, Kampondeni SD, Valim C, Potchen MJ, Milner DA, Muwali FW, Birbeck GL, Hammond CA, Bradley WG, DeMarco JK, Glover SJ, Ugorji JO, Latourette MT, Siebert JE, Molyneux ME, Taylor TE, 2012. Acute brain MRI findings in 120 Malawian children with cerebral malaria: new insights into an ancient disease. *AJNR 33*: 1740–1746.

85. Seydel KB, Kampondeni SD, Valim C, Potchen MJ, Milner DA, Muwali FW, Birbeck GL, Hammond CA, Bradley WG, Fox LG, Glover SJ, Heyderman RS, Chilingulo CA, Molyneux ME, Taylor TE, 2015. Brain swelling and death in pediatric cerebral malaria. *N Engl J Med* 372: 1126–1137.

86. Potchen MJ, Kampondeni SD, Ibrahim K, Bonner J, Seydel KB, Taylor TE, Birbeck GL, 2013. NeuroInterp: a method for facilitating neuroimaging research on cerebral malaria. *Neurology* 81: 585–588.

87. Ho M, White NJ, 1999. Molecular mechanisms of cytoadherence in malaria. *Am J Physiol* 276: C1231–C1242.

88. Wassmer SC, Combes V, Grau GE, 2003. Pathophysiology of cerebral malaria: role of host cells in the modulation of cerebral blood flow. *Transfusion* 43: 351–380.

89. Combes V, Costel N, Faure D, Wassmer SC, Grau GE, 2006. Cerebral malaria: role of microparticles and platelets in alterations of the blood-brain barrier. *Int J Parasitol* 36: 541–546.

90. Wassmer SC, Moxon CA, Taylor T, Grau GE, Molyneux ME, Craig AG, 2011. Vascular endothelial cells cultured from patients with cerebral or uncomplicated malaria exhibit differential reactivity to TNF. *Cell Microbiol* 13: 198–209.

91. Zehnbauer B, 2005. Population genetics in critical illness. *Crit Care Med* 33: 242–243.

92. Cowman AF, Crabb BS, 2006. Invasion of red blood cells by malaria parasites. *Cell 124*: 755–766.

93. Baruch DI, Adshead PS, Black BL, Singh HB, Bi X, Ma XC, Feldman M, Taraschi TF, Howard RJ, 1995. Cloning the *P. falciparum* gene encoding PFEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell 82*: 77–87.

94. Smith JD, Chitnis CE, Craig AG, Roberts DJ, Hudson-Taylor DE, Peterson DS, Pinches R, Newbold CI, Miller LH, 1995. Switches in expression of *Plasmodium falciparum* var genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell 82*: 101–110.

95. Su NZ, Heatwole VM, Wertheimer SP, Guinet F, Herrfeldt JA, Cowman AF, Crabb BS, 2006. Invasion of red blood cells by malaria parasites. *Cell 124*: 755–766.

96. Baruch DI, Adshead PS, Black BL, Singh HB, Bi X, Ma XC, Feldman M, Taraschi TF, Howard RJ, 1995. Cloning the *P. falciparum* gene encoding PFEMP1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell 82*: 77–87.

97. Smith JD, Chitnis CE, Craig AG, Roberts DJ, Hudson-Taylor DE, Peterson DS, Pinches R, Newbold CI, Miller LH, 1995. Switches in expression of *Plasmodium falciparum* var genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell 82*: 101–110.

98. Barry AE, Leliwa-Sytek A, Tavul L, Imrie H, Migot-Nabias F, Brown SM, McVean GAV, Day KP, 2007. Population genomics of the immune evasion (var) genes of *Plasmodium falciparum*. *PLoS Pathog* 3: e34.

99. Lavstsen T, Salanti A, Jensen AT, Arnott DE, Theander TG, 2003. Sub-grouping of *Plasmodium falciparum* 3D7 var genes.
CURRENT APPROACHES TO INVESTIGATE SEVERE MALARIA

55

based on sequence analysis of coding and non-coding regions. 

