Review

The pathophysiology of vivax malaria

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Long considered a benign infection, *Plasmodium vivax* is now recognized as a cause of severe and fatal malaria, despite its low parasite biomass, the increased deformability of vivax-infected red blood cells and an apparent paucity of parasite sequestration. Severe anemia is associated with recurrent bouts of hemolysis of predominantly uninfected erythrocytes with increased fragility, and lung injury is associated with inflammatory increases in alveolar-capillary membrane permeability. Although rare, vivax-associated coma challenges our understanding of pathobiology caused by *Plasmodium* spp. Host and parasite factors contribute to the risk of severe disease, and comorbidities might contribute to vivax mortality. In this review, we discuss potential mechanisms underlying the syndromes of uncomplicated and severe vivax malaria, identifying key areas for future research.

Vivax malaria: neglected and not benign

Plasmodium falciparum is responsible for the majority of severe and fatal malaria, thus overshadowing the public health importance and pathogenesis of vivax malaria [1,2]. However, outside of Africa, *Plasmodium vivax* accounts for almost half of malaria cases, with up to 390 million clinical infections each year [2]. In recent decades, *P. vivax* has usually been considered a benign infection; reports of severe vivax malaria have been confined to case reports and small case series [3]. Large studies from both halves of the island of New Guinea (Indonesian Papua [4,5] and Papua New Guinea, or PNG [6]) now show a strong association between *P. vivax* infection, severe disease and death [4–6]. These reports raise three major questions. How important a problem is severe and fatal vivax malaria outside of New Guinea? What proportion of severe disease and mortality associated with vivax infection is actually attributable to *P. vivax*? And what is the underlying pathogenesis of severe disease and death in vivax malaria? Some syndromes, such as severe vivax anemia, evoke several plausible mechanisms, whereas others (e.g. vivax coma) challenge our current understanding of pathogenesis caused by *Plasmodium* spp.

Because it has a longer evolutionary history of parasitizing primates than *P. falciparum, P. vivax* is probably better adapted to survival and replication without killing the host. This review compares the pathobiology of *P. vivax* with *P. falciparum* and discusses the potential mechanisms underlying the syndromes of uncomplicated and severe vivax malaria (Table 1) and the potential host and parasite factors that contribute to these.

Comparative pathobiology of *P. vivax*

Parasite biomass

Plasmodium falciparum invades red blood cells (RBCs) of all ages, progressing to high parasite burdens if uninhibited by treatment or host immunity. Conversely, *P. vivax* has a preference for infecting young RBCs, a property that seems to limit its reproductive capacity. Parasitemias in vivax malaria rarely exceed 2% of circulating RBCs [7], and high parasite burdens are not a feature of severe disease.

Why does P. vivax evoke a greater inflammatory response than P. falciparum?

A major feature that distinguishes *P. vivax* from *P. falciparum* is its lower pyrogenic threshold (the level of parasitemia associated with fever) [2,8]. Organ-specific studies have also shown that the inflammatory response during *P. vivax* infection is greater than that seen in *P. falciparum* infections with a similar or greater parasite biomass [9]. Cytokine production during *P. vivax* infections is higher than that in *P. falciparum* infections of similar parasite biomass [10,11]. Although the inflammatory correlates of the lower pyrogenic threshold have been described, the underlying mechanism(s) have not.

Are there differences in 'malaria toxin(s)' between the two species? Candidate *P. falciparum* 'toxins' thought to cause fever include glycosylphosphatidylinositol (GPI) [12] and *P. falciparum*-derived DNA associated with parasite hemozoin [13]. It is not known whether there are structural differences in the *P. vivax* GPI that make it more pyrogenic. Despite the AT-rich nature of the *P. falciparum* genome [14], recent studies have shown that when associated with hemozoin and internalized, *P. falciparum* CpG motifs stimulate toll-like receptor-9 (TLR9) and account for the pyrogenic properties previously attributed to hemozoin itself [13]. Because *P. vivax* has a GC-content that is more than double that of *P. falciparum* [15], greater concentrations of TLR9-stimulating CpG motifs within *P. vivax* hemozoin might account for greater pyrogenicity, although this hypothesis remains to be tested.

A lipid found in the cholesterol–triglyceride fraction of plasma at the time of paroxysmal fever has also been proposed as a putative malaria toxin and seems to be unique to *P. vivax* [16,17]. This lipid has greater activity

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Table 1. Pathobiology of *P. vivax* and potential mechanisms of severe disease

