VOLUME 19 SUPPL

Severe Malaria

Section 1: Epidemiology of severe falciparum malaria

When an individual has been inoculated with a *plasmodium* parasite, a variety of clinical effects may follow, within the sequence:

Infection→asymptomatic parasitaemia→uncomplicated illness→severe malaria→death.

Many factors influence the disease manifestations of the infection and the likelihood of progression to the last two categories. These factors include the species of the infecting parasite, the levels of innate and acquired immunity of the host, and the timing and efficacy of treatment, if any.

Plasmodium falciparum is the major cause of severe malaria

Progression to severe and fatal disease is largely but not entirely confined to *P. falciparum* infections; in this section and in most of this document, we will discuss severe malaria caused by *P. falciparum*. Although they contribute much less than *P. falciparum* to the global burden of severe malaria, both *P. vivax* and *P. knowlesi* can also cause severe disease and they do kill; these infections are discussed separately in Sections 13 and 14.

Problems in determining the epidemiology of severe malaria

An accurate description of the incidence and distribution of severe malaria requires identification of cases, and several factors make this problematic. (i) Malaria is most prevalent where there is poverty and where methods of disease identification, documentation and reporting are weakest. (ii) A large proportion of severe malaria illnesses and deaths occur in people's homes without coming to the attention of a formal health service: for children under 5 years of age, this proportion has been estimated at 90% in The Gambia (Greenwood *et al.* 1987) and at 49% more recently in Zambia (Mudenda *et al.* 2011). 'Verbal autopsies' have been used to identify causes of death in community surveys, but their accuracy for malaria is modest because severe malaria has no features by which it can be confidently distinguished from many other fatal febrile conditions in the absence of laboratory tests (Snow *et al.* 1992; Mudenda *et al.* 2011). (iii) Even when severe malaria is documented in a health facility, the diagnosis may be missed or wrongly applied to patients without malaria (Reyburn *et al.* 2004; Taylor *et al.* 2004). Recent estimates based on verbal autopsies suggest that there is a substantial mortality from malaria in older adults (Dhingra *et al.* 2010; Murray *et al.* 2012), but this is not borne out by hospital-based studies or clinical observation (Lynch *et al.* 2012; White *et al.* 2012). In endemic areas, severe malaria is very unusual in the elderly. [For discussion of problems in malaria diagnosis, see *Section 9*]. An alternative approach to counting malaria deaths is to assume a contribution from malaria to all-cause mortality based on data from countries with very good diagnostic and reporting systems (Black *et al.* 2010). Mathematical modelling can then be used to predict how various measurable indices might modify malaria mortality in different countries.

Estimated size and distribution of the problem of severe malaria

Recent data suggest that there were around 627 000 deaths from malaria worldwide in 2012 (World Health Organization 2013). These were deaths directly attributable to malaria (malaria also kills indirectly by reducing birthweight and debilitating children with repeated infections) and so would usually have been preceded by severe illness. With fewer than half of those who suffer severe malaria being able to reach a health facility, and assuming a case-fatality rate of 90% at home and 20% in hospital (Thwing *et al.* 2011), the global annual incidence of severe malaria can be estimated at approximately 2 million cases. In parts of the world where the transmission of *P. falciparum* is intense and stable, severe malaria is mainly a disease of children from the first few months of life to the age of about 5 years, becoming less common in older children and adults as specific acquired immunity gives increasing (although always incomplete) protection. About 90% of the world's severe and fatal malaria is estimated to affect young children in sub-Sahara Africa (Black *et al.* 2010). In areas of lower endemicity, severe malaria occurs in both adults and children. Non-immune travellers and migrant workers are vulnerable to severe

Publication of this supplement was sponsored jointly by the World Health Organization, the Medicines for Malaria Venture, Roll Back Malaria and The Wellcome Trust.

malaria, irrespective of the endemicity of the area where their infection was acquired. Early large-scale intervention studies with insecticide-treated bednets (ITN) suggested that malaria contributed to as much as half of all mortality in children aged between 1 month and 5 years (Alonso *et al.* 1993; Nevill *et al.* 1996). A later systematic literature review concluded that for the year 2000, an estimated 545 000 (uncertainty interval: 105 000–1 750 000) children under the age of 5 in sub-Saharan Africa were admitted to hospital for an episode of severe malaria (Roca-Feltrer *et al.* 2008).

Differences in clinical features of severe malaria between adults and children

The pattern of syndromes in severe malaria differs between children and adults (see Table 1). It is uncertain whether these differences reflect mainly the age of affected individuals or other differences between populations in the characteristics of host, parasite, pattern of exposure or provision of health services. There are few data on the pattern of clinical disease in children outside Africa (Dondorp *et al.* 2008b; Nanda *et al.* 2011).

Differing severe malaria syndrome patterns in African children according to transmission intensity

Studies of hospital admissions in different geographical sites within high transmission areas in Africa have consistently reported that the median age of patients is inversely proportional to transmission intensity (Slutsker *et al.* 1994; Modiano *et al.* 1998; Idro *et al.* 2006a; Okiro *et al.* 2009). In populations subjected to very high inoculation rates year-round, severe anaemia is the most common complication of *P. falciparum* infection, affecting mainly infants and very young children, while in areas with less intense or seasonal transmission, cerebral malaria in slightly older children may predominate (Snow *et al.* 1994, 2005; Slutsker *et al.* 1994; Modiano *et al.* 1998; Snow & Marsh 1998). Reyburn *et al.* (2005) described the distribution of severe malaria syndromes and fatalities among 1984 patients admitted with severe malaria to 10 hospitals serving populations living at elevations ranging from high (altitude >1200 m, very low *P. falciparum* transmission intensity) to low (altitude <600 m, very intense transmission) in north-eastern Tanzania. The mean age of severe malaria admissions was lowest in the most intense transmission area, where severe anaemia predominated, and highest in the low transmission area, where cerebral malaria predominated and case-fatality rates were highest. Systematic reviews of published articles reporting syndromes, ages and transmission patterns have confirmed this (Roca-Feltrer *et al.* 2008; Carneiro *et al.* 2010). In a study based in a Kenyan district hospital, a declining incidence of malaria admissions was accompanied by an increase in the mean age of children admitted with malaria and by an increase in the ratio of cerebral malaria to severe anaemia cases

Table 1 Severe manifestations of *Plasmodium falciparum* malaria in adults and children

Prognostic value (+ to +++)			Frequency (+ to +++)	
Children	Adults	Clinical manifestations	Children	Adults
+++	+++	Impaired consciousness	+++	++
+++	+++	Respiratory distress (acidotic breathing)	+++	++
+	++	Multiple convulsions	+++	+
+	+	Prostration	+++	+++
+++	+++	Shock	+	+
+++	+++	Pulmonary oedema (radiological)	+/−*	+
+++	++	Abnormal bleeding	+/−*	+
++	+	Jaundice	+	+++
		Laboratory indices		
+	+	Severe anaemia	+++	+
+++	+++	Hypoglycaemia	+++	++
+++	+++	Acidosis	+++	++
+++	+++	Hyperlactataemia	+++	++
++	++	Renal impairment†	+	+++
+/−	++	Hyperparasitaemia	++	+

*Infrequent.
†Acute kidney injury.

from 0.2 to 1.0 between 1999 and 2007 (O'Meara *et al.* 2008). This change in ratio resulted from a fall in the incidence of severe anaemia, not from an absolute increase in the incidence of cerebral malaria.

Impact of malaria control measures on the incidence and pattern of severe malaria

The estimated mortality from malaria of 781 000 in 2009 and 627 000 in 2010 represents a decline from 985 000 in the year 2000 (World Health Organization 2010b). The role of malaria control measures in causing this reduction cannot be assumed, but several lines of evidence point to a causal link between deployment of effective vector control measures (ITNs, insecticides) and effective drugs (ACTs) and declining mortality. Causality is likely when there are strong geographical associations of control measures with improvements, absence of changes in some other causes of death and consistency with predictions of the likely effects of malaria-specific interventions (Steketee & Campbell 2010). Akachi and Atun (2011) estimated that increased coverage with ITN and indoor residual spraying prevented around 240 000 child deaths in sub-Saharan Africa between 2002 and 2008. Hospital admissions and deaths, with identification of parasitaemia, provide the most dependable indicators of the likely incidence of severe malaria in a population, and dramatic falls in these events have been recorded in several African countries, notably Sao Tome & Principe, Madagascar, Eritrea, Ethiopia, Zambia and Zanzibar in Tanzania, between 2000 and 2009 (Bhattarai *et al.* 2007; Graves *et al.* 2008; Teklehaimanot *et al.* 2009; Steketee & Campbell 2010). The marked decline in malaria morbidity and mortality in Vietnam during the 1990s was attributed to deployment of artemisinin and improved access to treatment. On the island of Zanzibar, deployment of ACTs in late 2003 was associated with a 75% reduction in malaria-attributable mortality in children under 5 years over the next 2 years (Bhattarai *et al.* 2007). The containment of the 1995–2000 malaria epidemic in KwaZulu Natal was attributed to deployment of ACTs and indoor residual spraying with effective insecticide (Barnes *et al.* 2005). In some countries where there have been impressive reductions in hospital admissions and deaths due to malaria, there has been a later resurgence that may reflect lack of sustained resources for the local malaria control programme (Hamel *et al.* 2011). In some African countries, no decline in severe or fatal malaria is yet detectable (Roca-Feltrer *et al.* 2008). Reliable monitoring of hospital admissions and deaths will remain an important tool for measuring the impact of control measures on the incidence of severe and fatal malaria during the coming decades, as there is a worldwide effort to move towards malaria elimination and eventual eradication (Rajaratnam *et al.* 2010).

Section 2: Definitions of severe malaria

Severe malaria by definition is associated with a high mortality. From a clinical perspective, there is a continuum from asymptomatic malaria to uncomplicated illness through to severe and lethal malaria. Before artemisinin combination treatments (ACT) became widely available, *uncomplicated falciparum malaria* was associated with a case-specific mortality of approximately 0.1% when there was ready access to effective antimalarial drug treatment. Thus, 1 patient in 1000 who presented with apparently uncomplicated falciparum malaria would deteriorate despite treatment and die. With worsening resistance and/or delays in access to effective drugs mortality approached 1 in 100 (1%). The mortality with ACTs is lower than 0.1% as artemisinins are particularly effective in the group of patients with high ring-stage parasitaemias who may appear only mildly ill, but then deteriorate rapidly coincident with extensive parasite red cell sequestration. The mortality of *P. knowlesi* infections is higher, but the mortality associated with the other malarias is substantially lower than for falciparum malaria. The exception is on the island of New Guinea, where there is intense transmission of both *P. falciparum* and *P. vivax*, and recurrent infections result in severe anaemia.

Definitions of severe falciparum malaria

In a patient with a *P. falciparum* asexual parasitaemia and no other confirmed cause for their symptoms or signs, the presence of one or more of the clinical or laboratory features in Table 1 classifies that patient as suffering from severe malaria. Many of the features in Table 1 are not independent. It is not always clear which have the strongest prognostic significance, and not all can be recorded with equal ease. Inclusive definitions to guide clinical management are outlined in Tables 2 and 3, and a more specific definition of severe falciparum malaria for epidemiological or research purposes is detailed in Table 4. In recent years, several different prognostic scores have been developed both for adults and for children with severe malaria. These reflect that the two main determinants of outcome in both adults and children are the level of consciousness and the degree of metabolic acidosis (Molyneux *et al.* 1989b; Marsh *et al.* 1995; Mishra *et al.* 2007c; Helbok *et al.* 2009; Hanson *et al.* 2010; Jallow *et al.* 2012; von Seidlein *et al.* 2012, Newton et al 2013). Coma is assessed clinically by coma scales, and acidosis is reflected clinically by the rate and depth of breathing (English *et al.* 1996b) although, if possible, measurement of plasma bicarbonate, base deficit or plasma lactate is more precise.

Clinical approach to the patient with suspected severe malaria. Any patient with malaria, who is unable to take medications reliably, has any evidence of vital organ dysfunction or has a high parasite count, is at increased risk of dying. The exact risk depends on the infecting malaria parasite species, the degree of abnormality, the number of systems affected, age, background immunity,

Table 2 Outline bedside clinical classification of severe malaria in children in a high transmission area

Group 1	Prostrate children (prostration is the inability to sit upright in a child normally able to do so or to drink in the case of children too young to sit). Three subgroups of increasing severity should be distinguished: Prostrate but fully conscious Prostrate with impaired consciousness but not in deep coma Coma (the inability to localise a painful stimulus) Respiratory distress (acidotic breathing): Mild – sustained nasal flaring and/or mild intercostal indrawing (recession) Severe – the presence of either marked indrawing (recession) of the bony structure of the lower chest wall or deep (acidotic) breathing Shock compensated or decompensated (see definition above)
Group 2	Children who, although able to be treated with oral antimalarials, require supervised management because of the risk of clinical deterioration but who show none of the features of group 1 (above)*. These include children with any of the following: Haemoglobin <5 g/dl or haematocrit < 15% 2 or more convulsions within a 24-h period Haemoglobinuria (blackwater) Jaundice
Group 3	Children who require parenteral treatment because of persistent vomiting but who lack any specific clinical or laboratory features of groups 1 or 2 (above)

*If parasite counts are immediately available, a parasitaemia over 10% should be included in group 2.
Children are defined as <12 years old.

Table 3 Outline bedside clinical classification of severe malaria in adults

Group 1	Adults at increased risk of dying immediately who require parenteral antimalarials and appropriate supportive therapy Prostrated or obtunded adults (prostration is the inability to sit or to drink). Four subgroups of increasing severity should be distinguished: Prostrate but fully conscious Prostrate with impaired consciousness but not in deep coma (GCS > 11) Confusion and agitation (GCS > 11) Coma (the inability to localise a painful stimulus) (GCS < 11) Respiratory distress (acidotic breathing) Mild – sustained nasal flaring and/or mild intercostal indrawing (recession) Severe – the presence of either marked indrawing (recession) of the bony structure of the lower chest wall or deep (acidotic) breathing Shock (hypotension:systolic BP < 80 mmHg) Anuria Significant upper gastrointestinal haemorrhage
Group 2	Adults who, although able to be treated with oral ACTs, require supervised management because of the risk of clinical deterioration but who show none of the features of group 1 (above)*. This group includes adults with any of the following: Haemoglobin <7 g/dl or haematocrit <20% One or more convulsions within a 24-h period Haemoglobinuria (blackwater) Jaundice
Group 3	Adults who require parenteral treatment because of persistent vomiting but who lack any specific clinical or laboratory features of groups 1 or 2 (above)

*If parasite counts are immediately available a parasitaemia over 4% should be included in group 2.

Table 4 Epidemiological and research definition of severe falciparum malaria

For epidemiological and research purposes, severe malaria is defined as one or more of the following, occurring in the absence of an identified alternative cause, and in the presence of *P. falciparum* asexual parasitaemia:

Impaired consciousness:	A Glasgow Coma Score <11 in adults or a Blantyre coma score <3 in children
Acidosis:	A base deficit of >8 meq/l or, if unavailable, a plasma bicarbonate of <15 mM or venous plasma lactate >5 mM. Severe acidosis manifests clinically as respiratory distress – rapid, deep and laboured breathing
Hypoglycaemia:	Blood or plasma glucose <2.2 mM (<40 mg/dl)
Severe malarial anaemia:	A haemoglobin concentration <5 g/dl or a haematocrit of <15% in children <12 years of age (<7 g/dl and <20%, respectively, in adults) together with a parasite count >10 000/μl
Renal impairment (acute kidney injury):	Plasma or serum creatinine >265 μM (3 mg/dl) or blood urea >20 mM
Jaundice:	Plasma or serum bilirubin >50 μM (3 mg/dl) together with a parasite count >100 000/μl
Pulmonary oedema:	Radiologically confirmed, or oxygen saturation <92% on room air with a respiratory rate >30/min, often with chest indrawing and crepitations on auscultation
Significant bleeding:	Including recurrent or prolonged bleeding from nose gums or venepuncture sites; haematemesis or melaena
Shock:	Compensated shock is defined as capillary refill ≥3 s or temperature gradient on leg (mid to proximal limb), but no hypotension. Decompensated shock is defined as systolic blood pressure <70 mm Hg in children or <80 mm Hg in adults with evidence of impaired perfusion (cool peripheries or prolonged capillary refill)
Hyperparasitaemia:	*P. falciparum* parasitaemia >10%

pre-morbid and concomitant diseases, and access to appropriate treatment. The patient must be assessed and treated without delay. A practical bedside approach to the immediate assessment and classification of children with suspected severe malaria is shown in Table 2 and for adults in Table 3. Tests such as the parasite count, haematocrit and blood glucose may all be determined immediately at the point of care, but results of other laboratory measures, if any, may be available only after hours or days. The start of treatment must not wait. As severe malaria is potentially fatal, any patient considered at increased risk should be given the benefit of the highest level of care available. The attending clinician should not worry unduly about definitions; the severely ill patient

Table 5 A coma scale for children ('Blantyre coma scale'). This scale is for children, including those who have not learned to speak

Best motor response	Score
localises painful stimulus*	2
Withdraws limb from pain†	1
Non-specific or absent response	0
Verbal response	
Appropriate cry	2
Moan or inappropriate cry	1
None	0
Eye movements	
Directed (e.g. follows mother's face)	1
Not directed	0
Total	0–5

Unrousable coma ≤2.
*Painful stimulus: rub knuckles on patient's sternum.
†Painful stimulus: firm pressure on thumbnail bed with horizontal pencil.

requires immediate supportive care, and if severe malaria is a possibility, parenteral antimalarial drug treatment should be started without delay. In summary - if in doubt, treat as severe malaria.

Patients with a high parasite count (>4%) in a low transmission setting but none of the clinical or laboratory indicators of severe malaria should be monitored closely, preferably in hospital for the first day of treatment. Although oral treatment with an artemisinin derivative is highly effective, provided there is no vomiting (Luxemburger *et al.* 1995), if there is any clinical doubt, treatment should be started with parenteral artesunate followed by an ACT.

Impaired consciousness. Before the general use of coma scales, the term '*cerebral malaria*' was used for patients with malaria who were unrousable, that is, unable to localise a painful stimulus. This has been superseded by a Glasgow coma score of less than 11 of 15 in adults (Teasdale & Jennett 1974) or a Blantyre coma score of less than 3 of 5 in children who are too young to speak (Molyneux *et al.* 1989b; Newton *et al.* 1997a). Many patients with malaria recover full consciousness after a convulsion, and so it is important to exclude transient post-ictal coma. Assessment for clinical management purposes should be made immediately, but for classification, cerebral malaria is coma which persists for > 1 h after a seizure irrespective of anticonvulsant medications.

The *Blantyre coma score* (Table 5) requires careful local standardisation. Care must be exercised in applying the painful stimuli; it is unkind and unnecessary to test responses repeatedly. Testing should begin with a minimal stimulus, which should be increased only to the point where a clear response is obtained. A localising response must be distinguished from a brisk flexion response. The interpretation of 'appropriate cry' is difficult, some children are stoical, and the appropriateness of verbal response needs to be considered in the light of other responses and the age of the child. It is best to test directed eye movement by asking the mother to move her face across the child's field of vision, as the child may be less interested in looking at any other face or object.

Respiratory distress (acidotic breathing). Deep, laboured, noisy and often rapid breathing (Kussmaul's breathing) with increased inspiratory and expiratory chest excursion is the most important respiratory sign of severe malaria. Patients with acidosis may sometimes have such deep laboured breathing that their respiratory rates are slow. In children, sustained nasal flaring and indrawing (recession) of the bony structures of the lower chest wall on inspiration should also be noted.

Multiple convulsions. Generalised seizures are common, particularly in children, with severe malaria. Commonly, convulsions, especially recurrent seizures, are subtle (resulting in little or no movement of limbs), and care should be taken to detect minor manifestations such as twitching of a digit, repetitive jerky eye movements with deviation, increased salivation or abnormal respiratory patterns.

Prostration is the inability, because of extreme weakness, to sit unassisted, in an adult or a child who is normally able to do so. In children not old enough to sit up, prostration is defined as the inability to breastfeed. Prostration must always be recorded directly and not based on history. Many patients who have a fever and feel unwell prefer to lie or, in the case of children, to be carried but are capable of sitting if gently encouraged to do so.

Shock. Compensated shock is defined as capillary refill ≥3 s or temperature gradient on leg (mid to proximal limb), but no hypotension after adequate rehydration. Decompensated shock is defined as systolic blood pressure <70 mm Hg in a child and <80 mm Hg in an adult, with cool peripheries (this assessment may vary between observers) and prolonged capillary refill ≥3 s after adequate rehydration. Accurate blood pressure measurement in young children depends on using the correct sphygmomanometer cuff size.

Pulmonary oedema is suspected in any patient who develops tachypnoea (respiratory rate >30/min), dyspnoea and hypoxia (oxygen saturation <92% on room air) with

chest signs of diffuse wheeze or crepitations. It is confirmed radiologically. Abnormal bleeding is rare in severe malaria and may manifest by bleeding from the gums, nose, gastrointestinal tract or venepuncture sites.

Jaundice. This is detected clinically by examining the sclera and/or mucosal surfaces of the mouth. Jaundice may occur in adults with uncomplicated malaria so for a precise epidemiological classification of severe malaria jaundice is defined as a combination of elevated plasma bilirubin (>50 μM or 3 mg/dl) together with a parasite count >100 000/μl.

Severe anaemia is suspected clinically from pale mucosal surfaces and palms and confirmed by measurement of haemoglobin concentration or packed cell volume. Severe anaemia is defined as a haemoglobin <5 g/dl or a haematocrit of <15% in children (age <12 years) and a haemoglobin <7 g/dl or a haematocrit of <20% in adults. It should be specified whether results are from a finger prick or venous sample. Finger prick samples may underestimate the haemoglobin concentration by >1 g/dl if the finger or ear lobe is squeezed during blood collection. Anaemia is common in malaria-endemic areas, particularly in young children. To distinguish malaria-related anaemia from coincidental malaria for a precise epidemiological classification, severe anaemia is defined as above in combination with a parasite count >10 000/μl. It is difficult to distinguish chronic from acute anaemia. Chronic severe anaemia is common in areas of high malaria transmission, and areas with heavy hookworm burdens, and carries a better prognosis than rapidly developing anaemia associated with an acute malaria infection.

Hypoglycaemia is a whole blood or plasma glucose concentration of <2.2 mM (<40 mg/dl). For screening purposes, rapid tests are valuable, but their accuracy diminishes at blood glucose concentrations below 3 mM and may be affected by low haematocrit. For research purposes, hypoglycaemia should be confirmed on a venous sample measured with a glucose analyser.

Acidosis is defined as a plasma bicarbonate concentration <15 mM or base excess below −8 meq/l. In the absence of laboratory facilities, acidosis can be inferred from the presence of deep breathing with a clear chest on auscultation. Hyperlactataemia is defined as a plasma or whole blood lactate level >5 mM.

Renal impairment or acute kidney injury is not well defined from a single measurement. Changes in measures of glomerular filtration rate are more important, and these are incorporated in the widely used RIFLE and AKIN criteria, but an individual's previous values are seldom available in malaria-endemic areas. As most patients with severe malaria are children or younger adults with normal pre-morbid renal function, a plasma creatinine over 265 μM (3 mg/dl) is used as a criterion of severe malaria. This corresponds to a GFR of approximately <30 ml/min in a man and <25 ml/min in a woman (eGFR – calculator: http://www.renal.org/egfrcalc for patients aged 18 years or more). This plasma creatinine level corresponds approximately to a blood urea of 20 mM, a blood urea nitrogen of 57 mg/dl or blood urea of 122 mg/dl, although patients with severe malaria may be hypercatabolic and dehydrated which increase the urea/creatinine ratio. In the past, acute kidney injury has also been defined in terms of reduced urine output (<400 ml in adults, <12 ml/kg/24 h in children) despite rehydration, but this is much less useful as it requires accurate monitoring and waiting 24 h. Oliguric renal failure rarely complicates *P. falciparum* infection in young children. Prognostically, a blood urea measurement of >20 mM in children or adults with severe malaria identifies a high-risk group with a mortality over 30% (Dondorp *et al.* 2005a, 2010; Hanson *et al.* 2011a; von Seidlein *et al.* 2012).

Hyperparasitaemia. The relation of parasitaemia to severity of illness is different in different populations and age groups, and there has been considerable debate whether it should be included at all in definitions of severity. In children in areas of unstable endemicity, a peripheral parasitaemia of 4% or more (≥4% of circulating red cells contain parasites) carried an increased risk of death (Luxemburger *et al.* 1995). A 4% parasitaemia in non-immune children or adults should be considered an indicator of high risk requiring supervised management (Tables 2 and 3) but not by itself a criterion of severe malaria. In areas of stable endemicity, parasitaemia thresholds should be derived from local experience, but in the absence of local data, a parasitaemia >10% without the other signs of severity described above indicates severe malaria.

Epidemiological and research definition of severe vivax malaria

The criteria for severe vivax malaria are the same as for adults and children with severe falciparum malaria but with no parasitaemia density thresholds (and without the criterion of hyperparasitaemia) (see *Section 13*). Although the specificity is reduced without a parasitaemia threshold, parasite densities in vivax malaria are usually lower than *P. falciparum* (almost always <2% of total red cells). The

mechanisms of severe disease may differ from those in falciparum malaria and are not clearly related to parasite biomass. There is a 4- to 5-fold greater loss of uninfected red cells in *P. vivax* infection relative to *P. falciparum* infection at low parasite densities, so *P. vivax* may cause severe anaemia at lower parasitaemias.

Epidemiological and research definition of severe knowlesi malaria

Plasmodium knowlesi infections have a threefold higher risk of developing severe malaria than *P. falciparum* (Barber *et al.* 2012). The risk of severe malaria is 28-fold greater with a parasitaemia >100 000/μl than the risk in a *P. falciparum* infection of similar density. All the severity manifestations listed for severe falciparum malaria have been reported with knowlesi malaria, except for coma (see *Section 14*). Criteria for severe knowlesi malaria are the same as for the definitions for adults and children with severe falciparum malaria but with lower parasitaemia cut-offs for hyperparasitaemia and jaundice as follows:

- *P. knowlesi* hyperparasitaemia: Parasite density >100 000/μl
- Jaundice and parasite density >20 000/μl

Any patient with a *P. knowlesi* parasitaemia of >20 000/μl needs to be observed very carefully.

Section 3: Clinical features of severe falciparum malaria in children

Most of the estimated 0.6 million malaria deaths every year are in children up to 5 years old who live in areas of intense transmission of *P. falciparum*, especially in sub-Saharan Africa (World Health Organization 2013).

Severe malaria is rare in early infancy. Babies born to mothers who have malaria during pregnancy are at risk of having a lower birthweight than the community average, usually as a result of intrauterine growth retardation (see *Section 5*). Low birthweight is associated with increased mortality from all causes in infancy (Steketee *et al.* 1996). In endemic areas neonates may have cord blood and peripheral parasitaemia which usually disappears within hours or days. 'Congenital' malaria (illness in the neonate resulting from malarial infection) is uncommon, but may present as fever, anaemia, and/or neonatal jaundice 10–30 days after delivery. This may mimic neonatal sepsis. Over the next few months of life, parasitaemia appears in an increasing proportion of children, the rate of increase in prevalence being a measure of transmission intensity. The great majority of infections are oligosymptomatic or symptomless and are reflected in the high prevalence of asymptomatic parasitaemia among children in endemic areas. The likelihood of symptoms increases with the density of parasitaemia, allowing statistical calculations of malaria-attributable morbidity (Smith *et al.* 1994).

Importance of malaria as cause of severe disease and mortality in older infants and children

From the age of a few months onwards, infected infants may develop severe disease. One study estimated that about one infection in a hundred progressed to cause complications (i.e. became severe) in a population in The Gambia (Greenwood *et al.* 1991). These figures and the case-fatality rates of severe disease probably differ between populations dependent upon the transmission characteristics, health service provisions and availability, parasite drug sensitivities, and a variety of parasite and host factors. Malaria is one of the three commonest reasons for admission to hospital and is a major cause of hospital death in children aged 1–5 years, in many endemic areas (Roca-Feltrer *et al.* 2008; Black *et al.* 2010).

Relative importance of different syndromes in fatal malaria

Among the clinical syndromes that define severe malaria in children (Tables 1 and 2), the most commonly encountered fall into three main categories: severe anaemia, 'cerebral' malaria and metabolic acidosis. These may occur separately or in any combination. The presence of impaired consciousness *or* severe respiratory distress predicted 84.4% of 64 deaths among 1844 children admitted with malaria to a district hospital in coastal Kenya. In this study, severe anaemia was common but rarely fatal unless accompanied by impaired consciousness or severe respiratory distress (Marsh *et al.* 1995). Hypoglycaemia and jaundice are additional complications that may occur, usually in association with one or more of the above syndromes. Both hypoglycaemia and jaundice are associated with an increased case-fatality rate and were present in 31% and 16% of deaths, respectively, in the Kenya study. All of the deaths were among children with coma or respiratory distress. The pattern of clinical events associated with death may be different in the absence of hospital treatment. In Nigeria, 46.5% of 147 children with fatal *P. falciparum* infections had cerebral malaria (Elesha *et al.* 1993). In the Gambia, 43% of children admitted to hospital over a period of several years, and diagnosed as having severe malaria, were in coma (Waller *et al.* 1995); the proportion was similar – 50% – in a series in Burkina Faso (Modiano *et al.* 1995), but considerably smaller in a series in Madang, Papua New Guinea, where of 249 children admitted to hospital with severe malaria, 56 (22%) were comatose; in the same series, 155 (62%) of the children admitted had severe anaemia (Allen *et al.* 1997). Among 6200 children admitted to a rural hospital in Zambia, about 10% had severe malarial anaemia and half as many had cerebral malaria; but as the case-fatality rate in cerebral malaria was 19% and that of severe malarial anaemia was 9%, these two severe malaria syndromes contributed approximately equally to the malaria-related mortality (Biemba *et al.* 2000). In a prospective multicentre trial comparing artesunate with quinine in childhood severe malaria conducted in 11 centres in nine African countries, 5426 children were enrolled of whom 9.7% (527) died. There were five highly significant independent predictors of mortality: base deficit [adjusted odds ratio (AOR) 1.12, 95% CI 1.10–1.13], coma score (AOR 1.40, 95% CI 1.34–1.45), convulsions (AOR 1.72, 95% CI 1.30–2.30), BUN (AOR 1.02, 95% CI 1.02–1.03) and chronic illness (AOR 2.12, 95% CI 1.25–3.58) (von Seidlein *et al.* 2012). The relationships between 3 of these predictors and their associated case fatalities are illustrated in Figure 1.

Problems affecting the diagnosis of severe malaria in children

The difficulty of distinguishing clinically between severe malaria and pneumonia. Several studies have demonstrated the problem of distinguishing clinically between

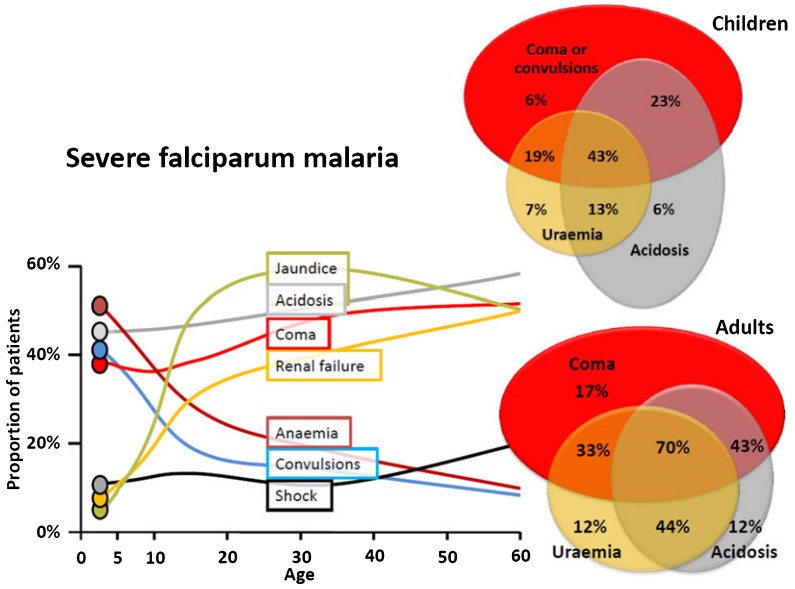


Figure 1 Data compiled from prospective series of severe falciparum malaria in 6189 children in studies conducted in Africa and 2605 adults in studies conducted in South-East Asia. Left side shows the prevalence of different features of severe falciparum malaria by age, and Venn diagrams on the right show the mortality in children and adults associated with manifestations of cerebral and renal impairment and metabolic acidosis alone or in combination. The sizes of the ovals are proportional to number of cases.

pneumonia and malaria. In Malawi, of 471 children attending a hospital outpatient department who fulfilled the WHO clinical definition for pneumonia, 449 (95%) also met the clinical definition of malaria (Redd *et al.* 1992). Among Gambian children with cough or breathing difficulty and a raised respiratory rate, 38% had only malaria (parasitaemia with normal chest radiograph) in the season of intense malaria transmission, compared to 6% during the season of low malaria transmission (O'Dempsey *et al.* 1993). In Kenya, 200 children with fever, cough, tachypnoea and additional features of respiratory distress, were compared with 26 children with definite pneumonia (radiological consolidation, no parasitaemia) and 38 children with definite malaria (normal chest radiograph, parasitaemia >100 000/μl); chest indrawing, unilateral signs and crackles or wheeze were significantly associated with pneumonia, and pallor and deep breathing with malaria, but no group of signs was entirely specific or sensitive for either diagnosis (English *et al.* 1996a).

These observations indicate the need to consider and treat for both malaria and pneumonia in children with fever and chest symptoms, in malarious areas, especially where either radiological or microscopy facilities are not available (Bloland *et al.* 1991). [See *Management of concomitant sepsis* p104 for discussion on the use of antibiotics in severe malaria]

The problem of diagnosis affects all syndromes that can resemble malaria. In a community with a high prevalence of asymptomatic parasitaemia, many febrile illnesses will also be accompanied by parasitaemia, yet have another cause. These illnesses include all of the syndromes that can be caused by severe malaria, each of which can have other causes. Vigilance for diagnoses other than malaria ('comorbidities'), even in the presence of parasitaemia, is important (Koram & Molyneux 2007). Indicators that malaria is a likely cause of the presenting illness include *high-density parasitaemia* and *thrombocytopenia*, although neither of these provide diagnostic certainty (Figure 2). In the comatose patient, the presence of *malarial retinopathy* is highly suggestive of a malarial aetiology of the illness [see *Section 8* (retinopathy)]. There have been few studies of the alternative causes of complicated febrile disease in children in malarious populations: in a recent hospital study in Malawi, of 513 children suspected to have meningitis who had no evidence of bacterial infection, 26% had PCR evidence of at least one virus in the cerebrospinal fluid (Mallewa *et al.* 2013). The development of bedside diagnostic tests for bacteraemias and viral infections would make an important contribution to both epidemiology and patient care in communities and peripheral hospitals in areas where incidental *P falciparum* parasitaemia is common.

Effects of HIV on childhood severe malaria. HIV-infected individuals of all ages are more susceptible to severe malaria (Otieno *et al.* 2006; Malamba *et al.* 2007; Imani *et al.* 2011). Severe malaria in HIV co-infected patients presents with higher parasite burden, more complications and more frequent comorbidity and carries a higher case-fatality rate (Hendriksen *et al.* 2012a).

Caution is needed when attributing complications to *P. falciparum* infection, as HIV immunosuppression also increases susceptibility to many of the opportunistic infections that cause clinical illnesses that might be mistakenly attributed to malaria in populations with a high prevalence of incidental parasitaemia.

Strengthening the definition of severe malaria. These various considerations have led to increasing attempts to standardise the definition of severe malaria in children, often fuelled by the need to assess the efficacy or effectiveness of interventions (severe malaria episodes being a primary endpoint) or to identify accurately patients to be recruited to pathogenesis or treatment studies (severe malaria as enrolment criterion). The Severe Malaria in African Children (SMAC) network aimed to quantify and describe severe malaria across a variety of epidemiological settings in order to design intervention studies with more precise sample size estimates (Taylor *et al.* 2006a). The network enrolled 20 333 parasitaemic children across five sites in sub-Saharan Africa with differing malaria transmission characteristics and identified the incidence of various severe syndromes in these diverse contexts. The programme to assess the malaria vaccine RTS,S gave rise to a widely deliberated case definition of severe malaria to be used in identifying the primary endpoint in the Phase 3 multicentre trials (Vekemans *et al.* 2011). The definition included: presence of one or more clinical and/or laboratory markers of disease severity; exclusion of four major comorbidities (pneumonia, meningitis, bacteraemia and gastroenteritis with severe dehydration); and a *P. falciparum* parasitaemia density threshold (to maximise the specificity of the case definition). This recommendation was largely based on a prior analysis of 4583 well children and 1361 children admitted to a district hospital in Kenya (Bejon *et al.* 2007). The latter study confirmed that increasing the parasitaemia threshold improved specificity but reduced sensitivity of severe malaria diagnosis and that the malaria-attributable fraction of diagnoses is considerably lower (61%) in areas of intense transmission than in areas of low or moderate transmission intensity (85%).

Syndromes defining severe malaria in children

Cerebral malaria (CM)

Impaired consciousness. A number of different disease processes may affect consciousness in the child with malaria, including convulsions, hypoglycaemia, hyperpyrexia, acidosis, severe anaemia and sedative drugs. However, coma may develop in the absence of any of these causes. In most published studies, the term *cerebral malaria* has been restricted to the syndrome in which altered consciousness, associated with a malarial infection, could not be attributed to convulsions, sedative drugs or hypoglycaemia alone or to a non-malarial cause. A child with loss of consciousness after a febrile convulsion should not be considered to have cerebral malaria unless coma persists for more than 1 h after the convulsion. Similarly in a child with malaria and hypoglycaemia who is comatose, diagnosis of cerebral malaria cannot be sustained if consciousness is promptly restored by administration of glucose. 'Cerebral malaria' is a clinical syndrome; the term is convenient for descriptive purposes.

The problem of diagnosis in 'cerebral malaria'. Finding retinopathy improves specificity. The syndrome of coma, commonly with convulsions, that is the hallmark of cerebral malaria is like all other syndromes that can complicate *P. falciparum* infection, one that has a number of other possible causes. Some of these are identifiable by bedside examination or tests – for example measles, bacterial meningitis – but many are not identifiable immediately, if at all, in most hospitals – for example other viral encephalitides, toxic syndromes, and intracranial vascular or mechanical events. Where asymptomatic parasitaemia is common, attributing coma to malaria is problematic. A study of fatal cerebral malaria in 27 parasitaemic Malawian children (Taylor *et al.* 2004) compared autopsy evidence of intracerebral parasite sequestration with ante-mortem clinical findings. In seven cases, autopsy revealed an alternative cause of death and no intracerebral sequestration, while in 20, there was intracerebral sequestration and no alternative cause of death. Nineteen of the 20 with sequestered parasites had retinopathy before death, while none of those without intracranial parasites had retinopathy. These results indicate (i) that the clinical diagnosis of CM is commonly wrong and (ii) that the presence of retinopathy is highly suggestive of the presence of intracerebral parasite sequestration, a characteristic histopathological feature of fatal CM. Several descriptions of malarial retinopathy have been published in both children and adults (Figure 2) (Lewallen *et al.* 1996; Hero *et al.* 1997; Hien *et al.* 2003; Beare *et al.* 2004, 2006; Harding *et al.* 2006; Maude *et al.* 2009a). Retinopathy can be seen by non-specialist clinicians using direct or indirect ophthalmoscopy through dilated pupils, but training is required to achieve dependable results (Beare *et al.* 2002; Mohammed *et al.* 2011). Retinopathy can include any of four features. Two of these are distinctive and specific to malaria: these are (i) patchy retinal whitening in the macula (especially peri-foveal) and/or in the peripheral retina; and (ii) white or orange discolouration of retinal vessels. Other features that may

accompany these but which may also be caused by non-malarial conditions are: (iii) white-centred haemorrhages and (iv) papilloedema. A system for classifying and grading these components has been proposed (Lewallen *et al.* 1999).

Retinopathy has been used to improve the classification of severe malaria (Lewallen *et al.* 2008) and to increase the specificity of the diagnosis of CM for studies of pathogenesis or treatment (Birbeck *et al.* 2010a). The severity of retinopathy has been found to be prognostic in children and adults with CM (Beare *et al.* 2004; Maude *et al.* 2009a).

Preceding symptoms in CM. In children with cerebral malaria who are admitted to hospital, the duration of febrile symptoms is usually short. In a series of 131 patients studied in Malawi, the mean length of reported history was 47 h (range 2 h to 7 days) (Molyneux *et al.* 1989b); in 195 Zambian children with cerebral malaria, the median duration of preceding fever had been 49.3 h (range 0–13 days) (Mabeza *et al.* 1995). The earliest symptom is usually fever, which is followed by failure to eat or drink. Vomiting and cough were reported in the majority of cases in Malawi; diarrhoea is an unusual symptom. Convulsions are common before or after the onset of coma (see below).

General features. Most but not all children with cerebral malaria are febrile. Rectal temperatures range from 36 to 41 °C or even higher. Dehydration is present in many cases (Waller *et al.* 1995; English *et al.* 1996d), but is rarely severe. The systolic blood pressure is usually normal, but there is a wide pulse pressure. Most patients have a tachycardia appropriate to their fever. Cardiac arrhythmias are rare. Breathing may be rapid or laboured; deep breathing suggests acidosis (see below). Examination of the heart and lungs is usually normal. A minority of patients have cold, clammy skin with a core-to-skin temperature difference >10 °C. Some of these patients are in a state of shock with systolic blood pressure <50 mmHg. Jaundice is less common in children than in adults with cerebral malaria (White *et al.* 1987a; Molyneux *et al.* 1989b). Hepatosplenomegaly is commonly found at presentation or during the course of the disease. Spontaneous bleeding into the skin or gastrointestinal tract is rare.

Neurological features. The depth of coma may be assessed by observing the response to standard painful or vocal stimuli (see Table 5). The gag reflex is usually but not invariably preserved. In patients with profound coma, corneal reflexes and oculocephalic 'doll's eye' reflexes may be abnormal, and there may be abnormalities of conjugate gaze. Corneal and pupillary light reflexes are usually retained. Abnormalities of muscle tone and posture are frequently seen (Molyneux *et al.* 1989b; Mabeza *et al.* 1995; Waller *et al.* 1995; Rey *et al.* 1966). These may take the form of muscular hypotonia or, more commonly, of decerebrate or decorticate posturings, which may be intermittent or sustained. In some children, extreme opisthotonos is seen which may misleadingly suggest a diagnosis of tetanus or meningitis. Bruxism (grinding of teeth) is common. Plantar reflexes are sometimes abnormal, and abdominal reflexes are almost invariably absent.

Convulsions. The majority of children with cerebral malaria have convulsions. Cerebral malaria was considered to be the cause of convulsions in one-third of children admitted to a Nigerian hospital in 1988 (Asindi *et al.* 1993). Convulsions may be generalised or focal, single or recurrent, and unlike febrile convulsions may occur in children of any age (Rey *et al.* 1966) and at any level of body temperature (Molyneux *et al.* 1989b). In some patients

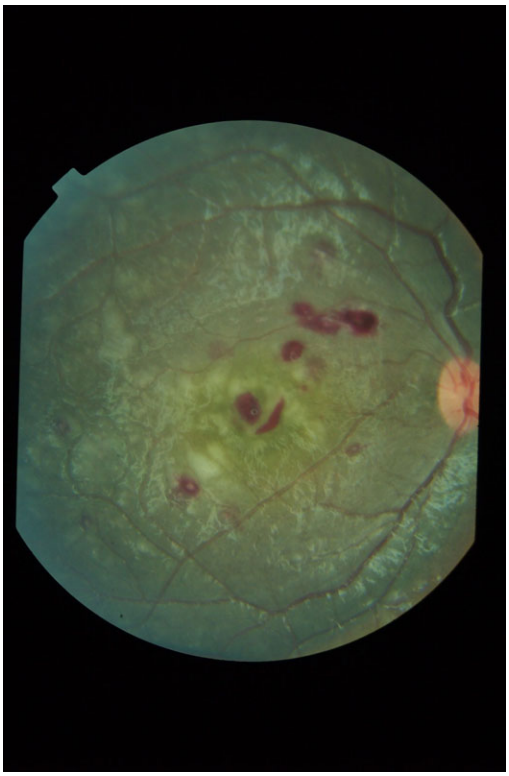


Figure 2 Retina in a child with cerebral malaria. On the macula there is extensive retinal whitening in patches, maximal around the fovea (centre of picture); and several white-centred haemorrhages. The optic disc is at the right edge of the picture.

(about 25% of children with cerebral malaria in a Kenyan hospital), seizure activity can be demonstrated by electroencephalography in the presence of only minor, if any, convulsive movements of limbs or facial muscles but with jerky eye movements with deviation, excessive salivation and irregular breathing patterns (non-convulsive status epilepticus) (Crawley *et al.* 1996); in a study in Malawi, electroencephalography revealed seizures in 7 (19%) of 36 children with cerebral malaria in whom there were no external signs of seizure activity (Birbeck *et al.* 2010b).

Intracranial pressure. The mean opening pressure at lumbar puncture is 160 mm CSF, which is similar to that in adults, but as the normal range is much lower in children, this means that approximately 80% of children have intracranial pressures above the normal range (Newton *et al.* 1991; Waller *et al.* 1991). The mechanism and pathophysiological importance of raised intracranial pressure are uncertain. Magnetic resonance imaging reveals evidence of cerebral oedema in many children with cerebral malaria, especially in those with retinopathy, and the presence of cerebral oedema is a strongly adverse prognostic indicator (Potchen *et al.* 2012). Whether raised intracranial pressure plays a causal role in coma or death remains to be determined. This topic is discussed more fully in the pathophysiology section (*Section 7*).

Recovery from cerebral malaria. The reported hospital case fatality of cerebral malaria in children has ranged from 10 to 40% (Dumas *et al.* 1986; White *et al.* 1987a; Molyneux *et al.* 1989b; Waller *et al.* 1995). In the recent large multicentre trial that compared intravenous artesunate and quinine in the treatment of 5425 children with severe malaria, mortality among those with cerebral malaria was 359/1825 (19.7%) (Dondorp *et al.* 2010). Most deaths of hospitalised children occurred within 24 h of admission, about two-thirds being from a cardiorespiratory arrest probably due to metabolic derangements, while in the remaining one-third, there was respiratory arrest, with clinical features of brainstem dysfunction that suggested raised intracranial pressure. In an audit of 306 children dying of cerebral malaria in Kenya, 196 (65%) had simultaneous cessation of cardiac and respiratory activity, while 110 (35%) initially had cessation of respiratory activity with good continuing cardiac activity (H. Bulstrode, personal communication). In survivors, the duration of altered consciousness after the start of treatment ranges from a few hours to several days, the mean being about 30 h in Malawi (Molyneux *et al.* 1989b) and a median of 12 h in the Gambia (White *et al.* 1987a). The transition from coma to full consciousness is sometimes very rapid and may not be witnessed unless frequent observations are made. In some children, recovery of consciousness may represent the end of a post-ictal state or emergence from a drug-induced stupor; these conditions cannot be distinguished from cerebral malaria, but the timing of recovery may be suggestive.

Neurological and cognitive sequelae after cerebral malaria in children. The majority of children who survive cerebral malaria make a full neurological recovery, but a significant number are left with neurological sequelae, while others develop later cognitive and behavioural difficulties or epilepsy (Figure 3).