Malar J 2: 27.

100. Kraemer SM, Kyes SA, Aggarwal G, Springer AL, Nelson 

SO, Christodoulou Z, Smith LM, Wang W, Levin E, 

Newbold CI, Myler PJ, Smith JD, 2007. Patterns of gene 

recombination shape var gene repertoires in Plasmodium 

falciparum: comparisons of geographically diverse isolates. 

BMC Genomics 8: 45.

103. Smith JD, Subramanian G, Gamain B, Baruch DI, Miller LH, 

Plasmodium falciparum. Semin Immunopathol 34: 169–180.

106. Fried M, Duffy PE, 1996. Adherence of 

Plasmodium falciparum for binding to human brain endothelial cells. 

Clin Microbiol Rev 26: 35–57.

109. Avril M, Brazier AJ, Melche R, Sampath S, Smith JD, 2013. 

DC8 and DC13 var genes associated with severe malaria bind 

avidly to diverse endothelial cells. PLoS Pathog 9: e1003430.

112. Claessens A, Adams Y, Chiu J, Wang CW, Berriman M, Marsh K, Bull PC, 2009. 

Global genetic diversity and evolution of var genes associated with placent 

al and severe childhood malaria. Mol Biochem Parasitol 148: 169–180.

115. Armar M, Raza A, Bozdech Z, Rowe JA, 2012. A subset of 

Plasmodium falciparum var genes encodes the malaria parasite ligands 

involved in pregnancy-associated malaria. Mol Microbiol 49: 179–191.

118. Naing C, Khittak MA, Nyunt Wai V, Mak JW, 2014. Is 

Plasmodium vivax malaria a severe malaria? A systematic 

review and meta-analysis. PLoS Negl Trop Dis 8: e3071.

121. van Wijngaarden ND, Grau GE, Gamage P, Carter R, Mendis KN, 

1992. Dynamics of fever and serum levels of tumor necrosis factor 

are closely associated during clinical paroxysms in Plasmodium 

vivax malaria. Proc Natl Acad Sci USA 89: 3200–3203.

124. Collins WE, Jeffery GM, Roberts JM, 2004. A retrospective 

examination of the effect of fever and microgametocyte count 

on mosquito infection on humans infected with Plasmodium 

vivax. Am J Trop Med Hyg 70: 638–641.

127. Tarwan GS, Khatri PC, Sengar GS, Kochar A, Kochar SK, 

Middha S, Tarwan G, Kochar N, Pakalpati D, Garg S, Das A, 

Kochar DK. 2011. Clinical profiles of 13 children with 

Plasmodium vivax cerebral malaria. Ann Trop Paediatr 31: 

351–356.

130. Savanurak S, Cooke BM, Dondorp AM, Silamut K, 

Sattabongkot J, White NJ, Udomsangpeet R, 2004. The 

deformability of red blood cells paralysed by Plasmodium 

falciparum and P. vivax. J Infect Dis 189: 193–194.

133. Carvalho BO, Lopes SC, Nogueira PA, Orlandi PP, Bargieri 

DY, Blanco YC, Mamoni R, Leite JA, Rodrigues MM, Soares 

IS, Oliveira TR, Wunderlich G, Lacerda MV, del Portillo HA, 

Araujo MO, Russell B, Suwanurak R, Souonou G, Renia L, 

Costa FT, Lopes SC, Albrecht L, Ataide R, Souque AM, Souza 

RM, Russell B, Renia L, Marinho CR, Lacerda MV, 2013. On 

cytoadhesion of Plasmodium vivax malaria: perspectives 

from the Brazilian field. Int J Parasitol 43: 1099–1105.

136. Sattar S, Wannapa J, Achariyarat P, Nair M, Arjun J, 

Costa FT, Lopes SC, Albrecht L, Ataide R, Souque AM, Souza 

RM, Russell B, Renia L, Marinho CR, Lacerda MV, 2013. On 

cytoadhesion of Plasmodium vivax malaria: perspectives 

from the Brazilian field. Int J Parasitol 43: 1099–1105.