Pathobiological process in *P. vivax*	Potential contribution to severe malaria
Destruction of non-infected RBCs	Severe anemia despite small parasite biomass
Invasion and destruction of reticulocytes	Very short RBC lifespan and failure to replace destroyed RBCs: contribution to severe anemia
Increased fragility of infected and non-infected RBCs	Severe anemia
Probable faster parasite growth rates in chloroquine-resistant *P. vivax*	Severe anemia
Possible pooling of RBCs in the spleen	Potential contribution to anemia
Increased deformability of infected RBCs	Unlikely to contribute to microvascular obstruction or severity
Relapse	Recurrent hemolysis and severe anemia
Recrudescence of chloroquine-resistant *P. vivax*	Recurrent hemolysis and severe anemia
Greater cytokine production relative to *P. falciparum*	Organ-specific inflammation, including increased alveolar-capillary membrane permeability and acute lung injury Hypothesized contribution to impaired utero-placental circulation and low birth weight Dyserythropoiesis and anemia Endothelial activation Greater hemodynamic compromise in setting of acute or chronic comorbidity
Rosetting	Potential contribution to hypothesized but as-yet uncharacterized microvascular obstruction and end-organ pathology
Leukocyte aggregation by paroxysm plasma	Possible organ-specific inflammation, including acute lung injury Possible contribution to hypothesized but as-yet uncharacterized microvascular obstruction and end-organ pathology
Maternal anemia	Low birth weight
Splenic hematoma (±trauma) and thrombocytopenia	Splenic rupture
Less well characterized processes	
Putative cytoadherence of *P. vivax*-infected RBCs	Possible targeting of inflammatory responses to the lung: acute lung injury Speculative contribution to hypothesized, but as-yet uncharacterized, microvascular obstruction and end-organ pathology
Endothelial activation, dysfunction and/or injury	Increased alveolar-capillary permeability and acute lung injury Impaired utero-placental circulation and low birth weight Possible contribution to hypothesized, but as-yet uncharacterized, microvascular obstruction and end-organ pathology
Altered thrombostasis, thrombocytopenia and microvascular thrombosis	Possible contribution to hypothesized but as-yet uncharacterized microvascular obstruction and end-organ pathology
Comorbidities	Potential for a non-fatal comorbidity to become fatal with the fever and anemia of acute vivax malaria exacerbating hypoxia and/or hemodynamic compromise.

than GPI-like phospholipids [16] and might also contribute to the pyrogenicity of *P. vivax*.

Do cytoadherence phenomena occur in P. vivax infections?

RBCs infected with late stages of *P. falciparum* cytoadhere to the microvascular endothelium, resulting in sequestration and microvascular obstruction, a central feature of severe disease and placental pathology in falciparum malaria [18]. Because all stages of *P. vivax* are visible in peripheral blood, *P. vivax* is not thought to sequester or cause end-organ dysfunction in the same manner as *P. falciparum*. However, based upon observations of a smaller proportion of circulating schizonts in relation to trophozoites, Shute [7] hypothesized (more than 50 years ago) that 'an appreciable proportion. . .complete their segmentation in the internal capillaries'. A report, yet to be published in full, indicates that *P. vivax*-infected RBCs cytoadhere *in vitro* to chondroitin sulfate A [19], a receptor expressed in placental [20], pulmonary [21] and cerebral [22] microvasculature. Indirect *in vivo* evidence indicates that *P. vivax*-infected RBCs might accumulate in certain organs, such as the lung [9], as has been shown in non-human *Plasmodium* spp. [23,24]. However, few post-mortem studies have been reported in *P. vivax,* particularly in modern times [25], and autopsy evidence for microvascular accumulation of vivax-infected RBCs is, at best, modest. Early 20th century autopsies reported 'intracapillary masses of swollen, infected erythrocytes and pigment' [26] and 'frequent. . .mature malaria parasite(s) within a red blood cell. . .taking up the entire lumen in immediate contact with the endothelial cell' [27] in at least some vessels in the brain, in addition to 'an unusually large number of infected red cells, young plasmodia and pigment' in intestinal submucosal vessels [27]. These findings have not been reported in other vivax autopsy studies [25] and might be confounded by the accuracy of diagnosis, representativeness of reported sections, comorbidities (e.g. neurosyphilis and sepsis) and unrecognized mixed-species infection. Larger modern autopsy series, including immunohistochemistry, electron microscopy and clinical correlates, are awaited with interest. *Plasmodium falciparum* parasites are no longer visible in autopsy microvasculature after three days of antimalarial treatment [28]; therefore, to address the question of sequestration, autopsies will be needed from patients dying with *P. vivax* before or shortly after commencing antimalarials. Nevertheless, if cytoadherence and/or sequestration of *P. vivax*-infected RBCs does occur, it will almost certainly be less widespread and of lesser magnitude than that with *P. falciparum*.

Other cytoadherence phenomena linked to the pathophysiology of severe falciparum malaria include rosetting,

adherence of non-infected to infected RBCs [29] and autoagglutination [30]. In vivax malaria, rosetting has been described *ex vivo* [31]; however, its role in pathophysiology is unknown. Autoagglutination/clumping of *P. falciparum*-infected RBCs requires binding to CD36 and P-selectin on platelets [30,32]. Conversely, *P. vivax*-infected RBCs do not seem to bind to platelets or CD36 [19,31], making similar platelet-mediated mechanisms unlikely in vivax malaria.

In vitro aggregation of leukocytes in plasma collected during febrile paroxysms [16,17] is a cytoadherence phenomenon specific to vivax malaria but of unknown clinical significance. Such leukocyte aggregation, comprising 80–90% neutrophils, is mediated by parasite-derived lipids in concert with host cytokines [16,17]. Microvascular accumulation of mononuclear leukocytes has been reported at autopsy in the lung, intestine and brain [27].

Deformability and fragility of parasitized erythrocytes

In contrast to *P. falciparum*, in which deformability of both infected and non-infected RBCs is impaired, the deformability of vivax-infected RBCs is increased [33,34]. This property might enable *P. vivax* to avoid destruction during passage through the ~2 μm slits between endothelial cells of the splenic sinusoids [34]. The rheological mechanisms underlying this are unknown [34]. Although reduced RBC deformability is thought to contribute to impaired organ perfusion in falciparum malaria, increased deformability of *P. vivax*-infected RBCs makes this unlikely in vivax malaria. However, such deformability is accompanied by increased fragility of both infected and non-infected RBCs [34].