Neurological sequelae. A combined analysis of 11 early African series which used varying methodologies to assess outcomes included 1341 children with cerebral malaria with a case fatality of 19% and neurological sequelae in 6.7% (Commey *et al.* 1980; Omanga *et al.* 1983; Schmutzhard & Gerstenbrand 1984; Molyneux *et al.* 1989b; Brewster *et al.* 1990; Bondi 1992; Sowunmi 1993; Sowunmi *et al.* 1993; Bojan *et al.* 1997). Studies from Malawi (Molyneux *et al.* 1989b), The Gambia (Brewster *et al.* 1990), Nigeria (Bondi 1992) and Kenya (Peshu, personal communication) used essentially similar criteria: in these studies, a total of 1060 children were reported with an overall incidence of neurological sequelae of 11.5% (13.3% in survivors) and a case fatality of 13.5%. As the children in these studies did not have pre-morbid assessments, they may include some with pre-existing neurological abnormalities. The most common sequelae were ataxia (43%), hemiplegia (39%), speech disorders (39%) and blindness (30%). Other sequelae on discharge include behavioural disturbances, hypotonia, generalised spasticity and a variety of tremors. Sowunmi (Sowunmi 1993) described two children with visual and auditory hallucinations a few days after recovery from cerebral malaria.

Several studies suggest that a significant proportion of reported neurological deficits actually reflect slow neurological recovery. In a prospective study of 624 children admitted with cerebral malaria to two hospitals in The Gambia, van Hensbroek *et al.* (1997) found that 23.3% of survivors had neurological sequelae on discharge from the hospital, but by 1 month, the proportion had decreased to 8.6%, and at 6 months, only 4.4% of survivors were found to have residual neurological sequelae. In the large artesunate versus quinine trial (AQUAMAT) in severe malaria, the proportion of children surviving cerebral malaria with neurological deficits [170/1466 (11.6%)] had also reduced significantly when the children were assessed 3–8 weeks later. Of the 170 patients, 129 were followed up between 3 and 8 weeks later and at

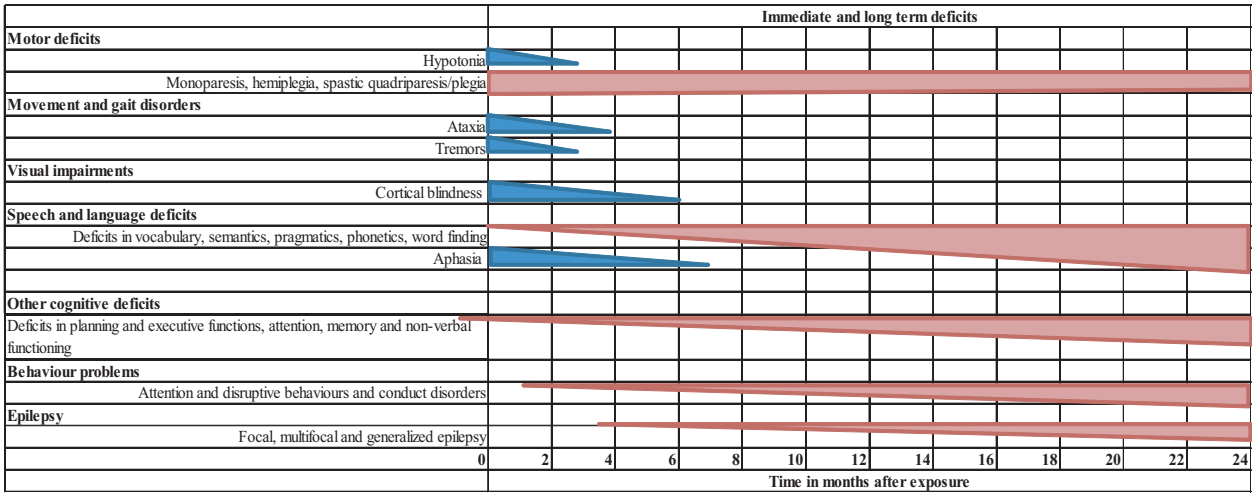


Figure 3 Manifestations and resolution of sequelae of cerebral malaria in children. Some sequelae of cerebral malaria (e.g. motor weakness and spasticity), blindness or loss of speech are apparent on recovery from the coma, while others such as behaviour difficulties and epilepsy may not appear until several weeks or months after discharge from hospital. Some deficits present on discharge such as generalised hypotonia, ataxia, aphasia and blindness may resolve over the months following discharge. Thickness of shaded area corresponds approximately to relative (i.e. not absolute) proportions.

this follow-up assessment, 68 (53%) had recovered fully, 18 were mildly or moderately impaired, and 43 had severe neurological deficits (Dondorp *et al.* 2010). A smaller study of 44 Ugandan children gave similar results; although neurological deficits were seen in 28.2% on discharge, rates decreased to 9.5% at 3 months and 0% at 6 months (Boivin *et al.* 2007). Many patients with persistent neurological sequelae – particularly blindness, ataxia and central hypotonia – also show considerable improvement with time (Schmutzhard & Gerstenbrand 1984; Bondi 1992; John *et al.* 2008), but other severe neurological symptoms persist (Carter *et al.* 2005a), while others such as epilepsy and behaviour difficulties may develop after discharge (Carter *et al.* 2004; Ngoungou *et al.* 2006a,b; Opoka *et al.* 2009; Birbeck *et al.* 2010a; Idro *et al.* 2010). Cortical blindness shows the most dramatic resolution; in the different series above, between 80 and 90% of children with cortical blindness recovered sight fully (Figure 3).

Epilepsy developing after severe malaria. A retrospective study in Kenya identified epilepsy in 14/152 (9.2%) children who had suffered cerebral malaria compared to 4/179 (2.2%) controls (Carter *et al.* 2004), while in Mali, 5/101 (4.9%) survivors of cerebral malaria had epilepsy compared to 1/222 (0.5%) controls (Ngoungou *et al.* 2006b). In a study in Malawi, 132 children with retinopathy-positive cerebral malaria and 264 age-matched, non-comatose controls were followed up for a median of 495 days (IQR 195–819) (Birbeck *et al.* 2010b). Twelve of 132 cerebral malaria survivors developed epilepsy versus none of 264 controls [odds ratio (OR) undefined; $P < 0.0001$]. In the same study, 28 of 121 cerebral malaria survivors developed new neurodisabilities, characterised by gross motor, sensory or language deficits, compared with two of 253 controls (OR 37.8, 95% CI 8.8–161.8; $P < 0.0001$). A similar delayed manifestation of epileptic seizures was described in Uganda where the cumulative incidence of seizures increased over time with a total of two of 76 children (2.6%) reporting seizures at 3 months, three of 74 children (4.1%) at 6 months and 11 of 68 children (16.2%) at 24 months (Opoka *et al.* 2009).

Longer term cognitive sequelae. Cognitive deficits are major sequelae of cerebral malaria (Figure 3). In Kenyan children who had suffered malaria with impaired consciousness, reviewed at least 42 months after exposure, impairments in executive functions, in particular the ability to initiate, plan and carry out tasks, were significantly more common than in controls (Holding *et al.* 1999). Carter *et al.* examined 152 children after cerebral malaria, 156 after malaria with multiple seizures and 179 controls, to identify developmental impairments (Carter *et al.* 2004, 2005a,b, 2006). Of children who recovered from cerebral malaria 24% had deficits in at least one domain and 42% of those with impairments had multiple impairments. Eighteen children (11.8%) of the cerebral malaria group, 14 (9%) of the malaria with seizures group and four (2.2%) of controls had language impair-

ment (Carter *et al.* 2006). Language deficits (in comprehension, syntax, pragmatics and word finding) and deficits in memory, attention, behaviour and motor skills were more pronounced in those with active epilepsy. Similar evidence of impaired language development has been reported among 83 Malawian children after recovery from cerebral malaria with retinopathy (Boivin *et al.* 2011). Other studies in Uganda and Senegal have described similar cognitive deficits especially in working memory and attention (Boivin 2002; Boivin *et al.* 2007). Although it has been reported, hearing loss has not been systematically examined as a sequela of severe malaria (Zhao & Mackenzie 2011). These developmental impairments have been confirmed in more recent studies in Malawian children in whom malaria retinopathy was used as an inclusion criterion to improve on the diagnostic accuracy of cerebral malaria. In this cohort, up to one-third (42/132; 32%) of children who survived cerebral malaria developed epilepsy or new neurobehavioural impairments (Birbeck *et al.* 2010b; Boivin *et al.* 2011). Adverse outcomes appeared to develop sequentially, with motor, sensory or language deficits being evident initially, followed by disruptive behaviour at a median of 150 days and lastly epilepsy at a median period of about 300 days. Post-cerebral malaria epilepsy manifested as localisation-related epilepsy with focal motor, complex partial, focal with secondary generalisation, multifocal or generalised tonic clonic seizures (Birbeck *et al.* 2010b).

Abnormal behaviour. Preliminary studies suggest that some children recovering from cerebral malaria may develop behavioural abnormalities – including hyperactivity, impulsiveness and inattentiveness – or conduct disorders such as aggressive, self-injurious and destructive behaviour. These defects are particularly seen in patients with other severe sequelae (Idro *et al.* 2010; Boivin *et al.* 2011). In the study of Malawian children, disruptive behaviour fulfilling the DSM IV criteria for attention deficit and hyperactivity disorder was described in 14/132 (10.6%) after a median follow-up period of 495 (IQR 195–819) days (Birbeck *et al.* 2010b). The pathogenesis is still poorly understood, and more detailed descriptions are awaited. The findings from all these studies suggest that (i) brain damage after cerebral malaria in children is more common than was originally thought; (ii) sequelae can take many forms including defects of movement and coordination, cognitive and behavioural impairments and epilepsy; and (iii) many of these sequelae are not evident at the time of discharge from hospital (Figure 3).

Prognostic factors for neurological sequelae. Prognostic factors associated with neurological sequelae are similar, but not identical, to those predicting mortality. The main risk factors include prolonged and repeated seizures, deep and prolonged coma, and intracranial hypertension (Molyneux *et al.* 1989b; Brewster *et al.* 1990; Crawley *et al.* 1996; van Hensbroek *et al.* 1997; Idro *et al.* 2004). Others are male gender and higher admission temperature (Birbeck *et al.* 2010b); age younger than 3 years (Molyneux *et al.* 1989b) and a biphasic clinical course characterised by recovery of consciousness followed by recurrent convulsions and coma (Brewster *et al.* 1990). The prevalence of sequelae increases with worsening depth of coma on admission. In a cohort of 143 surviving Kenyan children, 5/6 (83%) with admission Blantyre Coma Score 0 had impairments compared to 5/18 (28%) of those with a score of 1 and 24/119 (20%) of those with score 2 (Idro *et al.* 2006b). Children who were later found to have impairments had a slower recovery from coma, with a median time to full consciousness about 8 h longer than those without impairments. Although repeated seizures feature prominently as a risk factor for sequelae, the role of seizures in the causation of brain injury is not clear; it is possible that the seizures themselves are a manifestation of brain injury. Although high-dose prophylactic phenobarbitone significantly reduced seizures in cerebral malaria, it was associated with increased mortality (Crawley *et al.* 2000), and this intervention did not improve cognitive outcome in survivors (Abubakar *et al.* 2007). In a study to assess the memory outcomes of cerebral malaria, the number of seizures or seizure duration did not independently predict everyday memory (Kihara *et al.* 2009). It is likely that prolonged and recurrent seizures worsen an initial neural injury that is partly responsible for the seizures themselves. Intracranial hypertension may lead to reduced cerebral perfusion pressure, and impaired nutrient and oxygen delivery that result in global ischaemic injury and death, and contribute to clinical features suggesting herniation and brainstem compression (Walker *et al.* 1992; Newton *et al.* 1997b). There remains uncertainty how important these processes are overall as a cause of brain damage and death in cerebral malaria (see pathophysiology *Section 7* for discussion of raised intracranial pressure, and pathology *Section 8* for autopsy findings).

Syndromes defining severe malaria in children

Metabolic acidosis

In many severe disease states, including severe malaria, the presence of acidaemia is associated with a high case fatality (Stacpoole *et al.* 1994). Acidaemia is a manifestation of severe malaria, whether there is altered

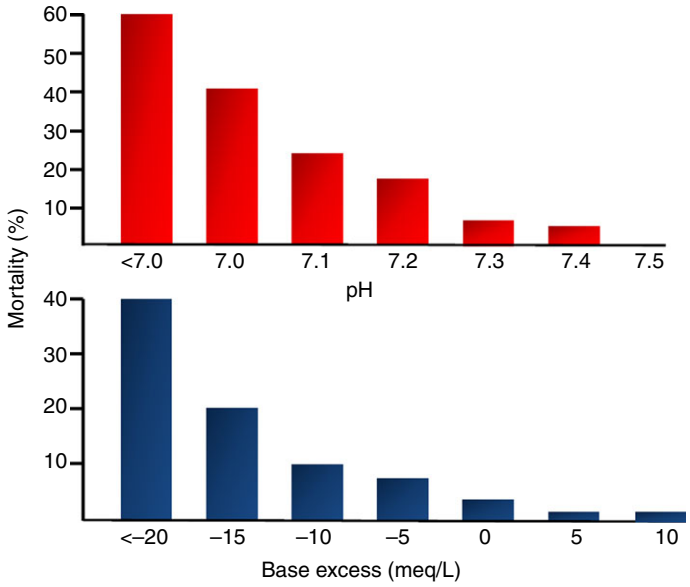


Figure 4 Relationship between mortality and acidosis (measured by venous pH and base deficit) in 5426 African children admitted with severe malaria (Dondorp *et al.* 2010; von Seidlein *et al.* 2012).

consciousness or not (Krishna *et al.* 1994; Marsh *et al.* 1995; Waller *et al.* 1995). In a selected group of Malawian children admitted to hospital with a primary diagnosis of malaria, 66/145 (46%) were found to be either acidaemic, with a pH < 7.3, or had a compensated metabolic acidosis (Taylor *et al.* 1993). 72% of children who subsequently died were acidotic on admission. 42% of children with cerebral malaria were acidaemic, and mortality in these children was 28% compared to 3% in the non-acidaemic group (relative risk of death for the acidaemic group = 8.5). Acidosis was the major independent risk factor for death and was also strongly associated with hypoglycaemia, a known risk factor for death in malaria; all 14 hypoglycaemic patients were acidotic. Lactic acidosis appears to be the major (but not the only) contributing mechanism of acidosis. Serial clinical and metabolic changes were measured in 115 Gambian children with severe malaria (Krishna *et al.* 1994) of whom 21 died. Mean venous blood lactate was almost twice as high in fatal cases as survivors. After treatment, lactate concentration fell rapidly in survivors but fell only slightly, or rose, in fatal cases. It is in the minority of patients whose hyperlactataemia does not resolve in the first few hours of admission that the prognosis is worst. In Kenya, severe metabolic acidosis (base excess equal to or less than minus 12) was strongly associated with risk of death (relative risk 8) in a series of 299 children with severe malaria. In the AQUAMAT trial in 5426 children with severe malaria, there was a progressive rise in mortality with increasing acidosis (Figure 4) (Dondorp *et al.* 2010, von Seidlein *et al.* 2012).

Respiratory distress. Respiratory distress is a summary description applied to children who have obviously abnormal breathing involving the expenditure of more effort than usual. There are two major components that may both contribute to this. *Deep breathing* involves an abnormally increased amplitude of chest excursion. It is a sign of metabolic acidosis. The increased depth of respiration may be accompanied by a degree of intercostal indrawing (recession) particularly in thin children with raised respiratory rates, but the intercostal indrawing is in proportion to the overall increase in effort and is not prominent. The second possible component is true indrawing of the bony structures of the lower chest wall. This can be a sign of lung disease, usually pneumonia, or of metabolic acidosis and is usually associated with increased use of accessory muscles. In practice, most cases of respiratory distress in association with malaria constitute deep breathing secondary to metabolic acidosis. In a small proportion of cases, there is concomitant pneumonia. The presence of either deep breathing or chest indrawing should be regarded as a danger sign. Although the respiratory rate will usually be raised above the age-related normal range, the actual degree of tachypnoea does not correlate well with the overall impression of distress, whether it results from metabolic acidosis or parenchymal lung disease. In fact, progressively severe metabolic acidosis may be associated with a decrease in respiratory rate, as the pattern of respiration changes from 'panting' to 'air hunger' (Kussmaul's breathing). Respiratory distress is a major risk factor for death in children with malaria (Lackritz *et al.* 1992). In the majority of cases, it results from severe

metabolic acidosis. Among 1844 children admitted consecutively to Kilifi Hospital on the Kenyan coast with a primary diagnosis of malaria, 14% had respiratory distress (nasal flaring, indrawing (recession) of the bony structure of the chest wall or deep breathing) of whom 14% died. Of all children with malaria who subsequently died, 55% had respiratory distress on admission. Over 80% of a subgroup of these children with respiratory distress were profoundly acidotic (Marsh *et al.* 1995).

Respiratory distress in severe malaria may be wrongly ascribed to congestive cardiac failure 'secondary' to severe anaemia. In a study of 24 intensively monitored Kenyan children with severe respiratory distress, 16 were severely anaemic (haemoglobin 2.1–4.9 g/dl) (English *et al.* 1996a). Lactate concentrations were significantly higher in the severely anaemic group, and plasma creatinine levels were higher in the children with low central venous pressures. Resuscitation with rapid transfusion of blood in the severely anaemic children (10 ml/kg over 1 h followed by 10 ml/kg over the next 1–4 h) was associated with rapid resolution of acidosis and clinical improvement in the majority. These findings suggest that respiratory distress in the severely anaemic child is commonly caused by acidosis resulting from inadequate oxygen delivery to poorly perfused tissues, rather than to congestive cardiac failure.

The relative importance of the clinical syndrome of respiratory distress and its overlap with impaired consciousness and severe anaemia in severe childhood malaria is shown in Figure 1 and Figure 5 (Marsh *et al.* 1995). The highest mortality occurred in children with both neurological impairment and respiratory distress. Deep breathing is an accurate clinical indicator of the presence of acidosis (English *et al.* 1996b), a fact that has prompted some investigators to argue that expensive attempts to measure plasma lactate concentration or acid/base balance are superfluous (Newton *et al.* 2005a). However, most studies suggest that laboratory measures are more sensitive and specific indicators of acidosis. Acidosis is largely due to the accumulation of both lactic acid and 3-hydroxybutyric acid as well as other unidentified organic acids (Sasi *et al.* 2007). The accumulation of lactic acid in the circulation results more from tissue anoxia and anaerobic glycolysis, together with impaired gluconeogenesis, than from the release of lactate by parasitised erythrocytes (Krishna *et al.* 1994).

The role of intravascular volume depletion in the pathogenesis of acidosis remains uncertain. Although some studies have reported an association of acidosis with apparent hypovolaemia and shock (reviewed in Maitland & Newton 2005), one study including 56 children with severe malaria found no evidence of volume depletion (Jarvis *et al.* 2006). A large randomised multicentre trial enrolling 3141 African children admitted to hospital with fever and impaired tissue perfusion showed that administration of additional fluid boluses, whether albumin or saline, *increased* mortality when compared to controls given routine fluid management (Maitland *et al.* 2011). Studies in adults strongly suggest that hypovolaemia is not a major contributor to acidosis in older patients (Hanson *et al.* 2012). It is likely that different factors may contribute to acidosis in different individuals, the principal mechanism being inadequate oxygenation of tissues, leading to anaerobic glycolysis. While the importance of hypovolaemia and shock remain uncertain, other contributory processes include sequestration in the microvasculature and severe anaemia, and exacerbating factors include hypoglycaemia, impaired hepatic function, renal impairment and recent convulsions (English *et al.* 1997) (see *Section 7*).

Syndromes defining severe malaria in children

Severe anaemia

Anaemia is an important and commonly life-threatening complication of falciparum malaria in children. The mean age of children presenting with severe malarial anaemia is about 1.8 years, compared to 3 years in those presenting with cerebral malaria (Figure 1). Anaemia may develop rapidly during the course of a malarial illness, especially if there is initial hyperparasitaemia (McGregor *et al.* 1956). While anaemia may be present or may develop in a child with any complication of *P. falciparum* infection, severe anaemia may be the only or predominant complication of malaria in a child presenting to a health facility; in this circumstance, and in a child found to have severe anaemia in a community survey, the rapidity of onset of anaemia cannot be determined. In a survey of children aged under 5 years living in villages in southern Tanzania, Schellenberg *et al.* (2003) reported a high prevalence of anaemia, especially among children in the second half of infancy, among whom 10% had severe anaemia (<5 g/dl). In the great majority of cases, anaemia was not suspected, and the infant was not considered by its mother to be ill.

Severe anaemia is often multifactorial and is attributable to malaria because of parasitaemia and the lack of an adequate alternative explanation. In an extensive study in Malawi, 381 pre-school children presenting to two hospitals with severe anaemia (<5 g/dl) were compared with 757 age- and location-matched controls without severe anaemia: this study showed that severe anaemia had multiple associations, including malaria, bacteraemia, HIV infection, hookworm and Vitamin A and B12 deficiencies (Calis *et al.* 2008). The importance

of malaria as a cause of severe anaemia is suggested by the seasonality of severe anaemia, the incidence of which tends to follow that of malaria; although nutritional and other factors may also change with season (Koram *et al.* 2000). In an area of very intense *P. falciparum* transmission in Western Kenya, 83% of 1116 paediatric hospital admissions were parasitaemic: severe anaemia was identified in 21% of admissions and in 12% of in-hospital deaths (Obonyo *et al.* 2007). Among 8195 febrile Gabonese children, almost all were anaemic to some degree, and the risk of severe anaemia was highest among infants (Bouyou-Akotet *et al.* 2009). Of 2433 children admitted to a Kenyan hospital in 1990, 684 (29%) had severe anaemia (Hb <5.0 g/dl), and this was strongly associated with *P. falciparum* parasitaemia. 18% of the severely anaemic patients died, compared to 8% of all admissions (Lackritz *et al.* 1992). In this and in other studies (Marsh *et al.* 1995; Bojang *et al.* 1997), the mortality among anaemic children was increased greatly when there was associated respiratory distress. In the review of 5426 African children admitted to the multicentre AQUAMAT trial (Dondorp *et al.* 2010), in which there was ready access to blood transfusion, mortality did not rise until the haemoglobin concentration fell below 3 g/dl (von Seidlein *et al.* 2012). Blood transfusion reduced the case-fatality rate in children with anaemia and respiratory distress (Lackritz *et al.* 1992). In that study, blood transfusion given after a delay of 2 days was not beneficial, suggesting that mortality due to severe anaemia may be considerably greater in the community than in hospital, where prompt treatment can be given. Community deaths from severe anaemia are difficult to identify in retrospect because of the poor sensitivity and specificity of verbal autopsies for the diagnosis of anaemia (Snow *et al.* 1992).

Syndromes defining severe malaria in children

Other complications

Hypoglycaemia. Children are more likely than adults to develop hypoglycaemia and may become hypoglycaemic solely as a result of fasting, particularly during any illness accompanied by fever and vomiting. Hypoglycaemia was found in 32% of Gambian children (White *et al.* 1987b) and 20% of Malawian children presenting with severe malaria (Taylor *et al.* 1988), and 43 (7%) of 603 children admitted to hospital in Mozambique, in association with a variety of clinical conditions including malnutrition, pneumonia, and malaria (Solomon *et al.* 1994). Hypoglycaemia (whole blood glucose <2.2 mM) commonly complicates severe malaria in children and is associated with an increased risk of dying or sequelae from the disease (White *et al.* 1987b; Taylor *et al.* 1988; Bassat *et al.* 2008; Camara *et al.* 2011; Nanda *et al.* 2011). Hypoglycaemia is particularly common in young children (<3 years), in those with convulsions or hyperparasitaemia, in patients with profound coma and those who have received quinine (Dondorp *et al.* 2010). It is associated with convulsions, lactic acidosis, and high concentrations of circulating tumour necrosis factor. Hypoglycaemia is easily overlooked clinically because the manifestations may be similar to those of cerebral malaria. In a child with impaired consciousness due to cerebral malaria, correction of hypoglycaemia with intravenous 50% dextrose does not, by definition, result in immediate restoration of consciousness. Hypoglycaemia may recur during treatment of severe malaria; these recurrences may be due to the disease (in which case plasma insulin concentrations are appropriately low) or quinine-induced hyperinsulinaemia. Recurrent hypoglycaemia may be difficult to detect clinically in the comatose child and may cause deepening coma or convulsions. An increase in the loading dose and frequency of administration of quinine in the treatment of severe malaria in children in a Kenyan hospital did not lead to any increase in the incidence or severity of hypoglycaemia (Ogetii *et al.* 2010). Factors associated with recurrent hypoglycaemia, apart from quinine, included coma (57%), circulatory failure (38%), and respiratory distress (21%), and less commonly, seizures (10%). Disruption of maintenance fluids and/or blood transfusion coincided with 42% of the hypoglycaemia episodes. Post-admission hypoglycaemia increased the odds of fatal outcome (24%) compared to children who remained euglycaemic (8%), odds ratio = 3.45.

Pulmonary and renal complications. Capillary blood pO_2 and arterial blood oxygen saturation are normal in nearly all patients with severe malaria. Plasma urea and creatinine concentrations may be elevated on admission, especially in the presence of dehydration; values revert to normal during treatment (English *et al.* 1996c). Biochemical evidence of renal impairment on admission is an independent risk factor for poor outcome even though acute kidney injury (acute renal failure) is rare in children (von Seidlein *et al.* 2012) (Figure 1). Hyponatraemia was found in 55% of 132 children with severe malaria in Kenya and was usually not attributable to inappropriate secretion of antidiuretic hormone secretion (English *et al.* 1996d). Chest radiographs are usually normal, even in the presence of deep or laboured breathing; this is further evidence that pulmonary oedema or 'acute respiratory distress syndrome' is rare in children. Hypokalaemia was present on admission in only four of 38 children with severe malaria and acidosis admitted to a Kenyan district

hospital, but 15 of these children became hypokalaemic within the first 4–8 h after admission, a change attributed to increased urinary loss of potassium (Maitland *et al.* 2004). The same group reported *hyperkalaemia* in 61/493 (12%) of Kenyan children with severe malaria, among whom there was a particularly high case-fatality rate (28%) (Maitland *et al.* 2003a).

Hyperpyrexia. High fever is a common feature of falciparum malaria in children, but there is no association between the degree of fever and the presence or likelihood of development of severe malaria. Convulsions in children with malaria may occur at body temperature, but the risk increases at temperatures above 38.5 °C (Familusi & Sinnette 1971; Patel 1971; Axton & Siebert 1982). Very high temperatures can contribute to altered consciousness or coma. High maternal temperature causes fetal distress in pregnant women with malaria (Looareesuwan *et al.* 1985).

Bacteraemia and other comorbidities. Children with clinically diagnosed severe malaria have a higher prevalence of bacteraemia than controls (Berkley *et al.* 1999, 2005). In many African studies, the commonest bacterial pathogen isolated from blood cultures has been non-typhi *Salmonella* (NTS). Bacteraemia is most common in infants and is associated not only with malaria but also with malnutrition and HIV infection. Bacteraemia cannot be identified with confidence by bedside examination or immediate tests, which is why it is now recommended that antibiotics be given in addition to antimalarials in children admitted with suspected severe malaria in high and moderate malaria transmission areas (Evans *et al.* 2004) (see section 'Management of concomitant sepsis' p. 72). The presence of bacteraemia in children with severe malaria has been found to be associated with a higher risk of death in some studies (Berkley *et al.* 2005; Were *et al.* 2011), but not in others (Bronzan *et al.* 2007).

Prognostic indices in children

Respiratory distress or altered consciousness predicted 84.4% of 64 deaths among 1844 Kenyan children admitted to hospital with malaria (Marsh *et al.* 1995). In a retrospective survey of 2911 children admitted with malaria to hospitals in Eastern Thailand, cerebral malaria was associated with the worst prognosis (21/96 died), but convulsions in the absence of coma were also associated with an increased risk of dying (4/225, compared to 5/2590 in children without coma or convulsions) (Wattanagoon *et al.* 1994). The density of peripheral parasitaemia is an indicator of risk, with mortality increasing greatly at very high levels of parasitaemia. In young children particularly from areas of high transmission, severe anaemia is the most important manifestation of malaria and mortality rises significantly if the haematocrit is below 13% (haemoglobin 4 g/dl) (Lackritz *et al.* 1993).

Among children with severe malaria, features associated with an increased risk of death include:

- depth of coma, witnessed convulsions, age <3 years and parasite density >1 million/μl in cerebral malaria (Molyneux *et al.* 1989b; Krishna *et al.* 1994; Mabeza *et al.* 1995);
- papilloedema and/or retinal oedema (Lewallen *et al.* 1993, 1996); malarial retinopathy (Beare *et al.* 2004);
- acidosis, evidenced biochemically by reduced plasma bicarbonate, increased plasma lactate concentration or clinically by acidotic breathing or severe respiratory distress (Krishna *et al.* 1994; Marsh *et al.* 1995; Maitland *et al.* 2003a; Issifou *et al.* 2007; Oduro *et al.* 2007; Orimadegun *et al.* 2007; Tripathy *et al.* 2007; Bassat *et al.* 2008; Ranque *et al.* 2008; Winkler *et al.* 2008; Camara *et al.* 2011; von Seidlein *et al.* 2012);
- increased cerebrospinal fluid lactate concentration (White *et al.* 1987a; Taylor *et al.* 1988);
- hypoglycaemia (White *et al.* 1987a,b; Taylor *et al.* 1988; Molyneux *et al.* 1989b; Walker *et al.* 1992; Krishna *et al.* 1994; Issifou *et al.* 2007; Orimadegun *et al.* 2007; Bassat *et al.* 2008; Ranque *et al.* 2008; Camara *et al.* 2011);
- blood glucose concentrations between 2.2 and 4.4 mM have also been associated with a worse prognosis than higher levels (Willcox *et al.* 2010; Nadjm *et al.* 2013);
- biochemical evidence of impaired renal function (Maitland *et al.* 2003a; von Seidlein *et al.* 2012).
- Whilst prognosis worsens with increasing parasite densities, the stage of parasite development is also important with a worse prognosis at any parasite density if more than 20% of parasites in the peripheral blood film contain visible malaria pigment (i.e. they are mature trophozoites or schizonts) (Silamut & White 1993; Waller *et al.* 1995);
- increased plasma or cerebrospinal fluid concentrations of tumour necrosis factor (Grau *et al.* 1989; Kwiatkowski *et al.* 1990; Esamai *et al.* 2003); and of other serum biomarkers including IL-10 (Armah *et al.* 2007) and elevated angiopoietin-2 with reduced angiopoietin-1 (Lovegrove *et al.* 2009);
- more than 5% of peripheral blood neutrophils contain malaria pigment (Nguyen *et al.* 1995); presence of pigment in peripheral blood polymorphs or monocytes (Lyke *et al.* 2003) and pigment in circulating

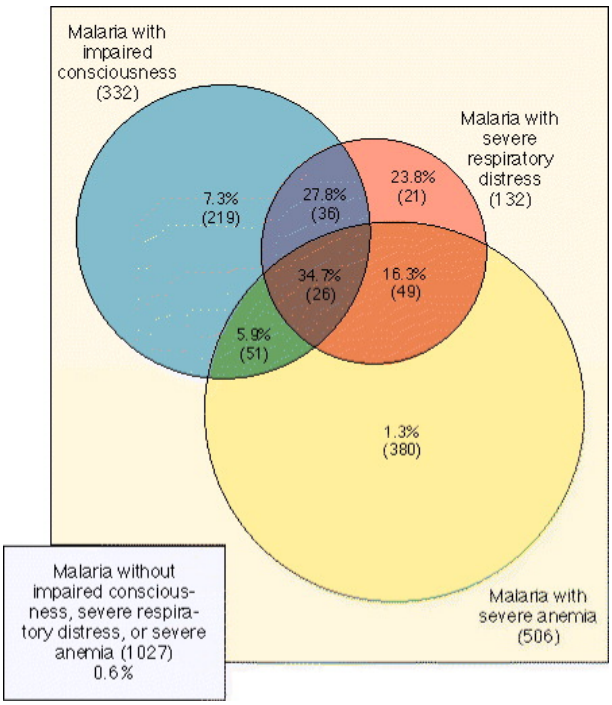


Figure 5 Prevalence, overlap and mortality for major clinical subgroups of 1844 Kenyan children with severe malaria (Marsh *et al.* 1995). Total numbers are given in parentheses, and mortality is given as a percentage.

monocytes (Mujuzi *et al.* 2006); although a study of 26 296 children admitted to six hospitals in Africa concluded, after multivariate analysis, that 'associations between pigment-containing cells (neutrophils, mononuclear cells and erythrocytes) and mortality were moderate to nonexistent in most sites' (Kremsner *et al.* 2009);

- degree of thrombocytopenia (Gerardin *et al.* 2002); although the level of thrombocytopenia was not related to outcome in two other studies (Ladhani *et al.* 2002; Chimalizeni *et al.* 2010);
- HIV infection (Malamba *et al.* 2007) (in children with severe malarial anaemia);
- bacteraemia (Berkley *et al.* 1999; Were *et al.* 2011);
- a prognostic score based on three of these indicators – coma, prostration and deep breathing, each of which has been shown to be prognostic alone – can predict outcome in a child with severe malaria with high sensitivity and specificity (Helbok *et al.* 2009). Similarly various combinations of coma, respiratory distress and severe anaemia carry a worse prognosis than any of the syndromes on their own (Marsh *et al.* 1995);
- raised plasma PfHRP2 concentration – this reflects the total parasite biomass and predicts progression to cerebral malaria in children presenting to hospital with uncomplicated malaria (Hendriksen *et al.* 2012a; Fox *et al.* 2013).

Section 4: Clinical features of severe falciparum malaria in adults

Epidemiology

Severe malaria is caused mainly by *Plasmodium falciparum*, although *P. knowlesi* and *P. vivax* may also cause severe illness. Severe malaria reflects an inability of host-defence mechanisms to control the infection so it occurs in patients with little or no effective immunity. In areas of moderate and high stable transmission, severe malaria is confined to childhood, whereas in areas of low unstable transmission, and in non-immune travellers to endemic areas, severe malaria may occur at any age. In the latter context, severe malaria is predominantly a disease of young men (reflecting their increased risk of malaria exposure). Women comprise between one quarter and one-third of cases. In low transmission settings, severe malaria is particularly likely and particularly dangerous in pregnancy (Wickramasuriya 1937). In some situations, epidemics with a very high mortality may occur (Christophers 1911). The risk of severe malaria is reduced in many haemoglobinopathies, and other inherited red cell abnormalities. As effective treatment of uncomplicated malaria prevents progression of the disease, the risk of severe malaria is determined largely by health-seeking behaviour and availability of efficacious antimalarials.

Clinical features

The symptoms and signs of acute falciparum malaria are non-specific. The illness usually starts with a general malaise followed by aching of the head, back and limbs, dizziness, anorexia, vague abdominal pain, nausea, vomiting or less commonly mild diarrhoea. Fever, which may precede or follow, is erratic, and there may be chills. An initial rigor is more commonly related to *P. vivax* or *P.ovale* infections than falciparum malaria. In addition to fever, physical signs may include anaemia, jaundice, postural hypotension and after some days tender hepatosplenomegaly. In adults, features of severe disease usually appear after 3–7 days of these non-specific 'flu-like' symptoms, although there are occasional reports of non-immune patients dying within 24 h of their first reported symptom, and some patients can deteriorate rapidly or fail to recover consciousness following a grand-mal convulsion. Agitation or confusion may occur during fever spikes but are ominous signs if they persist and often herald cerebral malaria. Once there is evidence of vital organ dysfunction progression may be rapid. In many patients, several of the manifestations of vital organ dysfunction occur together or evolve in rapid succession during the first few hours after admission to hospital. The clinical features in older children (>10 years) and adolescents are similar to those in adults (Dondorp *et al.* 2008b).

Cerebral malaria and other neurological abnormalities. Cerebral malaria refers to unrousable coma (Glasgow Coma Scale <11; see *Section 2*; definitions), in malaria having excluded, as far as possible, other causes of coma (see below) (Table 6). The proportion of patients with severe malaria who have cerebral malaria varies in time and place. In some parts of the tropics, cerebral malaria is the most common clinical presentation and the major cause of death in adults with severe malaria. In Thailand and Vietnam, about half of the cases of severe falciparum malaria over the past 30 years had cerebral malaria, although the proportion has steadily declined (Hien *et al.* 1996; Phu *et al.* 2010). In contrast, among Melanesian adults with severe falciparum malaria in Central Province, Papua New Guinea, only 17% presented with cerebral malaria (Lalloo *et al.* 1996). While *P. vivax* infections may be associated with some central nervous system dysfunction particularly during paroxysms, unrousable coma is extremely unusual (see *Section 13*; severe vivax malaria). This section refers to coma in severe falciparum malaria.

The diagnosis of cerebral malaria. In the past, patients with headache, neck stiffness, drowsiness, agitation, delirium, febrile convulsions, focal neurological signs or even behavioural disturbances who were not comatose were often described as having 'cerebral malaria'. In many of the published cases, focal signs and neurological sequelae may well have resulted from other central nervous system infections or vascular disease. In adults, high fever alone without direct involvement of the central nervous system can produce mild impairment of consciousness; variously referred to as delirium, obtundation, obnubilation, confusion and psychosis. In a febrile patient with impaired consciousness, it is important to exclude other encephalopathies, especially bacterial meningitis and, if possible, locally prevalent viral encephalitides. To allow comparability of clinical and therapeutic findings, a strict definition of 'cerebral malaria' has been developed (see *Section 2*; Definitions). A diagnosis of cerebral malaria requires definite evidence of malaria infection; asexual forms of *P. falciparum* in the blood film (or, less specifically, a positive rapid *P. falciparum* malaria test) and a Glasgow Coma Score of less than 11. 'Unrousable coma' is defined as a best motor response to noxious stimuli that is 'non-localising', and a best vocal response that is considered 'incomprehensible'. This level of unconsciousness is

chosen because the distinction between obtundation or drowsiness, and unrousable coma is clear, whereas lesser degrees of altered sensorium are difficult or impossible to separate from the effects of fever alone. In some patients with cerebral malaria, the eyes remain open, and so the eye signs in the Glasgow Coma Scale are of limited value in such cases. To distinguish cerebral malaria from transient post-ictal coma, unconsciousness should persist for at least 1 h after a convulsion, although in some patients following a seizure, a post-ictal state lasts for a few hours. In fatal cases, the diagnosis of cerebral malaria may be confirmed post-mortem by finding the cerebral capillaries and venules packed with erythrocytes containing mature *P. falciparum* parasites (mature trophozoites or schizonts) at autopsy or by needle necropsy of the brain. If the patient died after several days of treatment, then parasites may be scanty or absent although there is often residual pigment (trapped in cytoadherent residual red cell membranes).

Cerebral malaria is a diffuse and symmetrical encephalopathy. Focal neurological signs are unusual.

Coma. Cerebral malaria may start with a generalised convulsion followed by persisting unconsciousness or the level of consciousness may decline more gradually (Mishra *et al.* 2007b) (Table 6).

Meningism. Neck rigidity and photophobia are rare symptoms, but mild neck stiffness is not uncommon, and posturing with hyperextension of the neck and back may occur in severely ill adults.

Retinal abnormalities. Careful examination of the fundus is important in unconscious adults, just as in children, as distinctive abnormalities may confirm the diagnosis of cerebral malaria. Furthermore, the degree of retinopathy has prognostic significance. Retinal abnormalities are found in a small proportion of patients with uncomplicated falciparum malaria (<5%), 20% of patients with severe malaria, 40% of patients with cerebral malaria and 80% of fatal cases (Abu Sayeed *et al.* 2011). There are four components to malaria retinopathy: retinal whitening (macular or peripheral), vessel discoloration (white or orange), retinal haemorrhages (particularly with white centres – resembling Roth spots) and papilloedema (Figure 6). The first two of these are unique to severe falciparum malaria (see *Section 3*, severe malaria in children). Fluoroscein angiography reveals disruption of the retinal capillary network and microvascular obstruction. Retinal haemorrhages occur in approximately 40% of adults with cerebral malaria, papilloedema in approximately 10% and retinal whitening in approximately 5%. Vessel

Table 6 Coma scale for adults: modified Glasgow Coma Scale

	Score
Eyes open*:	
Spontaneously	4
To speech	3
To pain	2
Never	1
Best verbal response:	
Oriented	5
Confused	4
Inappropriate words	3
Incomprehensible sounds	2
None	1
Best motor responses:	
Obeys commands	6
Localises pain	5
Flexion/withdrawal to pain	4
Flexor (decorticate) posture	3
Extensor (decerebrate) posture	2
None	1
Total	3–15
Unrousable coma<9	Cerebral malaria <11

*In some patients with cerebral malaria who are unrousable, the eyes may remain open (scoring 4), so the definition of cerebral malaria also encompasses E4 V3 M4.

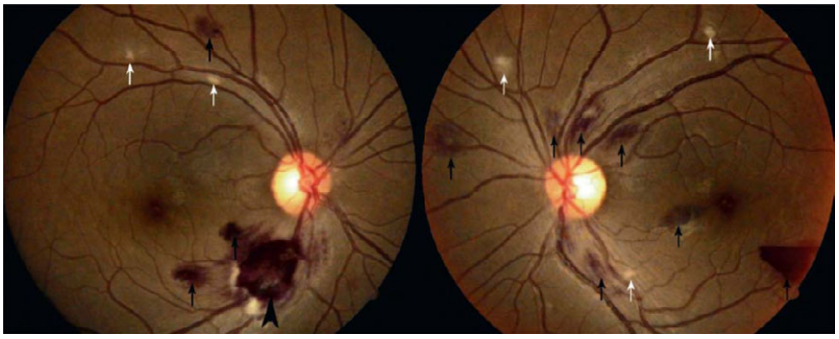


Figure 6 Fundus photographs of an adult with severe malaria showing a large white-centred haemorrhage (big black arrow), scattered patches of retinal whitening (white arrows) and haemorrhages (black arrows) in the right eye and multiple haemorrhages and patches of retinal whitening in the left eye from Abu Sayeed (2011).

discolouration is seen rarely in adults. Retinal whitening may occur without haemorrhages and can be difficult to see, particularly when it is in the peripheral retina, so can be easily missed on direct ophthalmoscopy. Although indirect ophthalmoscopy may reveal peripheral abnormalities, careful examination of the fundus by an experienced clinician is almost as sensitive. The retinal abnormalities resolve slowly over days or weeks but do not interfere with vision in survivors.

Other neurological signs. Corneal and eyelash reflexes are usually intact except in deep coma. The pupils are normal. Disorders of conjugate gaze are very common, the usual finding in adults being divergent eyes with normal oculocephalic ('doll's eye') and oculovestibular (caloric) reflexes. Convergence spasm, implying an upper brain stem lesion, transient ocular bobbing, horizontal and vertical nystagmus and sixth nerve palsies have been observed rarely. Forcible jaw closure and tooth grinding (bruxism) are common in cerebral malaria. The jaw jerk may be brisk. A pout reflex is usually elicited indicating 'frontal release', but other primitive reflexes, such as the grasp reflex, are almost always absent. The gag reflex is usually preserved. The neurological findings are usually symmetrical. Muscle tone and tendon reflexes are often increased, but may be variable or reduced. Ankle and sometimes patellar clonus may be elicited and the plantar responses are usually extensor. Abdominal and cremasteric reflexes are invariably absent and are a useful sign for distinguishing hysterical adult patients with fevers of other causes, in whom these reflexes are usually brisk. Various forms of abnormal posturing may be observed, occurring spontaneously or induced or accentuated by noxious stimuli (Plum & Posner 1972). These abnormal motor responses include abnormal flexor response in the arm(s) with extension of the leg(s) – 'decorticate rigidity' abnormal extensor responses in arm(s) and leg(s) – 'decerebrate rigidity' with or without opisthotonos and abnormal flexor responses of upper and lower limbs. Sustained upward deviation of the eyes, extension of the neck, pouting and periods of stertorous breathing may occur together. Extensor posturing may occur with hypoglycaemia, but is seen in both hypoglycaemic and normoglycaemic patients with cerebral malaria. Spontaneous movement implies lighter coma and therefore a better prognosis than immobility.

Convulsions. The incidence of convulsions in adult Thai and Vietnamese patients with cerebral malaria has fallen from 50% in the 1980s to less than 15% without obvious explanation (White 1995a) (Table 3). Seizures are usually generalised, but Jacksonian or persistent focal seizures are also observed. A variety of non-specific electroencephalographic abnormalities have been described both in uncomplicated and cerebral malaria (Collomb 1977).

Brain imaging. Computed tomography (CT) and magnetic resonance (MR) imaging are usually available only in urban centres, whereas adult cerebral malaria occurs usually in rural areas. Thus, reports are usually of travellers or unusual cases. CT of the brain in adults with cerebral malaria often shows evidence of cerebral swelling (Looareesuwan *et al.* 1983a; Mohanty *et al.* 2011; Patankar *et al.* 2002). Cerebral oedema is evident in a minority of cases and may be an agonal phenomenon (Looareesuwan *et al.* 1983b). Focal lesions, suggesting infarction or haemorrhage, have been demonstrated (Pham-Hung *et al.* 1990; Millan *et al.* 1993). In a prospective study of MRI of the brain, 22 of 24 adult Thai patients with cerebral malaria had no evidence of cerebral oedema, but MRI revealed that brain volume during acute cerebral malaria was slightly greater than during the convalescent phase (Looareesuwan *et al.* 1995). This difference was attributed to an increase in intracerebral blood volume. In two fatal cases in which assisted ventilation had been required, there was gross swelling of the brain in one and foramen magnum herniation in the other. Brain swelling, cortical infarcts and hyperintense white matter lesions, found in three of twelve patients with cerebral malaria, were interpreted as intravascular engorgement oedema and demyelination (Cordoliani *et al.* 1998). In a more recent series of three patients, focal hyperintensities in the bilateral periventricular white matter, corpus callosum, occipital subcortex and bilateral thalami were noticed on T2-weighted and FLAIR sequences. The lesions were more marked in the splenium of the corpus callosum (Yadav *et al.* 2008).

Neurological sequelae. Agitation and confusion may develop briefly as the patient recovers consciousness from cerebral malaria, and transient paranoid psychosis or delirium ('brief reactive psychosis') sometimes follows the acute illness (Anderson 1927; Kastl *et al.* 1968). In Vietnam, a post-malarial neurological syndrome was described in 22 patients, three of them children, all but one of whom had recovered from severe falciparum malaria. Symptoms of either an acute confusional state or psychosis developed in 13, six had one or more generalised convulsions, two had generalised convulsions followed by a prolonged period of acute confusion, and one developed a cerebellar tremor. In a randomised trial, 4.4% (10/228) of patients with severe malaria who received mefloquine after parenteral treatment developed

post-malarial neurological syndrome compared with 0.5% (1/210) of those who received quinine; relative risk 9.2 (95% CI 1.2–71.3, $P = 0.012$) (Nguyen *et al.* 1996). Similar cases have been reported in many different settings (Falchook *et al.* 2003; Prendki *et al.* 2008; Markley & Edmond 2009). A variety of rarer neurological abnormalities has been reported in the acute phase of illness including psychosis, confusion, ataxia, transient cranial nerve palsies and a variety of tremors.