139. Baird JK, 2013. Evidence and implications of mortality associ-

ated with severe Plasmodium vivax malaria. Clin Infect Dis 189: 193–194.

142. van Wijngaarden ND, Grau GE, Gamage P, Carter R, Mendis KN, 

1992. Dynamics of fever and serum levels of tumor necrosis factor 

are closely associated during clinical paroxysms in Plasmodium 

vivax malaria. Proc Natl Acad Sci USA 89: 3200–3203.

145. Collins WE, Jeffery GM, Roberts JM, 2004. A retrospective 

examination of the effect of fever and microgametocyte count 

on mosquito infection on humans infected with Plasmodium 

vivax. Am J Trop Med Hyg 70: 638–641.
reveals different subcellular localizations and cytoadherence to the ICAM-1 endothelial receptor. Cell Microbiol 14: 386–400.

132. Lacerda MV, Fragoso SC, Alcêmim MG, Alexandre MA, Magalhaes BM, Siqueira AM, Ferreira LC, Araujo JR, Mourao MP, Ferrer M, Castillo P, Martín-Jauar L, Fernandez-Becerra C, del Portillo H, Ordí J, Alonso PL, Bassat Q, 2012. Post-mortem characterization of patients with clinical diagnosis of Plasmodium vivax malaria: to what extent does this parasite kill? Clin Infect Dis 55: c67–c74.

133. Valecha N, Pinto RG, Turner GD, Kumar A, Rodrigues S, Dubhashi NG, Rodrigues E, Banaulikar SS, Singh R, Dash AP, Baird JK, 2009. Histopathology of fatal respiratory distress caused by Plasmodium vivax malaria. Am J Trop Med Hyg 81: 758–762.

134. Anstey NM, Russell B, Yeo TW, Price RN, 2009. The pathophysiology of vivax malaria. Trends Parasitol 25: 220–227.

135. Adams JH, Hudson DE, Torii M, Ward GE, Wellems TE, Aikawa M, Miller LH, 1990. The Duffy receptor family of Plasmodium knowlesi is located within the micronemes of invasive malaria merozoites. Cell 63: 141–153.

136. Menard D, Barnadas C, Bouchier C, Henry-Halldin C, Gray LR, Ratsimbasoa A, Thonier V, Carod JF, Domarle O, Colin Y, Bertrand O, Picot J, King CL, Grimberg BT, Mercereau-Puijalon O, Zimmerman PA, 2010. Plasmodium vivax clinical malaria is commonly observed in Duffy-negative Malagasy people. Proc Natl Acad Sci USA 107: 5967–5971.

137. King CL, Adams JH, Xianli J, Grimberg BT, McHenry AM, Greenberg LJ, Siddiqui A, Howes RE, da Silva-Nunes M, Ferreira MU, Zimmerman PA, 2011. Fy(a)/Fy(b) antigen polymorphism in human erythrocyte Duffy antigen affects susceptibility to Plasmodium vivax malaria. Proc Natl Acad Sci USA 108: 20113–20118.

138. Galinski MR, Medina CC, Ingravallo P, Barnwell JW, 1992. A reticulocyte-binding protein complex of Plasmodium vivax merozoites. Cell 69: 1213–1226.

139. Menard D, Chan ER, Benedet C, Ratsimbasoa A, Kim S, Chim P, Do C, Witkowski B, Durand R, Thellier M, Severini C, Legrand E, Musset L, Nour BY, Mercereau-Puijalon O, Serre D, Zimmerman PA, 2013. Whole genome sequencing of field isolates reveals a common duplication of the Duffy binding protein gene in Malagasy Plasmodium vivax strains. PLoS Negl Trop Dis 7: e2489.

140. Quispe AM, Pozo E, Guerrero E, Durand S, Baldeviano GC, Edgel KA, Graf PC, Lescano AG, 2014. Plasmodium vivax hospitalizations in a monoendemic malaria region: severe vivax malaria? Am J Trop Med Hyg 91: 11–17.