Relapse and tolerance

A fundamental difference between *P. vivax* and *P. falciparum* is the ability of *P. vivax* to relapse from dormant hypnozoites. In tropical regions, *P. vivax* is characterized by frequent relapses 3–6 weeks apart, whereas in temperate areas, these are fewer and delayed [35]. Differences in relapse patterns might account for geographic variation in vivax morbidity and disease severity. For example, the frequent relapses (every 3–4 weeks) found in Melanesia readily cause malaria tolerance, characterized by a higher fever threshold and attenuated symptomatology in second and subsequent infections [36,37]. A high proportion of vivax infections, therefore, are asymptomatic [8]. In such settings, recurring episodes of hemolysis and dyserythropoiesis are likely to contribute to vivax-associated severe anemia, particularly when the patient is afebrile and, thus, less likely to seek treatment [5,6]. Conversely, relapses in temperate regions occur up to 12 months after initial infection, leading to loss of tolerance and a greater likelihood of symptomatic recurrences.

Endothelial activation, dysfunction and injury and altered thrombostasis

Endothelial dysfunction and activation are key processes underlying impaired microvascular perfusion in severe falciparum malaria [38,39]. Although endothelial function has not been described in vivax malaria, endothelial 'stimulation' has been reported at autopsy [25,27], and concentrations of circulating endothelial activation markers are at least as high in uncomplicated vivax malaria as they are in falciparum malaria [40]. *P. vivax* infection is associated with elevated thrombomodulin [41], von Willebrand factor (VWF) [42] and procoagulant activity [10], and thrombotic microangiopathy [43] and ADAMTS-13 deficiency [42]. These altered hemostatic pathways could result in intravascular coagulation and endothelial inflammation through increased formation of ultra-large VWF and platelet aggregates. Weibel-Palade body exocytosis [39], hemolysis-associated nitric oxide quenching [38], altered thrombostasis, platelet activation [32], endothelial cell injury, and the impairment of vasomotor responses [38] and microcirculatory flow [44] all contribute to the pathophysiology of *P. falciparum* but have yet to be characterized in severe vivax malaria.

What causes the syndromes of severe vivax malaria?

Severe anemia

Plasmodium vivax is a major risk factor for severe anemia in vivax-endemic areas, particularly in young children [5,6]. The etiology is complex and confounded by intercurrent infection with *P. falciparum,* helminth infections, nutritional deficiencies and hemoglobinopathies [45], but in equatorial regions where >80% *P. vivax* infections relapse at 3–4 week intervals, progressive anemia is associated with recurrent bouts of hemolysis and dyserythropoiesis [2]. In areas of chloroquine resistance, this is exacerbated further by delayed parasite clearance and recrudescent infections [2].

The low parasite biomass of *P. vivax* indicates that severe anemia does not result from destruction of infected RBCs alone. Malariotherapy studies have shown that for every infected RBC destroyed during vivax malaria, ~32 non-infected RBCs are removed from the circulation [46]. This contrasts with the loss of only ~8 RBCs for every infected erythrocyte in falciparum malaria [47,48]. The mechanisms underlying this difference are not known; the proportion of uninfected RBCs destroyed compared to the proportion undergoing extravascular (e.g. splenic) pooling is also not known. Recent microfluidic studies demonstrate increased RBC fragility with *P. vivax*; ~50% of infected RBCs and ~15% of non-infected RBCs were destroyed after passage through a 2-μm channel mimicking splenic sinusoids [34]. Postulated mechanisms included oxidative damage or other host immune mediators [34]. Cytokine-related dyserythropoieis also probably contributes to anemia [49]. Although erythroblasts can be infected and lysed by *P. vivax in vitro*, bone marrow studies have not shown erythroblast infection *in vivo* [49].

Respiratory distress and acute lung injury

Plasmodium vivax commonly affects the lung, and a cough occurs in the majority of patients with vivax malaria [9,50]. Acute respiratory distress syndrome (ARDS) has been described in at least 22 adults with *P. vivax*, most of whom had *P. falciparum* co-infection ruled out using PCR [3]. As in acute lung injury in other disease settings, ARDS in vivax malaria probably results from cytokine-related increases in alveolar permeability and altered alveolar fluid clearance [3,51]. Pulmonary phagocytic cell activity

is increased in uncomplicated vivax malaria [50], and pulmonary microvascular leukocyte accumulation and/or trapping is likely in vivax–ARDS [9]. Most, but not all, occurrences of vivax-associated ARDS develop after the start of antimalarial chemotherapy [3,9], and gas-transfer studies show progressive deterioration in alveolar-capillary function after treatment [9]. These findings are consistent with exacerbation of the inflammatory response associated with parasite killing and/or hemozoin. Lung-function studies partitioning gas transfer at the onset of treatment indicate possible encroachment on the pulmonary capillary volume by parasitized erythrocytes and accumulation or sequestration of *P. vivax*-infected erythrocytes within the pulmonary microvasculature [9]. If so, killing of such parasites by host immune responses or antimalarial drugs might result in inflammatory responses being targeted to the lung more than other organs. As chloroquine-resistant *P. vivax* spreads and other treatment regimens without the anti-inflammatory effects of chloroquine become necessary [2,52], there might be an increased incidence of ARDS in vivax malaria [9].