Unusual neurological sequelae include cranial nerve lesions, focal seizures, extrapyramidal signs, ataxia (Abdulla *et al.* 1997) and greatly prolonged coma, but these are unusual (Warrell *et al.* 1982). In some cases, coma recurs after recovery of consciousness, and some of these patients have elevated cerebrospinal fluid protein and lymphocyte counts suggesting an immune-mediated pathology. The following neurological sequelae were described in 441 Indian adults with cerebral malaria, of whom 145 had died: psychosis in 15 (5.1%), cerebellar ataxia in 14 (4.7%), hemiplegia in five (1.7%), extrapyramidal rigidity in five (1.7%) and peripheral neuropathy in three (1.0%), and isolated sixth nerve palsy in one (0.33%) patient (Kochar & Shubhakaran Kumawat 2002). All survivors showed complete recovery on further follow-up.

Delayed cerebellar ataxia. Cerebellar ataxia, without impairment of consciousness or other signs of severe malaria, have been described in falciparum malaria, especially in India and Sri Lanka, but also, recently from the Sudan (Abdulla *et al.* 1997). Three or four weeks after developing transient fever attributable to falciparum malaria, patients present with unsteadiness of gait and of the upper limbs, vertigo, dysarthria and headache. On examination, there is ataxia of gait, intention tremor, dysmetria, dysdiadochokinesis, nystagmus and cerebellar dysarthria. Symptoms progress for up to 2 weeks but completely resolve 3–16 (median 10) weeks after their onset (Senanayake 1987).

Malarial psychoses. It is doubtful whether there is a specific 'malarial psychosis'. However, there are many references, especially in the older literature, to a variety of psychiatric manifestations attributed to malaria, both as the presenting feature of an acute attack of malaria and as a sequel, in convalescence, to an episode of severe or otherwise uncomplicated malaria. Limitations of many of these reports are failures to confirm the diagnosis of malaria and to exclude other causes of the psychiatric symptoms, such as antimalarial drugs, for example mepacrine, chloroquine (Akhtar & Mukherjee 1993) and mefloquine (Weinke *et al.* 1991; Luxemburger *et al.* 1994; Nguyen *et al.* 1996; Maguire *et al.* 2005). Other factors implicated in 'malarial psychosis' include alcohol, alcohol-withdrawal, narcotics, stresses associated with life or military service in tropical countries and exacerbation of pre-existing functional psychoses (Kastl *et al.* 1968). Psychiatric features include apathy, amnesia, depression, atypical depression, acute psychosis, personality change, paranoid psychosis and delusions, such as belief that family members have been killed (Kastl *et al.* 1968; Prakash & Stein 1990; Dugbartey *et al.* 1998). These symptoms rarely last for more than a few days, in contrast to those attributable to functional psychoses.

Anaemia. Anaemia is an inevitable consequence of severe malaria. On admission, approximately 10% of adults are severely anaemic (Hb <7 g/dl, haematocrit <20%), and 7% have Hb<5 g/dl and haematocrit <15% (Dondorp *et al.* 2005a) (Table 1). Anaemia develops very rapidly. In Thailand, haematocrits fell below 20% in approximately 30% of adult patients in one study (Phillips *et al.* 1986a). The degree of anaemia correlated with parasitaemia, schizontaemia, serum total bilirubin and creatinine concentrations. As in children, the prognosis of severe anaemia without other evidence of vital organ dysfunction is good with a mortality below 5%. Several large trials (Hien *et al.* 1996; Dondorp *et al.* 2005b) have therefore set the anaemia component of the severe malaria definition more strictly as a haematocrit <20% together with a parasite count >100 000/μl, as compared with the parasitaemia threshold of 10 000/μl suggested in this document.

Haemoglobinuria and Blackwater Fever. Classical descriptions of blackwater fever mention severe intravascular haemolysis with haemoglobinuria in patients with severe manifestations of *P. falciparum* infection, such as renal failure, hypotension and coma, but scanty or absent parasitaemia and mild or absent fever. The typical patient was an expatriate European who had lived in the endemic area for several months or longer, had had previous attacks of malaria and was taking quinine in an irregular fashion for prophylaxis and treatment. Symptoms associated with what was initially a typical attack of malaria included loin pain, abdominal discomfort, restlessness, vomiting, diarrhoea, polyuria followed by oliguria and passage of dark red or black urine. Signs included tender hepatosplenomegaly, profound anaemia and jaundice. The exaggerated haemolytic response in the absence of hyperparasitaemia was attributed to immune lysis of quinine-sensitised erythrocytes (Bruce-Chwatt 1987). However, direct evidence for auto-immune haemolysis in this condition was weak. Although the epidemiological evi-

dence strongly suggests a close link with quinine use, the pathophysiological mechanism has not been identified. In more recent times, intravascular haemolysis with haemoglobinuria has also been observed in Africa among patients who had repeatedly used quinine or halofantrine to treat febrile episodes (Vachon *et al.* 1992; Mojon *et al.* 1994). Massive haemolysis and methaemoglobinaemia were also well-recognised adverse effects of 8-aminoquinolines long before the discovery 60 years ago of glucose-6-phosphate dehydrogenase deficiency. As G6PD deficiency is prevalent throughout the malaria affected world, with gene frequencies typically of 5–15% (but up to 35% in some areas), oxidant haemolysis is an important cause of 'blackwater fever' independent of malaria. In a study of 50 patients with fever and haemoglobinuria in Vietnam, one-third of whom had malaria, quinine had been taken by more than half (Chau *et al.* 1996). In these patients, who typically had a greyish pallor, vomiting, loin pain and passage of black/red (Coca-cola™ coloured) urine were common symptoms. Self-treatment with quinine, often in inadequate doses, was until recently a common practice in Vietnam, as in the days when blackwater fever was first described in West Africa. In the context of severe malaria treatment, blackwater has been slightly more common in artesunate or artemether recipients than quinine recipients (Hien *et al.* 1996; Dondorp *et al.* 2005a; Phu *et al.* 2010). It seems that some patients with severe malaria and no known enzyme deficiency in oxidant defence systems have severe haemolysis sufficient to cause haemoglobinuria whichever antimalarial drug they receive.

Severe haemolysis has recently been reported following recovery from severe malaria in artesunate-treated patients (Caramello *et al.* 2012; Rolling *et al.* 2012; Centers for Disease Control & Prevention 2013). Most of these patients were hyperparasitaemic, and the haemolysis may be explained, at least in part, by the shortened survival of once infected 'pitted' erythrocytes (Newton *et al.* 2001a).

Jaundice and hepatic dysfunction. Jaundice is common in adult patients with malaria. In a study of 390 patients hospitalised with acute falciparum malaria in Thailand, one-third (124) were clinically jaundiced (total serum bilirubin >3 mg/dl or 57 μM) (Wilairatana *et al.* 1994). In severe malaria, over half the patients have admission values above this. Hyperbilirubinaemia is predominantly unconjugated (a combination of haemolysis and hepatic dysfunction). Jaundice is associated with cerebral malaria, acute renal failure, pulmonary oedema, shock and other severe complications. In Vietnamese adults, 63% of those with acute renal failure were jaundiced, compared to 20% of those without renal failure (Trang *et al.* 1992). Apart from jaundice, signs of hepatic dysfunction are unusual, although functional disturbances evidenced by altered metabolic clearance of antimalarial drugs are common (Pukrittayakamee *et al.* 1997). Clinical signs of liver failure with hepatic encephalopathy (such as asterixis or 'liver flap') are never seen unless there is concomitant viral hepatitis. Tender enlargement of the liver and spleen is, however, a common finding in all human malarias, especially in young children and non-immune adults. The low and falling serum albumin concentration is an important index of temporary hepatic dysfunction. Concentrations of aspartate and alanine aminotransferases may be increased up to tenfold, but never to the levels normally seen in viral hepatitis (Table 1). 5-nucleotidase and gamma glutamyl transpeptidase concentrations may also be moderately elevated. The prothrombin and partial thromboplastin times may be moderately prolonged particularly if there is associated coagulopathy (Clemens *et al.* 1994). Liver dysfunction also contributes to lactic acidosis (reduced lactate clearance) (Day *et al.* 1996b), hypoglycaemia (impaired gluconeogenesis) (White 1983, White 1987a) and changes in triglycerides, phospholipids, free fatty acids, cholesterol, esterified cholesterol and non-esterified fatty acids. Sometimes patients with malaria may take hepatotoxic herbal remedies resulting in liver dysfunction and hypoglycaemia.

Haemostatic abnormalities, thrombocytopenia. In the 1970s, bleeding was reported frequently in severe malaria, but in recent larger series, bleeding has been unusual (<5% of cases) at presentation (Dondorp *et al.* 2005a). Nevertheless, laboratory evidence of activated coagulation is very common (Clemens *et al.* 1994). Fewer than 10% of adult Thai patients with cerebral malaria showed clinically evident bleeding tendency with associated features of disseminated intravascular coagulation (Phillips & Warrell 1986). Bleeding gums, epistaxis, haematemesis, petechiae and subconjunctival haemorrhages were seen. If it does occur, bleeding usually accompanies renal, pulmonary or hepatic complications and is associated with severe thrombocytopenia and coagulopathy (Stone *et al.* 1972; Punyagupta *et al.* 1974). Stress-related gastrointestinal haemorrhage may occur in severely ill patients. Thrombocytopenia is a common feature of falciparum and vivax malarias and is not itself a sign of severity although profound thrombocytopenia (<20 000/μl) is more common in severe falciparum malaria (Horstmann *et al.* 1981). Altered platelet function has also been described (Srichaikul 1993). Thrombocytopenia is not related to other measures of coagulation (prothrombin time, partial thromboplastin time) or to

plasma fibrinogen concentrations. Thrombocytopenia does not, in itself, indicate disseminated intravascular coagulation and is usually not accompanied by bleeding. Plasma fibrinogen is usually in the high normal or slightly elevated range although its breakdown is accelerated (Devakul *et al.* 1966), so concentrations of fibrin degradation products are usually slightly elevated (Reid & Nkrumah 1972).

Renal dysfunction. Acute kidney injury is a common manifestation of severe falciparum malaria and in adults is often lethal. Although evidence of renal impairment is common at all ages, oliguric renal failure occurs almost exclusively in adults and older children. Malarial acute renal failure may be defined as a serum creatinine concentration > 265 μM (3 mg/dl) in patients who have asexual forms of *Plasmodium falciparum* in their peripheral blood smear. Plasma creatinine has the best prognostic value in terms of the subsequent need for renal replacement therapy and outcome (Hanson *et al.* 2011a, 2013). A 24-h urine output of <400 ml, in spite of rehydration, is a further indication. There are two categories of patients with acute renal failure: those with acute severe malaria and multiple organ involvement (a fulminant disease which carries a poor prognosis) and those who develop progressive renal impairment after successful treatment of malaria infection (whose prognosis is good, provided that renal replacement therapies are available). The latter group tends to be more anaemic and have a higher serum creatinine concentration on admission, reflecting a longer duration of illness before referral (Trang *et al.* 1992). Acute renal failure in malaria is usually oliguric (<400 ml/day), or anuric (<50 ml/day), but urine output may also be normal or rarely increased. The mortality of patients with renal impairment is approximately four times higher than in patients with normal renal function. In the SEAQUAMAT trial, 51 (38%) of 136 artesunate recipients and 76 (52%) of 146 quinine recipients with renal failure died, compared with 47 (9%) of 525 and 65 (13%) of 501, respectively, who did not have renal failure (Dondorp *et al.* 2005b). Patients with renal failure have a higher incidence of acidosis, hypoglycaemia and jaundice, and the duration of coma is significantly prolonged. Pulmonary oedema is a common terminal event.

Fluid balance. Some untreated patients with severe falciparum malaria are clinically hypovolaemic (low jugular venous pressure, postural hypotension, oliguria with high urine specific gravity), and those who have been febrile for days with inadequate water intake are dehydrated (reduced skin turgor) on admission. Other patients are not dehydrated, and in some cases, patients are referred having received excess intravenous saline. Clinical assessment, the central venous pressure and invasive haemodynamic monitoring are inaccurate in predicting the effective circulating blood volume and tissue oxygen delivery, probably because of extensive microvascular obstruction consequent upon sequestration (Hanson *et al.* 2011a, 2012, 2013). All this makes management of fluid balance difficult in severe malaria.

Electrolyte abnormalities. Mild hyponatraemia (serum sodium 125–135 mM) is common in falciparum malaria (Holst *et al.* 1994) and is often accompanied by mildly reduced plasma osmolality. The antidiuretic hormone concentrations are appropriate (Hanson *et al.* 2009). Approximately 25% of adults admitted with severe malaria have a plasma sodium <130 mM, and 25% also have a plasma potassium <3.5 mM, but very low values are rare (Dondorp *et al.* 2005a). Salt depletion through prolonged sweating or dilution from administration of intravenous dextrose solutions contribute. Hypocalcaemia has been observed in patients with severe falciparum malaria (Petithory *et al.* 1983), but may be explained in part by associated hypoalbuminaemia. In Thailand, in a cross-sectional study of 172 adults with acute falciparum malaria, 36% had mild asymptomatic hypocalcaemia (1.79–2.11 mM corrected). There was no difference between severe and uncomplicated cases. However, in 6 of 10 cases studied prospectively, hypocalcaemia was associated with inappropriately low intact parathormone concentrations in the serum (<5.0 pM) (Davis *et al.* 1991). Despite malaria-associated haemolysis, hypophosphataemia (<0.80 mM) was found in 43% of the 172 patients and 11 patients (6%) had values <0.30 mM. Hyperphosphataemia (>1.45 mM) was found in 9% (Davis *et al.* 1991). Severe hypophosphataemia could contribute to disturbances of cerebral, leucocyte and platelet function and to haemolysis and is exaggerated by administration of glucose and by quinine-induced hyperinsulinaemia.

Pulmonary oedema. This is a grave and often fatal manifestation of severe falciparum malaria in adults which may develop suddenly after one or two days of treatment. Some cases show evidence of fluid overload, with raised central venous or pulmonary artery wedge pressures and grossly positive fluid balance (Brooks *et al.* 1968). Other patients develop pulmonary oedema with normal or negative fluid balance and with normal or reduced pulmonary capillary wedge pressure. This indicates increased pulmonary capillary permeability, and so malaria pulmonary oedema resembles the acute

respiratory distress syndrome (ARDS) (Taylor *et al.* 2012b; Hanson *et al.* 2013). Patients with severe malaria are more vulnerable than those with sepsis to volume overload and readily develop ARDS. Hyperparasitaemia, renal failure, and pregnancy are predisposing factors. Hypoglycaemia and metabolic acidosis are commonly associated. The first indication of impending pulmonary oedema is usually an increase in the respiratory rate, which precedes the development of other chest signs. Without good facilities for emergency radiography, it may be difficult to differentiate acute pulmonary oedema from aspiration bronchopneumonia and metabolic acidosis, although in acidosis, auscultation of the chest is often normal. Metabolic acidosis and pulmonary oedema may also occur together. Although pulmonary oedema may develop at any stage of the acute illness, it tends to occur later than the other acute manifestations of malaria (Brooks *et al.* 1969). Hypoxia may cause convulsions and deterioration in the level of consciousness, and the patient may die within a few hours.

Cardiovascular abnormalities, shock ('algid malaria'). The blood pressure of patients with malaria is usually at the lower end of the normal range, although most patients are warm and well perfused. Approximately 10% of patients with severe malaria are considered clinically to be in shock (Dondorp *et al.* 2005a). Severe hypotension [systolic blood pressure less than 80 mmHg (10.7 kPa) in adults in the supine position] with features of circulatory failure (cold, clammy, cyanotic skin, constricted peripheral veins, prolonged capillary refill time) is seen in patients with pulmonary oedema, metabolic acidosis, bacteraemia (see below) and following massive gastrointestinal haemorrhage or splenic rupture. Dehydration may also contribute to hypotension, and some patients may be admitted severely dehydrated, hypotensive and oliguric having endured high fever and inadequate fluid intake for several days. However, most adult patients with severe malaria are not significantly dehydrated or hypovolaemic, and fluid resuscitation needs to be performed carefully to avoid fluid overload and pulmonary oedema (Hanson *et al.* 2013). Myocardial dysfunction, ventricular failure and cardiac arrhythmias are very rarely observed in severe malaria, despite the sequestration of parasitised erythrocytes in the myocardial vessels and the marked cardiac effects of many antimalarial drugs (Bethell *et al.* 1996). The clinical picture of 'algid malaria' (shock in severe malaria) resembles that of septic shock, and indeed, in many patients (but certainly not all), there is concomitant bacteraemia.

Acid base disturbances. Acidosis is a major cause of death from severe malaria, and the degree of acidosis is the single most powerful prognostic indicator (Day *et al.* 1996b; Hanson *et al.* 2012). Acidotic breathing (hyperventilation, Kussmaul's breathing) is a poor prognostic sign although it must be distinguished from pulmonary oedema and neurogenic hyperventilation. Metabolic acidosis may develop in severely ill patients as a result of microcirculatory obstruction combined with hepatic dysfunction, in patients who are shocked or are in renal failure. Hyperlactataemia with a high lactate/pyruvate ratio indicates an ischaemic basis for the lactic acidosis (Pukrittayakamee *et al.* 2002). Other unidentified organic ions also contribute (Dondorp *et al.* 2004a). Clinically, there is no distinction between the metabolic acidosis consequent upon microcirculatory obstruction, hepatic dysfunction, and renal impairment. On admission plasma lactate may be high for various reasons including recent seizures and very high catecholamine levels, which usually fall rapidly (Day *et al.* 2000a,b). Sustained elevation of plasma lactate carries a poor prognosis, whereas rapidly resolving hyperlactataemia carries a good prognosis. If possible, plasma concentrations should be checked 4 h after admission and after adequate rehydration.

Gastrointestinal symptoms. Nausea, vomiting (20–30% of patients), abdominal pain, which may be colicky and severe, and diarrhoea (10–20% of patients), which may be watery but does not contain blood or pus cells, all occur in adults with severe malaria and may lead to misdiagnosis of enteric infections. Patients who consistently vomit tablets should be transferred to a place where parenteral treatment can be given unless intrarectal artemisinin derivatives are available and can be given.

Hypoglycaemia. Hypoglycaemia is an important complication of falciparum malaria and its treatment (White *et al.* 1983a). Often hypoglycaemia is not suspected clinically so blood glucose concentrations must always be checked in severely ill patients. The incidence of hypoglycaemia at presentation has fallen as use of quinine has declined. In conscious patients, hypoglycaemia may present with classical symptoms of anxiety, breathlessness, a feeling of coldness, tachycardia and lightheadedness and signs of autonomic overactivity (sweating, or 'gooseflesh'). More severe signs include coma, deteriorating consciousness, abnormal posturing (decerebrate or decorticate rigidity, muscle spasms, pouting, stertorous breathing and opisthotonos) and generalised convulsions. The diagnosis of hypoglycaemia may be overlooked

because all these clinical features are also typical of severe malaria *per se*.

In severe malaria, many of the usual diagnostic features of hypoglycaemia may be absent and the diagnosis may be overlooked. Sweating is an inconstant sign, the pupils are frequently not dilated, the breathing may be cyclical or stertorous and deep, and there may be abnormal posturing of the arms and legs. There is usually a deterioration in the level of consciousness. Following treatment with intravenous 50% glucose, clinical improvement is very variable – from no apparent change to a change in the respiratory pattern and a lightening of coma. Hypoglycaemia complicates malaria in three clinical settings which may overlap: in patients with severe disease especially young children, in pregnant women and in patients given quinine. Quinine-induced hyperinsulinaemia is a common contributing cause of hypoglycaemia particularly in pregnant women (White *et al.* 1983a; Das *et al.* 1988), and hypoglycaemia in this context may be recurrent as glucose administration stimulates further hyperinsulinaemia. Hypoglycaemia develops during treatment of malaria significantly more commonly in patients (adults and children) treated with quinine than those treated with artesunate (Dondorp *et al.* 2005b, 2010) or artemether (Hien *et al.* 1996). Hypoglycaemia unrelated to quinine is associated with acidosis in severe malaria in adults and children and carries a poor prognosis. It is a direct result of the disease process (anaerobic glycolysis – the Pasteur effect).

Complicating and associated infections. Other severe and life-threatening infections may arise in patients with severe falciparum malaria. In some cases, there are complicating infections: for example, aspiration bronchopneumonia in patients who have had generalised convulsions; urinary tract infections in patients with indwelling urethral catheters; infected decubitus ulcers in patients with prolonged coma; and infection around intravenous cannulae. Nosocomial infection is an important contributor to death in patients who remain seriously ill for several days. In a recent study in Vietnam in a setting with good intensive care facilities, nosocomial infection was considered to contribute to a fatal outcome in 12 of the 37 deaths in the 370 adults enrolled in a clinical trial comparing artesunate and artemether (Phu *et al.* 2010). Bacteraemia may coexist with severe falciparum malaria without an evident focus. Unlike children in Africa, among whom the causative organisms are usually enteric bacteria, in Vietnam only one case of Gram-negative septicaemia was detected in 500 adult patients on admission with strictly defined severe malaria (Parry; personal communication).

Peripheral leucocytosis. The total white count is normal in the majority of patients (median approximately 8000/μl). Neutrophil leucocytosis may occur in severe falciparum malaria, even in the absence of detectable secondary bacterial infection. Approximately 25% of patients have a total white count on admission over 12 000/μl (Hien *et al.* 1996). Leucocytosis is associated with poor prognosis. Leukaemoid reactions have been reported (Stein 1987).

Peripheral gangrene/rhabdomyolysis. There are many reports of symmetrical peripheral gangrene occurring in acute malaria, both falciparum and vivax. Many of these have followed the treatment of severe falciparum malaria, and in some there has been associated coagulopathy (Alkizim *et al.* 2011). Generalised myalgia, myoglobinuria and histological evidence of inflammatory skeletal muscle necrosis have been reported (De Silva *et al.* 1988), but skeletal muscle damage sufficient to cause renal failure appears to be rare. Biochemical evidence of muscle damage is much more common (Miller *et al.* 1989).

Outcome and prognostic indices in adults

The prognosis in severe malaria is determined by the number of vital organ systems that are involved and the severity of their dysfunction. Mortality is increased in the elderly and also in pregnant women. While the quality of intensive care support is an important determinant of outcome, by far the most important factor is the specific antimalarial drug treatment. Treatment must be given as early as possible in the evolution of a potentially lethal infection. Artesunate reduces the mortality of severe malaria in adults by one-third compared with quinine (Newton *et al.* 2003; Dondorp *et al.* 2005a; Phu *et al.* 2010). Many studies have examined prognostic factors (Newton *et al.* 2013). Differences in predictive factors are explained by the different patient populations; for example, parasite density is clearly an important determinant of outcome in acute falciparum malaria; yet within strictly defined severe falciparum malaria, the density of parasitaemia has much less prognostic value. Delay in administering antimalarials is clearly a major determinant of progression to severe disease, but once severe disease has developed, outcomes are independent of the duration of preceding illness. Dangerous antecedent factors for the development of severe falciparum malaria include splenectomy, pregnancy, corticosteroid or cytotoxic drug use, immunosuppression, lack of previous malaria exposure and, to a much lesser extent, lapsed immunity.

Clinical indicators. Any degree of central nervous system dysfunction carries an increased mortality. Confusion, agitation, obtundation are all risk factors. In the large SEAQUAMAT study (N = 1461, 563 with cerebral malaria), unrousable coma or cerebral malaria (GCS < 11) carried a 37% mortality in adults treated with quinine and 30% in those treated with artesunate. In earlier series, mortality with quinine treatment was lower – approximately 20%. This is because mortality in comatose patients is very much determined by other vital organ dysfunction. In patients with 'pure' cerebral malaria, that is, no other vital organ dysfunction, the case fatality is <10%. Marked, often violent, agitation in young adults may precede rapid deterioration. Convulsions often progress to coma, but even with rapid recovery, convulsions define a group at increased risk in areas of low or unstable transmission (Wattanagoon *et al.* 1994). However, although cerebral malaria is undoubtedly a very important severe manifestation of malaria, patients of all ages may die without cerebral involvement.

During the past three decades in Vietnam and Thailand, the clinical pattern of severe malaria has changed. Over two decades ago, cerebral malaria was the predominant manifestation of severe malaria, whereas today the combination of jaundice and renal failure is more common. The incidence of convulsions in cerebral malaria has also declined from 50% to <20% (Warrell *et al.* 1982). Renal impairment is an indicator of severity, and the prognosis of acute renal failure is worsened by coexistent jaundice (Trang *et al.* 1992).The prognosis of cerebral malaria is worsened considerably if there is also renal failure. The signs of acidosis or uraemia represent a late stage in the progression of the disease. Oliguria or anuria are usually present, but may not have been recorded. Measurement of blood urea or creatinine is often the only method of diagnosis. In adults, severe anaemia is less prominent than it is in children. The prognosis of severe anaemia in the absence of other severe manifestations of malaria is good, probably because it often represents acute on chronic anaemia or a protracted course of illness. For this reason, recent large randomised trials have used a stricter threshold (haematocrit <20% plus parasitaemia 100 000/μl) than in the current document (where the parasitaemia threshold is 10 000/μl) as a criterion of severe falciparum malaria (Hien *et al.* 1996; Dondorp *et al.* 2010; Phu *et al.* 2010). Many studies have emphasised the prognostic importance of acidosis, reflected as acidotic breathing or measured as arterial pH, base deficit, plasma bicarbonate concentration, or as arterial, venous, or CSF lactate concentration (Day *et al.* 1996b; Hanson *et al.* 2010; White *et al.* 2013a). Rapid deep laboured breathing (reflecting acidosis, pulmonary oedema or aspiration pneumonia) often termed respiratory distress identifies a subgroup of patients at high risk of dying independent of cerebral malaria. Respiratory distress of this kind is usually a late sign in adults. Significant bleeding is associated with an increased risk of dying but is unusual. Jaundice carries a poor prognosis only if combined with other evidence of severe disease.

Laboratory indices. Laboratory indicators of poor prognosis reflect the size of the parasite burden (parasite count, stage of parasite development, neutrophil pigment), the degree of microvascular obstruction (lactate, bicarbonate) and the extent of vital organ dysfunction or damage (urea, creatinine, glucose, bilirubin, transaminases, haemoglobin, platelet count). The prognostic value of the parasite count, as a measure of parasite burden, depends on the age and degree of background immunity of the patient. The classical work of Field in Malaya (an area of relatively low seasonal transmission) first characterised the relationship between parasitaemia and prognosis (Field & Niven 1937; Field 1949). There was a correlation between parasite counts and prognosis with a significant increase in mortality with parasitaemias over 2%. In Field's original series, a parasitaemia over 500 000/μl was associated with >50% mortality. This loose relationship differs according to age and intensity of malaria transmission. In areas of higher transmission, the mortality associated with this parasitaemia would be considerably lower. In non-immune subjects, parasitaemias over 4% are considered sufficiently dangerous to warrant closely supervised treatment (Tables 2 and 3) (Luxemburger *et al.* 1995). In endemic areas, the threshold is higher. The prognostic value of the parasite count may be improved considerably by assessing the stage of parasite development in the peripheral blood film; at any parasitaemia, prognosis worsens if there is a predominance of more mature parasite stages. In general, if more than 50% of the peripheral blood parasites are at the tiny ring stage (where the diameter of the nucleus is less than half the diameter of the rim of cytoplasm), then the prognosis is relatively good, whereas if more than 20% of the parasites contain visible pigment (i.e. mature trophozoites or schizonts), then the prognosis is relatively bad (Silamut & White 1993). Assessment of peripheral blood polymorphonuclear leucocyte pigment proved to be a rapid and fairly accurate prognostic indicator in Vietnamese adults (Nguyen *et al.* 1995). As in children, recent studies in adults have shown the prognostic value of measuring plasma PfHRP2 concentrations as a surrogate measure of the parasite burden (Desakorn *et al.* 2005; Dondorp *et al.* 2005b). This has substantially better predictive

value than the parasite count (Fox *et al.* 2013; Hendriksen *et al.* 2013c). Several prognostic scores have been developed and their performance characteristics assessed. These scores are useful for rapid bedside assessments and triage. Acidosis (base deficit) and cerebral malaria (measured as Glasgow Coma Score) are the main independent predictors of outcome.

Based on data from 868 South-East Asian adults with severe malaria, Hanson *et al.* (2010) developed a simple 5-point Coma Acidosis Malaria (CAM) score derived from these two variables (Table 7). Mortality increased steadily with increasing score. A CAM score <2 predicted survival with a positive predictive value (PPV) of 95.8% [95% confidence interval (CI), 93–97.7%]. Of the 14 of 331 patients who died with a CAM score <2, 11 (79%) had renal failure and death occurred late after hospital admission (median, 108 h; range, 40–-360 h). Substitution of plasma bicarbonate as the measure of acidosis only slightly reduced the prognostic value of the model. Use of respiratory rate was inferior, but a score <2 still predicted survival with a PPV of 92.2% (95% CI 89.1–94.7%).

Table 7 Derivation of the Coma Acidosis Malaria (CAM) Score. Bicarbonate-Based CAM Score and Respiratory Rate-Based CAM Score (Assessed at Hospital Admission)

	Score		
Variable	0 (Normal)	1 (Deranged)	2 (Very deranged)
Base deficit	<2	2 to <10	>10
GCS	15	>10 to 14	≤10
Bicarbonate score	≥24	15 to <24	<15
Respiratory rate score	<20	20 to <40	≥40

CAM score	Mortality (%)
1	6–8
2	8–27
3	21–37
4	46–67

CAM score (0–4) is calculated as the base deficit score (0–2) plus the Glasgow Coma Score (GCS 0–2). Bicarbonate-based CAM score (0–4) is calculated as the bicarbonate score (0–2) plus the GCS (0–2). Respiratory rate-based CAM score (0–4) is calculated as the respiratory score (0–2) plus the GCS score (0–2) (Hanson *et al.* 2010).

Section 5: Severe malaria in pregnancy

In areas of low transmission, falciparum malaria is an important cause of maternal mortality (Desai *et al.* 2007; Rijken *et al.* 2012a). Severe malaria in late pregnancy is a devastating and fulminant disease with a high mortality for both mother and fetus that is very difficult to manage, requiring close liason between physicians, obstetricians and paediatricians. The case fatality is substantially higher in the second half of pregnancy than in non-pregnant adults, and with quinine treatment often reached 50% (Looareesuwan *et al.* 1985; Mengistu *et al.* 2006). Reduced host-defence mechanisms and extensive parasite sequestration in the placenta both contribute to the increased risk. Severe malaria is an important cause of abortion, stillbirth, premature delivery and fetal death (Desai *et al.* 2007; McGready *et al.* 2012b). In travellers from non-endemic areas and residents of low transmission areas who have little or no background immunity, pregnancy increases the risk that a *P.falciparum* infection will develop into severe malaria. Any of the syndromes observed in non-pregnant adults may occur in pregnant women, but two manifestations are particularly common: hypoglycaemia and pulmonary oedema (White *et al.* 1983b; Looareesuwan *et al.* 1987). The anaemia of severe malaria compounds pre-existing pregnancy-related anaemia (Phillips *et al.* 1986a; Fleming 1989). Fetal distress is usual, and fetal death is common. After delivery post-partum haemorrhage is common, and there is a high risk of puerperal sepsis. If the fetus does survive, there is intrauterine growth retardation.

Hypoglycaemia

Hypoglycaemia is a common presenting feature of severe malaria in pregnancy (Looareesuwan *et al.* 1985). Quinine is particularly dangerous in pregnancy because it causes marked hyperinsulinaemia. In the past, hypoglycaemia developed during the course of quinine treatment in 50% of pregnant women with cerebral malaria (White *et al.* 1983b). Artesunate reduces the mortality of severe malaria in adults by 35%, does not cause hypoglycaemia and is therefore the treatment of choice in all trimesters of pregnancy (World Health Organization 2010a). Hypoglycaemia may be associated with lactic acidosis in fulminant multisystem disease, or it may be an isolated complication. Hypoglycaemia may be asymptomatic or may present with sweating, behaviour change, altered consciousness or convulsions. There may be associated foetal bradycardia or other signs of fetal distress. Asymptomatic hypoglycaemia is commonly unrecognised, and cerebral effects may be mistakenly attributed to malaria. Hypoglycaemia in late pregnancy commonly recurs after correction by intravenous glucose therapy.

Acute pulmonary oedema

Pregnant patients with severe falciparum malaria are particularly prone to develop acute pulmonary oedema. The first signs are an increase in respiratory rate, which precedes the development of chest signs. Dyspnoea and hypoxia may be present on admission or develop suddenly several days after admission to hospital. Acute pulmonary oedema often develops immediately after delivery and may occur at any time in the first week post-partum. Women who are severely anaemic or fluid-overloaded when they go into labour may develop acute pulmonary oedema after separation of the placenta.

Severe anaemia is associated with maternal morbidity, an increased risk of post-partum haemorrhage and perinatal mortality.

Premature labour

Symptomatic falciparum malaria commonly induces uterine contractions and this may precipitate premature labour. The frequency and intensity of contractions are related to the height of fever (Looareesuwan *et al.* 1985). Fetal distress is usual. The foetal prognosis in premature labour associated with severe malaria is poor.

In areas of intense, stable transmission severe or complicated disease – with the exception of severe anaemia – is uncommon in pregnancy, and malaria is unusual as a direct cause of maternal death. The main presentation of severe malaria is severe anaemia. This is often multifactorial with a further reduction in haematocrit as a result of pregnancy-associated anaemia, iron and nutritional deficiency and sometimes folate deficiency (Fleming 1989). With increasing success of malaria control measures, transmission intensities are falling and this may result in an increase in the risk of maternal severe malaria. HIV infection and malaria synergise in their harmful effects on pregnancy. Severe malaria increases the risk of vertical transmission of HIV infection, as does the presence placental parasitaemia at parturition. Menendez *et al.* (2008) conducted a prospective autopsy study between October 2002 and December 2004 on the causes of maternal death in a tertiary-level referral hospital in Maputo, Mozambique: 65 of the 139 women (46.8%) who died were HIV-positive. Severe malaria was responsible for 14 (10.1%) of the maternal deaths.

Section 6: Severe malaria in the newborn

Babies are believed to have partial protection from malaria in the first few months of life, owing to passively acquired maternal IgG antibodies, the predominance of haemoglobin F (HbF) in their erythrocytes and the low levels of iron and para-amino benzoic acid (both required for parasite growth) in breast milk. Malaria infection in the first week of life is usually called 'congenital malaria', while that occurring in the next 3 weeks is sometimes referred to as 'neonatal malaria'. The former is assumed to be acquired from the mother, the latter by mosquito inoculation, although it is never possible for the clinician to distinguish between these routes of infection with confidence (D'Alessandro *et al.* 2012), and transplacental infection can present up to 2 months after delivery.

Parasitaemia in neonates

In areas with intense transmission of *P. falciparum,* newborn babies may have a low-grade *P. falciparum* parasitaemia that usually disappears without treatment by the third day of life. In a multicentre study in Nigeria, 5.1% of 1875 newborn babies were parasitaemic, all becoming aparasitaemic by day 3, in the majority without antimalarial treatment; about a third had fever and refusal to suck, possibly attributable to malaria and responding to antimalarial treatment, and none had severe malaria (Falade *et al.* 2007). Many other studies have reported prevalences of *P. falciparum* parasitaemia in newborns varying between 0% and nearly 50% (Runsewe-Abiodun *et al.* 2006; Falade *et al.* 2007; Orogade *et al.* 2008). Infants born to non-immune mothers who have malaria at the time of delivery may develop parasitaemia and illness in the first few weeks of life (Brabin 2007; Vottier *et al.* 2008; Mwaniki *et al.* 2010). Clinical features, which usually appear between the second and eighth weeks of life, include fever, anorexia, lethargy, anaemia, jaundice and hepatosplenomegaly. Occasionally, severe anaemia may develop, but other signs of severity are unusual.

Indirect effects of maternal malaria on neonates

Maternal malaria exerts important indirect effects on neonates. Low birthweight is common in babies born to mothers who have had malaria during pregnancy. The effects are greatest in primigravidae and secundagravidae, who are the most susceptible to malaria. In most affected babies, this low birthweight is due to intra-uterine growth retardation (the baby being 'small for dates'), but in some, it is due to premature delivery, especially if the mother had febrile malaria in the third trimester (Steketee *et al.* 1996; Yakoob *et al.* 2005; Mokuolu *et al.* 2009; Falade *et al.* 2010). Babies with low birthweight are at increased risk from all the infections that threaten children during infancy (Mokuolu *et al.* 2009).

Severe malaria in neonates

The definition of severe malaria in neonates is the same as in older children. Reported cases of severe malaria in the first month of life are very few, but both *P. falciparum and P. vivax* have been reported (Larkin & Thuma 1991; Ekanem *et al.* 2008; Poespoprodjo *et al.* 2010). As in older children, the condition is not distinguishable clinically from severe septicaemia (Piñeros-Jiménez *et al.* 2008). Tests for malaria parasites should be included in the routine screening of febrile babies with suspected septicaemia in malaria-endemic regions (Runsewe-Abiodun *et al.* 2006; Orogade *et al.* 2008). Conversely, neonates with severe illness and parasitaemia should have blood samples taken for culture, and in any case should be treated with antibiotics as well as antimalarial drugs.

Management

Supportive care appropriate to the age of the infant is crucial. Current standard treatment with parenteral artesunate (or if unavailable artemether) for the treatment of severe malaria should be used, although there are few data on appropriate dosages in this age group. Quinine is an alternative if an artemisinin derivative is not available. Antibiotics should be given, as bacteraemia cannot be excluded immediately. Studies are currently in progress to identify optimal antimalarial drug schedules and to monitor the safety and efficacy of these therapies.

Section 7: Pathophysiology of severe falciparum malaria

Pathology and pathophysiology

Uncomplicated and severe disease in malaria results solely from the blood stage of the infection. Disease is caused by the direct effects of red cell parasitisation and destruction by the asexual parasites, and the host's reaction to this process. In *P. falciparum* blood-stage infections, protuberances appear on the surface of the erythrocyte 12–15 h after cellular invasion. These membrane 'knobs' extrude a high molecular weight, antigenically variant, strain-specific adhesive protein (*Pf*EMP1) that mediates *cytoadherence,* or attachment of the parasitised erythrocyte to endothelial surface receptors in veins and capillaries, resulting in the *sequestration* of parasitised erythrocytes in these vessels. Under febrile conditions, which enhance P*f*EMP1 expression, cytoadhesion begins at approximately 12 h of parasite development; 50% of the maximum effect is obtained at 14–16 h, and adherence is highly effective in the second half of the parasite life cycle (Udomsangpetch *et al.* 2002). Once adherent, the infected red cells do not detach until schizont rupture. There is some ring-stage adherence via separate mechanisms. Of several potential receptors identified for cytoadhesion of erythrocytes infected with the more mature parasites, intercellular adhesion molecule 1 (ICAM-1) is probably the most important in the brain, chondroitin sulphate A in the placenta, and CD36 in most other organs (Cooke *et al.* 1994). The infected erythrocytes adhere to the vessel walls and eventually cause heterogeneous blockage of the microcirculation (MacPherson *et al.* 1985; Silamut *et al.* 1999). Parasitised erythrocytes may also adhere to each other (agglutination) (Pain *et al.* 2001) and to uninfected erythrocytes (rosetting) (Carlson *et al.* 1990). These adherence processes are central to the pathogenesis of falciparum malaria. They result in the sequestration of red cells containing mature parasites in vital organs (including the brain), where they interfere with microcirculatory flow and metabolism and the function of vascular endothelium (MacPherson *et al.* 1985, White *et al.* 2013b). Sequestered *P. falciparum* parasites develop safely away from splenic processing and filtration. Only the younger ring form *P. falciparum* parasites circulate in falciparum malaria, and so the peripheral parasite count may substantially underestimate the actual total parasite biomass. As the intraerythrocytic parasites mature, they make the infected red cells more spherical and rigid. Severe falciparum malaria is associated with reduced deformability of the uninfected erythrocytes, which may contribute to compromised flow through the partially obstructed capillaries and venules, and shortens their survival (Dondorp *et al.* 2002).

In the other malarias, significant sequestration does not occur, and all stages of the parasites' development are seen on peripheral blood smears. *P. vivax*, *P. ovale* and *P. malariae* show selective red cell invasion, and parasitaemias are usually less than 1%, whereas *P. falciparum* and *P. knowlesi* are less selective (Simpson *et al.* 1999) and may be associated with very high parasite densities. Initially, the host responds by augmenting splenic immunological and filtrative clearance functions, which accelerates the removal of both parasitised and uninfected erythrocytes. Schizont rupture releases parasite and red cell material into the blood that activates monocyte-macrophages and induces the release of pro-inflammatory cytokines, which cause fever and other pathologic effects. Temperatures of >40°C damage mature parasites. In untreated infections, these factors synchronise the asexual cycle, with eventual production of regular fever spikes, chills and rigors. These regular fever patterns, which were once used to classify the malarias (quotidian; fever spike daily, tertian; every 2 days, quartan; every 3 days), are rarely seen today in patients who receive prompt and effective antimalarial treatment.

Severe malaria

There is accumulating evidence that most of the major manifestations of severe malaria are caused by parasitised erythrocyte sequestration and consequent vital organ dysfunction. The sheer amount of pathological material obstructing microcirculatory flow is staggering; it has been estimated recently that the lethal parasite biomass on average in a 60-kg adult is approximately 270 ml (=3.4 × 1012 × 80 fL) of parasitised red cells (White *et al.* 2013b). In the brain in fatal cerebral malaria, 10–20% of the total parasite biomass may be sequestered, which represents an approximate intravascular volume of 50 ml. This is certainly enough to cause significant cerebral swelling independently of any vasogenic or cytotoxic oedema. Recent direct visualisation of the microcirculation in life in patients with falciparum malaria, and measurements of individual vessel flows, shows reversible heterogeneous microvascular obstruction in the retinal, buccal and rectal circulations with a pattern which mirrors closely the heterogeneous sequestration seen in the tissues examined from fatal cases (Beare *et al.* 2006; Dondorp *et al.* 2008a). The degree of microvascular obstruction observed in blood flow studies in life also parallels clinical severity and established prognostic measures such as plasma lactate and base deficit (Hanson *et al.* 2012).

Tissue hypoxia from microvascular obstruction is exacerbated by impaired microvascular function (Yeo *et al.* 2008a, 2012) and, in contrast to sepsis, increased oxygen demand (Day *et al.* 1996b; Yeo *et al.* 2012). Impaired

endothelial function may result from impaired bioavailability of nitric oxide (NO) (Anstey *et al.* 1996; Yeo *et al.* 2007) arising from hypoargininaemia (Lopansri *et al.* 2003; Yeo *et al.* 2007), impaired NO synthase expression (Anstey *et al.* 1996; Yeo *et al.* 2007), inhibition of NO synthesis by asymmetric dimethylarginine (Yeo *et al.* 2010a), local quenching of NO resulting from increased cell-free haemoglobin and haem (Yeo *et al.* 2009) and blockade or loss of local endothelial protein C receptors (Moxon *et al.* 2013). Reduced NO bioavailability exacerbates endothelial activation and exocytosis of intracellular Weibel-Palade bodies, which contain a variety of bioactive molecules including von Willebrand Factor (vWF) and angiopoietin-2. Plasma concentrations are both substantially elevated in severe malaria (Yeo *et al.* 2008b; De Mast *et al.* 2009), associated with a fatal outcome in both adults (Yeo *et al.* 2008b; Jain *et al.* 2011) and children (Erdman *et al.* 2011; Conroy *et al.* 2012). Angiopoietin-2 causes autocrine endothelial activation and may thereby exacerbate microvascular sequestration of parasitised red cells (Yeo *et al.* 2008b). Concentrations of ADAMTS13, which cleaves and inactivates UL-vWF, are low in patients with severe malaria (De Mast *et al.* 2009; Lowenberg *et al.* 2010). Ultralong vWF multimers could mediate cytoadherence and sequestration by binding activated platelets expressing CD36, the receptor for PfEMP1 (Bridges *et al.* 2010). However, vWF is not associated with either the degree of hyperlactataemia, metabolic acidosis or fatal outcome in severe malaria (Erdman *et al.* 2011; Phiri *et al.* 2011) and may have an earlier, more upstream, role in severe malaria pathogenesis.

Cerebral malaria. In patients who die in the acute phase of cerebral malaria, many of the cerebral capillaries and venules are packed tightly with parasitised erythrocytes, whereas other adjacent vessels are not obstructed (Newton *et al.* 1991; Silamut *et al.* 1999; Taylor *et al.* 2004; Dorovini-Zis *et al.* 2011; White *et al.* 2013a,b). The degree of packing and congestion of the cerebral microvessels with both infected and uninfected red cells is associated significantly with the level of pre-mortem coma and the interval to death (Pongponratn *et al.* 2003). A distinct and highly specific malaria retinopathy occurs in both children and adults with cerebral malaria (Beare *et al.* 2006; Maude *et al.* 2009a). There are many similarities between the retinal and cerebral microcirculations. Retinal whitening, haemorrhages, and whitening of the vessels (Figure 6) all reflect the microvascular pathology observed in post-mortem ultrastructural studies (White *et al.* 2001, 2009). Organ-specific and systemic lactate/pyruvate ratios are elevated in proportion to the severity of illness (a different profile is seen in the hypermetabolism of sepsis) (Day *et al.* 2000b). All these findings point to extensive microvascular obstruction and impaired perfusion as critical pathophysiological processes in cerebral malaria (White *et al.* 2013a,b). In adults, there is marked endothelial activation (Turner *et al.* 1994) but usually little inflammation in the brain (MacPherson *et al.* 1985). Intravascular leucocytes are more prominent in African children than in Asian adults who died from cerebral malaria (MacPherson *et al.* 1985; Taylor *et al.* 2004; Dorovini-Zis *et al.* 2011). There is a mild generalised increase in the systemic vascular permeability. The blood–brain barrier (BBB) is functionally intact in adult cerebral malaria (Warrell *et al.* 1986). Autopsy studies in African children show some increase in BBB permeability with a disruption of endothelial intercellular tight junctions (Dorovini-Zis *et al.* 2011). Imaging shows that although there is often cerebral swelling, mainly attributed to the intracerebral sequestered erythrocytes, most adults have no evidence of pronounced cerebral oedema. In African children, cerebral oedema is more common, particularly in the agonal stages (Potchen *et al.* 2012). The mechanisms underlying cerebral oedema in paediatric cerebral malaria are incompletely understood. Similarly, opening pressures on lumbar puncture are usually normal in adults, but are elevated in over 80% of children, although mean values (approximately 160 mm CSF) are similar in the two age groups (Newton *et al.* 1991, Waller *et al.* 1991). Summarising the evidence, raised intracranial pressure occurs more commonly in children and is more likely to develop in the later stages of cerebral malaria (Idro *et al.* 2005a; Medana *et al.* 2011). There is no evidence that pharmacological measures directed against oedema such as high-dose corticosteroids or mannitol are beneficial. Indeed a recent study in adults with cerebral malaria in India showed that mannitol as adjunctive therapy prolonged coma duration and increased mortality (Mohanty *et al.* 2011).