Acute lung injury is rare in young children, yet the majority of respiratory distress, in both falciparum and vivax malaria, occurs in this age group [5,6,53]. Therefore, whereas acute lung injury seems to be a major cause of vivax-associated respiratory distress in adults, other causes are likely to account for the distress in children. Metabolic acidosis [18,53], concurrent pneumonia [54], sepsis and severe anemia are important factors in respiratory distress in children with falciparum malaria, but prospective clinical studies are needed to determine the relative contributions of these processes in vivax-associated disease.

Coma

Coma associated with *P. vivax* is rare, and its etiology is the least well characterized of the syndromes associated with *P. vivax*. The recent large prospective studies from New Guinea did not exclude co-infection with *P. falciparum* by PCR, or other (e.g. bacterial or viral) infections or comorbidities [5,6]. The cytoadherence phenomena, described earlier, are thought to be central to the etiology of coma in falciparum malaria [18,44,55], but their role remains speculative in vivax-associated coma. Other potential factors include: concurrent infections, occult mixed plasmodium infections, metabolic changes, reversible local microvascular dysfunction, endothelial activation and injury, and microvascular thrombo-inflammatory responses.

Pregnancy-associated malaria and low birth weight

Although *P. vivax* is infrequently associated with severe malaria in pregnant women [56], it is responsible for maternal anemia and a reduction in birth weight (the birth weight being ~60% of that observed after maternal falciparum malaria) [56,57]. Because low birth weight is associated with higher infant mortality [58], *P. vivax* in pregnancy can be considered severe malaria from a public health perspective because it is responsible for substantial indirect delayed infant mortality. Low birth weight in falciparum malaria is thought to result from compromised materno–fetal circulation, arising from placental sequestration of *P. falciparum* [20]. By contrast, *P. vivax* does not seem to sequester in the placenta, although published data are limited [59]. Placental deposition of hemozoin is notable, but inflammatory and pathological changes are otherwise modest [59]. Maternal anemia, common in vivax malaria [56], is known to cause low birth weight [20]. Systemic and local inflammatory responses and microvascular dysfunction from vivax malaria might have additional deleterious utero-placental hemodynamic effects [20,59].

Other severe manifestations

Malnutrition

In past centuries, vivax malaria was associated with chronic relapsing–remitting debilitating fevers, hypoproteinemia, edema and kwashiorkor-like weight loss [60]. Malnutrition is clearly associated with vivax malaria in early childhood [61]. Although the underlying mechanisms are not clear, they are likely to involve deleterious catabolic responses associated with chronic relapsing–remitting inflammation.

Splenic rupture

Splenic rupture has long been recognized as a life-threatening complication of *P. vivax* infection but is uncommon in comparison to other manifestations of severe disease [1]. Rupture is frequently preceded by thrombocytopenia and splenic hematoma(s). The histopathology of ruptured spleens appears otherwise similar to the acute changes seen in malaria [25].

Thrombocytopenia

Vivax-associated thrombocytopenia is common [3], with multiple mechanisms resulting in peripheral destruction and splenic sequestration [62]. The roles of platelet activation, microvascular aggregation and microthrombotic processes in platelet destruction and severe vivax are not understood clearly.

Acute renal failure and shock

Only recently described [3,63,64], these manifestations warrant further prospective clinico-pathophysiogic and post-mortem characterization and exclusion of concurrent bacterial sepsis. Descriptions of vivax-associated renal failure associated with thrombotic microangiopathy [43] and hemolytic uremic syndrome [65] indicate that, in some circumstances, *P. vivax* might cause acute microvascular thrombosis, endothelial injury and thrombocytopenia similar to thrombotic-thrombocytopenic purpura.

Host, parasite and other factors increasing risk of severe disease

As in falciparum malaria [18], host, parasite, geographic and social factors are likely to explain variation, not only in the overall risk of severe disease in vivax malaria but also in differential risk in severe disease phenotype.

Host genetics

Some *P. vivax*-exposed populations (e.g. in New Guinea) have a higher risk of severe anemia than others. This might be explained, in part, by host genetics, including

differences in the prevalence of heritable hemoglobinopathies such as α^+-thalassemia. In *P. falciparum* infections, the microcytosis of homozygous α^+-thalassemia reduces the risk of severe anemia because hemolysis of a given proportion of RBCs results in a smaller overall reduction in hemoglobin [66]. Fowkes *et al.* [66] propose that protection against *P. vivax*-associated anemia will be similar, noting that the highest frequencies of α^+-thalassemia are found in *P. vivax*-endemic areas. Supporting this, the two largest studies of severe vivax-associated anemia have compared two different Melanesian populations in New Guinea, with very different prevalences of both α^+-thalassemia and severe vivax anemia [5,6]. The PNG population comprised lowland Melanesians with a high (>60%) prevalence of α^+-thalassemia [6]. By contrast, the Melanesian population studied in Indonesian Papua was mostly migrant highlanders [5] with a low prevalence of α^+-thalassemia [67]. Severe anemia was more prevalent in the vivax-infected highland population [5] than the vivax-infected lowland population [6]. Although differences in the prevalence of drug resistance are also likely to be important, variations in hemoglobinopathy prevalence probably contribute significantly to differential risk of severe anemia.