Although some patients may remain comatose for hours or days after cerebral sequestration should have cleared, full neurological recovery is the usual outcome. Transient disruption of axoplasmic transport provides a plausible explanation for the fully reversible coma and the persistence of unconsciousness after parasite clearance (Medana *et al.* 2002). In addition, after rupture of the schizont, residual erythrocyte membranes and malaria pigment may remain attached to the vascular endothelium for days providing a continued activation stimulus. Whereas adults rarely (i.e. in <3% of cases) suffer neurological sequelae, 3–15% of children surviving cerebral malaria – especially those with hypoglycaemia, severe anaemia, repeated protracted seizures and deep coma – have some residual neurological deficit when they regain consciousness; hemiplegia, cerebral palsy, cortical blindness, deafness and

impaired cognition and learning (all of varying duration) have been reported (Brewster *et al.* 1990; Dondorp *et al.* 2005a, 2010; Idro *et al.* 2007) (see Section 3). Approximately 10% of children surviving cerebral malaria have a persistent language deficit. The incidence of epilepsy is increased and the life expectancy decreased among these children (Birbeck *et al.* 2010b). The discrepancy between the microvascular pathology observed at autopsy and the large vessel territory strokes causing neurological deficit in cerebral malaria has not been explained satisfactorily.

Severe anaemia occurs most commonly in young children in areas of moderate and high transmission (Calis *et al.* 2008). Anaemia results from accelerated unparasitised red cell removal by the spleen and the obligatory erythrocyte destruction at parasite schizogony, compounded by ineffective erythropoiesis (Price *et al.* 2001; Buffet *et al.* 2011). Severe anaemia is often the end result of repeated infections which do not allow sufficient time for the bone marrow to recover. In severe malaria, both infected and uninfected erythrocytes become less deformable, and splenic clearance is increased (Dondorp *et al.* 1997, 1999; Safeukui *et al.* 2008; Buffet *et al.* 2011). Anaemia develops rapidly, and transfusion is often required. Slight coagulation abnormalities are also common in falciparum malaria, and thrombocytopenia is usual – and may be profound in severe malaria (a normal platelet count should challenge the diagnosis of malaria), but significant bleeding with evidence of disseminated intravascular coagulation is unusual (Clemens *et al.* 1994). Haematemesis from stress ulceration or acute gastric erosions may also occur rarely (Warrell *et al.* 1982).

Acidosis is an important cause of death from severe malaria and results from accumulation of organic acids including lactic acid (Day *et al.* 2000b). This may be compounded by ketoacidosis in children or acute renal failure in adults. Acidotic breathing, a major cause of respiratory distress, is a sign of poor prognosis in malaria (Marsh *et al.* 1995). It is often followed by circulatory failure refractory to volume expansion or inotropic drugs and ultimately by respiratory arrest. The plasma concentrations of bicarbonate or lactate are among the best biochemical prognosticators for death in severe malaria as they rise in proportion to disease severity (Krishna *et al.* 1994; Hanson *et al.* 2010; von Seidlein *et al.* 2012) (see *Section 10*). Lactic acidosis is caused by the combination of anaerobic glycolysis in tissues where sequestered parasites interfere with microcirculatory flow, lactate production by the malaria parasites, and a failure of hepatic and renal lactate clearance. Although hypovolaemia has been considered a contributor to hyperlactataemia, recent studies question its causative role in acidosis (Jarvis *et al.* 2006; Maitland *et al.* 2011; Hanson *et al.* 2012).

Hypoglycaemia is often associated with lactic acidosis and a poor prognosis. It is a particular problem in children and pregnant women. Hypoglycaemia results from a failure of hepatic gluconeogenesis combined with an increase in tissue consumption of glucose by both the febrile host (as a result of anaerobic glycolysis) and to a much smaller extent, the malaria parasites (Krishna *et al.* 1994). Hyperinsulinaemic hypoglycaemia is caused by quinine treatment and is particularly common in pregnant women. Quinine is a powerful stimulant of pancreatic insulin secretion. Hyperinsulinaemic hypoglycaemia occurs after treatment and may be recurrent despite glucose administration (White *et al.* 1983b).

Pulmonary oedema. Acute respiratory distress syndrome (ARDS) is a feared complication in adults with severe falciparum malaria, and it may also develop in acute vivax malaria. Significantly increased pulmonary capillary permeability develops after start of antimalarial treatment in up to a third of patients (Taylor *et al.* 2012b). As with ARDS in other settings the pathogenesis is not fully understood, although inflammatory mediated increased capillary permeability or endothelial damage is a central feature. Alveolar–capillary membrane function is significantly impaired in patients with severe malaria and deteriorates further during treatment (Maguire *et al.* 2005), and a post-treatment inflammatory response may explain the frequent development of ARDS after commencement of antimalarial therapy. The role of parasite sequestration in the pulmonary microvasculature is unclear. Pulmonary sequestration can be marked in *P. falciparum*. Careful fluid management is essential. Rapid infusion of large volumes of intravenous fluid in severe malaria may prove lethal (Maitland *et al.* 2011). If mechanical ventilation is not available, the mortality of ARDS exceeds 80% in falciparum malaria, but even with this treatment option, mortality exceeds 50% in most series (Taylor *et al.* 2012b). Mortality in vivax malaria ARDS, when there is commonly a single-organ failure, is much lower (see *Section 10*).

Acute Kidney Injury is a frequent feature of severe malaria. Oliguric renal failure is common in adults with severe falciparum malaria, but rare in children. It behaves clinically and pathologically as acute tubular necrosis. Hypertension and significant proteinuria never occur. The pathogenesis is still unclear, but reduced microcirculatory flow as well as inflammation likely contribute (Nguansangiam *et al.* 2007). Acute renal failure may occur with multiple vital organ dysfunction (carrying a high

mortality) or may develop as other disease manifestations resolve. In survivors, urine flow resumes in a median of 4 days, and serum creatinine levels return to normal in a mean of 17 days (Trang *et al.* 1992). Early haemofiltration or dialysis considerably improve the prognosis, particularly in acute hypercatabolic renal failure (Phu *et al.* 2002). Although a variety of glomerular abnormalities have been reported associated with malaria, the clinical features, urine sediment (not 'active'), and natural history of acute kidney injury do not suggest significant glomerulonephritis.

Severe jaundice is associated with *P. falciparum* infections; it is more common among adults than among children, and results from a combination of haemolysis, hepatocyte injury and cholestasis. Jaundice is often accompanied by renal impairment. Liver blood flow is reduced in severe malaria (Molyneux *et al.* 1989a; Pukrittayakamee *et al.* 1992), and hepatic gluconeogenesis is impaired (White *et al.* 1983a,b; White *et al.* 1987a,b; Taylor *et al.* 1988; Day *et al.* 2000a,b). Hepatic dysfunction contributes to hypoglycaemia, metabolic acidosis and impaired drug metabolism.

Interactions with bacterial infections and HIV/AIDS. Bacteraemia, especially with Gram-negative bacteria, may complicate severe malaria, particularly in children. African children with "slide-proven severe malaria" have a 4.6–7.8% prevalence of concomitant bacteraemia (Berkley *et al.* 2005; Bronzan *et al.* 2007). Given the limited sensitivity of blood culture (Gilman *et al.* 1975), the real number of children with malaria who have bacterial septicaemia could be twice as high. Falciparum malaria has been estimated to account for more than half of invasive bacterial disease in children living in malaria-endemic areas (Scott *et al.* 2011). Parasite digestive vacuoles, containing malaria pigment, are rapidly phagocytosed by polymorphonuclear granulocytes and cause a state of functional neutrophil exhaustion. This blunts their microbicidal activity upon bacterial challenge and may well contribute to the enhanced susceptibility of severe malaria patients to invasive bacterial infections (Dasari *et al.* 2011). Inhibition of neutrophil oxidative burst by induction of haem oxygenase (Cunnington *et al.* 2012a,b), and quenching of nitric oxide (Yeo *et al.* 2009), a key anti-*Salmonella* mediator, are also hypothesised to increase the risk of bacteraemia in malaria. Increased translocation of bacteria across the intestinal lining may also be an important contributor.

There is extensive misdiagnosis of severe malaria in children who have severe sepsis (septicaemia, pneumonia, meningitis) and incidental parasitaemia (Reyburn *et al.* 2004). This has confounded clinical studies and pathological interpretations. Plasma PfHRP2 concentrations, which can be used to estimate the number of sequestered *P. falciparum* parasites which release this protein, can distinguish between severe malaria and severe other febrile illnesses with incidental parasitaemia (Hendriksen *et al.* 2012b, 2013c; Seydel *et al.* 2012). HIV transmission and progression may be accelerated by malaria (Kublin *et al.* 2005; Abu-Raddad *et al.* 2006), whereas conversely, HIV infection increases the incidence of clinical malaria, severe malaria and malaria-related mortality in adults with deteriorating immune status (Whitworth *et al.* 2000; Kublin *et al.* 2005; Chalwe *et al.* 2009). In children with severe falciparum malaria, HIV-AIDS increases the severity, number of complications and mortality. (Patnaik *et al.* 2005; Berkley *et al.* 2009; Hendriksen *et al.* 2012a).

Other host factors

Many of the common inherited red cell disorders confer a relative protection against the development of severe malaria, and there is strong evidence that malaria has been the direct cause of their evolutionary persistence. The geographical distributions of sickle cell disease, haemoglobins C and E, ovalocytosis, the thalassaemias and glucose-6-phosphate dehydrogenase (G6PD) deficiency match that of malaria before the introduction of control measures, which suggests that these genetic disorders confer a survival advantage in the presence of malaria. Individuals who are homozygous for the sickle cell gene suffer from sickle cell disease, which confers a reduced life expectancy, whereas the heterozygous sickle cell carrier does not suffer from the haemoglobinopathy and in addition is protected from severe malaria. These contrasting clinical effects have resulted in a 'balanced polymorphism' during evolution (Williams 2012).

Several different malaria protective mechanisms have been identified: these include decreased parasite growth at low oxygen tensions (Hb AS), reduced cytoadherence (Hb AC, CC, AS), reduced invasion (ovalocytosis), reduced parasite densities (G6PD deficiency) and reduced multiplication at high densities (HbAE). The underlying mechanism has been worked out in detail for HbAS and HbS. In parasitised red blood cell with these haemoglobins, the trafficking of PfEMP1 and other proteins to the red blood cell surface is disturbed (Cyrklaff *et al.* 2011), causing aberrant knob formation leading to reduced cytoadherence of the infected erythrocyte (Cholera *et al.* 2008). Most of the haemoglobinopathies are associated with a reduced risk of severe malaria, but HbAS has also been associated consistently with reduced risk of uncomplicated malaria (Taylor *et al.* 2012a).

Section 8: Pathology of severe falciparum malaria

Pathology studies based on post-mortem and biopsy material are essential for a better understanding of the pathophysiology of severe *Plasmodium falciparum* malaria. Two large autopsy studies (one from Vietnam and the other from Malawi) conducted over the past twenty years have used detailed clinicopathological correlation with histological, ultrastructural and immunohistochemical features and are now applying more advanced molecular pathological techniques to the investigation of fatal malaria. However, several fundamental questions regarding the pathophysiology of fatal malaria remain controversial, such as the mode of death in malaria patients, and the factors causing coma in cerebral malaria. There are very few pathology reports of fatal vivax malaria (see *Section 13*).

The pathological features of cerebral malaria

Sequestration. The predominant histopathological feature of human cerebral malaria (CM) is the sequestration of erythrocytes containing *Plasmodium falciparum* trophozoites and schizonts in the capillaries and post-capillary venules of the brain, first recognised over a century ago (Marchiafava & Bignami 1892). Parasitised red blood cells (PRBC) sequester and reduce the diameter or completely fill the vascular lumen (Figures 7 and 8). Although sequestration is extensive in most cases of CM, the degree and intensity differ between organs and within areas of the brain.

Sequestration in cerebral microvessels tends to be more intense in the cerebral cortex, where vascularity is greater in the grey matter than in the midbrain or brainstem. The degree of sequestration measured histologically varies with duration of illness prior to death and with treatment. However, the measurement of sequestration using histology on brain sections (Aikawa *et al.* 1990; Pongponratn *et al.* 1991; Taylor *et al.* 2004; Dorovini-Zis *et al.* 2011; Ponsford *et al.* 2012), brain smears (Silamut *et al.* 1999; Milner *et al.* 2012a) or by electron microscopy (Pongponratn *et al.* 2003) (Figure 8) confirms that high levels are significantly associated with ante-mortem coma. Significantly more PRBC sequestration is seen in the brain of cerebral malaria patients compared to cases both without neurological complications and without clinical evidence of cerebral sequestration (i.e. malarial retinopathy), in both adult and paediatric cases, regardless of brain area, time from treatment to death or type of treatment (Pongponratn *et al.* 2003; Dorovini-Zis *et al.* 2011).

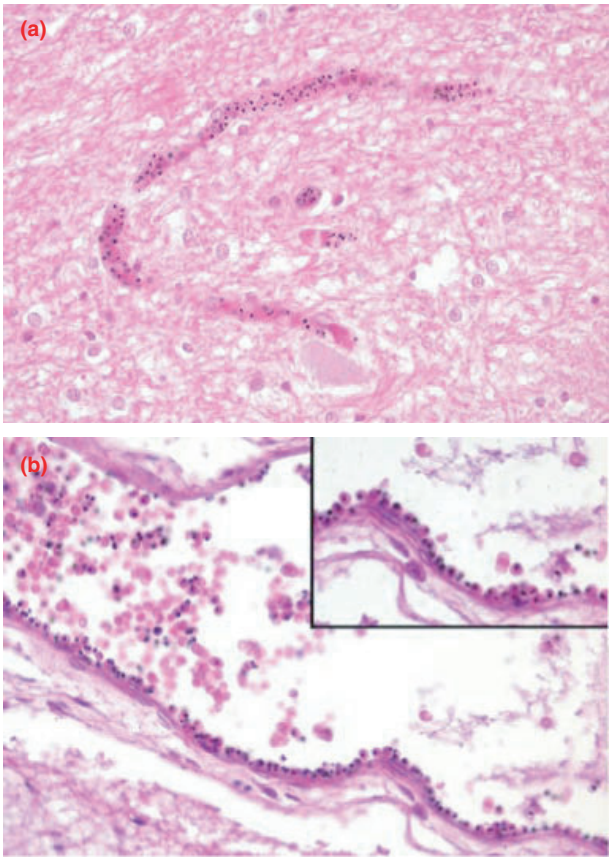


Figure 7 Sequestration of parasitised erythrocytes in cerebral microvessels in post-mortem brain tissue from fatal cases of cerebral malaria. Packing of small calibre capillaries and post-capillary venules is demonstrated in this section of cerebral cortex (a). Margination in larger calibre venules due to cytoadherence can also be demonstrated (b with inset) (haematoxylin and eosin, ×400).

The microvascular distribution of sequestration is not uniform and vessels may contain many parasites in one segment, but adjacent areas or neighbouring vessels may only demonstrate occasional parasites (Figure 9a). The developmental staging of parasites within a vessel on brain smears suggests that they arrive, adhere and subsequently develop in a synchronous manner and do not detach once adherent (Silamut *et al.* 1999; Milner *et al.* 2012a, White *et al.* 2013b). In addition to cytoadhesion of parasitised erythrocytes to microvascular endothelium, factors that contribute to microvascular sequestration in the brain include a reduction in RBC deformability (Dondorp *et al.* 2004b), clumping of PRBC or adhesion via platelet/von Willebrand factor strings (Bridges *et al.* 2010) and/or rosette formation (Ho *et al.* 1991; Chotivanich *et al.* 2000).

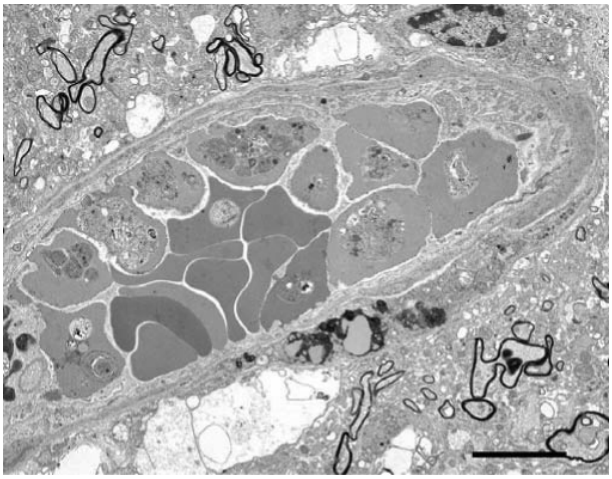


Figure 8 Transmission electron micrograph of a cerebral venule showing marginated cytoadherent parasitised erythrocytes, and uninfected erythrocytes within the narrowed vascular lumen. Tight interactions of the endothelium with parasitised red blood cells as well as between parasitised red blood cells and uninfected red blood cells have been seen at this level. Focal perivascular oedema is present in the Virchow-Robbins space. (Bar = 100 μm, courtesy of Prof Emsri Pongponratn, Mahidol University and Prof David Ferguson, Oxford University).

The calculation of a sequestration index in adult patients, comparing the parasite load within vessels with the concurrent peripheral blood smear, allows estimation of the degree of sequestration compared to the distribution expected in a free mixing model (Silamut *et al.* 1999). This shows that the load of infected erythrocytes in cerebral microvessels is up to 50 times greater in CM than non-CM patients and also that the load in cerebral vessels is greater than in other organs from the same patient such as the kidney (Pongponratn *et al.* 2003; Nguansangiam *et al.* 2007).

Sequestration is mediated by specific molecular adhesion between parasite-encoded molecules, notably erythrocyte membrane protein 1 (P*f*EMP-1) expressed on the surface of the infected erythrocyte, and endothelial cell ligands such as endothelial protein C receptor (EPCR, Moxon) and ICAM-1 (brain) (Turner *et al.* 1994, Moxon *et al.* 2013), CD36 (endothelium and platelets) and chondroitin sulphate A (CSA, placenta) (Craig & Scherf 2001). Field isolates of parasites from malaria cases most frequently show binding to CD36 (Cooke *et al.* 1994; Newbold *et al.* 1997), which is widely and constitutively expressed on all vascular beds except the brain (Turner *et al.* 1994). The expression of several potential endothelial adhesins such as VCAM-1, E-selectin and ICAM-1 is increased in cerebral malaria, reflecting diffuse endothelial activation in all vascular beds (Turner *et al.* 1998). Colocalisation of sequestered PRBC with ICAM-1 (Figure 12b) in brain microvessels (Turner *et al.* 1994) and the association of ICAM-1 binding phenotype with clinical risk of cerebral malaria suggest that this is a major receptor for sequestration in the brain during CM (Ochola *et al.* 2011). Using molecular pathological methods of PCR and RT-PCR from autopsy tissues, the genotype of parasites sequestered in different organs shows a narrower spectrum than circulating parasites, implying some selection of binding parasite phenotypes (Montgomery *et al.* 2006; Milner *et al.* 2012b). However, no specific

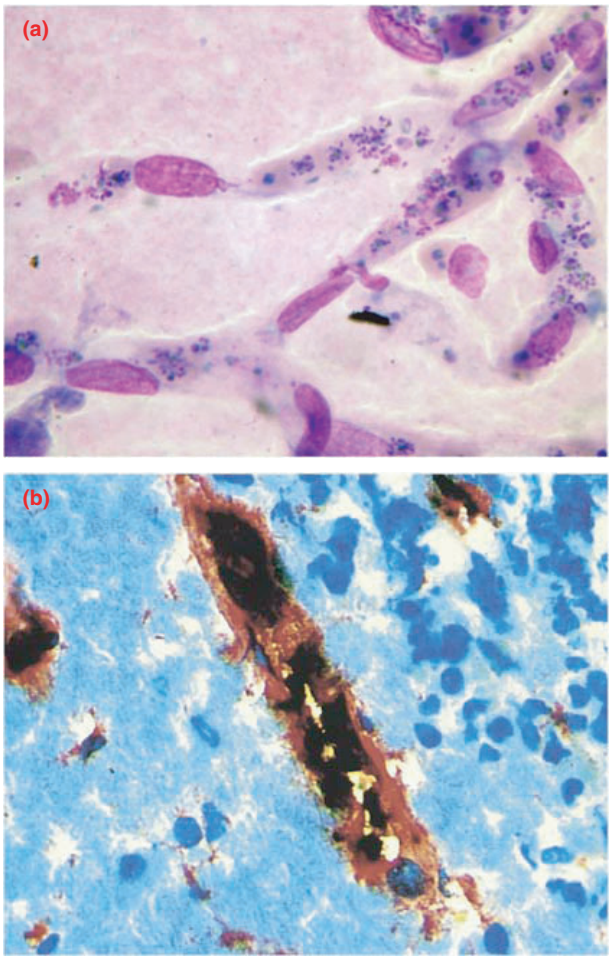


Figure 9 A brain smear from the cortex of a patient who died from cerebral malaria showing sequestration by multisegmented schizont-stage-parasitised erythrocytes, whereas other segments of the same vessels are relatively uninvolved (Giemsa stain ×1000). (b): A double immunohistochemical stain showing cerebral endothelial cells (ICAM-1, red) and parasitised erythrocytes (MSP-1, black) in a cerebellar venule (haematoxylin counter stain ×400).

relationship between the expression of specific *var* genes (which encode the main parasite adhesin, PfEMP-1) and sequestration in different organs was found (Montgomery *et al.* 2007).

Recent studies have provided evidence that host receptor and parasite adhesin expression patterns may influence sequestration and subsequent pathology in the brain in CM. The parasite var genes DC8 and DC13 allow binding to a variety of endothelial cell types from different organs and are associated with severe and cerebral malaria (Claessens *et al.* 2012; Avril *et al.* 2013; Bertin *et al.* 2013). Sequestration of PRBC to cerebral endothelial cells by binding to the endothelial protein C receptor and thrombomodulin colocalises with downregulation of EPRC, which might cause local microvascular fibrin and thrombin deposition and microhaemorrhages, linking sequestration of PRBC to local inflammatory and pro-coagulatory damage to the blood–brain barrier (Moxon *et al.* 2013). Therefore, binding of specific PRBC subsets determined by their var gene expression profile may be linked to organ-specific pathology.

Haemorrhages

The cut surface of the fresh brain in both adults and children may show multiple small petechial haemorrhages, often concentrated in the subcortical white matter, corpus callosum or cerebellum (Figure 12a). This type of haemorrhage may also be seen in other organs such as heart and lung in adult cases, but rarely in paediatric cases. Histologically, several types of haemorrhage are seen such as simple petechial haemorrhages, organised zonal ring haemorrhages (with central fibrin thrombi) and Dürck's granulomata, which are formed from astroglial responses to older haemorrhages (Figure 10). Haemorrhages are often most prevalent in watershed areas of vascular supply, which may reflect their genesis as a consequence of vascular obstruction and hypoxic–ischaemic injury. In paediatric cases, ring haemorrhages can contain fibrin thrombi with a zone free of red blood cells suggesting an earlier vessel breakdown and subsequent closure (due to fibrin).

Haemorrhages are not restricted to cerebral malaria but are also seen in non-cerebral cases and sometimes in other conditions such as bacterial infections, severe hypoglycaemia of the newborn, fat embolism or barotrauma (Spitz 1946). Although increased in number in the brain of CM patients, they are not quantitatively associated with ante-mortem coma or the presence of brain oedema in Vietnamese adults (Ponsford *et al.* 2012). Radiological studies such as MRI also detect small haemorrhages in patients who survive and make a full neurological recovery; hence, they seem a nonspecific feature of severe malaria that reflects focal compromise of vascular integrity. Furthermore, malaria retinopathy changes, which parallel ring haemorrhages

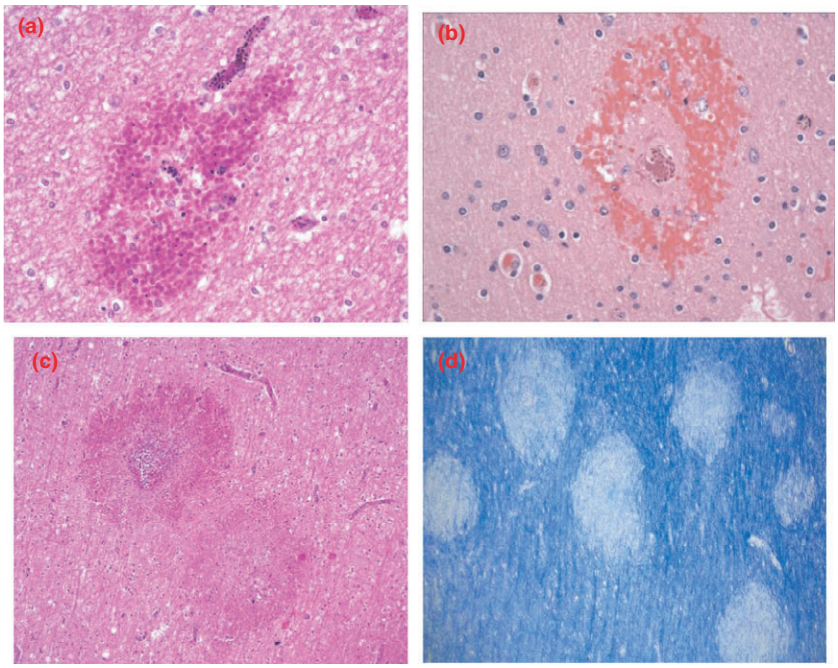


Figure 10 Examples of microhaemorrhages found in the brain in cerebral malaria are shown. A simple punctate haemorrhage (a) shows red blood cells surrounding a vessel (suggesting continued blood brain barrier breakdown or a reperfusion injury) in contrast to a ring haemorrhage (b) where there is a zone of damaged brain and no red blood cells along with a central fibrin thrombus suggesting coagulative occlusion after breakdown (H&E × 400). Ring haemorrhages (c) go through various stages of maturity (H&E × 200) and can be seen resolving if a patient has either survived the primary insult for an extended period or subsequently dies of another cause. Glial reaction to this process forms a lesion termed a Dürck's granuloma. As part of the damage induced by these lesions, demyelination can be found in white matter (d). (Luxol fast blue Cresyl Violet stain ×200).

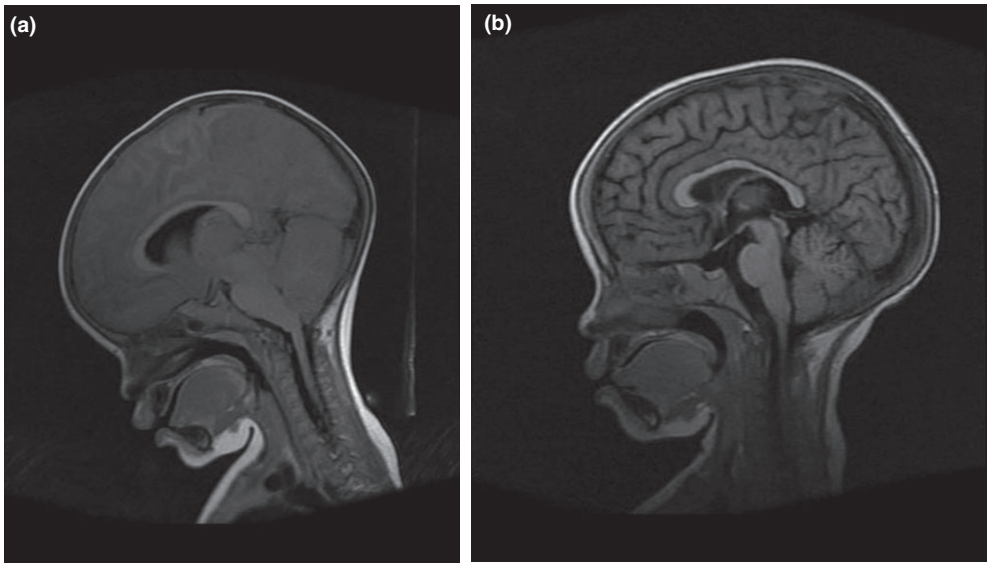


Figure 11 MRI imaging of the brain in paediatric cerebral malaria. Severe brain swelling occurred in this patient with effacement of the 4th ventricle, prepontine cistern and brainstem compression and tonsillar herniation (a); and normal child of similar age for comparison (b). Pictures supplied by Dr Sam Kampondeni.

in the brain, are commonly found in survivors, suggesting that in common with sequestration haemorrhages are not solely sufficient to cause mortality. However, unlike sequestration, the number of haemorrhages is not quantitatively linked to the presence of coma premortem.

Brain swelling and oedema

At autopsy, the brain may be swollen or normal in adults, whereas brain swelling is universal in African children (Potchen *et al.* 2010; Dorovini-Zis *et al.* 2011) (Figure 12a). Adult patients with coma may have no evidence of brain swelling either radiographically before death or at autopsy. Computerised tomography (CT) in Indian adults with cerebral malaria showed that brain swelling occurred but was not related to coma depth or fatal outcome (Mohanty *et al.* 2011). CT and magnetic resonance imaging (MRI) data in paediatric cases demonstrate oedema commonly in cerebral malaria patients with a strong association with outcome (Figure 11) (Seydel *et al.* 2011; Potchen *et al.* 2012). In a study of Nigerian children, the post-mortem findings suggested gross cerebral oedema and raised intracranial pressure in four of seven cases with petechial haemorrhages and small areas of focal necrosis (Walker *et al.* 1992). Computed tomography of the brain in 14 Kenyan children recovering from cerebral malaria with raised intracranial pressures revealed evidence of brain swelling in 6 and of ischaemic damage in 4, all of whom suffered from neurological sequelae (Newton *et al.* 1994). The pattern of injury was consistent with a critical reduction in perfusion pressure, and the convalescent scans showed cerebral atrophy. Acute CT findings in Malawian children included cerebral oedema and acute brain infarctions. In 11 children with subsequent neurological sequelae who had CT scans 7–18 months after the initial illness, 5/11 had focal and multifocal lobar atrophy correlating with regions of the brain affected by focal seizures during the acute illness (Potchen *et al.* 2010).

Evidence for raised intracranial pressure and subsequent frank brainstem herniation is uncommon in adults. Clinical and radiological evidence for brainstem herniation as a mode of death in CM has been reported in African children, although the specificity of the clinical signs is unclear (Pongponratn *et al.* 1991; Idro *et al.* 2005a; Seydel *et al.* 2011). In other words it has been unclear if the brainstem clinical signs resulted from brainstem compression or intrinsic dysfunction related to the disease process. Histological evidence of oedema is seen in over 60% of adult cases (Medana *et al.* 2011), and most if not all paediatric cases (Dorovini-Zis *et al.* 2011) (Figure 12b). Much of the increase in cerebral volume in adults in the acute phase of illness can be quantitatively explained by the intravascular load of sequestered parasites and uninfected erythrocytes (White *et al.* 2013b). Congestion of microvessels with PRBC and uninfected RBC is associated significantly with coma (Ponsford *et al.* 2012), but the degree and patterns

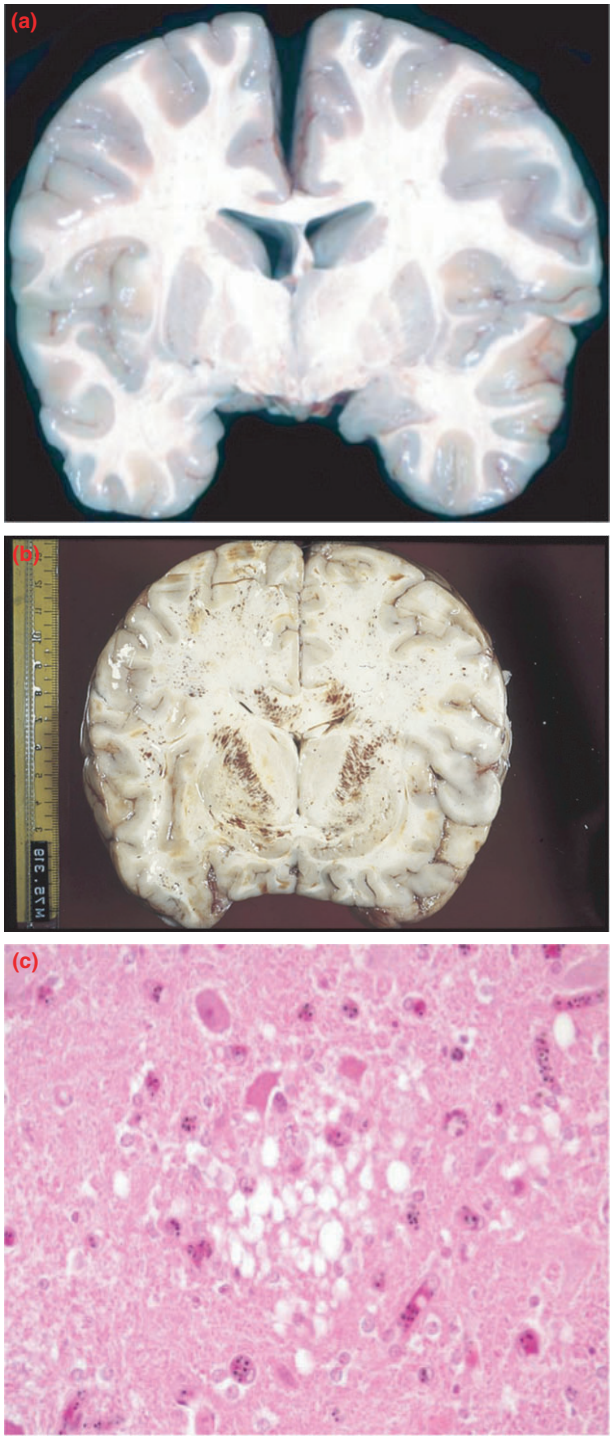


Figure 12 Macroscopic picture of brain swelling and petechial haemorrhages. An autopsy brain slice from a paediatric African case (a) of fatal CM shows swelling with obliteration of the sulci and ventricular system, (b) a brain slice from an adult case with less marked swelling, but multiple focal haemorrhages in the white matter, contrasting with a lack of haemorrhages in the grey matter (courtesy of Dr Peter King, SIAMR, Johannesburg). Histological evidence of perivascular or 'bubbly' parenchymal oedema (c) in the brain is seen predominantly in paediatric cases but may also be found in adults (H&E × 400).

of histological oedema are not associated with coma in adults (Medana *et al*. 2011). Brain swelling may therefore not be due solely to perivascular or parenchymal oedema.

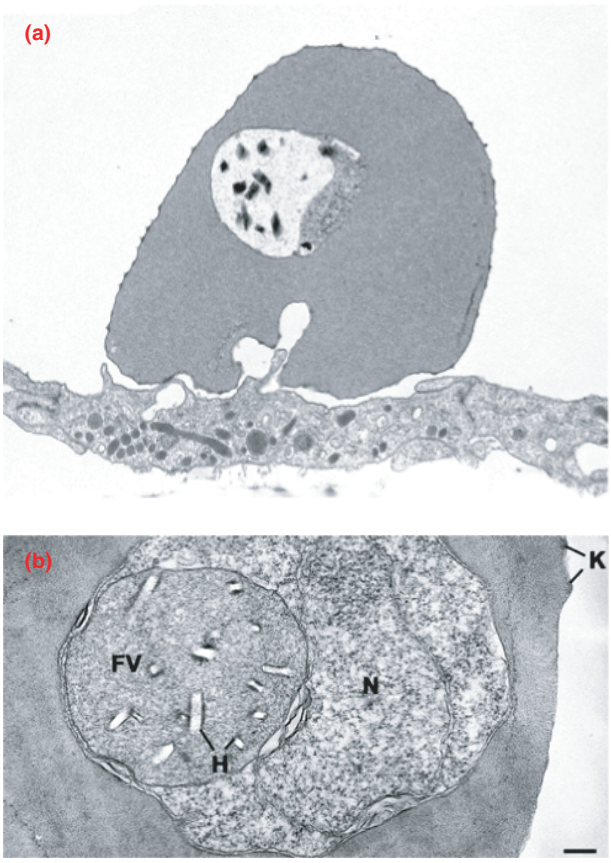


Figure 13 Pigment granules within an early trophozoite-stage *P. falciparum*-infected red cell (a), cultured *in vitro* and adherent to an endothelial cell as seen by transmission electron microscopy. Note the refractile quality of the pigment, which is both polarisable and paramagnetic. A transmission electron micrograph (b) showing the developing food vacuole (FV) containing crystalloids of haemozoin (H) – the FV is physically separated from the parasite nucleus (N); haemozoin is toxic to the parasite and thus sequestered in the food vacuole. This trophozoite-stage parasite shows surface knob proteins (K), which are the site of cytoadherence (×5000, courtesy of Prof Emsri Pongponratn & Prof David Ferguson).

In contrast, in paediatric cases, oedema and brain swelling are common at autopsy in children with other causes of coma as well as in cerebral malaria (Seydel *et al.* 2011).

Pigment deposition

The fresh cut surface of the brain may show a slate-greyish discolouration reflecting the heavy deposition of malaria pigment (haemozoin). Pigment can be seen both within vessels, often remaining attached to ruptured erythrocyte membrane ghosts following schizogony or phagocytised by host leucocytes (Figure 13, 15 and 16). Rarely, focal pigment granules can be seen within the brain parenchyma due to haemorrhage or passage across focal disruptions to the blood–brain barrier. Pigment loads in brain microvessels in African paediatric cases correlate with the degree of pigment in other organs such as the spleen and liver, suggesting that this is a surrogate measure both of current parasite biomass and of the number of life cycle replications that have happened within the body (Whitten *et al.* 2011).

Axonal injury

Acute axonal injury, detected both morphologically as swollen axonal sheaths and bulbs, or on immunohistochemistry by accumulation of beta-amyloid precursor protein, is found in post-mortem studies of CM patients in both adult and paediatric cases (Medana *et al.* 2002, Dorovini-Zis *et al.* 2011) (Figure 14). This likely represents a reversible but final common pathway to neurological impairment in CM.

Cellular immune responses

Studies in Malawian children demonstrated that 75% of cases have intravascular and perivascular pathology (haemorrhages, accumulation of pigmented leucocytes and thrombi), whereas leucocyte aggregation, vasculitis or thrombus formation are not commonly seen histologically in adult cases. In adults, individual vessels can show collections of leucocytes, predominantly monocytes and T cells on immunophenotyping, which can be marked (Patnaik *et al.* 1994). However, other vessels lack such collections of cells, and monocyte responses are seen mainly in cases who die later in the course of disease, where they show phagocytosis of pigment and ghosted erythrocyte membranes (Figure 15).

Within the Malawian cohort of children diagnosed by pathology as cerebral malaria, two distinct patterns were recognised. These included the classic pattern of cerebral malaria as seen in adults which demonstrates densely

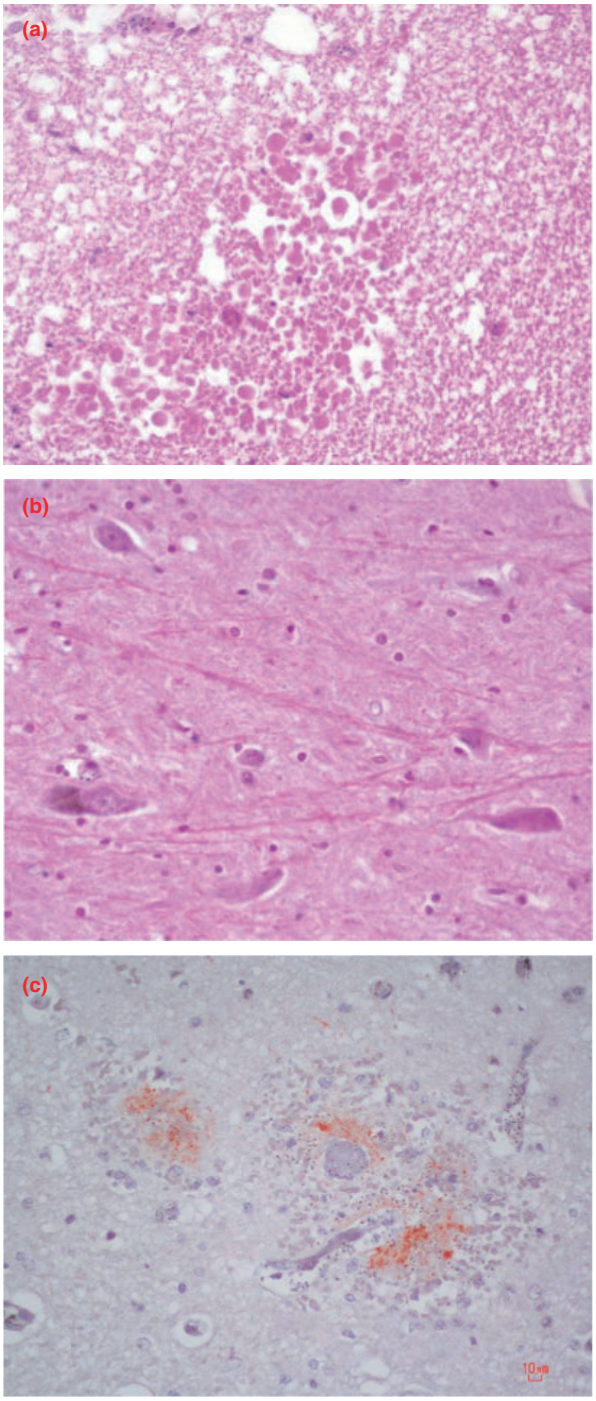


Figure 14 Axonal Injury: Swollen axonal bulbs in white matter with background oedema and sequestration of parasites in capillaries (a) demonstrate axonal injury. Swollen axonal sheaths within the white matter are also found (H&E staining ×400) (b). Immunohistochemistry for beta-APP, a marker of axonal injury, demonstrates damage in an African paediatric case (c).

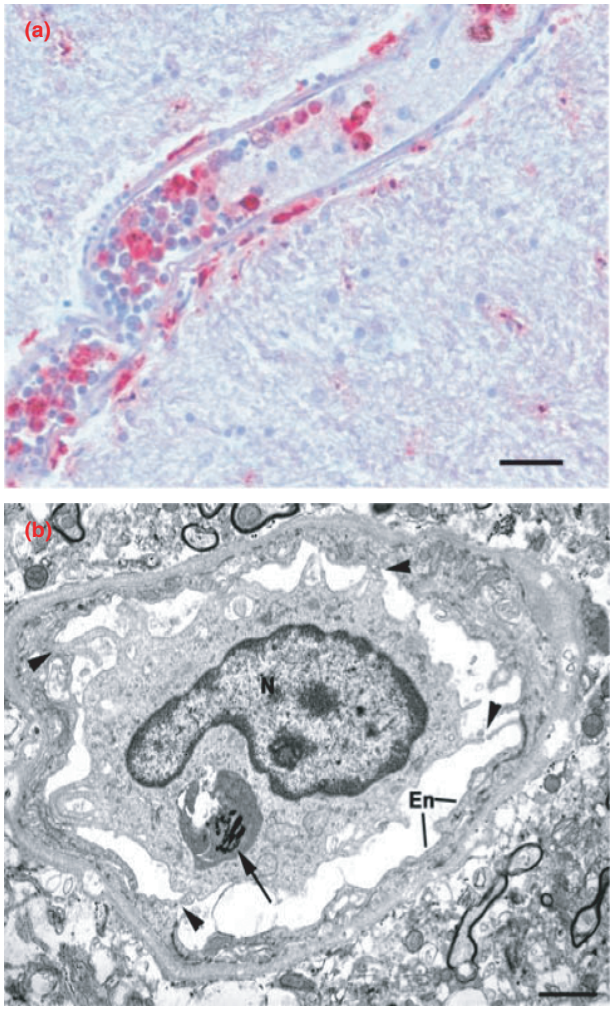


Figure 15 Evidence for cellular inflammatory responses in the brain during CM is presented. Immunohistochemistry with anti-CD68 (a) showing both intravascular monocytes, some of which contain phagocytosed malaria pigment and perivascular/parenchymal macrophages (haematoxylin counterstain, scale bar = 10 μm). (b): A transmission electron micrograph showing a cerebral vessel with a circulating monocyte (*N* = nucleus) which has phagocytosed a pigment granule (arrow). The cells are making multiple contacts via pseudopodia (arrowheads) with the lining endothelial cell (En) (scale bar = 1 μm, courtesy of Prof. Emsri Pongponratn and Prof. David Ferguson).

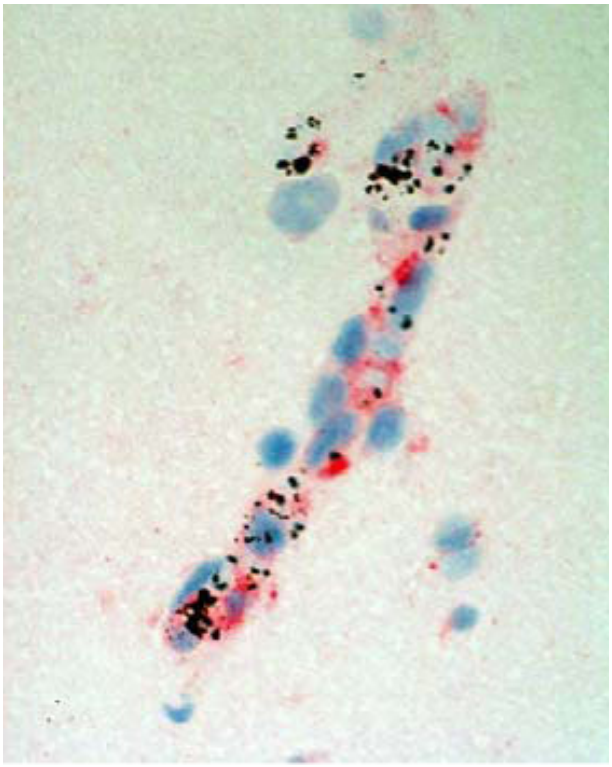


Figure 16 A brain capillary from a case of cerebral malaria demonstrates malaria pigment (black) within the vessel surrounded by endothelial cells and white blood cells (pale blue nuclei). Immunohistochemistry for CD36, a molecule not found in brain endothelium but ubiquitous on platelets, highlights the adherent clusters of platelets within areas of sequestration. Image at 630 × courtesy of Prof. Georges Grau, University of Sydney, Australia.

sequestered parasites within cerebral vessels, ring haemorrhages throughout the white matter of the cerebral cortex and excessive pigment within and outside of macrophages (Taylor *et al.* 2004). A second pattern showed densely sequestered parasites but little other pathology. Clinically, there was little difference between these two groups except for a higher incidence of HIV antibody positivity in the latter group. Detailed studies of these two pathologies with respect to HIV status are currently underway; however, the general consensus is that they may represent different temporal points in a continuum, with the question remaining why death has occurred at the particular and different time point in each patient. Review of the literature for all adult and paediatric cases published in the last century, suggests that both of these patterns were seen in both children and adults throughout this period, with little indication available to account for the different histological appearances (Milner & Taylor 2006).