Notwithstanding differences in α^+-thalassemia and vivax anemia prevalence between eastern and western New Guinea, it is unclear why the risk of severe vivax anemia is higher in Melanesians than in other vivax-endemic areas. In relation to other populations, Melanesian populations have lower erythrocyte expression of complement receptor-1 (CR1) [68]. During malaria, immune complexes and complement are deposited on the surface of infected and non-infected RBCs. CR1-associated removal of immune complexes by splenic macrophages enables RBCs to return to the circulation [69]. Accordingly, decreased CR1 expression leads to increased susceptibility to splenic phagocytosis and destruction and is associated with severe falciparum-associated anemia [69]. Reduced expression in Melanesians of CR1, the ligand responsible for rosetting, is hypothesized to reduce the risk of coma in older PNG children relative to African populations but increase the risk of severe anemia in younger children [68,69]. Higher RBC CR1 expression in *P. vivax*-exposed non-Melanesian populations [68,69] might contribute to their lower rates of vivax-associated severe anemia [5,6].

P. vivax merozoites are usually dependent on a single receptor, the Duffy antigen, to invade host erythrocytes, although they can have alternative invasion pathways [70]. The lack of the Duffy receptor in West African populations accounts for the low morbidity from *P. vivax* in this region [2].

Age

In most areas of mixed endemicity, *P. vivax* has a higher incidence of infection in infancy than *P. falciparum* [5,71]. In comparison with older children, young children produce lower concentrations of the pro-erythropoietic cytokine, interleukin-10, in response to inflammatory stimuli [72] and have lower expression of CR1 on their RBCs [73]. These age-related factors make it more likely that malaria in infancy will lead to anemia than malaria in older children and might contribute to the burden of *P. vivax*-associated severe anemia being highest in infancy [5].

Chloroquine resistance

In Africa, the rise of chloroquine resistance in *P. falciparum* paralleled an increase in severe malarial anemia and mortality [74]. The high prevalence of chloroquine-resistant *P. vivax* found in Indonesian Papua [52,75] is likely to be an important contributor to the incremental risk of severe vivax anemia [5] over areas with less chloroquine resistance [6]. As with *P. falciparum*, chloroquine resistance in *P. vivax* seems to be associated with faster parasite growth rates [76], a phenotype associated with severe falciparum malaria [77]. Faster growth rates in chloroquine-resistant *P. vivax* might similarly contribute to increased risk of severe disease.

Virulence factors?

Whether *P. vivax* possesses virulence factors that increase the risk of severe vivax malaria is unknown. Is there geographic and pathogenic variation among a hypothesized complex of *P. vivax* species [78]? What is the role of the *P. vivax* variant gene family, *vir*, in pathogenesis [15,79]? Studies of the genome [15] and transcriptome [80] of *P. vivax* from different disease phenotypes might shed light on these questions.

Mixed infections

Although earlier studies in Thailand showed that mixed infections with *P. vivax* seem to attenuate *P. falciparum* disease severity [81], recent studies in areas with drug resistance to both species demonstrate that mixed infections are associated with an increased risk of severe malaria, particularly severe anemia [5,6]. This might represent the additive effects of higher total parasite biomass and repeated exposures to each of these *Plasmodium* spp. from recrudescences, reinfections and relapses.

Geographic and social factors

As with falciparum malaria [18], sociocultural factors almost certainly play a key part in severe vivax malaria. Lack of access to primary health and antenatal care, inadequate prevention measures, ineffective antimalarial drugs, and inability to transfuse will contribute to disease severity and poor outcome. In Papua, migration of poorly immune adults from non-endemic regions has resulted in a high incidence of uncomplicated and severe vivax malaria in adults [5], an age group that is less commonly affected in areas of stable transmission without migration [6]. As with falciparum malaria, differences in transmission intensity might contribute to variation in the spectrum of severe disease [18], with higher vivax-transmission intensities seen in New Guinea than in other vivax-endemic areas.

Mortality from vivax malaria

Prospective studies from Papua have demonstrated a mortality rate of 1.6% among patients hospitalized with *P. vivax*, comparable to the 2.2% mortality rate seen with *P. falciparum* [5]. However, it is unclear how many of these deaths occur as a direct result of *P. vivax*. Further studies are needed to define *P. vivax*-attributable fractions for

Box 1. Morbidity from *P. vivax infection:* outstanding research questions

Spectrum of disease

What is the true spectrum of morbidity in vivax malaria?

What proportion of severe disease associated with *P. vivax* is actually attributable to *P. vivax*? What is the contribution of comorbidities to severe disease?

Is severe disease more common in Melanesia and why?

How often is *P. vivax* a primary rather than contributory cause of death or simply incidental?

Pathobiology

Does *P. vivax* cytoadhere and sequester to microvasculature and leukocytes?

If so, what are the ligands and receptors involved?

What is the clinical relevance of other *ex-vivo* cytoadherent phenomena (e.g. rosetting, leukocyte and platelet aggregation)?

What are the *P. vivax* toxin(s), and how does *P. vivax* GPI and hemozoin-associated DNA differ from that of *P. falciparum*?

Are some strains more virulent than others?

What is the role of the *vir* gene family in pathogenesis?

Do polyclonal infections select for increased virulence? [84]

Pathophysiology

What are the molecular mechanisms of the lower pyrogenic threshold in *P. vivax* vs *P. falciparum*?

What are the mechanisms of severe anemia in vivax malaria?

How does *P. vivax* cause respiratory distress in children? What is the role of acidosis, anemia and concurrent pneumonia?

How does vivax malaria cause coma?

How does *P. vivax* infection affect placental function and cause low birth weight?

What are the mechanisms by which *P. vivax* causes or contributes to death?

What is the role of endothelial activation and injury, thrombocytopenia, platelet activation and microvascular thrombo-inflammatory pathology?

What are the host genetic and other determinants of vivax morbidity?

Does co-infection with *P. vivax* and *P. falciparum* protect from or worsen severe malaria, and how?

severe and fatal disease [82] and the mechanisms underlying death.

Comorbidities

To what extent does vivax malaria contribute to death, rather than being the primary cause? Our unpublished data from Papua indicate that many infants who die with *P. vivax* have radiological evidence of pneumonia. Vivax-associated anemia is likely to exacerbate hypoxia and reduce the ability to survive pneumonia. Similarly, a review of all malaria deaths in the USA found that 5% were due to *P. vivax* associated with cardiac disease [83]. The hemodynamic consequences of vivax-associated fever or anemia might result in decompensation of chronic disease and a fatal outcome, whereas otherwise healthy people could have made an uncomplicated recovery. Comorbidities are common in malaria-endemic regions, and their importance in contributing to severe and fatal vivax malaria is likely to be underestimated.

A host of unanswered questions

Compared to the pathophysiology of falciparum malaria, there are remarkably large knowledge gaps for vivax malaria (Box 1). The true spectrum of clinical disease in vivax malaria in endemic areas remains unknown, as do the mechanisms underlying such disease. Prospective clinical, pathophysiological and autopsy studies are required in settings with different transmission and prevalence of chloroquine resistance to characterize the clinical spectrum and magnitude of morbidity and mortality from *P. vivax,* the role of comorbidities, and the mechanisms underlying severe disease (Table 1).

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References

1 Baird, J.K. (2007) Neglect of *Plasmodium vivax* malaria. *Trends Parasitol.* 23, 533–539

2 Price, R.N. *et al.* (2007) Vivax malaria: neglected and not benign. *Am. J. Trop. Med. Hyg.* 77, 79–87

3 Tan, L.K. *et al.* (2008) Acute lung injury and other serious complications of *Plasmodium vivax* malaria. *Lancet Infect. Dis.* 8, 449–454

4 Barcus, M.J. *et al.* (2007) Demographic risk factors for severe and fatal vivax and falciparum malaria among hospital admissions in northeastern Indonesian Papua. *Am. J. Trop. Med. Hyg.* 77, 984–991

5 Tjitra, E. *et al.* (2008) Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. *PLoS Med.* 5, e128

6 Genton, B. *et al.* (2008) *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. *PLoS Med.* 5, e127

7 Field, J. and Shute, P. (1956) *Plasmodium vivax*. In *The microscopuic diagnosis of human malaria. Vol. 2. A morphological study of the erythrocytic parasites*, Institute for Medical Research

8 Karyana, M. *et al.* (2008) Malaria morbidity in Papua Indonesia, an area with multidrug resistant *Plasmodium vivax* and *Plasmodium falciparum*. *Malar. J.* 7, 148

9 Anstey, N.M. *et al.* (2007) Lung injury in vivax malaria: pathophysiological evidence for pulmonary vascular sequestration and posttreatment alveolar-capillary inflammation. *J. Infect. Dis.* 195, 589–596

10 Hemmer, C.J. *et al.* (2006) Stronger host response per parasitized erythrocyte in *Plasmodium vivax* or *ovale* than in *Plasmodium falciparum* malaria. *Trop. Med. Int. Health* 11, 817–823

11 Karunaweera, N.D. *et al.* (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. U. S. A.* 89, 3200–3203

12 Boutlis, C.S. *et al.* (2005) Glycosylphosphatidylinositols in malaria pathogenesis and immunity: potential for therapeutic inhibition and vaccination. *Curr. Top. Microbiol. Immunol.* 297, 145–185

13 Parroche, P. *et al.* (2007) Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1919–1924

14 Gardner, M.J. *et al.* (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 419, 498–511

15 Carlton, J.M. *et al.* (2008) Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature* 455, 757–763

16 Karunaweera, N. *et al.* (2007) Plasmodium vivax: paroxysm-associated lipids mediate leukocyte aggregation. *Malar. J.* 6, 62