Platelet deposition

There is a marked difference between the degree of platelet deposition in the brain between paediatric African and adult Asian cases (Figure 16). Deposition of platelets in

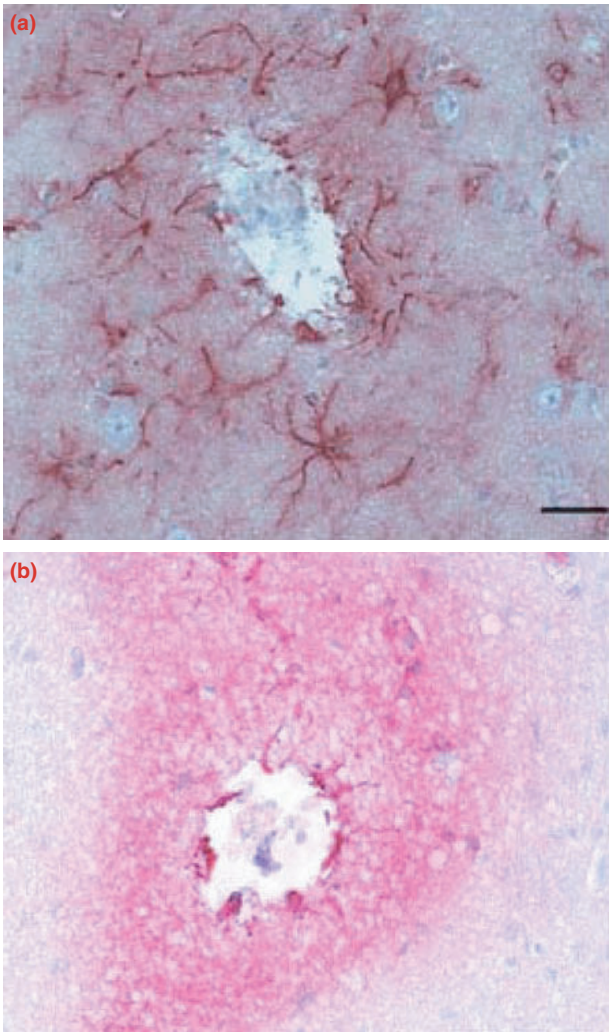


Figure 17 An immunohistochemical stain (a) for glial fibrillary acidic protein (GFAP, red) shows activation of perivascular and parenchymal astrocytes in a case of cerebral malaria (haematoxylin counter stain, ×200). An immunohistochemical stain (b) for fibrinogen (red) shows leakage around a small vessel into the brain parenchyma and associated uptake into astrocyte foot processes investing the abluminal border of the Virchow-Robbins space (haematoxylin counterstain, ×400).

areas of focal haemorrhage is associated with fibrin-platelet thrombi, intravascular clotting and perivascular haemorrhages in children (Grau *et al.* 2003; Dorovini-Zis *et al.* 2011). Thrombocytopenia is strongly associated with CM in these patients. This may reflect a systemic abnormality of clotting or the relative abundance of CD36 on the surface of platelets. However, the degree of thrombocytopenia counts was not associated with adverse outcomes in a large clinical study of over 5000 paediatric African malaria cases suggesting that, like ring haemorrhages, thrombocytopenia alone is not sufficient to explain mortality in fatal cerebral malaria (Dondorp *et al.* 2010).

Disruption of the blood–brain barrier

Cellular inflammatory responses are apparent in the intrinsic astroglial cells of the brain parenchyma (Figure 17a). Pathology studies have provided evidence for a direct link between sequestration of PRBC and dysfunction and local breakdown of the blood–brain barrier. There is leakage of plasma proteins such as fibrinogen into the perivascular space and activation of pericytes, perivascular astrocytes and microglial cells on immunohistochemistry (Figure 17b). Sequestration is associated with redistribution of endothelial cell junctional adhesion molecules such as ZO-1 and vinculin (Brown *et al.* 1999), focal disruption to the blood–brain barrier in adults (Brown *et al.* 2000) and Malawian children (Brown *et al.* 2000; Dorovini-Zis *et al.* 2011). Parasitised erythrocytes can stimulate intracellular signalling events in endothelial cells through direct adhesion to receptors such as CD36 or ICAM-1 (Jenkins *et al.* 2007; Tripathi *et al.* 2007). These events in turn affect cerebral endothelial cell structure and function, which may mediate changes to blood–brain barrier function in cerebral malaria (Medana & Turner 2006).

Heterogeneity of pathology and clinical misdiagnosis: What is a 'pathological' case definition of cerebral malaria?

Autopsy-based pathology studies often examine small numbers of cases, making statistical analysis difficult. In addition, the 'snapshot' effect – examining individual cases whose deaths occurred at different times in the parasites' maturation cycle and in the evolution of pathology – greatly complicates interpretation. Nevertheless, it can be seen that there are some differences between the pathology of CM in children from high endemicity settings and in adults from low endemicity settings. The clinical differences include the time to death (more rapid in children, within 48 h of admission) and the co-existence of other severe manifestations such as shock, acidosis, lung disease or renal failure (more prominent in adults). Levels of immunity, host polymorphisms and/or parasite virulence may play roles in these different pathologies in the individual patient. Pathologically, the differences include deposition of platelets and fibrin thrombi in children and the degree of host leucocyte reaction, neither of which is marked in adults. HIV co-infec-

tion is significantly associated with poorer prognosis in children and adults, and it has been noted that in HIV-infected children with fatal malaria, there is a lack of monocyte sequestration in the brain (Grimwade *et al.* 2004; Berg *et al.* 2008). Where the opportunity to examine potential differences due to treatment has presented, no differences in the type or degree of brainstem neuropathology were found in adult patients treated with different drugs (artemether versus quinine) (Hien *et al.* 2003).

Quantitative neuropathological examination using multivariate analysis in Vietnamese cases of CM shows that the presence of coma before death was associated with the neuropathological features of PRBC sequestration, microvascular congestion and axonal injury. Observed sequestration in brain microvessels was associated with time to death (i.e. duration of treatment), admission Glasgow Coma Score and density of peripheral parasitaemia. In African children, cerebral malaria was confirmed using a pathological cut-off of more than 21% of cerebral vessels showing sequestration at post-mortem (Taylor *et al.* 2004; Milner *et al.* 2012a). The sensitivity of diagnosis of malaria increases, and the prognosis worsens, when coma is seen in combination with malaria retinopathy (Beare *et al.* 2004, 2011; Taylor *et al.* 2004; Birbeck *et al.* 2010a; Dorovini-Zis *et al.* 2011; Milner *et al.* 2012a). The Malawi autopsy study showed that approximately 25% of clinically diagnosed CM cases did not have evidence of malaria parasite sequestration in the cerebral microvasculature and had other causes of death (Taylor *et al.* 2004). This large proportion of patients in which the clinical case definition failed has implications for both the reliability of verbal autopsy (Snow *et al.* 1992) and the interpretation of treatment or vaccine trials.

Lung pathology

Respiratory distress is a common feature of severe falciparum malaria in both children and adults (Taylor & White 2002; Taylor *et al.* 2006c). Metabolic acidosis is the most important cause of respiratory distress, but many other factors may contribute to it, including severe anaemia, pulmonary oedema and aspiration pneumonia. Frank pulmonary oedema is more common in adults than children and is a poor prognostic sign, although it is less common with improved ventilation

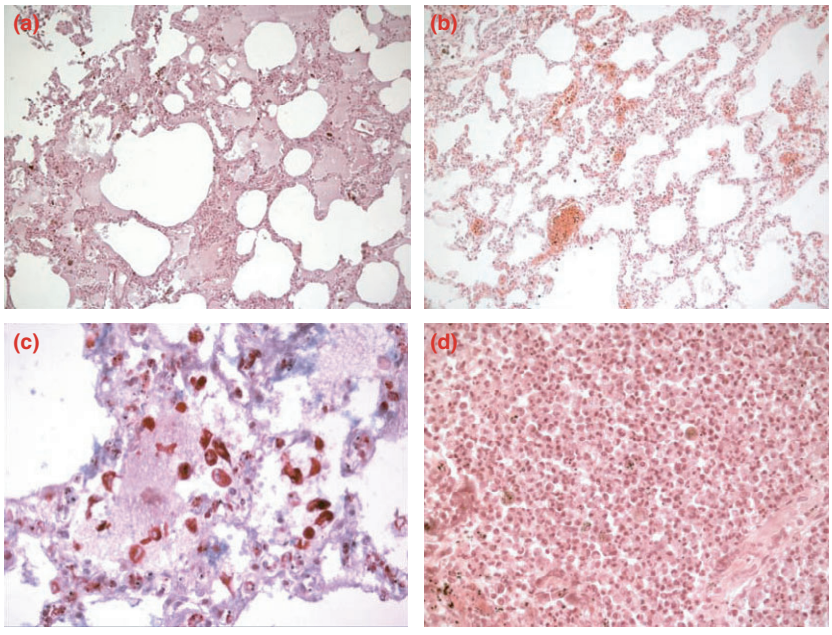


Figure 18 Examples of the pathology found in the lung during *Plasmodium falciparum* malaria. Patchy pulmonary oedema (a) with pigment deposition can be seen commonly in adults and in about 40% of paediatric cases (H&E × 100). Parasitised erythrocytes sequestered in alveolar capillaries alongside host lymphocytes and monocytes (b) are demonstrated in most patients (H&E × 100). Immunohistochemistry with anti-CD68 (c) shows both intralveolar macrophages and type 2 pneumocytes and an increase in circulating monocytes in alveolar capillaries, many of which contain phagocytosed malaria pigment (H&E × 400). In paediatric cerebral malaria, the amount of pigment within macrophages is significantly greater than in non-CM patients. A case of pyogenic pneumonia (d) is demonstrated as the cause of death in a patient who recovered from cerebral malaria but died later (H&E × 400).

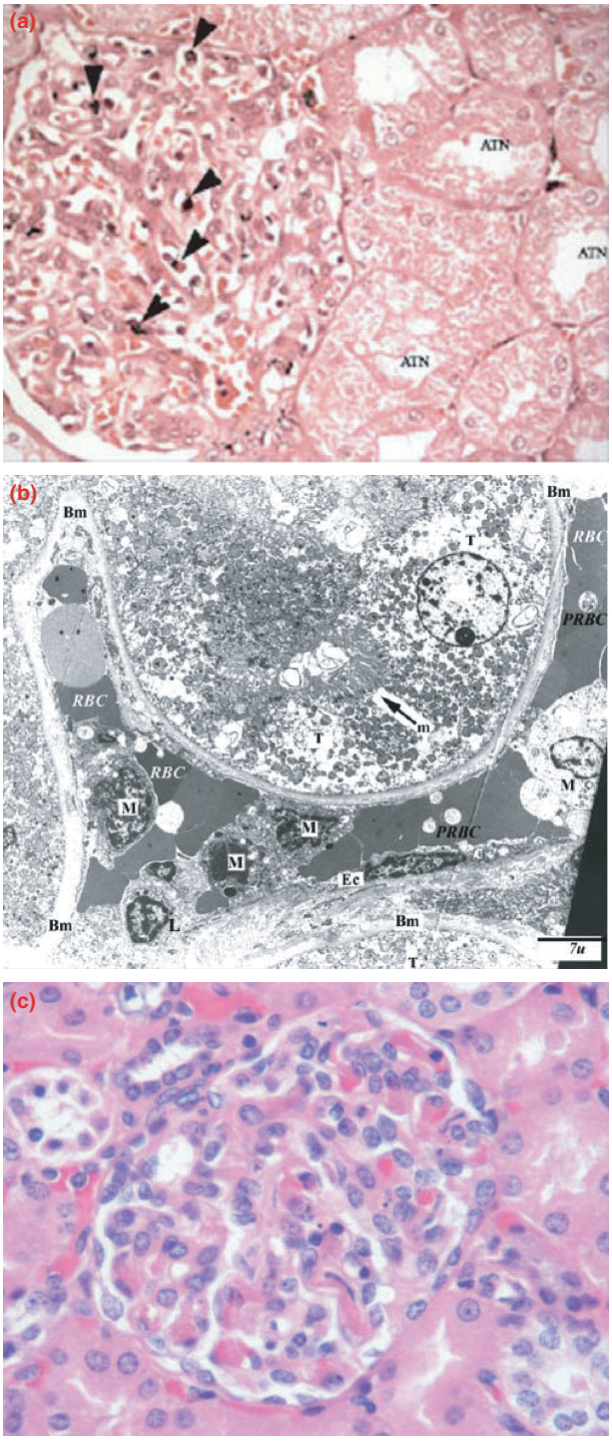


Figure 19 Manifestations of *Plasmodium falciparum* malaria infection in the kidneys are shown. Histology demonstrating sequestration of parasitised erythrocytes (a) in both glomerular (arrowheads) and peritubular capillaries. There is associated tubular epithelial cell swelling and degeneration consistent with acute tubular necrosis (ATN) (H&E staining ×200). A transmission electron micrograph (b) shows sequestered PRBC, uninfected red cells (RBC), and mononuclear leucocytes (M) in a peritubular capillary. The proximal tubular cell is separated by the basement membrane (BM) and is rich in mitochondria (Mi) (Scale bar = 2 μm, courtesy of Prof Emsri Pongponratn). Fibrin thrombi within glomerular capillaries (c) indicative of microangiopathic changes are seen in approximately 40% of classic paediatric cerebral malaria cases.

and ICU level care for adult patients (Brooks *et al.* 1969). Adult patients may develop radiological and clinical signs of acute respiratory distress syndrome (ARDS). At autopsy, the lungs may be normal or increased in weight with associated pulmonary oedema on sectioning. Pleural or intrapulmonary punctuate haemorrhages can be seen in adults (these are common in many organs in adults with fatal malaria). In contrast, haemorrhages outside of the brain in paediatric cases have not been found. Alveolar capillaries may show sequestration of PRBC, causing congestion of pulmonary capillaries, and there is sometimes associated intra-alveolar haemorrhage. The degree of host leucocyte response is greater in the lung than the brain, with monocytes, neutrophils and lymphocytes seen in alveolar capillaries (Duarte *et al.* 1985; MacPherson *et al.* 1985). In some cases, the inflammatory response resembles a pneumonitis (Figure 18).

The pathological features include thickened alveolar septa showing monocytes and neutrophils (often containing phagocytised particles of malaria pigment), patchy intra-alveolar haemorrhage and pulmonary oedema. In individual adult cases, hyaline membrane formation can be seen as an indicator of diffuse alveolar damage; this pattern has not been observed in children. Pyogenic consolidation may be found in some cases, indicating that pneumonia was the true diagnosis in a patient with incidental parasitaemia whose illness was mistakenly attributed to malaria, or it may be a secondary complication of coma, convulsions or intubation in patients suffering from cerebral malaria.

Renal pathology

Renal failure is a common clinical manifestation of severe falciparum malaria in adults, but it is uncommon in children (Trang *et al.* 1992; Mehta *et al.* 2001; Dondorp *et al.* 2005a). There is a clinical association between jaundice and acute renal failure in adults (Day *et al.* 2000a). Patients may have shock, anaemia and other complications potentially reducing renal oxygenation. A study of renal blood flow and haemodynamics in Vietnamese patients with severe malaria showed only a small decrease in renal

blood flow in patients with severe malaria, compared to patients with sepsis and to controls (Day *et al.* 2000a). These findings suggest that malaria-associated acute renal failure (MARF) is not caused by shock alone. Nevertheless, both ultrastructural (Nguansangiam *et al.* 2007) and histological studies in the Vietnam autopsy series showed that in patients dying with MARF, there was a high prevalence (64%) of tubular epithelial cell degeneration and acute tubular injury, features that are known to occur in shock, and so may result from impaired microvascular perfusion. Acute renal failure is rare in children, but some degree of renal dysfunction has been reported in series from Africa, although this may result from dehydration and pre-renal failure, which usually resolves rapidly with appropriate fluid management (Maitland *et al.* 2004; Anochie & Eke 2005). Recent evidence in the Malawian autopsy series has revealed thrombin within the renal glomerular microvasculature, possibly as a part of a systemic coagulopathy, which may cause a thrombotic microangiopathy in the kidney (D. Milner personal communication) (Figure 19).

Pathology studies in Asian patients have reported a number of different findings (Boonpucknavig & Sitprija 1979; Sitprija 1988; Barsoum 2000) correlated with the ante-mortem diagnosis of MARF, including acute tubular injury (termed acute tubular necrosis but potentially less florid), sequestration of PRBC within glomerular and peritubular blood vessels, accumulation of host leucocytes in peritubular capillaries and a mild endocapillary proliferative glomerulonephritis (Figure 19). A study cohort of South-East Asian adults (Nguansangiam *et al.* 2007) showed no evidence for established immune complex-mediated glomerulonephritis in falciparum malaria. Earlier studies of renal pathology (Boonpucknavig & Sitprija 1979; Boonpucknavig & Soontornniyomkij 2003) described an active, immune complex-mediated glomerulonephritis in acute falciparum malaria. Several have recorded marked glomerular hypercellularity, mesangial proliferation and both immunofluorescence and EM evidence of immunoglobulins, complement components and malarial antigen deposition in capillary loops. Immune complexes were cleared rapidly and the glomerular injury was reversible, in contrast to immune complex nephritis in *P. malariae* (Kibukamusoke & Hutt 1967). Other clinical studies (Barsoum 2000; Boonpucknavig & Soontornniyomkij 2003; Eiam-Ong 2003) emphasised that the clinical and biochemical findings in MARF were more in keeping with an ischaemic nephropathy or acute tubular necrosis (Trang *et al.* 1992), with little or no proteinuria. The absence of associated hypertension, the rapid resolution without residual renal impairment, together with the "inactive" urinary sediment findings, all suggested that renal failure in severe malaria results from acute tubular injury, rather than an acute tubulointerstitial nephritis or glomerulonephritis.

Liver pathology

Jaundice is a common finding in adult patients presenting with severe malaria. Jaundice may be due to severe intravascular haemolysis or disseminated intravascular coagulation (DIC), usually with the addition of hepatocyte dysfunction. 'Malarial hepatitis' is an erroneous term, because neither the clinical nor the histopathological features resemble those of viral or toxic hepatitis. Severe liver dysfunction occurs occasionally in severe malaria in association with multiorgan failure and poor

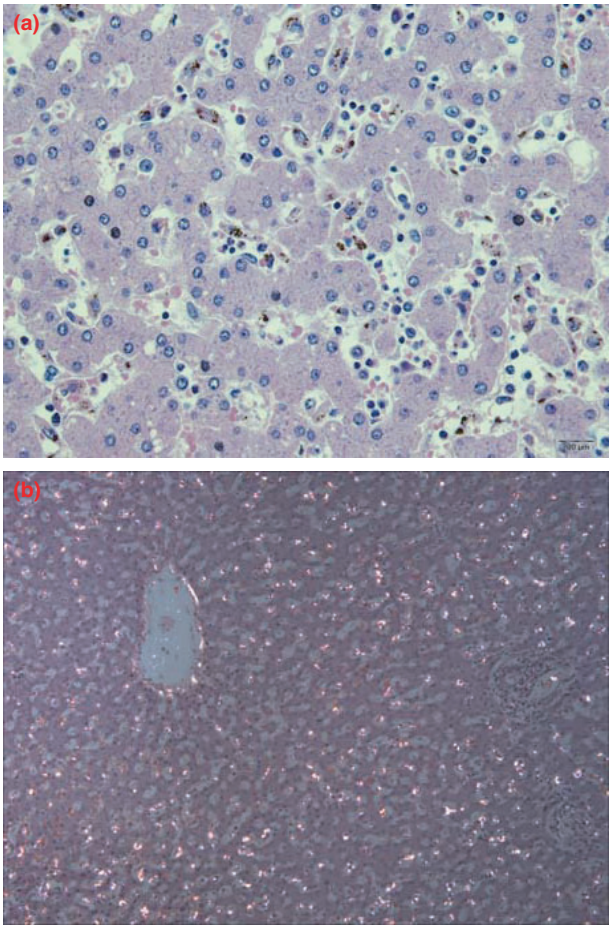


Figure 20 Histology of the liver (a) in *P.falciparum* malaria infection, showing dilated sinusoids, red cell congestion, host leucocytes and pigment uptake by Küppfer cells. There is minimal cellular hepatocyte change (H&E × 400). Diffuse pigment distribution (b) and load is strongly associated with the presence of cerebral malaria in paediatric cases (pigment is seen here as refractory granules under polarisation).

prognosis (Das *et al.* 2007). In adult, non-immune patients in South-East Asia and India, jaundice and liver dysfunction occur in up to 50% of cases in severe malaria, almost always as part of multiorgan disease. Jaundice is associated with predominantly conjugated hyperbilirubinaemia, unconjugated hyperbilirubinaemia and mixed patterns being less common. There may be prolonged prothrombin times and low concentrations of albumin in the serum. Elevations of liver cytoplasm enzymes are common, including raised aspartate and alanine aminotransferases (AST and ALT) and alkaline phosphatase, but these are never increased to the levels seen in viral hepatitis (Anand *et al.* 1992; Anand & Puri 2005).

Histopathological examination of liver biopsies during life or autopsy samples reveals a spectrum of changes in adults (Kochar *et al.* 2003; Das *et al.* 2007; Rupani & Amarapurkar 2009). These include PRBC sequestration within hepatic sinusoids and adhesion of parasitised erythrocytes to sinusoidal endothelial cells, Küppfer cell hyperplasia and the retention of malaria pigment (Figure 20). Host leucocyte responses are variable. There is some conflicting evidence as to the extent of direct parenchymal hepatocyte damage, but several series report hepatocyte swelling and necrosis, host leucocyte infiltrates and focal centrilobular hepatic necrosis. Fatty change is uncommon, and cholestasis is rarely seen. In contrast, paediatric cases of cerebral malaria have revealed relatively normal appearing liver with a predominance of pigment-laden macrophages and little sequestration of PRBCs (Whitten *et al.* 2011). A finding of widespread hepatic necrosis at autopsy should raise the possibility of hepatotoxicity induced by traditional medicines taken to treat malaria before presentation. An ultrastructural study of post-mortem liver samples from adults (Prommano *et al.* 2005) showed that the degree of jaundice, hepatomegaly and liver enzyme abnormalities correlates with the overall parasite load in the body. Parasitised red cell sequestration in the liver was quantitatively associated with liver weight, serum bilirubin and AST levels.

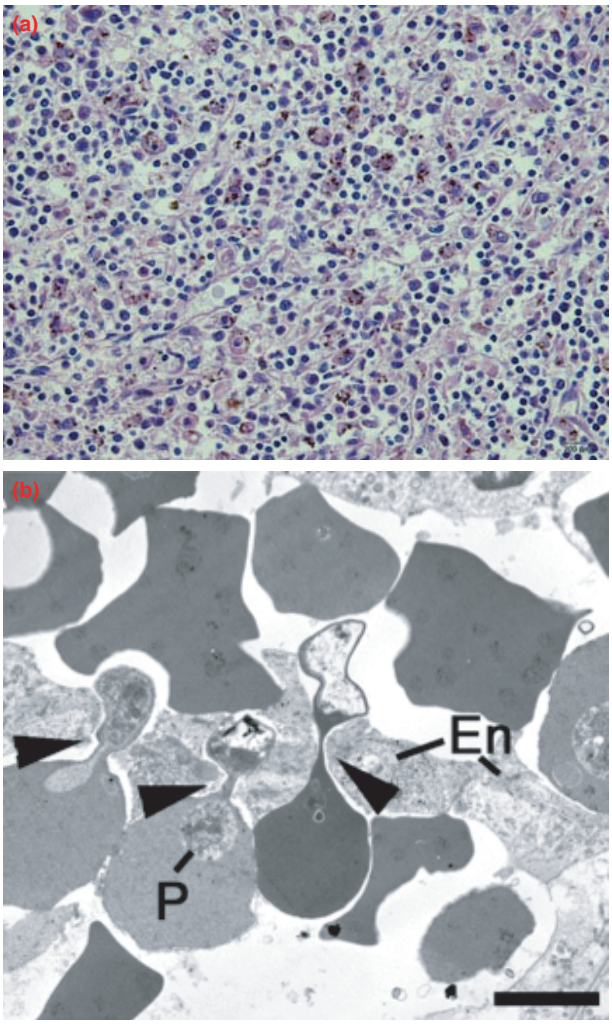


Figure 21 Histological appearances of the spleen (a) in acute *Plasmodium falciparum* malaria, showing engorgement of the red pulp sinusoids by infected erythrocytes, and numerous pigment-laden monocytes (H&E × 400). (b): An electron micrograph showing 'pitting', whereby the *Plasmodium* parasite (P) is removed from the erythrocyte as it passes between the narrow slits between the endothelial cells (En) lining the sinusoids (courtesy of Prof Emsri Pongponratn).

Splenic pathology

The spleen is commonly enlarged in severe malaria and can occasionally be so engorged with PRBC and uninfected RBC that acute splenic rupture occurs with death due to haemorrhagic shock, although this is rare (Imbert *et al.* 2009). Chronic, repeated or relapsing malaria infection can result in chronic splenic enlargement, pigmentation, and is a cause of the syndrome known as hyperreactive malarial splenomegaly (formerly 'tropical splenomegaly syndrome'). Histological findings in the acutely infected spleen include an increase in the red to white pulp ratio, due to engorgement of sinusoids with red cells and pigment (Figure 21). The germinal centres (white pulp) show a degree of dissolution, which reflects trafficking of leucocytes in response to malaria infection, and there are increases in the numbers of dendritic cells within the marginal zones (Urban *et al.* 2005). Recent studies of an *ex vivo* model of human splenic per-

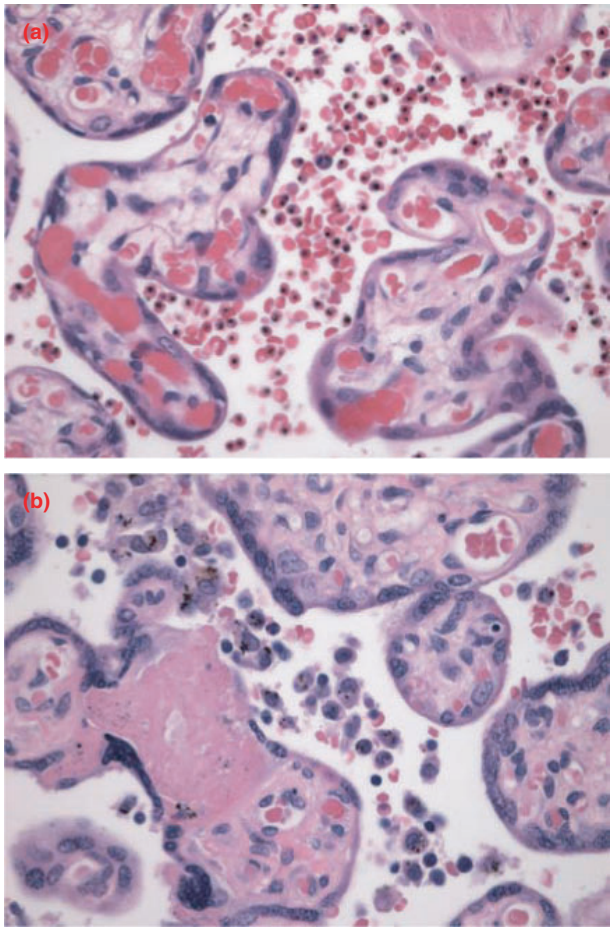


Figure 22 Histological features of placental malaria (a): Acute, active placental malaria infection, showing sequestration of infected erythrocytes in the maternal vascular space, some adhering to the syncytiotrophoblast cells lining the chorionic villi, with focal intervillous fibrin formation. (b): Chronic placental malaria infection, with clearance of parasitised erythrocytes, numerous monocytes within the maternal vascular space and fibrin deposition within a villus. (haematoxylin and eosin staining ×400, images courtesy of Dr Atis Meuhlenbachs, CDC, USA).

fusion with PRBC (Safeukui *et al.* 2008) have indicated that red cells containing late-stage parasites are removed in the splenic sinusoids and that non-adherent ring-stage PRBC can also be removed, a process that is assisted by the slowness of sinusoidal circulation in the spleen. Removal of infected red cells may contribute to both splenic pathology and the immune response to falciparum malaria (Buffet *et al.* 2011).

The pathology of placental malaria

The pathological features of placental malaria depend on the timing of infection in relation to delivery (Figure 22). In active malaria infection of the placenta, sequestration of PRBC is seen in the intervillous blood space. Sequestration in the placenta is mediated by binding to chondroitin sulphate A (CSA), which is expressed by the syncytiotrophoblast lining the placental intervillous space (Fried & Duffy 1996). Placental PRBC binding to CSA is mediated by a unique P*f*EMP-1 protein VAR2CSA that is immunologically distinct from variant surface antigens (VSA) on other PRBC. This unique pregnancy-specific VSA is recognised by antibodies which may then be boosted when a malaria infection with the same VSA occurs in a subsequent pregnancy (Fried *et al.* 1998). The absence of such antibodies in first pregnancies could explain in part the higher susceptibility of primigravidae to placental malaria. The close association between a specific parasite binding phenotype and placental sequestration offers the hope for immunisation against this specific disease phenotype using vaccines modelled on the VAR2CSA protein (Orogade *et al.* 2008).

Active infection may be associated with marked inflammation, with a predominantly mononuclear cell infiltrate of monocytes and lymphocytes and with some neutrophils. The degree of inflammation varies but is often considerable and can lead to a picture of massive intervillositis. Host monocytes can secrete chemokines (Abrams *et al.* 2003), and the inflammatory infiltrate may continue to affect the function of the materno-foetal unit after clearance of parasitaemia, leading to adverse pregnancy outcome (Rogerson *et al.* 2003). Deposition of malaria pigment and fibrin in the intervillous space and phagocytosis of syncytiotrophoblastic cells by monocytes can also be seen. With effective treatment, the placenta can become cleared of acute malaria infection, but subsequent episodes of clinical malaria can cause further pathological changes, which are not specific to malaria. These include excessive fibrin deposition within and between villi, thickening of the trophoblastic basement membrane, loss of villous vascularity and obliteration of arterioles in the terminal stem villi, and occasional focal fibrinoid necrosis of villi. Several histological grading schemes have been suggested to detail the pathological changes in placental malaria and relate them to maternal and fetal outcomes in pregnancy (Bulmer *et al.* 1993, Ismail *et al.* 2000, Meuhlenbachs *et al.* 2010).

Section 9: Clinical and laboratory diagnosis of severe malaria

Clinical diagnosis

Severe malaria is clinically similar to other severe febrile illnesses (Gwer *et al.* 2007). Hence, the specificity of clinical diagnosis is low. Each clinical syndrome of severe malaria (e.g. coma, severe anaemia, acidosis) can have other causes (e.g. meningitis, sickle cell disease, septicaemia) (Berkley *et al.* 1999; Gwer *et al.* 2007; Pfeiffer *et al.* 2008; Poschl *et al.* 2010; Hendriksen *et al.* 2012a). Obtaining a parasitological diagnosis does not resolve the diagnostic problem, especially in high transmission areas, where asymptomatic parasitaemia is common and may be incidental in any severe illnesses (Gwer *et al.* 2007).

Because of the fatal consequences of missing the diagnosis of severe malaria, a traveller with a severe febrile illness who has been in a malarious area within the previous 2 months must be considered to have malaria unless proven otherwise. Similarly, in patients living in endemic countries who present with a fever, the suspicion of malaria should be high.

In countries where malaria is common, overdiagnosis is another possibility, diverting attention from other infectious causes of severe febrile illness. Overdiagnosis of malaria has been shown to contribute to mortality (Reyburn *et al.* 2004). Overdiagnosis is commonly due to neglect of a negative blood film or to failure to obtain a blood film or rapid diagnostic test (RDT) at all (English *et al.* 1996a; Molyneux *et al.* 1998; Berkley *et al.* 2005). A parasitological diagnosis should be obtained whenever possible (Hendriksen *et al.* 2011), but the relevance of parasitaemia to the current illness must always be considered carefully (Koram & Molyneux 2007). In the absence of diagnostic facilities, antimalarial treatment should not be delayed if the patient is severely ill but should be started based on a clinical suspicion while other diagnoses are also considered.

Parasitological diagnosis

Microscopy remains the reference standard for the diagnosis of falciparum malaria, but this requires the availability of a good microscope, significant technical skills, good-quality reagents and clean slides. The diagnostic quality of microscopy is very variable in routine hospitals in sub-Saharan Africa (McMorrow *et al.* 2008; Nankabirwa *et al.* 2009).

Peripheral blood films. Malaria is diagnosed by examination of thick and thin blood smears stained with supravital dyes. Blood smears are usually stained by the methods described by Wright, Field or Giemsa. Thin films are quicker to obtain, provide accurate counts of high parasite densities, and allow prognostic assessments based on staging of parasite development and assessment of the proportion of neutrophils containing malaria pigment. On the thick film, in which many layers of red cells are overlaid and lysed by water in the staining procedure, parasitaemias as low as 30–50/μl may be detected.

Reliable examination of the peripheral blood slide (both thick and thin films) requires a degree of technical precision in the preparation of the blood slide, its handling and staining, and are dependent on the optical quality of the microscope and the illumination, as well as competence, care and time on the part of the microscopist. Common pitfalls include artefacts (e.g. dye precipitation) mistaken for parasites, Maurer's clefts confused with Schüffner's dots (causing *P. falciparum to* be misdiagnosed as *P. vivax)*, Babesia and Bartonella infections mistaken for malaria and young gametocytes of *P. falciparum* mistaken for *P. vivax*. In SE Asia *P. knowlesi* ring stages may be mistaken for *P. falciparum* and *P. knowlesi* mature trophozoites may be mistaken for *P. malariae*.

In *P. vivax*, *P. ovale* and *P. malariae* malaria, all stages of parasite development may be seen in the peripheral blood film, whereas in *P. falciparum* infections, only

Table 8 Criteria for staging the development of *Plasmodium falciparum* assessed by light microscopy

Stage development	Cytoplasm	Nucleus	Pigment
Tiny ring	Ring form, width <1/2 of diameter of the nucleus	1–2, round at one side of cytoplasm	No
Small ring	Ring form, width ≥ 1/2 of diameter of the nucleus	1–2, round at one side of cytoplasm	No
Large ring	Ring form, width ≥ diameter of the nucleus	1–2, round or elongated	No
Early trophozoite	Spherical	1–2, inside cytoplasm	Faint, pale brown
Middle trophozoite	Spherical, enlarged, stained dark	1–2, pale inside cytoplasm	Brown, clumps
Late trophozoite	Spherical, ≅ 1/2 of host RBC, stained dark	1–2, pale inside cytoplasm	Dark brown clumps
Early schizont	Spherical, >1/2 of the host RBC, stained dark	3–5, irregular, inside cytoplasm	Dark brown clumps
Late schizont	Spherical, nearly fills the host RBC, stained dark	>5, round or oval, inside cytoplasm	Dark brown clumps

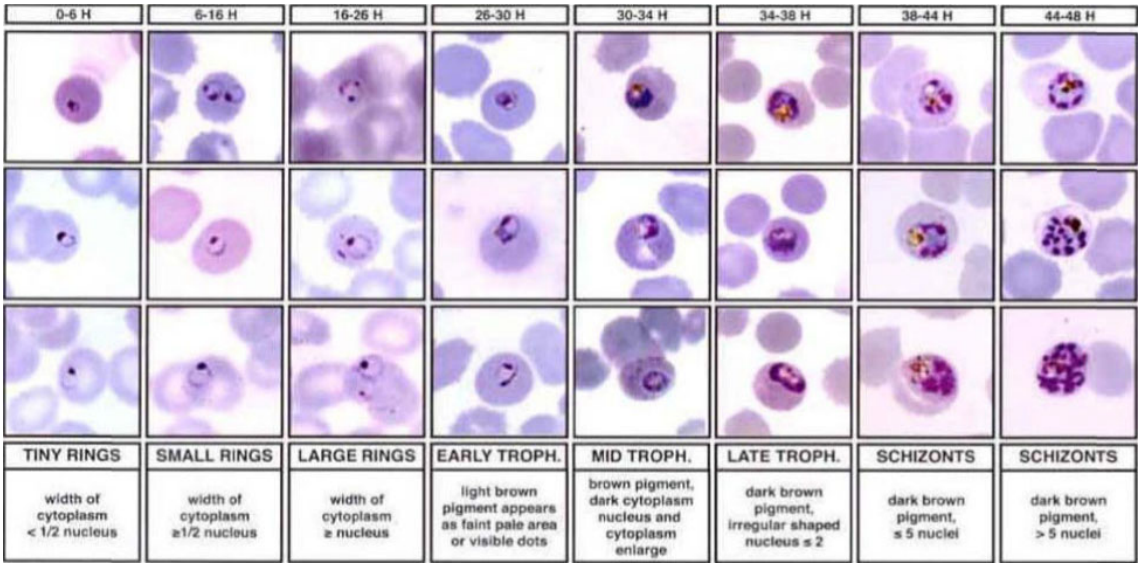


Figure 23 *Plasmodium falciparum* in the thin blood smear: stages of asexual parasite development (Silamut *et al.* 1999). Pigment-containing trophozoites and schizonts is sequestered and so rarely seen on peripheral blood smears. Finding these mature stages comprise >20% of peripheral blood parasites carries a poor prognosis in severe falciparum malaria.

parasites in the first 24 h of the 48 h asexual life cycle are usually visible (Figure 23 and Tables 8 and 9). Sexual stages of the parasites (gametocytes) may also be seen but do not indicate acute infection, as their period of maturation and subsequent clearance is considerably slower than that of asexual stages.

Patients with uncomplicated malaria without clinical severity symptoms have on average lower peripheral blood parasitaemias than patients with severe disease. As an example, one study from Thailand found a geometric mean parasitaemia of 62 574/μl (50 095–78 144/μl) in hospitalised patients with uncomplicated disease, versus 206 395/μl (156 458–272 332/μl) in patients with severe disease (Dondorp *et al.* 2005b). However, within the patient group with severe disease, peripheral blood parasitaemia varies greatly and is only a weak predictor of mortality in falciparum malaria. This is because the less pathogenic circulating stages can be counted in the peripheral blood slide, whereas the more pathogenic sequestered mature parasitised erythrocytes are not seen. As a consequence of sequestration, two identical peripheral blood parasite counts can represent a 100-fold difference in sequestered parasite biomass (White & Krishna 1989). The clinician should thus not be reasssured by the laboratory reporting a low peripheral blood parasite count when the patient has symptoms of severe disease. Although severe malaria can present with a low parasite count, high counts without signs or symptoms of severity are associated with increased risk. In a low transmission setting a peripheral blood slide showing ≥ 4% infected RBCs (around 150 000/μl) without signs of severity is associated with a mortality of around 3% (Luxemburger *et al.* 1997).

In areas of high malaria transmission, children may tolerate higher levels of parasitaemia without severe symptoms, and low level parasitaemia can be detected in a high proportion of asymptomatic children. The probability that a severe febrile illness is attributable to malaria increases with higher parasitaemias. A Kenyan study calculated that among children under 2 years old with severe disease and over 2500 parasites/μl, the malaria-attributable fraction of severe disease exceeded 85% in moderate- and low transmission areas, but 61% in a high transmission area (Bejon *et al.* 2007). In children with cerebral malaria, parasitaemias in excess of 1 000 000/μl were significantly associated with a fatal outcome (Molyneux *et al.* 1989b). Both histidine-rich protein 2 (*Pf* HRP2) and malaria pigment phagocytosed by monocytes clear more slowly than malaria parasites and indicate recent infection in a parasite negative patient, which is a not uncommon scenario in patients that have been pre-treated with antimalarial drugs before hospital admission (Day *et al.* 1996a).

Table 9 Criteria for identifying developmental stages of *Plasmodium falciparum* in thick blood smears as assessed by light microscopy modified from Silamut *et al.* (1999)

Stage development	Cytoplasm	Nucleus	Pigment
Ring	Blue, ring shape	Red dot; can be elongated, 1–2 dots	No
Trophozoite	Blue, round shape, stained darker than ring (on the same slide)	1–2 red, big dots or irregular shape inside cytoplasm. Sometimes cannot see	From pale brown to clumped and dark brown, inside cytoplasm
Schizont	Blue, round shape, size up to that of red cell	At least 3 nuclei, purple-blue colour	Dark brown, clumps in the middle of the parasite
Gametocyte	Crescent shape like a banana	Not obvious	Elongated, like a chopstick, in the middle

The blood slide should be examined not only for parasites but also for malaria pigment, a characteristic coal-coloured refractile material, inside peripheral blood leucocytes. In Vietnamese patients with severe malaria finding over 5% of peripheral blood neutrophils containing visible pigment indicates a poor prognosis (Nguyen *et al.* 1995). In severe malaria, the stage of parasite development on the peripheral blood smear also has prognostic importance (Silamut & White 1993). The presence of late-stage parasites in the peripheral blood reflects a larger proportion of sequestered late-stage parasites. In patients with clinically severe disease but a low peripheral blood parasitaemia, a high proportion of late-stage parasites may be observed in the thin blood film.

A theoretical scenario is that a patient with a highly synchronised infection presents at the moment that all the parasites are in the late stage of erythrocytic development and thus all sequestered in the microcirculation, resulting in a negative peripheral blood slide. Although this possibility has been commonly proposed, it has not been encountered with certainty in untreated patients with severe malaria by the authors of this supplement. A negative blood smear in an untreated patient thus makes malaria a very unlikely diagnosis.

Causes of apparent blood film negativity in severe malaria.

- Recent drug treatment, especially with an artemisinin-containing therapy
- Inadequate examination of blood film – quality of slides, duration of examination, expertise of microscopist (Figure 24)
- Reliance on thin film and failure to examine thick films
- Identification of non-falciparum parasites, leading to failure to search further for *P. falciparum*

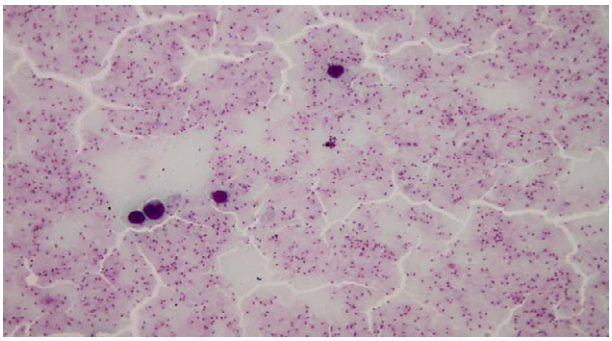


Figure 24 Heavy *Plasmodium falciparum* parasitaemia on the thick film. Accurate parasite counting of high parasite densities is not possible on thick films. Occasionally, particularly if stains and microscopes are poor quality, microscopists will misread such a slide as negative – a disastrous mistake.

If there is still uncertainty, the test should be repeated every 12 h for 48 h. Parasitaemia may fluctuate widely in the first 24 h of treatment and may rise alarmingly despite the administration of adequate antimalarial drugs. The relationship between peripheral circulating ring-form-infected erythrocytes and sequestered infected cells is a function (predominantly) of the stage of development (synchronicity) and, to a much smaller extent, of the parasite multiplication rate. There may be wide differences between the number of infected cells circulating and the number sequestered, and rapid changes may be anticipated in synchronous infections (White & Krishna 1989). Microscopy with fluorescent staining of the buffy coat (quantitative buffy coat analysis or QBC) has a higher sensitivity to detect low parasitaemias, but few microscopists are familiar with this method.

Rapid diagnostic tests (RDTs). Compared with microscopy, malaria rapid diagnostic tests (RDTs) do not

require extensive training or well-maintained equipment. They are increasingly used for malaria diagnosis.

Malaria RDTs are immunochromatographic tests that identify either malaria antigens [most commonly *Plasmodium falciparum* histidine-rich-protein 2 (PfHRP2)], or the parasite enzyme Plasmodium lactate dehydrogenase (pLDH) in a drop of blood. A disadvantage of all RDTs to date is that they cannot provide a quantitative result. PfHRP2-based tests can remain positive for up to a month after a *P. falciparum* infection, while pLDH tests are positive only while there are living parasites in the blood.

As PfHRP2 can remain in the circulation for several weeks after parasite clearance depending on the parasite burden, there was a concern that PfHRP2-based RDTs would have limited specificity, especially in areas of intense transmission and therefore frequent infections (Iqbal *et al.* 2004; Mayxay *et al.* 2001; Swarthout *et al.* 2007). In a large trial evaluating RDTs for the diagnosis of severe falciparum malaria in African children, with 'expert' microscopy as reference test, the sensitivity of the PfHRP2 test was 94%, but the specificity only 71% (Hendriksen *et al.* 2011). Both sensitivity and specificity of the pLDH test were 88%, but the sensitivity of the pLDH test was unacceptably poor for parasite densities of <1000/μl. Both RDTs performed better than did the routine slide reading in a clinical hospital laboratory.

In a patient with severe falciparum malaria who has received antimalarials before presentation and now has a negative blood film, a PfHRP2 based RDT will still be positive confirming the diagnosis.

There is need to continue to monitor the diagnostic value of the PfHRP2 antigen. The PfHRP2 gene is highly polymorphic, with deletion of the entire gene reported in both laboratory and field isolates, and this could potentially affect expression of the protein, compromising the sensitivity of PfHRP2-based RDTs. Reassuringly in a recent study evaluating sequence variation in African children with severe malaria, no deletion could be identified in the PfHRP2 gene in patients with low PfHRP2 concentrations, and sequence variation in the gene did not confound the measurement of plasma PfHRP2 concentrations (Ramutton *et al.* 2012).

Malaria-attributable disease in high transmission settings

In areas of high transmission (sub-Saharan Africa), a high background prevalence of peripheral parasitaemia can hamper the diagnosis of 'cerebral malaria'. A positive blood slide in a febrile comatose child does not adequately exclude other possible diagnoses. Bacteraemia can be present in up to 20% of these patients (Berkley *et al.* 2005) and is associated with young age and severe anaemia (Bronzan *et al.* 2007).

Among 31 children with slide-positive fatal cerebral malaria who came to autopsy, 24 had intracerebral sequestration and no other cause of death, while 7 (23%) had no sequestration and an alternative cause of death (Taylor *et al.* 2004). Ophthalmoscopy during life had identified malarial retinopathy in 23 of the 24 cases with intracerebral sequestration, and in none of the seven without it, indicating that retinopathy is strongly associated with cerebral malaria and can serve as an additional diagnostic criterion (Beare *et al.* 2011). The sensitivity of this finding is 96% in Malawian children and around 63% in Asian adults (Taylor *et al.* 2004; Maude *et al.* 2009b).

PfHRP2 is released into the plasma compartment at the moment of schizont rupture, and its concentration in plasma can serve as a measure of the total parasite biomass. Plasma PfHRP2 concentrations have proved to be a promising tool to distinguish children in high transmission settings with slide-positive severe falciparum malaria from those with severe febrile illness from other causes). Slide-positive patients with low plasma PfHRP2 concentrations represent children with a low sequestered biomass in whom the clinical disease is likely to have a cause other than malaria. Modelling data from a large study of clinically diagnosed severe malaria indicated that the probability of malaria-attributable disease dropped below 50% with plasma PfHRP2 < 174 ng/ml. A semi-quantitative PfHRP2 rapid test with well-chosen cut-offs, if one could be developed, would be a useful clinical tool, prompting the physician to look for alternative diagnoses in case the plasma PfHRP2 is low.

Peripheral blood parasite density can also be used to calculate malaria-attributable disease in slide-positive severe malaria in African children. A study by Bejon (Bejon *et al.* 2007) found that the case definition for severe malaria is improved by applying a parasite density threshold (2500/μl in the Kenyan setting), and by excluding children with meningitis, lower respiratory tract infection (clinician's diagnosis), bacteraemia and gastroenteritis with severe dehydration, but not by excluding children with HIV or malnutrition. Of course, this requires accurate parasite counting. These criteria are useful for selecting patients in clinical trials with a higher chance of 'true' severe malaria, but because of limited specificity, they are less valuable for individual patient care.

Whenever possible, blood cultures should be obtained in the African child presenting with severe febrile illness, even when the peripheral blood slide shows the presence of malaria parasites. Aerobic culturing is generally sufficient to detect the most common pathogens causing bac-

teraemia. Positive blood culture results have been reported in 4.6–12.6% of African children with slide-positive severe falciparum malaria (Berkley *et al.* 1999, 2009; Evans *et al.* 2004; Bronzan *et al.* 2007; Were *et al.* 2011). The sensitivity of blood cultures to detect bacteraemia is low, and thus, the true prevalence of bacteraemia is likely to be more than twice as high as the prevalence of culture positivity. Bone marrow cultures (1 ml) are more sensitive than blood cultures for recovery of *Salmonella typhi* in typhoid fever (Gilman *et al.* 1975). Common pathogens include non-typhoidal *Salmonella* species, *S. pneumoniae*, *E. coli*, *S. aureus*, Group A streptococci and in babies, Group B streptococci.

Other tests

HIV testing

Severe malaria in HIV co-infected patients presents with higher parasite burden, more complications and comorbidities, and a higher case-fatality rate than severe malaria in HIV-uninfected patients. Identifying HIV infection prompts a more extensive search for additional pathogens and for opportunistic infections upon recovery (Gwer *et al.* 2007).

Lumbar puncture

Should lumbar puncture (LP) be performed routinely in the evaluation of patients with *P. falciparum* parasitaemia and altered consciousness? Experts differ as to whether an LP should be carried out on admission. Most agree that LP is contraindicated if the patient's breathing is precarious or if there is papilloedema, but is otherwise safe. The lumbar puncture is performed to exclude alternative or additional specifically treatable conditions such as *H. simplex* encephalitis and cryptococcal, tuberculous and bacterial meningitis. Few studies have evaluated specifically the diagnostic benefit of LP in this situation. If there is any doubt about the diagnosis, then a lumbar puncture should be performed. Some physicians prefer to defer LP until the patient recovers consciousness and meanwhile to cover at least with antibiotics according to local protocols for meningitis (Pinhas-Hamiel *et al.* 1993).

Assessment of parasite stage development in thin smears

Thin smears that contain *P. falciparum* parasites can be assessed for stage development using criteria from Silamut *et al.* (1999), which divide the developmental cycle of the parasite into 8 stages based on morphology of cytoplasm, the appearance of malarial pigment and number of nuclei (Figure 23). Schizonts are not normally seen in the peripheral blood in *P. falciparum* infections, but they may appear in severe disease.

Assessment of parasite stage development in thick smears

This is more difficult than in the thin film. Normally, the parasites appear compact and it is not easy to identify their stages. This method may be used for low parasitaemias (<0.1% or 1/1000 rbc) or for slides that have only thick smears.

Thick blood smears containing *P. falciparum* parasites can be assessed for developmental stage using modified criteria from Silamut *et al.* (1999) which divide the parasites into three asexual developmental stages and one sexual stage (gametocytes) based on the morphology of the cytoplasm, the appearance of malarial pigment and the number of nuclei.

Section 10: Management of severe malaria

Introduction

As severe malaria potentially affects multiple organ systems, general care and supportive treatments are crucially important.

In severe malaria, the prompt administration of an effective antimalarial drug, preferably by a parenteral route, is essential. Artesunate is the treatment of choice.

Chemotherapy is discussed in *Section 11*. This section gives recommendations for supportive treatment and nursing care as well as for the treatment of concomitant diseases. The recommendations are as much evidence based as possible. Where specific studies in severe malaria have not been carried out, some treatment strategies are extrapolated, when appropriate, from the 'surviving sepsis campaign' guidelines (Dellinger *et al.* 2008; Dunser *et al.* 2012).

General considerations

Assessment and reassessment of patients with severe malaria. Most deaths in severe malaria occur during the first 48 h following admission. The condition of a patient with severe malaria can worsen unexpectedly. In particular, in patients with cerebral malaria, the degree of coma may worsen and the patient may have convulsions, respiratory depression or respiratory arrest. In adult patients, pulmonary oedema with respiratory insufficiency can develop even several days after start of antimalarial therapy. The patient may become haemodynamically unstable, which can indicate concomitant bacterial sepsis. Development of acute kidney injury is an important threat in adults with severe malaria. The patient must be observed frequently by a nurse (or equivalent) well instructed on when to call for help. Implementation of an 'early warning system' has proved to be an important intervention. Ideally, the patient should be admitted to an intensive care unit with 24-h presence of trained nurses and physicians. A system of patient-centred care, where each nurse has the responsibility for one or a limited number of patients, is better than a task-orientated approach, which dilutes responsibilities. If no intensive care unit is available, care of these severely ill patients should occur in a dedicated unit or area of a ward where intense monitoring can be assured.

Clinical examination. Thorough physical examination often reveals important information beyond that obtained by electronic monitoring, imaging technologies and laboratory tests. The examination should be carried out systematically and vital organ functions should be checked regularly, including assessments of the level of consciousness (Glasgow Coma Score/Blantyre Coma Score), presence of seizures (which can be subtle), respiratory rate and work of breathing, blood pressure, pulse rate, peripheral perfusion and urine output. Clinical examinations should be performed several times a day – at least every 6 h, but more frequently in the early phases of admission, in case of instability, or whenever there is a change in the patient's condition. Hypoglycaemia should always be considered when the patient has seizures, behaviour disturbance or a deterioration in the level of consciousness, and blood glucose should be monitored at least every 6 h in all comatose patients. A team or family member's concern about the patient is always a reason to reassess the patient carefully.

Patient monitoring. Whenever available, a continuous patient electronic monitor should be used and alarm limits should be set appropriately. Patient monitors allow for continuous surveillance of vital parameters and reduce staff workload. However, monitors can never replace continuous attendance of experienced healthcare staff or repeated clinical examinations. Intensive care unit staff must be familiar with the monitor and its technology. It is reasonable to focus on a basic set of parameters such as heart rate (preferably measured by electrocardiography), blood pressure (generally measured non-invasively in the resource-poor setting), respiratory rate (usually by impedance methods) and transmission oxygen saturation (pulse oximetry). While alarm limits set too liberally put the patient at risk by delaying recognition of changes, limits set too tightly lead to over-reporting measurement deviations and critically they 'desensitise' medical staff for truly important alarms. When these facilities are not available, regular patient observations of respiratory rate, effort of breathing, oxygen saturations (if a pulse oximeter is available), heart rate, blood pressure and conscious level should be prioritised. At every clinical review, the patency of intravenous cannulae, the access site, and the rate and volume of intravenous fluids should be checked.

Data documentation. A medical record including relevant information about demographic data, allergies, medical history and current disease processes should be kept for every patient. Vital signs should be documented regularly on a dedicated patient record form to allow rapid assessment of the disease course and to interpret changes in the patient's condition. Depending on the phase of the disease, vital signs should be documented at

intervals from hourly in unstable patients to every 6 h in stabilised patients. Body temperature, peripheral perfusion and fluid balance should be recorded at least once per shift, but more frequently when abnormal. Whenever a deterioration occurs, it is crucial not only to treat symptomatically (e.g. treat seizures, stabilise haemodynamic and/or respiratory function), but also to identify possible underlying causes. Important and common causes explaining a worsened or persistently unstable condition of a patient with severe malaria are recurrent hypoglycaemia, seizures, inadequate or excessive fluid resuscitation, concomitant septicaemia and development of ALI/ARDS or acute kidney injury with metabolic disturbances.

Good collaboration between all physicians and nurses with an adequate handover between shifts conveying all essential information is crucial (Reader *et al.* 2009). Systematic adherence to a standardised protocol, adapted to the local setting, can improve the quality and completeness of information flow. Clear definition of daily goals for each patient using a 'daily goal form' increases the proportion of team members understanding the goal of care for the day and shortens the intensive care unit length of stay (Pronovost *et al.* 2003). In case of an emergency, lives can be saved by having essential drugs and equipment immediately available. These should be kept readily to hand on each ward where severely ill patients are cared for.

Patient Transfer. When resources are limited, inter-hospital transfer to a unit with higher-level facilities may save lives. However, the risks of transfer must be critically weighed against the potential benefits. Whenever possible, a patient being transferred should be accompanied by a physician or other experienced medical staff. Common conditions in the patient with severe malaria warranting transfer to a higher-level care facility include:

- development of respiratory insufficiency requiring intubation and mechanical ventilation,
- acute kidney injury requiring renal replacement therapy,
- haemodynamic instability requiring vasoactive drugs and haemodynamic monitoring.

In many malaria-endemic countries, patients with cerebral malaria but no other organ involvement are frequently taken care of in settings with only basic facilities. Despite the lack of electronic monitoring, reported case-fatality rates in this group of patients can be low.

Hygiene precautions. The rate of hospital-acquired infections in middle- and low-income countries is 3–5 times higher than international standards (Rosenthal *et al.* 2006), and these infections increase length of stay, costs of care, morbidity and mortality (Allegranzi *et al.* 2011). Two important pillars in preventing hospital infections are good hand hygiene and aseptic measures during invasive procedures with the patient. The hands of medical personnel are the main culprits in transmitting bacteria from one patient to another (Pittet *et al.* 2009), and washing hands before and after each patient contact is essential to reduce cross-infections in the hospital. Educational programmes to implement strict hand hygiene and the correct technique of hand washing have reduced the rate of hospital-acquired infections (Pittet *et al.* 2000; Caniza *et al.* 2009; Allegranzi *et al.* 2010). Invasive procedures increase the risk of infection, but this risk can be minimised by applying sterile, full barrier precautions including skin disinfection with chlorhexidine or alcohol-based rather than iodine-based disinfectants (Darouiche *et al.* 2010). Equally important is the use of sterile draping, hand disinfection, and wearing a cap, surgical mask, sterile gloves and gown (Pronovost *et al.* 2006). Venous access sites should be checked frequently. Urinary (Foley) catheters should be removed when no longer needed, because removal is associated with reduced rates of catheter-associated urinary infections (Apisarnthanarak *et al.* 2007).

Stress ulcer prophylaxis. In adult patients, stress ulcer prophylaxis should be considered. No studies have been performed specifically in patients with severe malaria, but stress ulcer prophylaxis has been shown to be effective in reducing upper gastrointestinal haemorrhage in general ICU populations in resource-rich settings. Stress ulcer prophylaxis can be provided using H_2 blockers such as ranitidine or proton pump inhibitors such as omeprazole (Levy *et al.* 1997).

Deep vein thrombosis prophylaxis. In adult patients, deep vein thrombosis prophylaxis should be considered, although thrombosis risk in patients with severe malaria has not been assessed to date. It has long been assumed that in resource-limited settings, the prevalence of deep venous thrombosis is low, and thus, routine antithrombotic prophylaxis is not indicated (Osime *et al.* 1976). However, other studies show important morbidity and mortality associated with deep venous thrombosis in this setting (Colin *et al.* 1975; Lee *et al.* 2009a). A large Nigerian study identified sepsis and prolonged (> 4 days) immobility as risk factors for deep vein thrombosis (Sotunmbi *et al.* 2006). Clearly, more information is needed. Subcutaneous low molecular weight heparin is the preferred DVT prophylaxis. In settings where no

heparin is available, either antithrombotic stockings or elastic bandages can be applied on both legs (Amaragiri & Lees 2000). It is important to check for correct application of elastic bandages as unevenly applied bandages may provoke thrombosis.

Mobilisation. Prolonged bed rest and immobilisation lead to numerous unwanted effects, including muscular atrophy, prolonged weakness, respiratory compromise, autonomic dysfunction, hypovolaemia, gastrointestinal paralysis, deep venous thrombosis and delirium (Brower 2009). Early mobilisation may prevent or counteract these effects and facilitate recovery (Schweickert *et al.* 2009). As soon as the patient is stable, mobilisation in and outside of the bed should be encouraged actively.

Specific supportive treatments

Care of the unconscious patient with cerebral malaria. In severe malaria patients presenting with coma, an alternative cause of the reduced level of consciousness should always be considered, including hypoglycaemia, meningitis, septic shock, a post-ictal state after a febrile convulsion (much more common in children) and use of sedative drugs. Unconscious adult patients or older children should be nursed in the lateral 'recovery' position. To prevent pressure sores, the patient's position needs to be changed at least every 2 h. In adults, an oropharyngeal airway (Guedel) can be inserted if the lateral position alone does not maintain airway patency. A patient's inability to clear the airway is associated with a high risk of aspiration of saliva or regurgitated gastric contents. Unconscious or semi-conscious patients with severe malaria often vomit. If feasible and safe, the comatose patient should be intubated to protect the airway and enable mechanical ventilation. This needs to be performed swiftly and efficiently as respiratory compromise and transient hypercapnoea during a difficult intubation further increase intracranial pressure. In hospitals without access to ventilation, insertion of a nasogastric tube and regular suction may protect from aspiration in patients who are unable to protect their airway (see below). Oral hygiene (tooth brushing and cleansing with an oral antiseptic at least twice daily), repetitive suctioning of oropharyngeal secretions and elevation of the head of the bed (also in the lateral position) can help prevent hospital-acquired pneumonia (Tantipong *et al.* 2008). A nasogastric tube should be passed, and the stomach contents aspirated to reduce the risk of aspiration pneumonia. Enteral feeding through the nasogastric tube will eventually be necessary, but in the non-intubated adult patient, it should not be started before 60 h from admission, as earlier nasogastric feeding carries a 33% risk of aspiration pneumonia (Maude *et al.* 2011). If nasogastric feeding is needed after this time, the position of the nasogastric tube should be checked (by X-ray, or by inflation and auscultation of a bolus of air injected through the tube), and the head should be tilted 15° above the horizontal. In an adult patient with prolonged coma, a possible scheme is to start feeding with 100 ml pureed feeding every 4 h and measure gastric retention just before next feeding. If there is no gastric retention, the feeds can be increased stepwise until 300 ml is given every 4 h, providing around 2000 kcal per day. If gastric retention exceeds 200 ml, the feeds should discontinue and prokinetic drugs added, for example metoclopramide suppositories 20 mg, three times daily. Metoclopramide can have extrapyramidal side effects. Cisapride and domperidone are no longer recommended because of potential cardiotoxicity (QT_c prolongation). The eyes should be irrigated with saline or artificial tears (methyl cellulose) and the lids kept closed with eye pads. In adults, a urethral ('Foley') catheter should be inserted and urine output recorded accurately.

In settings where scanning is available, deterioration in the level of consciousness and appearance of new neurological features (in the absence of hypoglycaemia) including lateralisation of neurological symptoms should prompt a CT-scan or MRI of the brain to differentiate intracerebral bleeding from raised intracranial pressure, cerebral oedema and cerebral/medullary herniation. There is no evidence to support the use of mannitol in the treatment of raised intracranial pressure in patients with cerebral malaria. In a placebo-controlled trial in Ugandan children involving 156 children, a single dose of mannitol had no significant impact on the clinical outcome of cerebral malaria compared to placebo (Namutangula *et al.* 2007). Although mannitol controlled intracranial pressure in patients with intermediate intracranial hypertension, it did not prevent the development of intractable intracranial hypertension in those with severe intracranial hypertension (Newton *et al.* 1997b). In adults, mannitol is not recommended, because it prolongs coma recovery time and might increase mortality (Mohanty *et al.* 2011). Dexamethasone failed in both adults (Warrell *et al.* 1982) and children (Hoffman *et al.* 1988) to alleviate coma or to improve survival, although sample sizes were relatively small for definitive conclusions.

Treatment of seizures. Generalised convulsions occur in over 80% of children on admission and they recur in the majority (>60%) of children during hospitalisation; 30% have status epilepticus (Crawley *et al.* 1996; Idro *et al.*

2005b). In contrast, seizures occur in fewer than 20% of adults with cerebral malaria. Aspiration pneumonia is a common immediate complication, while recurrent convulsions are a risk factor for neurological sequelae (Molyneux *et al.* 1989b; van Hensbroek *et al.* 1997; Idro *et al.* 2004, 2006b). In children, convulsions frequently herald the onset of coma or are followed by neurological deterioration. Hypoglycaemia should always be excluded as a causative factor, and hyperthermia can also be a trigger. In a randomised double-blind trial in adults, convulsions were prevented by a single 3.5 mg/kg intramuscular dose of phenobarbital sodium (White *et al.* 1988). A higher dose was considered necessary for seizure prophylaxis in children (Winstanley *et al.* 1992) but in a large randomised trial in Kenyan children without access to mechanical ventilation, prophylaxis with phenobarbital (phenobarbitone) 20 mg/kg increased mortality, probably through respiratory depression. This was particularly likely if the drug was combined with multiple doses of benzodiazepines (Crawley *et al.* 2000). Fosphenytoin (20 mg phenytoin equivalents/kg body weight), a less respiratory depressant drug, did not prevent seizures effectively (Gwer *et al.* 2013). In both trials, prophylaxis against seizures was not associated with improved neurological or cognitive outcome (Abubakar *et al.* 2007). Thus, routine seizure prophylaxis is currently not recommended in patients with cerebral malaria. Active convulsions should be controlled with a benzodiazepine (diazepam, midozalam or lorazepam) given by slow intravenous injection. Diazepam emulsion is less irritant to veins. In adults, the dose of intravenous diazepam is 10 mg, and for lorazepam is 4 mg. In children, intravenous diazepam 0.3 mg/kg body weight or lorazepam 0.1 mg/kg body weight is given as a slow bolus injection over 2 min. In the absence of intravenous access, diazepam may be given intrarectally (0.5 mg/kg of body weight) although absorption from the rectum may be erratic and seizure recurrences more frequent (Ogutu *et al.* 2002). Lorazepam and midazolam can be administered by the buccal, sublingual or intranasal routes (Mpimbaza *et al.* 2008). Intravenous benzodiazepines may be repeated if seizure activity does not stop after 10 min. The alternative is paraldehyde, 0.2 mg/kg of body weight, given by deep intramuscular injection (or 0.4 mg/kg body weight intrarectally) using a sterile glass syringe because paraldehyde is adsorbed to plastic surfaces. Disposable plastic syringes may be used if the injection is given immediately after the paraldehyde is drawn up and the syringe never reused. Multiple doses of diazepam may produce severe respiratory depression and so should be avoided unless respiratory support is available. Respiratory monitoring is important in comatose patients receiving benzodiazepines.

Patients with recurrent seizures or those whose seizures are not terminated by 2 doses of benzodiazepine (or one dose each of benzodiazepine and paraldehyde) given 10 min apart should be considered to have status epilepticus and given intravenous phenytoin at a loading dose of 18 mg/kg over 20 min. This should be followed by maintenance doses of 5 mg/kg/day for 48 h. Phenobarbital, loading dose 15 mg/kg body weight and maintained at a dose of 4–8 mg/kg/day for 48 h, may be used instead of phenytoin. Because phenobarbital is a strong respiratory depressant, and prophylactic use has been clearly associated with increased mortality (Crawley *et al.* 2000), respiratory monitoring is essential in these patients. Respiratory support (intubation, ventilation, general anaesthesia) may well be needed, particularly if phenobarbital is given or seizures are refractory to treatment.

Treatment of seizures in severe malaria

Intravenous diazepam (0.3 mg/kg – maximum 10 mg –preferably as emulsion which is less irritant) or lorazepam (0.1 mg/kg-maximum 4 mg) slow bolus injection over 2 min.

In the absence of intravenous access, diazepam may be given intrarectally (0.5 mg/kg of body weight), and lorazepam and midazolam can be given by the buccal, sublingual or intranasal routes.

Recurrent seizures despite benzodiazepines may require parenteral phenytoin or phenobarbitone preferably with artificial ventilation and support. Phenobarbitone may cause lethal respiratory depression, so careful respiratory monitoring is essential.

Management of shock. Definitions for shock vary between different guidelines and between adults and children.

Adults. Shock is the presence of hypotension (systolic blood pressure <80 mmHg) with evidence of impaired peripheral perfusion.

Children compensated shock. Clinical markers of impaired perfusion [these include 2 or more of delayed capillary refill time (CRT) ≥3 s, weak pulse volume, severe tachycardia and cool peripheries (cold hands and feet)] with a normal blood pressure.

Decompensated shock. One or more of the above features together with a low blood pressure (systolic pressure <70 mm Hg). While there is divergent opinion on the pathological relevance of hypovolaemia and its contribution to acidosis and death in severe malaria, there is now agreement that rapid fluid loading in adults or children with severe malaria is dangerous. In children with severe malaria (defined by impaired consciousness or deep breathing), signs of compensated hypovolaemic shock occurred in 57% of cases (in-hospital mortality 18%), hypotension in 13% (case fatality 26%), and severe dehydration in 6% (case fatality 28%) (Maitland *et al.* 2003a). Studies in Kenyan children showed that low central venous pressure (CVP) (a measure of preload) is present in many children with metabolic acidosis with features of impaired perfusion and that CVP increased into the normal range (7–10 mmH_2O) with 20–40 mls/kg bolus of isotonic fluids (Maitland *et al.* 2003b). Evidence of mild to moderate myocardial impairment and inferior vena caval collapsibility also improved on fluid resuscitation (Yacoub *et al.* 2010). This led to a series of phase II clinical trials comparing different colloids and 0.9% saline which suggested that outcome was improved in children receiving albumin boluses compared to any other fluid (Akech *et al.* 2010). These studies were then extended into a large pragmatic randomised controlled trial (FEAST trial) in 6 centres in East Africa. Children with severe infection (including malaria and other infections) and with impaired peripheral perfusion clinically and treated with standard baseline fluid therapy were randomised to receive either 20–40 ml boluses of normal saline or 20–40 ml boluses of 5% albumin or no boluses. The trial was stopped early because of *increased* mortality in the intervention groups; 48-h mortality was substantially *higher* in the combined groups receiving fluid boluses (10.6%) than controls (7.3%) – a relative risk (RR) for any bolus vs. control of 1.45 (95% CI 1.13–1.86) (P =0.003) (Maitland *et al.* 2011). Children who received fluid resuscitation were more likely to no longer be in shock after 1 h than controls (no bolus), but this did not translate into improved survival. Cardiovascular collapse was described as the main cause of death (Maitland *et al.* 2013). In the subgroup with *P. falciparum* malaria, mortality in the bolus groups was 51% higher in the intervention groups; 9.2% compared to 5.8% [(RR 1.51(1.17–1.95)]. Moderate hypotension (systolic BP if <12 months: 50–75 mmHg; or if 12 months to 5 years: 60–75 mmHg; or if >5 years: 70–85 mmHg) was also associated with increased mortality in the intervention groups. Signs of severe dehydration were also associated with increased risk of poor outcome in the bolus groups (21%) compared to the control group (13%), as was lactic acidosis (>5 mM); 20.5% versus 15%.

Fluids in severe malaria

These are general guides to fluid replacement, but each patient needs individual assessment of their fluid requirements. Hypoglycaemia, which is particularly common in children and pregnant women, should be corrected immediately. Then, the following fluid management recommendations should be adjusted to individual needs.

Children: Correct fluid deficits over 3–4 h with 0.9% ('normal') saline at 3–5 ml/kg/h, then switch to maintenance 5% dextrose (2–3 ml/kg/h). If solutions containing 0.45% saline/5% dextrose are available, then these are preferred for initial resuscitation.

Adults: Start with 0.9% ('normal') saline 3–5 ml/kg/h during the first 6 h of admission with frequent assessment of vital signs. Then reassess and in most cases switch to alternate 500 ml bags or bottles of 5% dextrose and 0.9% saline for maintenance (2–3 ml/kg/h).

Do NOT use colloids.

In refractory or recurrent hypoglycaemia, after correction of the low blood glucose with intravenous hypertonic dextrose (0.3–0.4g/kg) check that the intravenous line is patent and fluid infusion rate is correct, or stop blood transfusions and restart 5% dextrose infusion. Rarely, it may be necessary to give 10% glucose infusions for maintenance after correction of hypoglycaemia. These can be prepared by dilution of 50% glucose (usually 50 ml) in 450–500 ml of 5% dextrose.

Management of shock and dehydration in children with severe malaria

Bolus fluids (fluid resuscitation) should not be given to any child with severe malaria including those with moderate hypotension, severe dehydration or metabolic acidosis. Severe dehydration should be corrected *slowly*; the optimum rates and volumes recommended in IMCI Guidelines are 5 ml/kg/h with frequent clinical evaluation (see box above). Acidosis and shock should be managed with maintenance therapy (see section below).

Maintenance fluids

For children who are unable to take or retain oral fluids (including children with impaired consciousness, respira-

tory distress, shock and/or vomiting), 5%-dextrose-containing maintenance fluids should be provided at a rate of 3–4 ml/kg/h until the child is able to drink. Children with severe malaria need continuous glucose to prevent hypoglycaemia, and saline to slowly restore circulating volume and replace extracellular fluid losses. Suspected or confirmed hypoglycaemia should be corrected first with a bolus of 20% glucose (2 ml/kg over 10 min) or alternatively if 20% glucose is unavailable, 50% glucose, 1 ml/kg over 10 min. If an intravenous saline infusion has started or can be instituted without delay, the dextrose can be injected into the rubber tubing of the intravenous infusion as hypertonic dextrose is sclerosant. Ideally, initial resuscitation should start with a 0.45% saline and 5% dextrose mixture 'dextrose-half normal saline', but this is often not readily available. The only two crystalloid solutions that are widely available are 0.9% 'normal' saline, and 5% dextrose, in which case fluid deficits should be corrected over 3–4 h with 0.9% ('normal') saline, at 3–5 ml/kg/h. Then, the maintenance infusion can be switched to 5% dextrose for maintenance (infusion rate: 2–3 ml/kg/h) until oral fluids are tolerated. Intravenous fluid administration should be monitored carefully to ensure that the line remains patent and the rate of administration is correct.

Fluid therapy in adult patients with severe malaria

Optimal fluid resuscitation in the non-intubated adult patient with severe falciparum malaria is difficult. As a general rule, the old adage to 'keep the patient on the dry side' is still valid in the majority. For example, 84% of SEAQUAMAT participants, in a large pragmatic trial of all-cause severe malaria (SEAQUAMAT 2005) did not present with decompensated shock (defined as low blood pressure with cold extremities), and thus, fluid management was conservative. Fluid therapy in adult patients with severe malaria should be individualised, because intravascular requirements can vary considerably between individuals and development of pulmonary capillary leakage is unpredictable. A study in adult severe malaria on fluid resuscitation targeting the global end-diastolic right heart volume index ('GEDI' assessed with PiCCO invasive monitoring) recorded infusion volumes varying between 2 and 7 litres to reach this target (Hanson *et al.* 2013). This fluid deficit should be corrected slowly. The danger of correcting this volume deficit rapidly is that, even with invasive monitoring, approximately one-third of patients will develop pulmonary oedema a variant of the acute respiratory distress syndrome (ARDS). ARDS often develops after the start of antimalarial treatment, irrespective of the fluid resuscitation strategy followed. Once the patient develops ARDS, characterised by significant pulmonary capillary leakage, liberal fluid therapy will worsen pulmonary oedema and respiratory insufficiency. Patients who will develop ARDS later during admission cannot be recognised on admission. In addition to the dangers of developing pulmonary oedema, optimising the intravascular filling status only partly restores tissue perfusion, probably because microvascular sequestration of parasitised red blood cells is quantitatively a more important contributor to impaired perfusion (Hanson *et al.* 2013). Blood pressure is not compromised in the majority of patients with severe malaria, justifying restricted fluid management.

However, keeping the patient severely dehydrated is also dangerous, reducing tissue perfusion and increasing the risk of acute kidney injury (AKI) from acute renal tubular necrosis. At the current stage of knowledge, it is difficult to provide more concrete guidance for fluid management than the general advice to 'keep the patient (slightly) dry'. Invasive measurement of CVP or assessments of the JVP on physical examination are inaccurate measures of intravascular filling status in patients with severe malaria (Hanson *et al.* 2011b). Measurement of CVP or JVP, together with assessment of the skin turgor, mucosal membranes, etc., can only give a global indication about filling status. Based on expert opinion, the following fluid management is suggested.

In the absence of invasive monitoring and with no or limited possibilities for intubation and mechanical ventilation, a general recommendation for patients who are not shocked, do not appear severely dehydrated, and are not anuric on admission, is to start fluid resuscitation with 3–5 ml/kg/h of normal saline (250 ml/h in the average 50 kg patient) during the first 6 h of admission (total 18–30 ml/kg) while checking for basal crepitations or an increase in work of breathing every 2 h. After this, the fluid status of the patient should be assessed, and further fluids administration should be individualised to the needs of the patients (see Box). There is no evidence for benefit, and some evidence for harm, from using colloids in resuscitation (Perel *et al.* 2007). When the patient is clearly severely dehydrated on admission with a urine output <0.5 ml/kg/h (25 ml/h in the average 50 kg patient), which is only possible to assess when a urinary catheter is *in situ*, the initial fluid administration can be more liberal and start with 10 ml/kg/h (or 500 ml/h in the average 50 kg patient) during the first 2 h, with reassessment of the patient after this, including auscultation of the chest and observation of urine output. If there is still no diuresis of more than 0.5 ml/kg/h and provided

there is no increase in the respiratory rate or work of breathing and no chest crepitations signifying pulmonary oedema, another litre of fluid can be given over 4 h (5 ml/kg/h for 4 h).

Fluid therapy and vasopressive drugs in adult malaria patients with shock

Haemodynamic shock occurs in a minority of adults with severe malaria (approximately 16% of cases) and should prompt the clinician to explore alternative explanations for cardiovascular instability (e.g. concomitant bacterial sepsis or, much more rarely, gastrointestinal haemorrhage or splenic rupture). In case of haemodynamic shock, not caused by haemorrhage, the patient should be treated according to the 'surviving sepsis' guidelines. A revision tailored for resource-limited settings has been prepared (Dunser *et al.* 2012). Rapid infusion of intravenous normal (0.9% w/v) saline of 20 ml/kg is recommended continuously guided by the patient's response to fluid loading. It may be necessary to repeat this. As soon as adequate perfusion is achieved the infusion should be stopped, as excess fluid may precipitate pulmonary oedema. Some adult patients may require several litres of fluids during the first 24–48 h to maintain adequate blood pressure. If the patient is not rapidly responsive to fluid therapy, vasopressive drugs should be started. Although evidence is limited, norepinephrine (noradrenaline) is preferred to dopamine or epinephrine (adrenaline) preferably infused through a central venous catheter. Adrenaline aggravates the lactic acidosis in patients with severe malaria (Day *et al.* 1996b). If central venous catheters are unavailable or the medical staff has insufficient experience handling them, a peripheral venous cannula, preferably placed in a large bore vein, can be used instead. It is important to check the site of infusion regularly for signs of drug extravasation, because this may cause substantial skin necrosis. Norepinephrine (noradrenaline) or dopamine should be administered continuously using a syringe or infusion pump. When pumps are unavailable or power cuts frequently occur, dopamine can be diluted in crystalloid solution (250 mg in 500 ml) and infused using a drop regulator or micro-infusion set. Dosing should be tailored to the clinical response so in patients requiring vasopressive drugs, blood pressure and heart rate should be measured frequently. As shock is rare in adult patients with severe malaria and can indicate concomitant bacterial sepsis, prompt start of broad-spectrum antibiotics is important, after first obtaining blood cultures in settings with microbiology laboratories.

Management of metabolic acidosis

Severe metabolic acidosis is a frequent feature of both adults and children presenting with severe falciparum malaria and has a strong prognostic significance for mortality. It is mainly a lactic acidosis, although other acids can also play an important role (Dondorp *et al.* 2004a). The most important cause of the lactic acidosis is microvascular obstruction through sequestration of parasitised red blood cells in the microcirculation (Hanson *et al.* 2012; White *et al.* 2013b). Hypovolaemia or shock, hypoglycaemia and seizures can also contribute. Therapy should target these causes, optimise fluid status and correct hypotension. Prompt therapy with parenteral artesunate will itself contribute to the control of acidosis as parasites are killed and further sequestration is reduced. Bicarbonate infusion is not generally recommended and is only indicated if the blood pH drops below 7.10 as, at this pH, myocardial and other vital functions are endangered and the liver becomes a net exporter of lactic acid. Clinical trials in septic patients with a blood pH above 7.10 have not shown a beneficial effect on outcome with bicarbonate infusion, whereas there are potential adverse effects of bicarbonate infusion. These include: an increase in hepatic lactate production (probably through an increase in hepatic intracellular pCO2); a fall in brain and intracellular pH (through pCO2 generation from the infused bicarbonate and more rapid transfer across the blood–brain barrier and cell membranes than HCO3 ion); and a risk of sodium overload, as bicarbonate is given as the sodium salt. In patients with bacterial sepsis, a consensus paper (Cariou *et al.* 2004) recommends giving bicarbonate infusion if the blood pH is $\leq$7.10, in a dose of 1–2 meq/kg/h (corresponding to 1 ampoule of 100 meq HCO_3^- in a 50 kg patient), given over 1–2 h. Faster infusion risks a drop in intracellular and cerebral pH, through the generation of CO_2. This recommendation can also apply to patients with severe malaria.

Treatment of malaria-related severe anaemia

For blood transfusion issues of blood safety, adequate supply, equitable access and rational use still remain major challenges throughout the malaria-endemic world. WHO estimates the blood requirement for countries in the Africa region to be 10–20 units per 1000 population per year (Tapko *et al.* 2007) which compares with an average of 3.4 units of blood donated per 1000 population for malaria-endemic sub-Saharan Africa. For anaemia from other causes in both adults (in the absence of acute myocardial infarction or unstable angina) and children, a restrictive strategy of transfusing at a threshold of 7 g/dl is

at least as effective as, and possibly superior to, a more liberal transfusion strategy (Hebert *et al.* 1999; Lacroix *et al.* 2007). Over the past decade, the capacity of transfusion services to meet the need for blood in malaria-endemic countries has greatly increased owing to steady declines in the intensity of malaria transmission that have led directly to reductions in hospitalisation of children with malaria, and indirectly to reduced utilisation of blood transfusion services (Pedro *et al.* 2010). Despite these declines, the outcome of the disease in children presenting with severe malarial anaemia remains unchanged (Pedro *et al.* 2010). Clinical studies in Kenya (Lackritz *et al.* 1992; English *et al.* 2002) have shown that profound anaemia (Hb < 4 g/dl) is independently associated with death (OR = 2.5), as is severe anaemia (Hb < 5 g/dl) complicated by reduced consciousness (OR = 7.4) or respiratory distress (OR = 4.1). Many deaths in children with severe malarial anaemia occur within 48 h of admission, with 25–50% (Bojang *et al.* 1997; English *et al.* 2002; Ernest *et al.* 2002) occurring within 6 h.

Recommendations for blood transfusion

Blood transfusion in children with severe malaria. To avert overuse of blood products, the WHO advocates a conservative transfusion policy (World Health Organization 2005b), reserving whole blood (20 ml/kg) or packed cells (10 ml/kg) for all children with a Hb of <4 g/dl. Children with <5 g/dl warrant transfusion if they have respiratory distress or cardiovascular instability. This is a general policy. However children with severe malaria or high parasite counts and Hb <5 g/dl will usually have a further drop in haemoglobin, so transfusion will usually be necessary. Thus, the recommended transfusion threshold in severe malaria is 5 g/dl and not 4 g/dl for children in areas of moderate or high transmission. There is no evidence to support the need for furosemide and/or digoxin in children with severe malaria anaemia – studies indicate that children with these complications usually have signs of hypovolaemia and not of myocardial failure (English *et al.* 1996c).

Indications for blood transfusion in severe malaria

In areas of moderate to high transmission, such as most of sub-Saharan Africa, children should be transfused if the haemoglobin concentration falls below 5 g/dl.

For adults and for children in other settings, patients should be transfused if the haemoglobin concentration falls below 7 g/dl.

Blood transfusion in adults with severe malaria. In countries where pathogen-free compatible fresh blood is available in sufficient supply, that is >10 units per 1000 population per year, transfusion should be considered if the haematocrit of a normally hydrated patient falls below 7 g/dl. In the presence of high parasitaemia, transfusion could be prepared for at a higher haemoglobin level because of the expected further fall in haemoglobin with the loss of the parasitised erythrocytes and accelerated destruction of unparasitised red cells. In patients with symptoms related to severe anaemia (dyspnoea, chest pain, shock, cardiac failure, extreme lethargy), the threshold should also be higher. Concerns about shortage of adequate blood supply or about blood borne pathogens should restrict the use of transfusion, especially in areas where HIV is prevalent and facilities for screening are inadequate. In patients with chronic anaemia predating the acute severe malaria episode, a low haematocrit is often much better tolerated. An absolute threshold where a blood transfusion is indicated is a haemoglobin concentration below 5 g/dl or an haematocrit <15%, although there are no formal studies in adult severe malaria to support this recommendation.

Fully cross-matched packed cells, or when not available whole blood, should be used. Transfusion should be carefully monitored, and in severely anaemic patients, the respiratory rate should be monitored frequently and the lung fields should be checked for crepitations to detect hypervolaemic pulmonary oedema. If circulatory overload is suspected, an intravenous dose of furosemide (40 mg in an adult patient with normal renal function) should be given, but furosemide should not be standard treatment when a blood transfusion is given. In patients with circulatory overload, anaemia is best corrected by exchange transfusion (Maitland *et al.* 2003a). Many patients improve after transfusion, but repeated transfusions may be required if there is abnormally rapid haemolysis of donor erythrocytes. Iron and folic acid supplementation may be necessary when oral medication can be taken, particularly in pregnant patients and in those who may also have hookworm anaemia (in which case anthelmintic therapy is also indicated).

Exchange transfusion. Exchange transfusion (ET) was first reported as an adjunct to the treatment of severe malaria in 1974 (Gyr *et al.* 1974). Numerous reports, based on small case series, have been published subsequently advocating its use as a supportive treatment in severe malaria. Recently, the role of red cell exchange transfusion has also been advocated (Macallan *et al.* 2000). For the majority of reports, poor study design

including the use of historical control groups, controls from different centres or lack of uniformity in the definitions of severe malaria makes interpretation and generalisability difficult. Although advocated for use in cases with hyperparasitaemia (>10%) in adult ICU settings, there is evidence that exchange transfusion may not confer a better outcome (Riddle *et al.* 2002). The advent of the artemisinins in management of severe malaria, which specifically target hyperparasitaemia, further questions the role of this invasive and potentially unsafe procedure. A recent meta-analysis of 8 comparative studies and a large case–control study did not find an association between exchange transfusion and survival outcome (OR 0.84; 95% CI 0.44–1.60) (Tan *et al.* 2013).

Nevertheless, there are a number of theoretical advantages of exchange transfusion: the provision of new red cells, thus improving the rheological properties of circulating red cell mass; the removal of plasma contain high concentrations of cytokines and other physiologically active agents; and the provision of fresh plasma in the donor blood that may correct deficiencies (e.g. fibrinogen). Exchange transfusion may be considered as an adjunctive therapy in patients with persistent acidosis and/or multiorgan impairment unresponsive to first-line treatment, or in the subgroup of children with sickle cell disease, owing to the inherent propensity of HbS containing red cells to become rigid under stress, which makes this a group in whom the prognosis is much more guarded. The value of exchange transfusion or red cell exchange transfusion should be regarded as experimental until further data become available.

Management of haemoglobinuria and blackwater fever. In patients with haemoglobinuria, it is important to continue full-dose antimalarial treatment in patients with proven malaria, despite the possible association between these drugs and haemolysis. Transfusion of fresh blood or (preferably) packed cells should aim to maintain a haematocrit above 20%, while fluid overload is avoided by monitoring respiratory rate and checking for chest crepitations. Cross-matching may be difficult. The patient should be monitored closely. To prevent exacerbating renal injury, it is important to keep the patient with haemoglobinuria adequately hydrated to maintain urine output. It has been suggested that alkalinisation with bicarbonate infusion could be beneficial in these patients, but this has never been trialled. Alkalinisation of the urine can be accomplished by administration of 5% dextrose to which 100–150 meq/l of sodium bicarbonate have been added.

Management of acute kidney injury (AKI) in adults with severe malaria. Development of renal failure during severe malaria is much more common in adult patients than in children, although in children a raised blood urea on admission also has prognostic significance (von Seidlein *et al.* 2012). Acute renal failure in severe malaria is usually accompanied by oliguria, but is non-oliguric in approximately one-third of cases. In the latter, urine volume is not representative of the glomerular filtration rate, which determines renal function, and monitoring urine output will not detect declining renal function. Serum or plasma creatinine concentrations should always be measured on admission and then daily if serum creatinine >2 mg/dl (>177 μM). Blood urea or blood urea nitrogen (BUN) is more prone to confounding factors such as the hydration and metabolic status of the patient. Oliguria is defined as urine output (with a Foley catheter *in situ*) <0.5 ml/kg/h, which corresponds to 25 ml/h in an average 50 kg adult patient. A patient who is oliguric and clinically dehydrated should receive rehydration with 0.9% saline 10 ml/kg/h (or 500 ml/hr in the average 50 kg patient) during the first 2 h (total 1000 ml). To prevent fluid overload, respiratory rate, lung auscultation and jugular venous pressure measurement and also if possible pulse oximetry and central venous pressure measurements should be performed after every 200 ml of fluid. If a central venous catheter is in place, a CVP between 0 and +5 cm is recommended, although the accuracy of the CVP as a measure of fluid status has been questioned recently (Hanson *et al.* 2013).

Diuretics in oliguric patients. Loop diuretics have been used frequently in different forms of AKI, in an attempt to convert oliguric into non-oliguric renal failure (Anderson *et al.* 1977; Bellomo & Ronco 1998; Klahr & Miller 1998). The most common loop diuretic used is furosemide (frusemide). If there is no urine output after fluid replacement, an intravenous dose of furosemide can be given, 40 mg initially then increasing the dose to 100 mg, 200 mg and 400 mg at half-hour intervals. If oliguria persists despite furosemide therapy, this makes the diagnosis of acute renal failure highly likely, and preparation for renal replacement therapy should be started. If it is clear that fluid replacement and furosemide have failed to restore diuresis, it is then critical to restrict intravenous fluids (500 ml per day but more if the patient is perspiring profusely) to avoid overhydration and pulmonary oedema. Excessive fluid administration is one of the commonest errors of management in malaria-associated acute kidney injury.

Once a diagnosis of acute renal failure is established, loop diuretics have no further useful role, as has been

shown in AKI of non-malarial causes. In a placebo-controlled clinical trial in 338 dialysis-requiring patients (none with malaria), the use of high-dose loop diuretics caused no improvements in patient survival, renal recovery rates, number of dialysis sessions required or time on dialysis, despite an increase in urine output (Anderson *et al.* 1977; Van Der Voort *et al.* 2009). High doses of loop diuretics are associated with an increased risk of ototoxicity.

Dopamine in patients with renal failure. Dopamine has also been used to treat acute renal failure (Lumlertgul *et al.* 1989). This vasoactive drug can increase glomerular filtration rate (GFR) and sodium and water excretion through direct effects on renal blood flow and function. These effects are evident in patients with normal renal function, but in severe malaria with renal impairment, dopamine increases renal blood flow but does not increase renal oxygen delivery (Day *et al.* 1996b, 2000a). In patients with early renal dysfunction (serum creatinine > 2 mg/dl or urine output < 0.5 ml/kg/h), dopamine did not alter subsequent peak serum creatinine or the need for renal replacement therapy (RRT). This was confirmed in a meta-analysis assessing the efficacy of dopamine in stopping the progression of AKI, decreasing the need for RRT, or the mortality of AKI (Bellomo *et al.* 2000; Gambaro *et al.* 2002; Friedrich *et al.* 2005). Therefore, low-dose dopamine currently has no role in the treatment or prevention of AKI especially in malaria.

Malaria-related AKI is particularly difficult to manage compared with most other AKI, because of the hypercatabolic state and concomitant multiorgan dysfunction (coma, hepatic dysfunction, etc.). To avoid rapid deterioration after admission, if renal replacement therapy (RRT) is indicated, then it should be initiated if possible within 2 h. The precise indications for starting RRT for malaria AKI have not been well defined. In a prospective multicentre trial involving 54 ICUs in 23 countries, timing of RRT was stratified into 'early' or 'late' by median blood urea at the time RRT started and also categorised temporally from ICU admission into early (<2 days), delayed (2–5 days) or late (>5 days). Timing by blood urea showed no significant differences in mortality. However, when timing was analysed in relation to ICU admission, late RRT (this may also be late AKI) was associated with greater mortality. Overall, late RRT was associated with a longer duration of RRT and stay in hospital and greater dialysis dependence (Bagshaw *et al.* 2009). Current indications for RRT for AKI in general (i.e. not specific for malaria) are as follows (Elhassan & Schrier 2011):

- Oliguria (urine output < 200 ml/12 h)
- Anuria (urine output 0–50 ml/12 h)
- [Urea] > 35 mM
- [Creatinine] > 400 μM
- $[K^+]$ > 6.5 mM or rapidly rising
- Pulmonary oedema unresponsive to diuretics
- Uncompensated metabolic acidosis (pH <7.1)
- $[Na^+]$ < 110 mM and >160 mM

If one criterion is present, RRT should be considered. If two criteria are simultaneously present, renal replacement therapy is strongly recommended.

As AKI in the acute phase of severe malaria develops rapidly and is often compounded by severe metabolic acidosis, RRT should be instituted *earlier rather than later* in the diseases process. Sensitive indicators of the need for dialysis include anuria, severe metabolic acidosis, arterial blood lactate concentration > 4 mM and a rapidly rising plasma or serum creatinine concentration (>2.5–3 mg/dl/day or 220–265 uM) (Trang *et al.* 1992; Phu *et al.* 2002). In contrast, some patients will pass small volumes of urine sufficient to maintain fluid balance. Serum creatinine may rise slowly, while other manifestations of severe malaria resolve. If other indications for dialysis do not arise, these patients can be managed conservatively, but they will need frequent assessment. Timing of RRT may play an important role in determining patient outcomes. We recommend that physicians should assess changes in renal function (BUN, serum creatinine, potassium) and decide on RRT as early as possible, rather than waiting for complete renal shut down.

Patients with established acute renal failure should be referred if possible to an intensive care unit or centre for RRT because close nursing care is needed. Haemofiltration or haemodialysis is superior to peritoneal dialysis (Phu *et al.* 2002), but if peritoneal dialysis is the only option, then large amounts of dialysate (50–60 l/day) need to be prepared. A positive fluid balance after the onset of AKI is strongly associated with mortality (Grams *et al.* 2011). It is critical that intravenous fluids should be restricted so that the CVP is maintained between 0 and +5 cm H_2O, and the body weight falls about 0.5 kg/day. Excessive fluid administration is one of the commonest errors of management leading to lethal complications such as pulmonary oedema. While arrangements for RRT are being made patients with very severe hyperkalaemia (>7 mM or with alterations on the ECG) should be treated immediately with (i) intravenous calcium, (ii) glucose plus insulin or (iii) intravenous sodium bicarbonate. Calcium and bicarbonate must not be mixed, because of precipitation of calcium carbonate!

Continuous veno-venous haemofiltration (CVVHF). In a study in 2002, CVVHF was shown to be superior to peritoneal dialysis and significantly reduced mortality in the treatment of infection-associated AKI in Vietnam (Phu *et al.* 2002). Of the 70 patients randomised, 48 had malaria-associated renal failure, and mortality was reduced from 47% to 15%. Haemofiltration corrected acidosis and azotaemia more rapidly and more completely than peritoneal dialysis and was associated with a shorter duration of treatment (44 vs 88 h) (Phu *et al.* 2002). It was also less expensive. Haemofiltration has proved particularly effective in very severe metabolic acidosis. There have been no comparisons of haemodialysis and haemofiltration in acute malaria. In acute renal failure from other causes, a multicentre randomised controlled trial in France compared intermittent haemodialysis continuous renal replacement with haemofiltration using the same polymer membrane and bicarbonate-based buffer. A total of 360 patients were randomised, and survival was similar in the two groups (32 versus 33%). Current evidence does not support continuous renal replacement therapy over intermittent therapies in the treatment of AKI (Vinsonneau *et al.* 2006; Lins *et al.* 2009). Concerning the dialysis membranes, biocompatible membranes may reduce immune reactions but do not consistently improve AKI outcomes (Alonso *et al.* 2008). Regarding the dialysate and haemofiltration fluid, a recent study showed that bicarbonate fluids led to a more rapid fall in lactate and greater improvement in base excess during CRRT, but not to an overall improvement in the control of acidosis (Agarwal *et al.* 2011). As a consequence, the use of bicarbonate buffer is now preferred.