17 Karunaweera, N.D. *et al.* (2003) The paroxysm of *Plasmodium vivax* malaria. *Trends Parasitol.* 19, 188–193

18 Miller, L.H. *et al.* (2002) The pathogenic basis of malaria. *Nature* 415, 673–679

19 Chotinavich, K. *et al.* (2003) The adhesion receptors of *P. vivax*-infected red cells. *Exp. Parasitol.* 105, 33–34

20 Rogerson, S.J. *et al.* (2007) Malaria in pregnancy: pathogenesis and immunity. *Lancet Infect. Dis.* 7, 105–117

21 Traore, B. *et al.* (2000) Cytoadherence characteristics of *Plasmodium falciparum* isolates in Thailand using an *in vitro* human lung endothelial cells model. *Am. J. Trop. Med. Hyg.* 62, 38–44
22 Robert, C. *et al.* (1995) Chondroitin-4-sulphate (proteoglycan), a receptor for *Plasmodium falciparum*-infected erythrocyte adherence on brain microvascular endothelial cells. *Res. Immunol.* 146, 383–393
23 Desowitz, R.S. *et al.* (1969) The sites of deep vascular schizogony in *Plasmodium coatneyi* malaria. *Trans. R. Soc. Trop. Med. Hyg.* 63, 198–202
24 Lovegrove, F.E. *et al.* (2008) Parasite burden and CD36-mediated sequestration are determinants of acute lung injury in an experimental malaria model. *PLoS Pathog.* 4, e1000068
25 Clark, H. and Tomlinson, W. (1949) The pathologic anatomy of malaria. In *Malariology* (Boyd, M., ed.), pp. 874–903, WB Saunders
26 Billings, F. and Post, W. (1915) Fatal malaria due to the tertian parasite. *Trans. Chicago Path. Soc.* 9, 209–215
27 Bruetsch, W. (1932) The histopathology of therapeutic (tertian) malaria. *Am. J. Psychiatry* 12, 19–65
28 Pongponratn, E. *et al.* (2003) An ultrastructural study of the brain in fatal *Plasmodium falciparum* malaria. *Am. J. Trop. Med. Hyg.* 69, 345–359
29 Carlson, J. *et al.* (1990) Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet* 336, 1457–1460
30 Pain, A. *et al.* (2001) Platelet-mediated clumping of *Plasmodium falciparum*-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1805–1810
31 Udomsanpetch, R. *et al.* (1995) Rosette formation by *Plasmodium vivax*. *Trans. R. Soc. Trop. Med. Hyg.* 89, 635–637
32 Wassmer, S.C. *et al.* (2008) Platelet-induced clumping of *Plasmodium falciparum*-infected erythrocytes from malawian patients with cerebral malaria – possible modulation *in vivo* by thrombocytopenia. *J. Infect. Dis.* 197, 72–78
33 Suwanarusk, R. *et al.* (2004) The deformability of red blood cells parasitized by *Plasmodium falciparum* and *P. vivax*. *J. Infect. Dis.* 189, 190–194
34 Handayani, S. *et al.* (2009) High deformability of *Plasmodium vivax*-infected red blood cells under microfluidic conditions. *J. Infect. Dis.* 199, 445–450
35 Baird, J.K. *et al.* (2007) Prevention and treatment of vivax malaria. *Curr. Infect. Dis. Rep.* 9, 39–46
36 Boyd, M. (1938) The threshold of parasite density in relation to clinical activity in primary infections with *Plasmodium vivax*. *Am. J. Trop. Med. Hyg.* 18, 497–503
37 Boutlis, C.S. *et al.* (2006) Malaria tolerance – for whom the cell tolls? *Trends Parasitol.* 22, 371–377
38 Yeo, T.W. *et al.* (2007) Impaired nitric oxide bioavailability and L-arginine reversible endothelial dysfunction in adults with falciparum malaria. *J. Exp. Med.* 204, 2693–2704
39 Yeo, T.W. *et al.* (2008) Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria. *Proc. Natl. Acad. Sci. U. S. A.* 105, 17097–17102
40 Jakobsen, P.H. *et al.* (1994) Increased plasma concentrations of sICAM-1, sVCAM-1 and sELAM-1 in patients with *Plasmodium falciparum* or *Plasmodium vivax* malaria and association with disease severity. *Immunology* 83, 665–669
41 Ohnishi, K. (1999) Serum levels of thrombomodulin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin in the acute phase of *Plasmodium vivax* malaria. *Am. J. Trop. Med. Hyg.* 60, 248–250
42 de Mast, Q. *et al.* (2009) ADAMTS13 deficiency with elevated levels of ultra-large and active von Willebrand factor in malaria. *Am. J. Trop. Med. Hyg.* 80, 492–498
43 Saharan, S. *et al.* (2008) Thrombotic microangiopathy associated with *Plasmodium vivax* malaria. *Pediatr. Nephrol.* 24, 623–624
44 Dondorp, A.M. *et al.* (2008) Direct *in vivo* assessment of microcirculatory dysfunction in severe falciparum malaria. *J. Infect. Dis.* 197, 79–84
45 Calis, J.C. *et al.* (2008) Severe anemia in Malawian children. *N. Engl. J. Med.* 358, 888–899
46 Collins, W.E. *et al.* (2003) A retrospective examination of anemia during infection of humans with *Plasmodium vivax*. *Am. J. Trop. Med. Hyg.* 68, 410–412
47 Jakeman, G.N. *et al.* (1999) Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. *Parasitology* 119, 127–133
48 Price, R.N. *et al.* (2001) Factors contributing to anemia after uncomplicated falciparum malaria. *Am. J. Trop. Med. Hyg.* 65, 614–622
49 Wickramasinghe, S.N. *et al.* (1989) Dyserythropoiesis and ineffective erythropoiesis in *Plasmodium vivax* malaria. *Br. J. Haematol.* 72, 91–99
50 Anstey, N.M. *et al.* (2002) Pulmonary manifestations of uncomplicated falciparum and vivax malaria: cough, small airways obstruction, impaired gas transfer, and increased pulmonary phagocytic activity. *J. Infect. Dis.* 185, 1326–1334
51 Suratt, B.T. and Parsons, P.E. (2006) Mechanisms of acute lung injury/acute respiratory distress syndrome. *Clin. Chest Med.* 27, 579–589
52 Baird, J.K. (2004) Chloroquine resistance in *Plasmodium vivax*. *Antimicrob. Agents Chemother.* 48, 4075–4083
53 Marsh, K. *et al.* (1995) Indicators of life-threatening malaria in African children. *N. Engl. J. Med.* 332, 1399–1404
54 O'Dempsey, T.J. *et al.* (1993) Overlap in the clinical features of pneumonia and malaria in African children. *Trans. R. Soc. Trop. Med. Hyg.* 87, 662–665
55 Taylor, T.E. *et al.* (2004) Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nat. Med.* 10, 143–145
56 Poespoprodjo, J.R. *et al.* (2008) Adverse pregnancy outcomes in an area where multidrug-resistant plasmodium vivax and *Plasmodium falciparum* infections are endemic. *Clin. Infect. Dis.* 46, 1374–1381
57 Nosten, F. *et al.* (1999) Effects of *Plasmodium vivax* malaria in pregnancy. *Lancet* 354, 546–549
58 Luxemburger, C. *et al.* (2001) Effects of malaria during pregnancy on infant mortality in an area of low malaria transmission. *Am. J. Epidemiol.* 154, 459–465
59 McGready, R. *et al.* (2004) The effects of *Plasmodium falciparum* and *P. vivax* infections on placental histopathology in an area of low malaria transmission. *Am. J. Trop. Med. Hyg.* 70, 398–407
60 Hutchinson, R.A. and Lindsay, S.W. (2006) Malaria and deaths in the English marshes. *Lancet* 367, 1947–1951
61 Williams, T.N. *et al.* (1997) *Plasmodium vivax*: a cause of malnutrition in young children. *QJM* 90, 751–757
62 Erhart, L.M. *et al.* (2004) Hematologic and clinical indices of malaria in a semi-immune population of western Thailand. *Am. J. Trop. Med. Hyg.* 70, 8–14
63 Kochar, D.K. *et al.* (2005) *Plasmodium vivax* malaria. *Emerg. Infect. Dis.* 11, 132–134
64 Chung, B.H. *et al.* (2008) Predictors of *Plasmodium vivax* malaria-induced nephropathy in young Korean men. *Nephron* 110, c172–c177
65 Sharma, J. *et al.* (1993) *Plasmodium vivax* malaria presenting as hemolytic–uremic syndrome. *Indian Pediatr.* 30, 369–371
66 Fowkes, F.J. *et al.* (2008) Increased microerythrocyte count in homozygous α^{+}-thalassaemia contributes to protection against severe malarial anaemia. *PLoS Med.* 5, e56
67 Hill, A. *et al.* (1989) Haemoglobin and globin gene variants in the Pacific. In *The colonization of the Pacific. A genetic trail* (Hill, A. and Serjeanstson, S., eds), p. 248, Clarendon Press
68 Cockburn, I.A. *et al.* (2004) A human complement receptor 1 polymorphism that reduces *Plasmodium falciparum* rosetting confers protection against severe malaria. *Proc. Natl. Acad. Sci. U. S. A.* 101, 272–277
69 Kai, O.K. and Roberts, D.J. (2008) The pathophysiology of malarial anaemia: where have all the red cells gone? *BMC Med.* 6, 24
70 Ryan, J.R. *et al.* (2006) Evidence for transmission of *Plasmodium vivax* among a duffy antigen negative population in Western Kenya. *Am. J. Trop. Med. Hyg.* 75, 575–581
71 Maitland, K. *et al.* (1996) The interaction between *Plasmodium falciparum* and *P. vivax* in children on Espiritu Santo island, Vanuatu. *Trans. R. Soc. Trop. Med. Hyg.* 90, 614–620
72 Nussenblatt, V. *et al.* (2001) Anemia and interleukin-10, tumor necrosis factor alpha, and erythropoietin levels among children with acute, uncomplicated *Plasmodium falciparum* malaria. *Clin. Diagn. Lab. Immunol.* 8, 1164–1170
73 Waitumbi, J.N. *et al.* (2004) Age-related changes in red blood cell complement regulatory proteins and susceptibility to severe malaria. *J. Infect. Dis.* 190, 1183–1191