Peritoneal dialysis. When dialysis is indicated, the peritoneal catheter[1] insertion should be performed in an operating theatre under full sterile conditions. The exchange volume of dialysate[2] is 1500–2000 ml with a cycle time of 60 min: inflow 10 min, dwell time 30 min, outflow 20 min. This should be modified in each individual as necessary: the duration of exchange of dialysate (standard or shorter dwell period) should be governed by biochemical investigations, and fluid overload should be managed by the use of high glucose dialysate (this is also an excellent way to control hypoglycaemia). Potassium chloride (2–4 mM) must be added to the dialysis solution if the patient is hypokalaemic or normokalaemic. Heparin (200 U) is usually added to each litre of dialysis fluid to prevent obstruction of the catheter by fibrin clots. The duration of each dialysis session depends on the patient's urine output and plasma creatinine concentration and on the stage of the disease and varies from 26 to 82 h (Trang *et al.* 1992). Daily reassessments of clinical status, biochemistry values (electrolytes, blood urea and creatinine) and 24 h fluid balance are needed. Peritoneal dialysis should be stopped when these variables return to within the normal range, and the urine output is above 400 ml/day. However, 30% of dialysed patients required repeated dialysis (T.T Hien 1995, unpublished data). Complications are rare: infection has been the most frequent. If the dialysis effluent becomes cloudy, a cell count, Gram stain and culture of peritoneal fluid should be carried out, and antibiotics should be given by both the intraperitoneal and systemic routes. The results of the Gram stain of concentrated peritoneal effluent guide the initial choice of antibiotic while cultures are awaited.

In Brazil, a prospective randomised study of daily intermittent haemodialysis (IHD) versus peritoneal dialysis (PD) in 120 AKI patients showed no difference in survival or recovery of renal function (Gabriel *et al.* 2009), but mortality in both groups was very high (>50%). In a recent study from India of patients with malaria and AKI, the mortality was 20% in patients receiving peritoneal dialysis and 36% in patients who received haemodialysis (Mishra *et al.* 2007a; Mishra & Das 2008; Mishra & Mahanta 2012).

Unfortunately, facilities for haemofiltration or haemodialysis are seldom available in the rural tropics. Therefore, peritoneal dialysis is still a valuable option for developing countries where malaria occurs. It reduces the incidence of malaria-associated hypoglycaemia and avoids the risk of parenteral use of anticoagulant. Initial doses of antimalarial drugs should not be reduced in patients with renal failure. Doses of artemisinin and derivatives do not need to be altered in renal failure. The doses of quinine should be reduced after 2 days in patients with renal failure. The additional clearance of quinoline antimalarial drugs by peritoneal or haemodialysis is small and should not affect dosage schedules.

Prognosis. Without dialysis, death occurs rapidly in 50–75% of patients with acute renal failure (Hien *et al.* 1990). If complications can be overcome by dialysis, most patients' renal function will return to normal over a period of several weeks. The prognosis in anuric patients is significantly worse both in mortality and rate of recovery of renal function. Overall, the mortality rate of severe malaria with renal dysfunction requiring dialysis is around 25%; a fatal outcome being associated significantly with anuria, a short history of illness, multisystem

[1]Any acute dialysis catheter.

[2]Dialysis solution contains (meq/l) sodium 135; chloride 101; calcium 3.5; magnesium 1.5; acetate 45; glucose 1.5 g/100 ml (standard) or 4.25 g/100 ml (hypertonic).

involvement and high parasitaemia (Trang *et al.* 1992). In the series from Vietnam, most patients died during the dialysis suggesting that they were admitted to hospital too late or dialysis was started too late. Recovery of renal function was unrelated to density of parasitaemia or haemoglobinuria; the median (range) time until urine output exceeded 20 ml/kg/day was 4 (0–19) days, and the time (mean ± SD) for serum creatinine level to return to normal was 17 ± 6 days (Trang *et al.* 1992).

Management of concomitant sepsis (for fluid management: see earlier paragraph). Severe malaria by itself constitutes a risk factor for invasive bacterial infection such as pneumonia or bacteraemia, although in adults concomitant invasive bacterial infection is less common than in children. Patients with a blood film positive for *P. falciparum* who also present with a clinical syndrome compatible with serious bacterial infection (meningitis, malnutrition or severe pneumonia) should receive parenteral antibiotics together with antimalarial treatment. In case of concomitant severe sepsis or septic shock, the 'surviving sepsis' guidelines should be followed, which have recently been adapted for use in resource-poor settings (Dunser *et al.* 2012). In African children with severe malaria, blood culture positivity rates are between 5.4 and 12%, and there is no way to distinguish this bacteraemic group clinically or with basic laboratory tests (Berkley *et al.* 1999; Evans *et al.* 2004; Bronzan *et al.* 2007; Berkley *et al.* 2009; Were *et al.* 2011). Falciparum malaria has been estimated to account for more than half of invasive bacterial disease in children living in malaria-endemic areas (Scott *et al.* 2011). Common pathogens include non-typhoidal *Salmonella* species, *S. pneumoniae*, *E. coli*, *S. aureus*, Group A streptococci and in infants, Group B streptococci. As the sensitivity of blood culture is limited, the real proportion could be as high as 10–20%. Most studies (but not all) report higher mortality in the children with concurrent invasive bacterial diseases. A general recommendation arising from these data is that all children presenting with severe malaria in areas of intermediate and high transmission should be given broad-spectrum antibiotics in addition to antimalarial drugs. If at all possible, blood cultures should be taken prior to the start of the antibiotic therapy: a positive result may guide antibiotic therapy, but a negative result does not rule out bacteraemia, and continued therapy should be guided by clinical indications. The antibiotic regimen will depend on local hospital guidelines, and the prevailing patterns of antimicrobial susceptibility, but could include ampicillin/gentamicin combination, co-amoxiclav, chloramphenicol or ceftriaxone.

Antibiotics in severe malaria
All children presenting with severe malaria in areas of intermediate and high malaria transmission should be given broad spectrum antibiotics in addition to antimalarial drugs.

Management of respiratory distress and hypoxia

General treatment of respiratory distress in patients with severe malaria. Respiratory distress develops in up to 25% of adults with severe falciparum malaria (Taylor *et al.* 2012b). Its aetiology is diverse, including respiratory compensation of metabolic acidosis, non-cardiogenic pulmonary oedema (ARDS – rare in children), concomitant pneumonia (including aspiration pneumonia) or severe anaemia. These causes have to be distinguished, because their respective treatments obviously differ. A physical examination helps distinguish deep breathing (Kussmaul-type breathing) related to metabolic acidosis, detects pulmonary oedema and identifies severe pallor. However, if at all possible, additional diagnostics are indicated, including pulse oximetry, a full blood count and basic biochemistry, and a chest X-ray. Clinical signs of hypoxaemia (e.g. cyanosis) occur only late (e.g. when oxygen saturation by pulse oximeter falls to <80%) and may be difficult to recognise in patients with a dark complexion. Clinical signs of respiratory distress (dyspnoea, increased work of breathing) reflect changes in respiratory mechanics and may not be reliable gauges of hypoxaemia. Monitoring severe malaria patients with a pulse oximeter is thus highly recommended (Walker *et al.* 2009). Patients presenting with hypoxaemia should receive oxygen to achieve an oxygen saturation >90%, as soon as possible. If no pulse oximeter is available, oxygen should be administered empirically in all severe malaria patients with respiratory distress.

Depending on the condition of the patient, different oxygen sources can be used. Oxygen concentrators usually supply flow rates up to 4–6 l/min and are appropriate to treat septic children and adults with mild hypoxaemia. The use of oxygen concentrators is recommended in areas with reliable electric power supply. In case of frequent or lengthy power cuts, oxygen cylinders should be available as a backup source of oxygen. If oxygen flow rates >6 l/min are required, an oxygen cylinder or, when available, a hospital-based pressurised oxygen system should be used. Alternatively, two oxygen concentrators can be combined with a 'Y' connector. Considering that severely hypoxaemic patients (with min-

imal tolerance to interruptions of oxygen supply) commonly require high oxygen flow rates, sufficient oxygen stores must be assured. High oxygen flow rates may empty an oxygen cylinder rapidly!

Management of malaria-related ARDS in adult patients with severe malaria. ALI/ARDS may be evident at presentation but often occurs within a few days of treatment when parasitaemia is falling (Krishnan & Karnad 2003; Maguire *et al.* 2005). Cerebral malaria and malaria in pregnancy are associated with an increased risk of ARDS. The treatment of ARDS in malaria does not differ essentially from that in ARDS from other infections and has been reviewed elsewhere (Putensen *et al.* 2009). Mechanical ventilation is often difficult in the severely diseased lung. Lung compliance is markedly reduced and unevenly distributed, ventilation/perfusion is mismatched, and gas diffusion is compromised. Lung protective ventilation includes the application of pressure support ventilation with positive end-expiratory pressure (PEEP), avoidance of both high tidal volumes (6 mg/kg ideal body weight) and prolonged periods of high inspiratory oxygen pressures. Permissive hypercapnia is not recommended because this exacerbates the increased intracranial pressure and brain swelling resulting from increased intravascular blood volume of sequestered parasitised red blood cells (Ponsford *et al.* 2012). For the same reason, rapid sequence intubation should be carried out to prevent hypercapnia with a subsequent further rise in intracranial pressure.

Good respiratory care is also important, with intermediate ballooning and suction of secretions, as well as appropriate recruitment procedures. In refractory hypoxaemia, reversal of the inspiration/expiration ratio is indicated. Putting the patient in the prone position can dramatically improve oxygenation, and a recent trial has shown that early application of prolonged prone-positioning sessions in patients with severe ARDS decreases 28-day and 90-day mortality (Guerin *et al.* 2013). Some case studies report the successful treatment with non-invasive positive pressure ventilation in patients with vivax malaria-associated ARDS (Agarwal *et al.* 2007), but it failed in 16 of 32 falciparum-infected patients (Bruneel *et al.* 2010). It is probably only indicated for milder respiratory compromise.

Glucose control. Hypoglycaemia is a common complication of severe falciparum malaria. The incidence is less in adult patients than in children. Quinine therapy is a risk factor, because quinine stimulates pancreatic insulin production. However, most children presenting with hypoglycaemia have not received any quinine. Other risk factors include pregnancy, malnutrition and chronic liver disease. Blood glucose levels should be checked promptly in any patient with altered consciousness. If it is not possible to check blood glucose immediately in a patient with impaired mental state, a presumptive diagnosis of hypoglycaemia should be made and intravenous glucose administered. After admission, blood glucose in patients with cerebral malaria should be checked regularly, at least every 6 h, as in contrast with patients who are awake, a change in the level of consciousness cannot be used as an indicator of hypoglycaemia. Frequent monitoring of the coma score allows detection of a sudden deterioration, which should prompt an immediate check of the blood glucose level. Discontinuation of an intravenous dextrose infusion has been associated with recurrence of hypoglycaemia, especially in children unable to take oral fluids, so blood glucose should be checked and carefully monitored in these circumstances. Blood glucose should also be checked in the event of convulsions or metabolic acidosis. Hypoglycaemia (<2.2 mM) should be treated promptly, because it can cause irreversible cerebral damage and is associated with residual neurological deficit in survivors. Treatment of hypoglycaemia is with a bolus of 20% glucose, 2 ml (0.4 g) per kg over 10 min or alternatively, if 20% glucose is not available, 50% glucose, 1 ml (0.5 g) per kg over 10 min. Thereafter, blood glucose levels should be checked frequently (at least every hour), because there can be a rebound hypoglycaemia. The aim should be to keep blood glucose concentration >4 mM (>70 mg/dl) by providing an adequate glucose calorie source. In critically ill adult patients, tight glucose control, which includes the treatment of moderate hyperglycaemia with insulin therapy, is not recommended (Brunkhorst *et al.* 2008).

Treatment of hypoglycaemia

Give an intravenous a bolus of 20% glucose, 2 ml per kg over 10 min.

If 20% glucose is not available give 50% glucose, 1 ml per kg over 10 min, preferably piggy-backed into an intravenous infusion. Thereafter blood glucose levels should be checked frequently (at least every hour), as rebound hypoglycaemia is common. Maintenance with 10% dextrose may be necessary.

Management of hepatic dysfunction. Severe jaundice is common in adult patients with severe malaria, but overt hepatic dysfunction is not and rarely needs special attention. Jaundice is uncommon in children with severe

malaria, but its presence is associated with a worse prognosis (Marsh *et al.* 1995). Hepatic biotransformation is significantly impaired, so metabolic drug clearance is reduced in severe malaria.

Management of bleeding disorders. Thrombocytopenia is always present in patients with severe malaria, but bleeding complications are surprisingly uncommon. In case of bleeding disorders, disseminated intravascular coagulation (DIC) should be suspected, which can be an indication of concomitant bacterial sepsis. Antibiotics should already have been prescribed in all children with severe malaria in areas of moderate or high malaria transmission. Low-dose heparin, antithrombin or recombinant activated Protein C therapy are no longer recommended for the treatment of DIC, nor is the use of fresh frozen plasma to correct laboratory clotting abnormalities unless there is bleeding (Dellinger *et al.* 2008). If available, platelets can be administered when the platelet counts are <5000/mm^3 (5×10^9/l) regardless of bleeding or if there is significant bleeding and counts are below 30 000/mm^3 (30×10^9/l).

Treatment of hyperpyrexia. Patients with severe malaria often have a high body temperature (>38 °C), which is uncomfortable, exacerbates dehydration, and may contribute to impaired consciousness and seizures. Paracetamol is an effective and inexpensive antipyretic. The adult dose is 1000 mg (or 15 mg/kg) every 6 h (maximum daily dose 4000 mg), given orally or via a nasogastric tube (as powdered tablets or suspension which have generally good bioavailability) (Ismail *et al.* 1995; Wilairatana *et al.* 1995). In children aged 3–6 months, the dose is 60 mg, from 6 months to 24 months, it is 120 mg, from 2 to 4 years, it is 180 mg, from 4 to 8 years, it is 250 mg, from 8 to 10 years, it is 375 mg, and from 10 to 12 years, it is 500 mg – all given every 6 h. The rectal dose, as paracetamol suppositories, is the same as the oral dosage and can be used in patients with vomiting, but the time to maximum plasma concentration and overall plasma concentrations (AUC) are lower than with oral administration (Kolloffel *et al.* 1996). There is also a paracetamol formulation for intramuscular administration, but adequate pharmacokinetic data are lacking and in sick patients with peripheral shutdown, the relative bioavailability of i.m. paracetamol may be reduced (Douglas *et al.* 2013). More recently, a formulation for intravenous administration has become available, but this is not widely available in malaria-endemic countries (Duggan & Scott 2009). Aspirin and nonsteroidal anti-inflammatory agents (NSAID) are effective, but carry a risk of causing gastrointestinal bleeding and may impair renal function in the already compromised patients with hypovolaemia. In febrile children, the use of salicylates or NSAIDs can cause Reye's syndrome, and they should not be used. In addition to antipyretic medication, removing of clothing, tepid sponging and fanning can be used to lower the body temperature, although in contrast with paediatric patients, these have not been formally assessed for their efficacy in adults.

Emergency assessment and management of severe malaria in children

As for any sick child presenting to hospital, initial management of a child presenting with suspected severe malaria should be guided by a rapid, structured, triage assessment, aimed at identifying emergency and priority signs. The WHO Emergency Triage, Assessment and Treatment (ETAT) advocates prioritising care for cases with emergency signs identified by the ABCD assessment (airway/breathing/circulation/dehydration and level of consciousness) (World Health Organization 2005a). Many children with severe malaria will have emergency signs including: a compromised airway (convulsions/deep coma); altered breathing pattern; perturbations of circulatory or hydration status and/or impaired consciousness. Immediate management of these complications should not be delayed, while the diagnosis of malaria is confirmed.

Management of coma and seizures

The general and supportive management of coma and seizures are similar and will be described together. Initial management should include maintenance of the airway, support of breathing and immediate correction of hypoxia and hypoglycaemia – metabolic disorders which may contribute to a depressed level of consciousness or seizure. If the child is not breathing or has difficulty in maintaining the airway, use a Guedel airway and provide short-term support by bag-and-mask ventilation. To manage the airway of a convulsing child, do not try to insert anything in the mouth to keep it open. Once stabilised, place the unconscious child in the recovery position to prevent aspiration and if possible insert a nasogastric tube to empty the stomach contents. General nursing care and regular monitoring are essential, including turning the unconscious child every 2 h, attention to pressure spots and oral and eye care (World Health Organization 2005a). For those with prolonged coma, nasogastric feeding must be planned. Other CNS infections or intracranial haemorrhage should be considered as alternative diagnoses, especially in a child with neck stiffness or a full fontanelle.

Section 11: Antimalarial drug treatment of severe malaria

The artemisinin derivative artesunate is now firmly established as the treatment of choice for severe malaria. The largest randomised clinical trials ever conducted in severe falciparum malaria have shown a highly significant reduction in mortality with intravenous or intramuscular artesunate compared with parenteral quinine (Dondorp *et al.* 2005a, 2010). This reduction in mortality was not at the expense of an increase in neurological deficit, indeed there were fewer neurological sequelae in artesunate recipients. Furthermore, artesunate was simpler and safer to use than the previous 'gold standard' and proved highly cost-effective.

Artemisinin derivatives

Various artemisinin derivatives have been used in the treatment of severe malaria. These include artemether, artemisinin (rectal), artemotil (arteether) and artesunate. These drugs have similar intrinsic pharmacodynamic properties. The ether derivatives artemether and artemotil are two- to threefold less active than dihydroartemisinin, their main metabolite and also the main biologically active metabolite of artesunate. The ethers are also metabolised to a lesser extent, and in severe falciparum malaria, the concentrations of parent compounds predominate. A artesunate, by contrast, is hydrolysed rapidly and almost completely to dihydroartemisinin, which provides the main antimalarial effect. The ethers are water insoluble, lipid soluble compounds that are formulated in oil for intramuscular injection. They are absorbed slowly and erratically following intramuscular administration in severe malaria (Murphy *et al.* 1997; Hien *et al.* 2004), whereas artesunate is instantly bioavailable after intravenous injection and is absorbed rapidly and reliably after intramuscular injection. These pharmacological advantages presumably explain the clinical superiority of parenteral artesunate over artemether in severe malaria (Phu *et al.* 2010).

Artesunate versus quinine. Randomised trials comparing artesunate and quinine from South-East Asia show clear evidence of substantial life-saving benefit with artesunate. In the largest multicentre trial from the region, the SEAQUAMAT study (Dondorp *et al.* 2005a), which enrolled 1461 patients (including 202 children <15 years old), mortality was reduced by 34.7% (95% CI = 18.5 to 47.6%; $P = 0.0002$) in the artesunate group compared to the quinine group. The results of this and smaller trials (Hien *et al.* 1992; Win *et al.* 1992) are consistent and showed clearly and unequivocally that artesunate is the treatment of choice for adults with severe malaria. The AQUAMAT trial (Dondorp *et al.* 2010) was a multicentre comparison of parenteral artesunate and parenteral quinine in severe malaria, which enrolled 5425 children

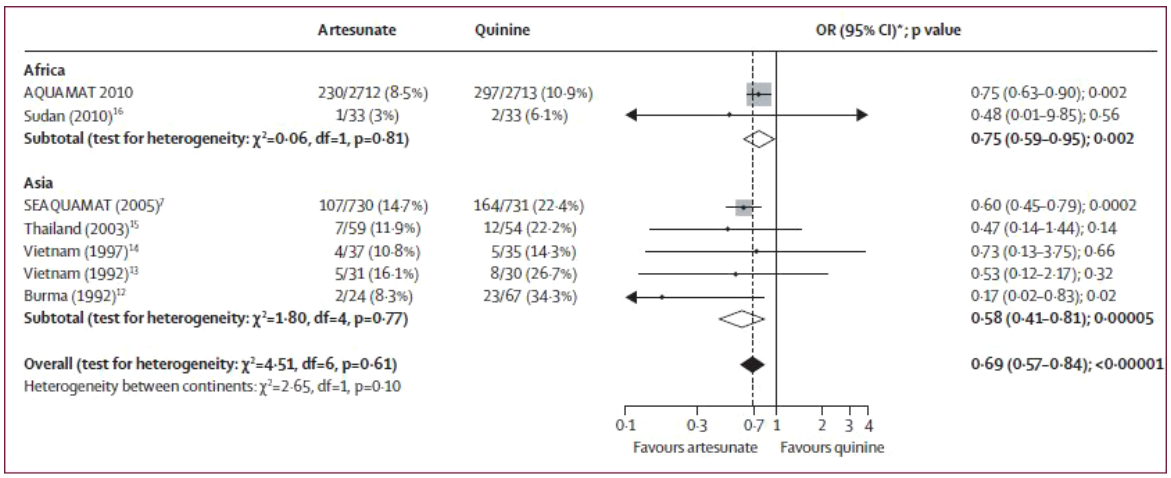


Figure 25 Randomised controlled trials comparing artesunate and quinine in severe malaria (Dondorp et al 2010). The solid vertical line represents equality of the two groups; the dashed line is the overall treatment difference. The horizontal lines and the width of the diamonds show the CIs for the odds ratios. The size of the squares is proportional to the size and therefore the weight, of the trial. OR = odds ratios. *99% CIs for totals.

<15 years of age across Africa. Mortality was 22.5% (95% CI = 8.1 to) lower in the artesunate recipients than in those children who received quinine ($P = 0.022$). The incidences of convulsions, coma, and hypoglycaemia developing after hospital admission were also significantly reduced. Importantly, there was no significant difference in the incidence of severe neurological sequelae in any of these studies. A meta-analysis of all trials comparing mortality in artesunate and quinine recipients provided an odds ratio of 0.69 (95% CI = 0.57 to 0.69) in favour of artesunate ($P < 0.00001$) (Figure 7) (Win *et al.* 1992; Cao *et al.* 1997; Dondorp *et al.* 2005a, 2010; Eltahir *et al.* 2010). Parenteral artesunate is therefore also the treatment of choice for children with severe malaria.

The advantage of artesunate over quinine is probably a result of rapid killing and clearance of ring stages from the circulation, thus preventing sequestration and microvascular obstruction (Udomsangpetch *et al.* 1996). The higher risk of severe hypoglycaemia and hyperinsulinaemia resulting from quinine therapy, seen in adults children and especially in pregnant women (Hien *et al.* 1996; Dondorp *et al.* 2005b, 2010), is an additional disadvantage of quinine (White *et al.* 1983b).

Artemether versus quinine. In an individual patient data meta-analysis of 1919 patients enrolled in randomised controlled trials of artemether and quinine, the mortality in artemether recipients was 14% compared with 17% in quinine recipients ($P = 0.08$) (Bhattacharya *et al.* 2013). The combined 'adverse outcome' of either death or neurological sequelae was significantly less common in the artemether group [odds ratio 0.77 (95% CI 0.62 to 0.96), $P = 0.02$]. Among the adults with severe malaria, the mortality in artemether recipients was significantly lower than in quinine recipients, whereas in African children, there was no significant difference in mortality.

Artesunate versus artemether. There has been only one randomised controlled trial comparing intramuscular artesunate and artemether in severe malaria (Phu *et al.* 2010). This enrolled 370 Vietnamese adults; there were 13 deaths in the artesunate group and 24 in the artemether group ($P = 0.052$), and parasitaemia declined more rapidly in the artesunate group.

Rectal artesunate. The risk of death from severe malaria is greatest in the first 24 h. In malaria-endemic areas, severe malaria is commonly suspected in rural or remote locations, where parenteral treatment cannot be given. Referral to a centre where parenteral therapy is possible may involve considerable delay or 'referral time'. During this delay, the patient may deteriorate and even die. In a very large multicentre *placebo*-controlled randomised trial, a single dose of rectal artesunate given at the primary healthcare level as pre-referral treatment reduced the risk of death or permanent disability in young children by 25%, but in the smaller group of older children and adults, mortality was inexplicably higher in artesunate recipients. The benefits from rectal artesunate were greatest in children who took more than 6 h to reach a health facility (Gomes *et al.* 2009). Until the uncertainties over adults are resolved, pre-referral rectal artesunate is recommended currently only for children ≤6y.

If referral is impossible, rectal treatment should be continued until the patient can tolerate oral medication. (*In this situation, continued efforts must still be made to achieve referral – repeated rectal artesunate is not a satisfactory home treatment for possible severe malaria*). The administration of an artemisinin derivate by the rectal route as pre-referral treatment has been found feasible and acceptable at the community level (Machado Siqueira *et al.* 2012; Baird 2013).

Apart from the unexplained apparent increase in mortality in adults and older children receiving rectal artesunate, these very large trials (by far the largest ever conducted in severe malaria) provide consistent evidence. They show unequivocally that artesunate is the best drug for the treatment of severe malaria in all patients. Artemether is second choice; for although it has comparable antimalarial activity, its erratic absorption following intramuscular injection particularly in shocked patients (Murphy *et al.* 1997; Hien *et al.* 2004) results in some patients having inadequate plasma concentrations in the critical early phase of treatment. Quinine is now third choice. For pre-referral treatment of patients with severe malaria, or those unable to

Treatment of severe malaria

- Artesunate (i.v. or i.m.) 2.4 mg/kg* immediately, then at 12, 24 h and daily until oral medication can be taken reliably. For children <20 kg the parenteral artesunate dose is 3 mg/kg.

If unavailable

- Artemether (i.m.) 3.2 mg/kg stat followed by 1.6 mg/kg daily

If unavailable

- Quinine dihydrochloride (20 mg salt/kg) by slow intravenous infusion over 4 h or by i.m. injection split to both anterior thighs, followed by 10 mg salt/kg 8 h.

*Young children need a higher dose to achieve comparable exposures to adults – see Table 10

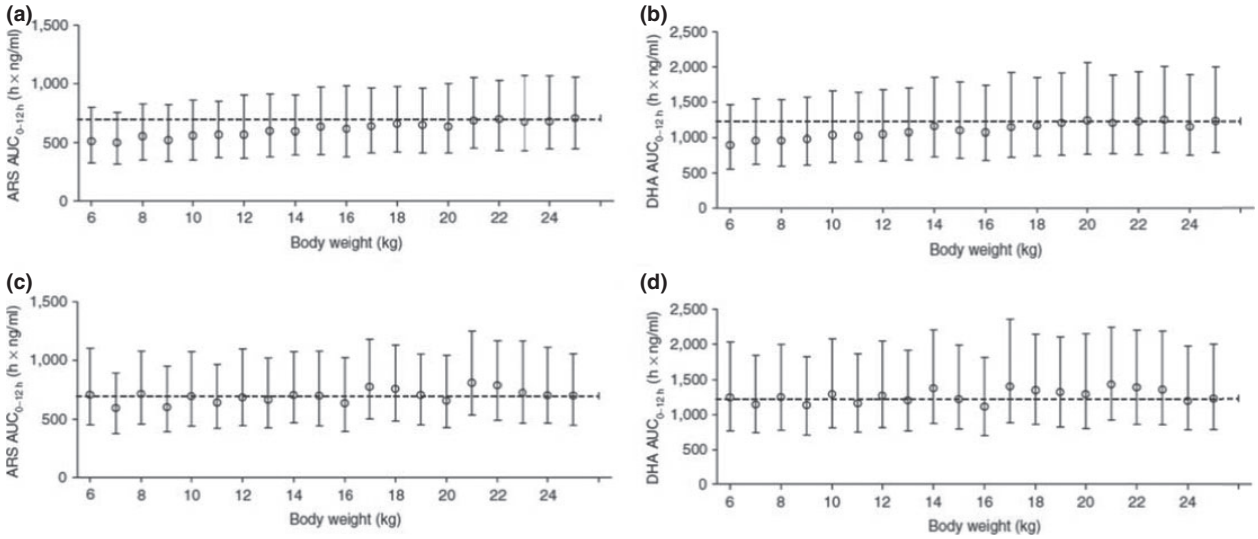


Figure 26 Simulated total first-dose exposure levels (AUC0–12 h) of (a) artesunate and (b) DHA after the standard 2.4 mg/kg parenteral dosing in children at different body weights (ref is Hendriksen *et al.* 2013b). Simulated total first-dose exposure levels (AUC0–12 h) of (c) artesunate and (d) DHA after the suggested adjusted dose regimen (Table 3). Open circles represent median values, and bars indicate the 25th–75th percentiles of simulations (1000 simulations at each body weight). The broken line represents the median exposure for the largest weight group (i.e. 700 and 1230 h ng/ml for artesunate and DHA, respectively). ARS, artesunate; AUC0–12 h, area under the concentration–time curve from time points 0–12 h; (DHA, dihydroartemisinin).

take oral medications reliably, parenteral (intramuscular) artesunate is also the drug of choice. If injections cannot be given, then rectal artesunate is indicated in young children, but not in older children (>6 y) and adults until further evidence of safety is obtained.

Drug treatment. Severe malaria is a medical emergency. After rapid clinical assessment and confirmation of the diagnosis, full doses of parenteral antimalarial treatment should be started without delay with any effective antimalarial first available. Artesunate (i.v. or i.m.) is the treatment of choice, artemether (i.m.) is second choice, and quinine (i.v. or i.m.) is third choice.

Artesunate should be given in a dose of 2.4–3 mg/kg BW preferably by intravenous route or by intramuscular injection on admission (time = 0), then at 12 h and 24 h, then once a day until the patient can reliably take oral medication. As children require higher doses to achieve equivalent exposures to adults (Hendriksen *et al.* 2013b) (Figure 26, Table 10), a simple recommendation is to give parenteral artesunate at a dose of 3 mg/kg to patients <20 kg, and 2.4 mg/kg for heavier patients. All the large clinical trials have been performed with one artesunate formulation, in which a lyophilised powder of artesunic acid is dissolved first in 1 ml 5% sodium bicarbonate, and this solution is then diluted with 5% dextrose or 0.9% sodium chloride and given by intravenous or intramuscular injection. The pharmacological properties, notably the bioavailability of the active metabolite DHA, were found to be similar for i.m. and i.v. artesunate (Nealon *et al.* 2002), whereas rectal artesunate needs to be given at fourfold higher doses to reach similar bioavailability (Ilett *et al.* 2002a). Other formulations are in development. This dose is unchanged in renal impairment, liver dysfunction, pre-treatment, and the elderly. Any uncertainties over the safety of artemisinins to

Table 10 Bodyweight-adjusted i.m. artesunate dosing regimen for children that provides similar exposure to that in adults receiving 2.4 mg/kg

Weight (kg)	Dose i.m. (mg)	Prepared solution (ml)*	Dose i.m. (mg/kg)
6–7	20	2	2.86–3.33
8–9	25	2.5†	2.78–3.13
10–11	30	3†	2.73–3.00
12–13	35	3.5†	2.69–2.92
14–16	40	2	2.50–2.86
17–20	50	2.5	2.50–2.94
21–25	60	3	2.40–2.86

*For children <14 kg dilute to 10 mg/ml, for children ≥14 kg dilute to 20 mg/ml.
†Divide over both thighs.

the developing fetus are outweighed in severe malaria by their enormous life-saving benefit and better safety profile in pregnancy.

Artemether 3.2 mg/kg BW should be given by intramuscular injection to the anterior thigh on admission then 1.6 mg/kg BW daily thereafter until the patient can reliably take oral medication. This dose is unchanged in renal impairment, liver dysfunction, pre-treatment, infants, children and the elderly. Intramuscular artemether is, however, absorbed very slowly in patients with acute malaria (Hien *et al.* 2003) and also with great variability in children with severe malaria (Mithwani *et al.* 2004). The generally recommended regimen for intramuscular *artemotil* (available only in India) is a larger initial dose of 4.8 mg/kg followed by the same maintenance dose of 1.6 mg/kg BW daily thereafter, until the patient can reliably take oral medication (Li *et al.* 2004).

Quinine 20 mg dihydrochloride salt/kg BW on admission (4-h IV infusion or divided IM injection – 10 mg salt/kg given into each anterior thigh). This is followed by 10 mg/kg BW every 8 h. The intravenous infusion rate should not exceed 5 mg salt/kg BW per hour.

Follow-on treatment. Parenteral antimalarials in the treatment of severe malaria should be given for a minimum of 24 h, once started (irrespective of the patient's ability to tolerate oral medication earlier). This should be followed by a full course of oral antimalarial treatment, once the patient can tolerate oral therapy. The previously recommended options for follow-on oral treatment are as follows (World Health Organization 2010a):

- artemether plus lumefantrine
- artesunate plus amodiaquine
- dihydroartemisinin plus piperaquine
- artesunate plus sulphadoxine–pyrimethamine
- artesunate plus clindamycin or doxycycline*
- quinine plus clindamycin or doxycycline

Most countries have their own first-line treatment policy for uncomplicated malaria – this should be given in full as follow-on therapy after initial parenteral treatment for severe malaria. The only caveat is that mefloquine should be avoided as a component of follow-on therapy if the patient has had impaired consciousness, because of an increased incidence of neuropsychiatric complications associated with mefloquine following cerebral malaria (Nguyen *et al.* 1996).

*Doxycycline is preferred to other tetracyclines because it can be given once daily and does not accumulate in renal failure. Where available, clindamycin should be substituted in children up to age of 7 years and pregnant women because doxycycline should not be given to these groups.

Section 12: Community diagnosis, management and referral

Severe malaria must be treated promptly to prevent death and disability. Severe malaria is always best managed at the highest possible level of health care, where staff and equipment are available. Unfortunately, most cases occur in remote areas, and patients present to facilities that have only minimal staff and laboratory capability for managing severe malaria. Patients must then be referred to a sufficiently resourced hospital, but the delay in arranging or accomplishing the referral can be dangerous for a patient with severe malaria. Giving rectal artesunate immediately to 'cover' the time spent in the referral process, has been shown to improve survival in children (but not adults) in this situation (Gomes *et al.* 2009).

Recognising severe febrile illness at the community and peripheral health facility levels

In many countries, community and peripheral health facility workers have been trained through the Integrated Management of Childhood Illness (IMCI) and Integrated Management of Adolescent and Adult Illness (IMAI) initiatives to recognise syndromes of severe illness requiring referral and pre-referral treatment. Most often these include the presence of fever or history of fever in the past 2 days, the presence of palmar pallor in children under 5 years, and one or more of the danger signs listed in Table 11.

Identifying parasitaemia in cases being prepared for pre-referral treatment

Global policies now recommend parasitological confirmation as part of case management for malaria. A parasitological diagnosis can be accomplished at a peripheral level by rapid diagnostic tests (RDTs) that detect malaria parasite antigens. Increasingly, RDTs are being deployed at community and small health facility levels where microscopy is not possible. In the case of a severe febrile illness, it is important that life-saving treatment be initiated as soon as possible, even if an RDT is not available. Where possible, community health and peripheral health workers should be provided with malaria RDTs and should be taught how to use them.

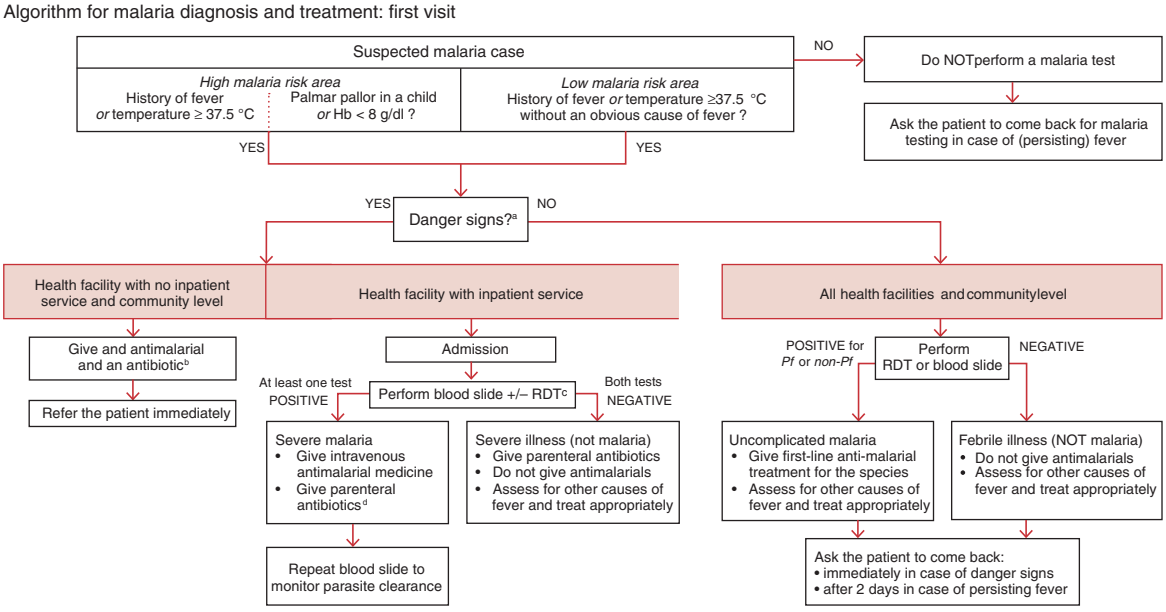
In areas where malaria transmission occurs, clinical features of severe malaria often overlap with invasive bacterial illness. For this reason, both specific antimalarial and antibacterial treatments should be started immediately once a severe febrile illness is suspected – even before laboratory testing, admission or referral. Figure 27, below shows a simplified algorithm for recognising and responding to severe febrile illness (World Health Organization 2011a,b).

Selecting the antimalarial drugs for pre-referral treatment for severe malaria

Malaria-specific treatment can be initiated with parenteral or rectal artesunate, parenteral artemether, or parenteral or rectal quinine. The choice of initial pre-referral treatment depends on what is available at the point of presentation and the training and skills of the health worker initiating a pre-referral treatment. For example, many community health workers and clinicians at peripheral health facilities may only be trained to deliver rectal medicines, while others may be trained in delivering initial intramuscular doses, or even establishing intravenous access (although the latter is generally restricted to hospitals and points of definitive care). Depending on the availability of medicines and trained staff, pre-referral treatment should be initiated with one of the following, in descending order of preference:

Table 11 General danger signs suggesting severe febrile illness as criteria for referral from peripheral health facilities, adapted from IMCI and IMAI algorithms

IMCI for children 2–59 months in areas of malaria transmission	IMAI for older children and adults in areas of malaria transmission
Fever or history of fever in the past 24 h OR palmar pallor plus one or more of the following danger signs:	Fever or history of fever in the past 24 h plus one or more of the following danger signs:
Unable to drink or breastfeed	Very weak or unable to stand
Vomiting everything	Convulsions
Multiple convulsions	Lethargy
Lethargy	Unconsciousness
Unconsciousness	Stiff neck
Stiff neck	Respiratory distress
Chest indrawing or stridor	Severe abdominal pain



[a] The following general danger signs are considered criteria for referral at peripheral level [adapted from Integrated Management of Childhood Illness (IMCI) and Integrated Management of Adolescent and Adult Illness (IMAI)]:
in children: unable to drink or breastfeed, vomit everything, have convulsions, are lethargic or unconscious and present with neck stiffness, chest indrawing or stridor;
in adults: are very weak or unable to stand, are lethargic or unconscious or have neck stiffness, convulsions, respiratory distress or severe abdominal pain
[b] Pre-referral treatment as recommended by WHO 2010 *Guidelines for the treatment of malaria* and by Integrated Management of Childhood Illness (IMCI) and Integrated Management of Adolescent and Adult Illness (IMAI): rectal artesunate or intramuscular quinine, artesunate or artemether and intramuscular ampicillin plus gentamicin or intramuscular ceftriaxone
[c] RDT is performed while waiting for the result of the blood slide to decide earlier on treatment and to document malaria in patients who have received pre-referral antimalarial treatment (and might thus have already cleared their parasites).
[d] Because of the possibility of concomitant bacterial infection in severe malaria patients, especially in children, antibiotics should be given beside antimalarials until bacterial infections have been ruled out (including bacteremia by blood cultures if available).

Figure 27 A practical algorithm for the diagnosis, assessment and management of malaria at the clinic level.

- IM artesunate
- PR artesunate (only for children ≤6 years)*
- IM artemether
- IM quinine
- PR quinine

*In the large multicentre study of rectal artesunate vs *placebo* (Gomes *et al.* 2009), the significant benefit of rectal artesunate in children was not seen in adults, in whom the provision of rectal artesunate was detrimental.

Initial doses for each of these regimens are provided in the treatment Table 12. WHO no longer recommends oral treatments for the pre-referral management of severe febrile illness or suspected severe malaria, although if no parenteral or rectal formulation is available, and the patient can still swallow then oral treatment should be started.

The addition of pre-referral antibacterial drugs

It is impossible to distinguish clinically between severe malaria and severe invasive bacterial infection. African children with severe malaria are at high risk of concomitant bacteraemia [see *Section 10*]. **IMCI and IMAI guidelines recommend pre-referral treatment for both malaria and bacterial infections.** Some countries recommend antibacterial treatment for all severe febrile illness; others for the presence of specific danger signs such as stiff neck, nasal flaring or chest indrawing. Nevertheless, children with severe febrile illness in malaria-endemic Africa seldom receive initial treatment with appropriate antibacterial drugs, either for pre-referral or for definitive care. Broad-spectrum antibacterial drugs recommended for pre-referral treatment of severe febrile illness differ depending on local policies, age of the patient, antimicrobial resistance patterns and other patient characteristics (such as immunisation and HIV status). These recommendations are not elaborated here, but they are a critical element of pre-referral care. Clinicians are advised to refer to their national treatment guidelines for antibacterial medicine recommendations.

Ensuring referral and definitive management of patients who have received pre-referral treatment

Pre-referral treatments can often result in a dramatic clinical improvement, but it remains essential that

Table 12 Pre-referral treatment of severe malaria.

Severe malaria is a medical emergency Treatment should never be delayed. If there is any doubt, the physician or healthcare worker should treat as severe malaria pending definitive diagnosis. Ideally, antimalarials should be given parenterally in severe malaria, but if that is not possible, preferral rectal artesunate should be given to children ≤6 years. If nothing else is available, and it will take many hours to reach a health facility, attempted oral treatment is better than nothing.

The following are the recommendations for immediate antimalarial drug treatment at a village or health post before referral to hospital for definitive diagnosis and treatment

Choice of drug

Order of preference	Children <6 years	Older children and adults
1	Artesunate i.m.	Artesunate i.m.
2	Rectal artesunate	Artemether i.m.
3	Artemether i.m.	Quinine i.m
4	Quinine i.m	

Doses

Drug	Dose
Artesunate i.m.	2.4 mg/kg*
Rectal artesunate	10 mg/kg
Artemether i.m.	3.2 mg/kg
Quinine i.m	20 mg salt/kg

*3 mg/kg is recommended in children <20 kg.

Intramuscular injections should be given to the anterior thigh. Quinine dihydrochloride is acidic and painful. It should be diluted to 60–100 mg/ml, and the dose split such that half is given to each thigh. If rectal artesunate is administered, the child should be examined to check it has been retained. If the rectocap is expelled, the dose should be repeated

patients complete referral and transport to a higher level of care for definitive management as promptly as possible. Generally, this is a district hospital or a high-level health centre with personnel, laboratory and facilities for managing severe illness 24 h a day. Community health workers who are likely to provide pre-referral care should know where to refer severely ill patients from their catchment areas. Providing transportation costs and carefully involving family members in the referral plan may improve the completion of referral in a timely way. The referring unit should provide a referral note outlining the patient's condition including initial assessment, any diagnostic tests and what pre-referral treatments have been initiated and when. Upon arrival at the referral centre, assessment and management of severely ill patients should proceed as described in the remainder of this document.

Where referral is delayed or impossible

A number of national policies include special recommendations for the rare situation when referral must be delayed or is impossible. Frequently, these recommendations involve repeated IM or PR doses of pre-referral medications. There can be no reasonable doubt that continued treatment is better than none, even though there is no evidence to support this practice. But completed referral for definitive care is always the preferred course of action, and every effort should be made to achieve it. Attempting, as some do, to complete the whole course of treatment at home is not acceptable practice, because the diagnosis may have been wrong and complications and comorbidities cannot be identified (Walter *et al.* 2009).

Section 13: Severe vivax malaria

Plasmodium falciparum causes the majority of severe and fatal malaria cases and has overshadowed the public health importance of vivax malaria (Baird 2007; Price *et al.* 2007). *Plasmodium vivax* is less pathogenic than *P. falciparum* in otherwise healthy patients, but can cause complicated and severe disease (Price *et al.* 2007, 2009; Baird 2009; Bassat & Alonso 2011; Anstey *et al.* 2012). In the malaria therapy era, acute mortality during *P. vivax* therapy of neurosyphilis averaged 5–10% overall (Swellengrebel & De Buck 1938) and up to 10–14% with the Madagascar strain (James 1933), but these were debilitated patients with a fatal underlying disease. *Plasmodium vivax* infection has been associated with severe and fatal disease in endemic areas, including Indonesia (Barcus *et al.* 2007; Tjitra *et al.* 2008; Lampah *et al.* 2011; Nurleila *et al.* 2012), Papua New Guinea (Genton *et al.* 2008; Manning *et al.* 2011), India (Kochar *et al.* 2010; Yadav *et al.* 2012), Brazil (Andrade *et al.* 2010; Lacerda *et al.* 2012), Venezuela (Rodriguez-Morales *et al.* 2008), Thailand (Luxemburger *et al.* 1997), Malaysia (Barber *et al.* 2012) and Sudan (Mahgoub *et al.* 2012). Severe manifestations associated with *P. vivax* infection in these series include severe anaemia, respiratory distress and acute lung injury (ALI), acute kidney injury (AKI), splenic rupture, metabolic acidosis, jaundice, multiorgan dysfunction, shock and rarely coma. *P. vivax* also causes substantial morbidity, particularly severe anaemia (Genton *et al.* 2008; Tjitra *et al.* 2008; Poespoprodjo *et al.* 2009; Price *et al.* 2009; Manning *et al.* 2011; Nurleila *et al.* 2012) and low birthweight (Nosten *et al.* 1999; Poespoprodjo *et al.* 2008; Rijken *et al.* 2012a). While many recent series report PCR exclusion of mixed *Plasmodium* infection, investigation for concurrent infections or comorbidities has been mostly incomplete. Evidence is strong that *P. vivax* can cause severe anaemia (Price *et al.* 2009; Douglas *et al.* 2012), acute respiratory distress syndrome (ARDS) (Tan *et al.* 2008; Valecha *et al.* 2009; Taylor *et al.* 2012b), splenic rupture (Imbert *et al.* 2009) and in some areas acute kidney injury (Chung *et al.* 2008; Kute *et al.* 2012b; Sinha *et al.* 2012). For other reported severe malaria syndromes, the extent to which they are attributable to *P. vivax* is not yet clear: infectious and non-infectious comorbidities may be additive or synergistic contributors to severe disease or alternative causes (Kitchen 1949b; Anstey *et al.* 2009; Price *et al.* 2009; Lampah *et al.* 2011; Anstey *et al.* 2012; Barber *et al.* 2012; Lacerda *et al.* 2012). However, the presence of comorbidities does not exclude a key role for *P. vivax* in the pathophysiology of severe disease.