74 Trape, J.F. *et al.* (1998) Impact of chloroquine resistance on malaria mortality. *Comptes rendus de l'Academie des Sciences* 321, 689–697
75 Suwanarusk, R. *et al.* (2007) Chloroquine resistant *Plasmodium vivax*: *in vitro* characterisation and association with molecular polymorphisms. *PLoS One* 2, e1089
76 Russell, B. *et al.* (2008) Determinants of *in vitro* drug susceptibility testing of *Plasmodium vivax*. *Antimicrob. Agents Chemother.* 52, 1040–1045
77 Chotivanich, K. *et al.* (2000) Parasite multiplication potential and the severity of falciparum malaria. *J. Infect. Dis.* 181, 1206–1209
78 Li, J. *et al.* (2001) Geographic subdivision of the range of the malaria parasite *Plasmodium vivax*. *Emerg. Infect. Dis.* 7, 35–42
79 Fernandez-Becerra, C. *et al.* (2009) *Plasmodium vivax* and the importance of the subtelomeric multigene vir superfamily. *Trends Parasitol.* 25, 44–51
80 Bozdech, Z. *et al.* (2008) The transcriptome of *Plasmodium vivax* reveals divergence and diversity of transcriptional regulation in malaria parasites. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16290–16295
81 Snounou, G. and White, N.J. (2004) The co-existence of *Plasmodium*: sidelights from falciparum and vivax malaria in Thailand. *Trends Parasitol.* 20, 333–339
82 Anstey, N.M. and Price, R.N. (2007) Improving case definitions for severe malaria. *PLoS Med.* 4, e267
83 Stoppacher, R. and Adams, S.P. (2003) Malaria deaths in the United States: case report and review of deaths, 1979-1998. *J. Forensic Sci.* 48, 404–408
84 Havryliuk, T. and Ferreira, M.U. (2009) A closer look at multiple-clone *Plasmodium vivax* infections: detection methods, prevalence and consequences. *Mem. Inst. Oswaldo Cruz.* 104, 67–73

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