Epidemiology of complicated and severe vivax malaria

Outside of Africa, *P. vivax* causes almost half of all malaria cases, with 70–390 million clinical infections each year (Price *et al.* 2007). In countries endemic for both major *Plasmodium* species, *P. vivax* infection can account for up to 38% of patients hospitalised with malaria (Buck *et al.* 1983; Gopinathan & Subramanian 1986; Maitland *et al.* 1997; Carrara *et al.* 2006; Tjitra *et al.* 2008). In Indonesian Papua, *P. vivax* accounted for 24% of malaria admissions in all age groups, but 47% (415/887) of infants (Tjitra *et al.* 2008). The need for hospitalisation indicates significant morbidity and at least moderately severe disease (Anstey *et al.* 2012). This ranges from vomiting and inability to tolerate oral therapy, through to prostration and those with disease manifestations fulfilling the severity criteria described earlier for falciparum malaria (*Section 2*). The risk of severe disease from single *P.vivax* infections is very low in otherwise healthy adults and older children without comorbidities, with ready access to early diagnosis and effective treatment. (Price *et al.* 2009; Anstey *et al.* 2012). In endemic areas, the risk of severe disease is associated with young age, higher transmission intensity, early and frequent relapse, less access to early diagnosis and treatment and/or greater prevalence of comorbidities including bacterial co-infections and malnutrition (Price *et al.* 2009; Anstey *et al.* 2012).

In the only modern autopsy series, at least four of 17 (23.5%) vivax-associated deaths in Brazil were attributable to alternative causes, including meningitis and yellow fever (Lacerda *et al.* 2012). This proportion is similar to the 23% of *P. falciparum*-associated deaths attributable to other non-falciparum aetiologies at autopsy in Malawi (Taylor *et al.* 2004). An additional 41% of the Brazilian fatal vivax cases had major underlying comorbidities contributing to death at autopsy (Lacerda *et al.* 2012), an association also recognised in the early literature (James 1925; Kitchen 1949a). The population-based risk of severe and fatal disease is not well defined. In Indonesian Papua, where transmission is high, the risk of severe disease and death from *P. vivax* was estimated at 1 in 270 and 1 in 3959 clinical infections, respectively, compared to estimates for *P. falciparum* of 1 in 185 and 1 in 1742, respectively (Tjitra *et al.* 2008). Mortality rates in those hospitalised in Indonesian Papua with microscopy-diagnosed *P. vivax* were reported as 0.8–1.6%, similar to that of *P. falciparum* infection (1.6–2.2%) (Barcus *et al.* 2007; Tjitra *et al.* 2008; Price *et al.* 2009). The adjusted odds ratio of death from severe anaemia in Papua was 5.9 for those with *P. falciparum* and 4.4 for those with *P. vivax* infection. Case-fatality

rates in Indian children hospitalised with PCR-confirmed *P. vivax* mono-infection (3.9%) were also comparable to that seen in PCR-confirmed *P. falciparum* infection (3.2%) (Kochar *et al.* 2010). In marked contrast, substantially lower vivax mortality (0.22%) has been reported in children hospitalised in Thailand (Wattanagoon *et al.* 1994), and only one of 1000 sequential adult admissions with strictly defined severe malaria in Vietnam had *P. vivax* malaria (TT Hien, personal communication). Furthermore, in contrast to the reports from some parts India, acute kidney injury from vivax malaria is very unusual in South-East Asia. The mortality of vivax malaria in low transmission areas is uncertain with some recent case series reporting significant mortality and other series reporting very low mortalities. Thus, whereas the spectrum of falciparum malaria severity in relationship to age and transmission intensity appears similar across the world, there appear to be marked differences in the reported severity of vivax malaria between different areas.

Vulnerable groups

Young children. In co-endemic areas, morbidity, including severe disease from *P. vivax*, usually occurs at a younger age than from *P. falciparum* (Michon *et al.* 2007; Tjitra *et al.* 2008; Kochar *et al.* 2010; Lin *et al.* 2010). Most severe disease in children, especially severe anaemia, is reported from high transmission areas in children under 5 years (Genton *et al.* 2008; Tjitra *et al.* 2008; Poespoprodjo *et al.* 2009; Alexandre *et al.* 2010; Kochar *et al.* 2010; Lanca *et al.* 2012). This risk is highest in infancy (Douglas *et al.* 2013). In Indonesian Papua, more infants are hospitalised with vivax malaria than falciparum malaria (Tjitra *et al.* 2008; Poespoprodjo *et al.* 2009) including a 2.4-fold higher proportion with severe anaemia if hospitalised with vivax compared with falciparum malaria (Tjitra *et al.* 2008). Infant risk starts *in utero*, with *P. vivax* infection in pregnancy associated with abortion, low birthweight, congenital malaria (Poespoprodjo *et al.* 2011) and a greater risk of clinical disease and severe anaemia in the neonatal period (Poespoprodjo *et al.* 2009; Lanca *et al.* 2012).

Malnutrition. As in falciparum malaria (Berkley *et al.* 2009), malnutrition is a likely major contributor to severe disease in *P. vivax* infection. In India, malnutrition was found in 69% of children with severe vivax malaria and 75% of the vivax-associated deaths (Kochar *et al.* 2010). In Brazil, 17% of vivax-infected children requiring intensive care admission had malnutrition (Lanca *et al.* 2012).

Other comorbidities. Acute and chronic infectious and non-infectious comorbidities probably contribute to severe and fatal disease in *P. vivax* infection (Anstey *et al.* 2009; Price *et al.* 2009; Anstey *et al.* 2012; Lacerda *et al.* 2012). Haemodynamic effects of the systemic inflammatory response to *P. vivax* and anaemia may contribute to decompensation of concurrent acute and chronic disease and subsequent death, whereas otherwise healthy children and adults may make an uncomplicated recovery (James 1925; Kitchen 1949a). In the Manaus (Brazil) autopsy series, 10 of the 13 *P. vivax*-associated deaths had concurrent comorbidities, including pneumonia, emphysema, diabetes, haemorrhagic stroke, decompensated cirrhosis and congestive heart failure (Lacerda *et al.* 2012). HIV co-infection was found in one case (Lacerda *et al.* 2012). HIV infection is a risk factor for severe disease and death in *P. falciparum* infection (Bejon *et al.* 2007; Berkley *et al.* 2009), but the risk in *P. vivax* infection is not yet known. In the United States, deaths from *P. vivax* have occurred in patients with pre-existing cardiac disease (Stoppacher & Adams 2003). Among 24 *P. vivax*-infected Brazilian children requiring intensive care admission, 25% had concomitant acute gastroenteritis, and in total, 58% had a concurrent acute or chronic comorbidity that may have contributed to severe illness (Lanca *et al.* 2012). Such comorbidities are in malaria-endemic regions, and their contribution to severe and fatal disease in *P. vivax* infection is probably underestimated (Anstey *et al.* 2009; Price *et al.* 2009; Anstey *et al.* 2012).

Severe malaria syndromes

Series of severe vivax malaria have described a broad range of severe manifestations in children and adults, using criteria developed for severe falciparum malaria.

Severe anaemia. The major severe manifestation in most series of vivax malaria in children is severe anaemia, defined as a haemoglobin concentration of <5 g/dl in children and <7 g/dl in adults (Luxemburger *et al.* 1997; Rodriguez-Morales *et al.* 2008; Tjitra *et al.* 2008; Poespoprodjo *et al.* 2009; Alexandre *et al.* 2010; Kochar *et al.* 2010). There are few data on the confounding effects of other causes and the effect of successive episodes of malaria caused by reinfection and relapses. Despite these limitations, the association between vivax infection and severe anaemia is strong, particularly in infancy (Michon *et al.* 2007; Genton *et al.* 2008; Tjitra *et al.* 2008; Ladeia-Andrade *et al.* 2009; Poespoprodjo *et al.* 2009; Lin *et al.* 2010; Douglas *et al.* 2013). Among patients presenting to hospital in Indonesian Papua,

where transmission of malaria is high, the adjusted population fraction of severe anaemia attributable to *P. falciparum* was 15.1% compared to 5.8% for *P. vivax* and 5.9% for mixed infections. The fraction of severe anaemia attributable to *P. vivax* was highest in infancy, when it rose to 30.4% compared to 20.5% for *P. falciparum*. (Douglas *et al.* 2013). In Brazil, 9% of children up to 14 years old requiring intensive care unit admission with vivax malaria had severe anaemia, but there were no deaths (Lanca *et al.* 2012). Even in adults, severe anaemia can be a common manifestation of severe vivax disease. In Indonesian Papua, 10% of all adults hospitalised with *P. vivax* infections between 2005 and 2007 had a haemoglobin <5 g/dl (Tjitra *et al.* 2008). In other series from India and Brazil, severe anaemia accounted for almost a third of all cases of severe adult vivax malaria (Kochar *et al.* 2009; Andrade *et al.* 2010). Conversely, in a hypoendemic region of north-western Thailand, none of 1978 adults and children >5 years diagnosed with acute vivax malaria over a 3-year period required hospitalisation for severe anaemia or blood transfusion (Luxemburger *et al.* 1997). Infection with gastrointestinal helminths can cause anaemia through chronic blood loss. While mixed infections with hookworm and *P. falciparum* cause an additive reduction in haemoglobin in preschool children (Brooker *et al.* 2007), in the one study co-infection with hookworm, *Ascaris* and *Trichuris* in Brazil attenuated the reduction in haemoglobin associated with vivax malaria (Melo *et al.* 2010).

Acute lung injury (ALI) and respiratory distress. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are well-recognised complications of *P. vivax* malaria. Initial reports were of cases mostly managed in non-endemic country hospitals, with a very low case-fatality rate and with the majority occurring after commencement of antimalarial drug treatment (Taylor *et al.* 2012b). In a single case report from India, ARDS arising pre-treatment in vivax malaria had a fatal outcome (Valecha *et al.* 2009). More recently reported ALI/ARDS in adults have mostly been part of larger series of severe vivax malaria from endemic settings, generally with less-clearly defined ARDS criteria, occurring as part of multiple organ dysfunction/failure (Kochar *et al.* 2005, 2009; Alexandre *et al.* 2010; Andrade *et al.* 2010; Lacerda *et al.* 2012). Clinical ARDS/'respiratory failure' occurred in 10–32% of patients with severe vivax malaria and had case-fatality rates of 50–67% (Kochar *et al.* 2005, 2009; Alexandre *et al.* 2010; Andrade *et al.* 2010), much higher than the very low fatality rate seen in reports of single-organ ARDS (Taylor *et al.* 2012b). Lactic acidosis appears less common than in falciparum malaria, but has also been reported in adult severe vivax malaria (Lampah *et al.* 2011). In the Brazilian autopsy series, 88% had respiratory distress prior to death, again mostly appearing after the start of antimalarial drug treatment. ARDS and/or lung oedema was identified as the commonest complication contributing to death, occurring in 35%, and in three of the four patients in whom *P vivax* was considered the direct cause of death (Lacerda *et al.* 2012). In two of the latter cases, lung oedema occurred in conjunction with other major pathology (one each with coma/encephalitis and splenic rupture (Lacerda *et al.* 2012).

Respiratory distress is also a common manifestation in most series of severe paediatric vivax malaria (Genton *et al.* 2008; Tjitra *et al.* 2008; Kochar *et al.* 2010; Yadav *et al.* 2012). As in adults, definitions used for respiratory distress vary considerably between series (Taylor *et al.* 2006c), complicating comparisons. Respiratory distress occurred more frequently in vivax malaria (60%) than falciparum malaria (41%) in Papua New Guinean children, and with substantially higher proportions than those reported in hospitalised children in Indonesia Papua, a reflection of broader inclusion criteria. In Indonesian Papua, rates were similar between *P. vivax* (2.3%) and *P. falciparum* (2.5%) (Tjitra *et al.* 2008). In India, respiratory distress was more frequent in children hospitalised with falciparum malaria (18%) than those with vivax malaria (11%); however, this ratio was reversed in children younger than 5 years (2% and 15%, respectively) (Kochar *et al.* 2010).

Both hypoxaemia and metabolic acidosis have been described in children with *P vivax*-associated respiratory distress (Kochar *et al.* 2010; Lanca *et al.* 2012). The relative contributions of metabolic acidosis, lung injury, pneumonia, sepsis and severe anaemia to the respiratory distress associated with *P. vivax* in children is unclear. Respiratory distress was the commonest complication (67%) among Brazilian children admitted for intensive care with severe disease associated with *P. vivax*, with 12.5% overall having strictly defined ARDS (Lanca *et al.* 2012); none died. One child with respiratory distress (6%) had pneumonia and empyema identified as the underlying cause (Lanca *et al.* 2012). One of 65 Indian children with PCR-confirmed severe vivax malaria had pulmonary oedema in association with multiorgan dysfunction (Kochar *et al.* 2010).

Acute kidney injury. Evidence of acute kidney injury (AKI) has been reported in several series of adult vivax malaria and not in others (Kochar *et al.* 2005; Chung

et al. 2008; Kochar *et al.* 2009; Alexandre *et al.* 2010; Andrade *et al.* 2010; Kute *et al.* 2012b; Sinha *et al.* 2012). There appear to be geographical differences in risk and severity, with little or no vivax-associated AKI being reported in returned travellers (Tan *et al.* 2008) or from some vivax-endemic areas, such as Thailand (Luxemburger *et al.* 1997; Piyaphanee *et al.* 2007) and Vietnam (Hien *et al.* 1996). AKI is reported, but is rarely severe, in Korean cases of vivax malaria (Chung *et al.* 2008). Across northern India, severe dialysis-requiring AKI and/or AKI-related death is increasingly reported (Prakash *et al.* 2003; Kochar *et al.* 2005, 2009; Kute *et al.* 2012a, b; Sinha *et al.* 2012), but only the Rajasthan series was *P. vivax* mono-infection confirmed by PCR. AKI in PCR-confirmed mono-infection has also been reported from Brazil (Alexandre *et al.* 2010; Andrade *et al.* 2010; Lacerda *et al.* 2012). No population-based data on risk of AKI have been reported from any region. It is unclear why there are such marked differences in the apparent incidence of renal complications between different areas.

In those series reporting AKI in vivax malaria, this was most commonly associated with multiorgan dysfunction and was a risk factor for fatal outcome (Kute *et al.* 2012b). Shock was also a common association. Renal biopsies in four patients with vivax-associated AKI reported patchy cortical necrosis in three cases and acute tubular necrosis in the other (Kute *et al.* 2012b). The presentations reported frequently mimicked AKI seen with sepsis and/or shock, but the contribution of concomitant bacterial sepsis to these syndromes is not known. In the Brazilian autopsy series of deaths associated with *P. vivax* infection, all six cases with AKI were associated with pre-existing comorbidities predisposing to AKI or multiorgan dysfunction (heart failure, sickle cell haemolytic crisis, chronic liver disease) or alternative aetiologies (primaquine-induced haemolysis or yellow fever) (Lacerda *et al.* 2012). AKI, with varying definitions, has also been reported increasingly as a manifestation of severe vivax malaria in children (Kochar *et al.* 2010; Manning *et al.* 2011; Jat *et al.* 2012; Kaushik *et al.* 2012; Yadav *et al.* 2012) occurring in 3–15% of severe cases in these series, usually as part of multiorgan dysfunction. This contrasts with severe falciparum malaria, in which acute kidney injury is markedly less common and is less severe among children than in adults (Anstey *et al.* 1996; Hien *et al.* 1996; Weber *et al.* 1999; Dondorp *et al.* 2008b). In paediatric series describing both severe falciparum and severe vivax malaria, renal impairment occurred with a lower (Kochar *et al.* 2010) or similar (Manning *et al.* 2011; Yadav *et al.* 2012) frequency in those with *P. vivax* infection. Mortality rates of up to 30% have been reported in children from north-west India with PCR-confirmed *P. vivax* mono-infection-associated AKI, with all deaths having associated multiorgan dysfunction (Kochar *et al.* 2010). In contrast, in Vietnam and Thailand, where the two infections have similar prevalence, excluding massive haemolysis in G6PD deficiency, AKI in paediatric vivax malaria is not seen (TT Hien, NJ White; personal communication). The reasons for these substantial differences are unclear.

Other *P. vivax* series have reported AKI associated with thrombotic microangiopathy (Saharan *et al.* 2008; Sinha *et al.* 2012) in both adults and children, and haemolytic-uraemic syndrome (HUS) in children (Sharma *et al.* 1993). In the largest series (four adults and five children), persistent vivax-associated AKI was associated with thrombocytopenia, bleeding, anaemia, splenomegaly, with schistocytes on peripheral film in 77% (Sinha *et al.* 2012); one of the patients with schistocytes had concomitant coma (Sinha *et al.* 2012). Biopsies showed universal ischaemia and endothelial injury and arteriolar thrombi in two cases, consistent with thrombotic microangiopathy (Sinha *et al.* 2012). Fatal AKI associated with crescentic glomerulonephritis (Patel *et al.* 2012) and nephrotic syndrome (more commonly associated with mesangioproliferative and membranoproliferative glomerulonephritis from *P. malariae* (Gilles & Hendrickse 1960, 1963 ; van Velthuysen & Florquin 2000) has also been reported in association with *P. vivax* infections (Bircan *et al.* 1997; David *et al.* 2009). Acute glomerulonephritis in malaria (of any species) is very rare, so the pathological role of *P.vivax* in these latter cases remains uncertain.

Shock and multiorgan dysfunction. Shock has been associated with adult (Kochar *et al.* 2005, 2009; Song *et al.* 2007; Alexandre *et al.* 2010; Barber *et al.* 2012) and paediatric (Kochar *et al.* 2010; Kaushik *et al.* 2012) *P. vivax* infections usually as part of multiple organ dysfunction. Culture of blood and other fluids was not reported, and bacterial co-infection and bacterial sepsis are possible synergistic or alternative causes. Shock was present in 54% of Brazilian children requiring ICU admission in Brazil in which negative pre-antibiotic blood cultures were reported; however, 38% of these had an identified additional infectious comorbidity potentially contributing to shock (Lanca *et al.* 2012).

Bacterial co-infection and bacteraemia. The bacteraemia risk in severe *P. vivax* infection has not been studied systematically. A large prospective blood culture and malaria microscopy study in Kolkata, India, showed that 6 of 89 uncomplicated vivax malaria cases [6.7% (95%

CI: 3.1–13.9%)] had concomitant bacteraemia, with 5/6 (83%) of the bacteria isolated being Gram-negative (one *S. typhi*, one *S. paratyphi* A, three other enterobacteriaceae) (Bhattacharya *et al.* 2013). Of the two cases of *Salmonella* bacteraemia reported from Thailand in association with PCR-confirmed *P. vivax* mono-infection, one patient had multiorgan dysfunction (renal failure, jaundice and hypotension), with concurrent serogroup D *Salmonella* bacteraemia, and the other uncomplicated, *P. vivax* case had non-typhoidal *Salmonella* bacteraemia (Piyaphanee *et al.* 2007). Gram-positive (*Streptococcus pneumoniae*) bacteraemia has also been reported in association with hypotension in adult vivax malaria (Barber *et al.* 2012).

Coma and other vivax-associated neurological complications. Coma is much less common in vivax than falciparum malaria. Over 100 cases of *P. vivax* mono-infection in adults and children associated with coma have been described in reports or series from 1921 to 2011; however, only studies in the last 15 years have been able to exclude mixed species infection with *P. falciparum* by PCR methods (Beg *et al.* 2002; Kochar *et al.* 2005; Thapa *et al.* 2007; Sarkar & Bhattacharya 2008; Harish & Gupta 2009; Kasliwal *et al.* 2009; Kochar *et al.* 2009; Parakh *et al.* 2009; Thapa *et al.* 2009; Kochar *et al.* 2010; Lampah *et al.* 2011; Tanwar *et al.* 2011). In Indonesian Papua, coma associated with PCR-confirmed *P. vivax* mono-infection (and without overt comorbidities) occurred 23 times less frequently than with falciparum malaria and was estimated as occurring in one in 29 500 infections (Lampah *et al.* 2011). In Thailand, the risk of hospitalisation with impaired consciousness with microscopy-diagnosed *P. vivax* (not-PCR-confirmed) was 1 in 858 infections, with the risk being 15.2-fold less than that with *P. falciparum* (Luxemburger *et al.* 1997). In over 1000 prospectively studied patients with severe malaria studied in a specialist unit in Vietnam, one patient (with coma) had vivax malaria (TT Hien, personal communication). Most other reports of coma in association with PCR-confirmed *P. vivax* mono-infection have been from the Indian subcontinent; however, denominators of the surveillance and population-based risks were not reported. Only one case with no identified alternative aetiology has been reported from South America, a PCR-confirmed *P. vivax* mono-infection (Lacerda *et al.* 2012).

No reports of coma in vivax malaria, including those with PCR-confirmed *P. vivax* mono-infection, have reported complete, systematic microbiological and radiological investigation to exclude bacterial and viral infection and other causes of coma. Such studies are needed. In autopsies in two Brazilian children with vivax-associated coma, encephalitis was identified in one (Lanca *et al.* 2012) and could not be excluded in the other (Lacerda *et al.* 2012). In a prospective series of 24 cases of coma associated with *P. vivax* infection diagnosed by microscopy, 75% had PCR evidence of *P. falciparum* (co)infection or other bacterial or non-infective causes of coma; almost all PCR-confirmed cases of coma in *P. vivax* mono-infection in this series were in young adults with low parasitaemia, no other organ dysfunction and good outcomes (Lampah *et al.* 2011). *Plasmodium vivax* -associated coma has been associated with thrombotic thrombocytopenic purpura in one (Sinha *et al.* 2012), but not another series (Lampah *et al.* 2011). Other adult series with *P. vivax* mono-infection have described coma in association with multiorgan dysfunction (Kochar *et al.* 2005, 2009). A post-malarial neurological syndrome with tremor and myoclonus has also been reported after recovery from PCR-confirmed *P. vivax* coma (Lampah *et al.* 2011), similar to that occasionally seen following coma in falciparum malaria (Nguyen *et al.* 1996).

Other, rarer, neurological complications reported in association with *P. vivax* infection include facial diplegia (Kochar *et al.* 2007; Sim *et al.* 2010), acute inflammatory polyneuropathy (Chakravarty *et al.* 2004), acute disseminated encephalomyelitis (Koibuchi *et al.* 2003) and anterior ischaemic optic neuropathy (Flowe *et al.* 2011). As in many of these observations and associations, causality is uncertain. While not seen in a series of uncomplicated adult vivax malaria in Bangladesh (Abu Sayeed *et al.* 2011), cases of retinal haemorrhages in pure vivax malaria have been described elsewhere without central nervous system complications (Choi *et al.* 2004; Lee *et al.* 2010b).

Splenic rupture and infarction. Splenic rupture is a life-threatening but probably under-reported complication in adults (Moses 1945; Kapland *et al.* 1946; Kitchen 1949a; Lubitz 1949; Imbert *et al.* 2009; Lacerda *et al.* 2012). In a systematic review of 55 cases of malaria-associated splenic rupture, the mortality rate was 22%, with *P. vivax* accounting for approximately half of all cases (Imbert *et al.* 2009). In the 2012 Brazilian *P. vivax* autopsy series, three patients (18%) had splenic rupture,

all adults, two of whom also had pulmonary oedema (Lacerda *et al.* 2012). Of the four deaths considered directly caused by *P. vivax*, splenic rupture occurred in two (Lacerda *et al.* 2012).

Other complications. Jaundice is common in both adult [36–57%; (Kochar *et al.* 2005, 2009; Alexandre *et al.* 2010; Andrade *et al.* 2010)] and paediatric (Alexandre *et al.* 2010; Kochar *et al.* 2010; Jat *et al.* 2012; Lanca *et al.* 2012) series of severe vivax malaria, often in association with other severe manifestations. However, in Brazilian children, jaundice did not predict need for ICU admission (Lanca *et al.* 2012). Severe epistaxis associated with thrombocytopenia requiring blood and platelet transfusions was reported in 5% of adults with severe vivax malaria (Kochar *et al.* 2009), and thrombocytopenia was associated with other severe manifestations (Kochar *et al.* 2005; Kochar *et al.* 2009; Andrade *et al.* 2010; Alexandre *et al.* 2010). Fatal pulmonary haemorrhage and haematemesis have been reported (Jat *et al.* 2012). Hypoglycaemia (blood glucose <2.2 mM or <40 mg/dl) (World Health Organization 2010a) was found in 12.5% of children with vivax malaria requiring admission to intensive care unit in Brazil (Lanca *et al.* 2012). Less common disease associations with *P. vivax* include haemoglobinuria in the absence of G6PD deficiency (Kochar *et al.* 2010) and peripheral gangrene (Raghunandan *et al.* 2012). Acalculous cholecystitis has been described in both adults and children (Curley *et al.* 2011), with gall bladder wall oedema and periportal oedema described in 32% and 35% of 34 vivax malaria patients undergoing CT scanning for abdominal pain (Kim *et al.* 2010). Series from the 1940s reported patients with acute lower abdominal pain associated with vivax malaria, but not with splenomegaly, mimicking appendicitis and other acute surgical conditions (Most & Hayman 1946; Kitchen 1949b).

Vivax malaria in pregnancy

Effects on mother. In contrast to *P. falciparum*, *P. vivax* rarely causes severe malaria in pregnant women (Nosten *et al.* 1999; Poespoprodjo *et al.* 2008; McGready *et al.* 2012a,b; Rijken *et al.* 2012a), and in a large series from the Thailand–Myanmar border, no maternal deaths were associated with vivax malaria over a 25-year period (McGready *et al.* 2012a). Despite its rarity in very large series from Thailand and Indonesia (Nosten *et al.* 1999; Poespoprodjo *et al.* 2008; McGready *et al.* 2012b), severe vivax-associated maternal malaria, including severe anaemia, has been reported in small series from India (Kochar *et al.* 2005; Nayak *et al.* 2009) with poor pregnancy outcomes but again with no maternal deaths. In all endemic regions, less severe vivax-associated maternal anaemia is common, being approximately twice as likely in pregnant women infected with *P. vivax* than without (Nosten *et al.* 1999; Poespoprodjo *et al.* 2008).

Effects on fetus and neonate. Plasmodium vivax infection in pregnancy causes a reduction in birthweight (median 108 g) (Nosten *et al.* 1999; Poespoprodjo *et al.* 2008; Rijken *et al.* 2012a) approximately 70% of that observed following maternal falciparum malaria (median 150–192 g). With low birthweight contributing to greater infant mortality (Luxemburger *et al.* 2001), *P. vivax* in pregnancy is thus responsible for substantial indirect mortality in the first year of life. A single episode of *P. vivax* infection in the first trimester also increases risk of miscarriage 2.7-fold and fourfold with asymptomatic and symptomatic malaria, respectively (McGready *et al.* 2012b), a rate approximating that with *P. falciparum* (McGready *et al.* 2012b).

Congenital malaria. Plasmodium vivax can cause congenital malaria (McGready *et al.* 2004; Valecha *et al.* 2007; Vottier *et al.* 2008; Del Punta *et al.* 2010; Poespoprodjo *et al.* 2011; Liu *et al.* 2012), through transplacental infection *in utero* or during delivery (Poespoprodjo *et al.* 2011; Rijken *et al.* 2012a). In Papua, *P. vivax* congenital infection (alone or mixed) occurred in 1.6 per 1000 live births (Poespoprodjo *et al.* 2011); congenital malaria was independently associated with low birthweight and was mostly asymptomatic at birth (Poespoprodjo *et al.* 2011). Like congenital falciparum malaria (Poespoprodjo *et al.* 2010), congenital vivax malaria can cause severe illness mimicking neonatal sepsis (Del Punta *et al.* 2010).

Severe and fatal complications of primaquine

Morbidity and mortality secondary to adverse effects of primaquine are likely to be underestimated in populations with unmonitored use of primaquine and high prevalence of G6PD deficiency (Baird & Surjadjaja 2010). Primaquine-induced haemolysis in patients with G6PD deficiency can cause life-threatening AKI and severe anaemia, accounting for 8% of *P. vivax*-associated intensive care admissions in Brazil (Lanca *et al.* 2012) and 12% of deaths in the Manaus autopsy series (Lacerda *et al.* 2012). Severe haemolysis results from multiple dosing in radical treatment regimens. Overall, 14 deaths from primaquine toxicity have been reported over the past

60 years (Recht *et al.* 2013) despite over 11 million documented exposures (mainly in the course of mass drug administrations).

Risk factors for severe vivax malaria

As in falciparum malaria (Miller *et al.* 2002), host, parasite and socio-geographical factors likely contribute to risk of severe disease and death in vivax malaria, as well as specific severe manifestations.

Host and parasite genetics. *P. vivax* uses the red cell Duffy antigen as its main receptor for red cell invasion, so people who are Duffy negative (such as those in most of sub-Saharan Africa) are largely protected against vivax malaria. Genetic polymorphisms have been associated with both increased risk [alpha- and beta-thalassaemia (Williams *et al.* 1996; O'Donnell *et al.* 2009)] and decreased risk [Duffy antigen negativity (Miller *et al.* 1976), G6PD deficiency (Leslie *et al.* 2010) and ovalocytosis (Rosanas-Urgell *et al.* 2012)] of *P. vivax* parasitaemia. The role of ovalocytosis in protecting against vivax anaemia and other severe disease syndromes is less well defined. Alpha-thalassaemia has been associated with reduced risk of severe anaemia from *P. falciparum* (Fowkes *et al.* 2008), attributed to a lesser reduction in haemoglobin from the loss of microcytic cells, and has been hypothesised to protect against vivax anaemia through a similar mechanism (Fowkes *et al.* 2008). Whether naturally acquired *P. vivax* strains vary in virulence and risk of severe vivax malaria is unknown.

Chloroquine resistance. A high prevalence of chloroquine-resistant *P. vivax* (Sumawinata & Bernadeta Leksana 2003; Ratcliff *et al.* 2007) has been associated with a high risk of severe vivax anaemia (Tjitra *et al.* 2008). The prevalence of chloroquine-resistant *P. vivax* is increasing across vivax-endemic areas (Douglas *et al.* 2010), and its role in contributing to severe vivax disease warrants further investigation (Price *et al.* 2009).

Mixed Plasmodium infections. In areas of low malaria endemicity such as Thailand, mixed infections with *P. vivax* appear to attenuate *P. falciparum* disease severity (Luxemburger *et al.* 1997; Price *et al.* 2001; Mayxay *et al.* 2004; Snounou & White 2004). Conversely, in areas with higher endemicity of both species, mixed infections are associated with an increased risk of severe malaria (Genton *et al.* 2008; Tjitra *et al.* 2008), including severe anaemia (Genton *et al.* 2008; Tjitra *et al.* 2008; Douglas *et al.* 2013), coma (Manning *et al.* 2011) and death (Manning *et al.* 2011; Yadav *et al.* 2012).

Pathogenesis of disease in vivax malaria

Comparative pathobiology of *Plasmodium vivax*

There are significant differences in pathobiology between *P. vivax* and *P. falciparum* that are important in understanding vivax pathophysiology (Kitchen 1949b; Anstey *et al.* 2009).

Parasite biomass. In contrast to the invasion of red cells of all ages by *P. falciparum*, *P. vivax* has a very strong predilection for infecting red blood cells that have emerged from the bone marrow within the preceding 2 weeks (Kitchen 1949b; Simpson *et al.* 1999), particularly early in the course of infection (Kitchen 1938). This property contributes to the lower parasite biomass seen in *P. vivax* infections. Unlike *P. falciparum* infections, parasitaemia densities in vivax malaria rarely exceed 2% of circulating erythrocytes (Ross & Thomson 1910; Kitchen 1949b; Field & Shute 1956). Although a high *P. vivax* parasite burden (140 000/μl) has been reported in fatal vivax malaria (Valecha *et al.* 2009), this is very unusual. Although series reporting parasite counts in severe vivax malaria have documented moderately high parasitaemias, all others have been less than 100 000/μl (about 2% parasitaemia): (Kochar *et al.* 2005; Alexandre *et al.* 2010) (Kochar *et al.* 2009), (Kochar *et al.* 2010; Barber *et al.* 2012). Nevertheless, an association between disease severity and semi-quantitative parasitaemia (1+ to 4+) has been reported (Lanca *et al.* 2012; Nurleila *et al.* 2012). It has been hypothesised that total parasite biomass in uncomplicated and severe malaria may be higher than that represented by peripheral parasitaemia due to accumulation of parasitised red cells in organs such as the spleen (Machado Siqueira *et al.* 2012; Baird 2013); however, this requires further investigation.

Relapse. A fundamental difference from *P. falciparum* is that *P. vivax* can relapse from dormant hypnozoites to cause repeated episodes of clinical and subclinical infections. Frequent recurrent infections may result in insufficient time for adequate haematological recovery from each episode. Multiple recurrences are associated with greater anaemia (Douglas *et al.* 2013). In contrast, in temperate areas, relapses are fewer and delayed, and the haematological impact of each recurrence is less (Song *et al.* 2003; Goller *et al.* 2007; Price *et al.* 2009).

Inflammatory responses. *Plasmodium vivax* has a lower pyrogenic threshold than *P. falciparum* (Ross & Thomson 1910; Kitchen 1949a). Cytokine production (Karunaweera *et al.* 1992; Hemmer *et al.* 2006; Yeo *et al.* 2010b; Goncalves *et al.* 2012), degree of endothelial activation (Yeo *et al.* 2010b) and pulmonary inflammatory responses (Anstey *et al.* 2007) are higher during and after *P. vivax* infections than in *P. falciparum* infections with similar parasitaemias. Although the inflammatory correlates of the lower pyrogenic threshold have been described, the underlying mechanism(s) have not. Hypothesised reasons include differences between the two species in candidate 'malaria toxin(s)' (Anstey *et al.* 2012). A lipid unique to *P. vivax* (Karunaweera *et al.* 2003, 2007) may also contribute to the greater pyrogenicity of *P. vivax*. It is possible that *Plasmodium* spp. priming of the innate immune response to bacterial products (Franklin *et al.* 2009) may be greater in *P. vivax* than in *P. falciparum* infections, although this remains to be demonstrated. Although *P. vivax* is capable of eliciting greater concentrations of both pro- and anti-inflammatory cytokines than *P. falciparum* (Hemmer *et al.* 2006; Yeo *et al.* 2010b; Goncalves *et al.* 2012), relationships with disease severity may be different in vivax malaria.

Cytoadherence and rosetting. As all stages of *P. vivax* are visible in peripheral blood, albeit with partial depletion of mature stages (Rudolf & Ramsay 1927; Field & Shute 1956), sequestration is not thought to occur to a significant degree in vivax malaria; so microvascular obstruction causing end-organ dysfunction as in *P. falciparum* is not thought to occur (Anstey *et al.* 2009). Despite *in vitro* evidence of cytoadherence to ICAM-1 (Carvalho *et al.* 2010) and chondroitin sulphate A (CSA) (Chotivanich *et al.* 2012), evidence for sequestration-mediated pathology in vivax malaria *in vivo* is absent (Valecha *et al.* 2009) or at best modest (Ewing 1901; Billings & Post 1915; Bruetsch 1932; Anstey *et al.* 2007; Lacerda *et al.* 2012; Anstey *et al.* 2012 for review), although splenic accumulation of infected and uninfected red cells as in falciparum malaria seems likely (Machado Siqueira *et al.* 2012; Baird 2013). Published placental histology is even more limited, but has shown an absence (McGready *et al.* 2004) or limited presence (Mayor *et al.* 2012) of placental *P. vivax*-infected red cells. Taken together, histological findings suggest that significant microvascular obstruction from sequestration of parasitised red cells does not occur in vivax malaria, although it is possible that in some circumstances and in some organs, more limited cytoadherence to endothelial cells may occur. Rosetting, adherence of non-infected to infected RBCs *in vitro* (Udomsanpetch *et al.* 1995) is a consistent finding in *P. vivax* malaria, although its role in vivax pathophysiology is unknown.

Deformability and fragility of parasitised erythrocytes. In contrast to *P. falciparum* (Dondorp *et al.* 1999), the deformability of vivax-infected RBCs is increased (Suwanarusk *et al.* 2004; Handayani *et al.* 2009). This may enable *P. vivax* to pass through the narrow interendothelial slits of the splenic sinusoids (Handayani *et al.* 2009; Deplaine *et al.* 2011). Increased deformability may, however, be accompanied by increased fragility of both *P. vivax*-infected and non-infected RBCs (Handayani *et al.* 2009), although the extent to which this is an artefact of the *ex vivo* microfluidic system used is unknown.

Endothelial activation and altered thrombostasis. Concentrations of circulating endothelial activation markers are as high (ICAM-1 and E-selectin) or higher (angiopoietin-2), in uncomplicated vivax malaria than in falciparum malaria (Jakobsen *et al.* 1994; Yeo *et al.* 2010b). Endothelial dysfunction and impaired NO bioavailability may be significant contributors to severe falciparum malaria (Yeo *et al.* 2007, 2009, 2010a), but their role in severe vivax malaria is unknown. Endothelial activation and damage have been described in fatal vivax-associated ARDS (Valecha *et al.* 2009). Increased procoagulant activity (Hemmer *et al.* 2006), vWF (De Mast *et al.* 2009) and ADAMTS-13 deficiency (De Mast *et al.* 2009) occur in uncomplicated vivax malaria; however, the role of altered haemostatic pathways, intravascular coagulation and endothelial inflammation through increased formation of ultra-large VWF and platelet aggregates in severe vivax malaria is not known.

Pathophysiology of specific syndromes of severe vivax malaria

Severe vivax anaemia. The aetiology of vivax-associated anaemia is complex. Anaemia results from loss of both vivax-infected and uninfected red cells from the circulation and impaired RBC production (Wickramasinghe *et al.* 1989; Anstey *et al.* 2009; Douglas *et al.* 2012). Malaria therapy data demonstrated that removal of red cells from the circulation is particularly pronounced during acute infection (Kitchen 1938; Collins *et al.* 2003), over and above the rate modelled from reticulocyte loss and impaired supply of mature red cells (McQueen & McKenzie 2004; Antia *et al.* 2008). The removal of red cells occurs from both extravascular loss in the spleen and from intravascular red cell loss (Douglas *et al.* 2012). Despite the lower parasite biomass of *P. vivax* relative to *P. falciparum*, red cell removal is comparable

because of the greater proportional removal of uninfected red cells following *P. vivax* infection. In vivax malaria, approximately 34 uninfected red cells are removed for every infected red cell (Collins *et al.* 2003; Douglas *et al.* 2012) compared to approximately 8 uninfected red cells for every infected red cell in falciparum malaria (Jakeman *et al.* 1999; Price *et al.* 2001). Mechanisms for this difference are not clear. Although greatest during the early stages of infection, enhanced removal of uninfected red blood cells has been shown to persist for at least 5 weeks after antimalarial treatment (Woodruff *et al.* 1979; Looareesuwan *et al.* 1987). An additional contributory factor in the anaemia of *P. vivax* is relapse. In tropical latitudes, relapses at 3- to 4-week intervals are associated with progressive anaemia from recurrent episodes of haemolysis and dyserythropoiesis before haematological recovery from preceding infections can occur (Price *et al.* 2009). In some regions, severe vivax anaemia is common in children less than 2 months of age (Poespoprodjo *et al.* 2009); multiple relapses cannot explain severe anaemia at such an early age. Sustained parasitaemia, initially subclinical, from congenital malaria may the explanation. In areas of chloroquine resistance, anaemia is exacerbated further by delayed parasite clearance, and recrudescent and chronic infections (Price *et al.* 2007). Impaired production of red cells is also likely to contribute to vivax-related anaemia, particularly with chronic and repeated infections, but mechanisms are unclear. Cytokine-related dyserythropoiesis has been demonstrated in *P. vivax* malaria in adults (Wickramasinghe *et al.* 1989). In adults, erythroblasts can be infected by *P. vivax in vivo* (Ru *et al.* 2009).

Acute lung injury. Even in uncomplicated vivax malaria, clinical pulmonary function testing shows increased pulmonary phagocytic cell activity associated with reduced gas transfer at the alveolar–capillary membrane (Anstey *et al.* 2002, 2007). Autopsy findings in *P. vivax* ARDS have shown heavy intravascular monocytic infiltrates with diffuse endothelial and alveolar damage (Valecha *et al.* 2009) or interstitial infiltrates with predominantly neutrophils (Lacerda *et al.* 2012). While alveolar–capillary parasites after peripheral blood clearance have been reported at autopsy in vivax-associated acute lung injury (Lacerda *et al.* 2012), another severe vivax ARDS autopsy did not show sequestration of parasitised red blood cells in the pulmonary vasculature (Valecha *et al.* 2009). As in acute lung injury in other disease settings, ARDS in vivax malaria probably results from soluble mediators, diffuse alveolar-endothelial capillary damage, exacerbated by shock, with increases in alveolar permeability and altered alveolar fluid clearance (Suratt & Parsons 2006; Tan *et al.* 2008; Valecha *et al.* 2009). Onset of most but not all vivax-associated ARDS occurs after starting antimalarial treatment (Anstey *et al.* 2009; Taylor *et al.* 2012b) with gas transfer studies showing progressive deterioration in alveolar–capillary function following treatment (Lomar *et al.* 2005; Anstey *et al.* 2007).

Acute kidney injury. Both the true incidence and the mechanisms underlying AKI in vivax malaria are not clear. In prospective studies of adult severe malaria conducted in South-East Asia, with the exception of haemoglobinuric renal failure in G6PD deficiency, acute renal failure has never been observed in vivax malaria (Trang *et al.* 1994; T.T. Hien & N. J. White, unpublished observations). Whether acute tubular necrosis underlies AKI in vivax malaria in the setting of multiorgan dysfunction or acute cortical necrosis, as reported in one series (Kute *et al.* 2012b), requires further study. Over a third of fatal cases in Manaus, Brazil, had AKI (as part of multiorgan dysfunction) (Lacerda *et al.* 2012). The extent to which thrombotic microangiopathy causes vivax-associated AKI (Sharma *et al.* 1993; Saharan *et al.* 2008; Sinha *et al.* 2012) is also not known. Elevated vWF and low levels of the vWF-cleaving protein ADAMTS13 occur even in uncomplicated in vivax malaria (De Mast *et al.* 2009) although they are linked to disease severity in falciparum malaria (Larkin *et al.* 2009) and so might conceivably underlie vivax thrombotic microangiopathy in these patients.

Coma. The true incidence and the aetiology of the coma associated with *P. vivax* are not known, and the role of co-infections remains unclear (Anstey *et al.* 2009; Lampah *et al.* 2011). The rarity of coma in *P. vivax* relative to *P. falciparum* and its absence in *P. knowlesi* (Daneshvar *et al.* 2009; William *et al.* 2011; Barber *et al.* 2012) makes it unlikely that the cerebrovascular sequestration of parasitised red cells characteristic of *P. falciparum* occurs in the same way with these other parasites. This contention is supported by the absence of *P. vivax* DNA, albeit post-treatment, in a single Brazilian autopsy case of coma attributed to *P. vivax* (Lacerda *et al.* 2012) and brain aspirates from three PNG children with fatal coma associated with mixed *P. falciparum* – *P. vivax* infection (Manning *et al.* 2012).

Multiorgan dysfunction and shock. The extent to which multiorgan dysfunction and shock are attributable to sepsis-like inflammatory responses to *P. vivax*, concurrent bacteraemia (Sur *et al.* 2006; Piyaphanee *et al.* 2007; Barber *et al.* 2012), or both, requires further

study. Negative blood cultures reported in some series are limited by widespread pre-hospitalisation use of antibiotics in the community, and the known insensitivity of pre-antibiotic blood cultures in bacterial sepsis (Davis *et al.* 2011).

Pregnancy-associated malaria and low birthweight. There are few reports of placental histopathology, showing absent (McGready *et al.* 2004) or modest (Mayor *et al.* 2012) placental parasite burden. Placental histopathology shows little (McGready *et al.* 2004) or no (Mayor *et al.* 2012) hemozoin deposition, and largely absent inflammatory changes (McGready *et al.* 2004). Pathological mechanisms are unknown, but vivax-associated microvascular dysfunction may cause deleterious uteroplacental haemodynamic effects and foetal growth restriction (McGready *et al.* 2004; Rogerson *et al.* 2007; Umbers *et al.* 2011; Rijken *et al.* 2012b). Maternal anaemia, common in vivax malaria (Poespoprodjo *et al.* 2008; Rijken *et al.* 2012a), is likely to be an additional contributor to low birthweight (Rogerson *et al.* 2007).

Diagnosis and definition of severe vivax malaria

The recent series from Brazil suggests that criteria in previous WHO Guidelines for severe falciparum malaria (World Health Organization 2000, 2010a) are sensitive in identifying children requiring intensive care admission (Lanca *et al.* 2012) and in identifying patients at risk of death (Lacerda *et al.* 2012). Criteria for severe vivax malaria (Box) are the same as for severe falciparum malaria with the exclusion of parasite density thresholds. This is because of the unclear association to date between parasitaemia and disease severity and outcome in *P. vivax* malaria, and the greater propensity for *P. vivax* to destroy uninfected red cells.

Microscopy remains the gold standard for the immediate diagnosis of *P. vivax* infection in severe disease. The sensitivity of parasite LDH-based rapid antigen tests for *P. vivax* has improved recently (who.int/tdr/publications/tdr-research-publications/rdt_round3/pdf/rdt3.pdf), with high sensitivity reported in severe vivax malaria (Barber *et al.* 2013a). *P. falciparum* -specific HRP2 rapid antigen tests have proved useful in identifying occult mixed species infections (Mayxay *et al.* 2001, Lampah *et al.* 2011), with PCR used as a delayed gold standard in excluding misdiagnosis and mixed infections. Aggressive microbiological and radiological investigations for alternative causes (both infectious and non-infectious) of severe disease are essential.

Management of severe vivax malaria

In the absence of comparative drug trials in severe vivax malaria, when it does occur, severe disease in vivax malaria has been managed in the same way as severe falciparum malaria (World Health Organization 2010a; Price *et al.* 2011). Both quinine (Tjitra *et al.* 2008) and artesunate (Tjitra *et al.* 2008; Kochar *et al.* 2010; Lampah *et al.* 2011; Barber *et al.* 2012) have been used successfully. Therapeutic responses in uncomplicated vivax malaria are significantly faster following artesunate. Given the importance of starting highly effective schizontocidal treatment as quickly as possible in severe falciparum malaria, the magnitude of the benefit of artesunate compared to quinine in severe falciparum malaria, the difficulties of discriminating between *P. vivax* and *P. falciparum*, the relative safety of artesunate compared with quinine, the high sensitivity of *P. vivax* to artemisinins and ease of use and the operational benefits of a unified approach to the treatment of vivax and falciparum malaria (Douglas *et al.* 2010), the current unified policy of intravenous artesunate for severe malaria from all *Plasmodium* species is pragmatic and appropriate (Price *et al.* 2011). Other supportive care for severe malarial manifestations, including transfusion, intravenous antibiotics, vasopressor support, dialysis and invasive ventilation as needed, should be given as per recommendations for severe falciparum malaria.

Epidemiological or research definition of severe vivax malaria

As for the criteria for adults and children with severe falciparum malaria but with no parasitaemia thresholds and without a criterion of hyperparasitaemia.

Section 14: Severe knowlesi malaria

The simian parasite, *Plasmodium knowlesi,* was first shown to be transmissible to humans in 1932 (Knowles & Das Gupta 1932) and has more recently been identified as a common cause of human malaria in Malaysian Borneo (Singh *et al.* 2004; Cox-Singh *et al.* 2008; Daneshvar *et al.* 2009; William *et al.* 2011; World Health Organization 2011a,b; Barber *et al.* 2012; Rajahram *et al.* 2012), with isolated reports from elsewhere in South-East Asia. The potential of *P. knowlesi* to cause severe disease was originally suggested by experimental simian and human infections (Ciuca *et al.* 1955, 1964; Chin *et al.* 1968; Coatney *et al.* 1971; White 2008). The full asexual life cycle takes only 1 day, so expansion of the infection can be very rapid. In total, 86 human cases of severe and/or fatal knowlesi malaria have been reported since 2008 (Cox-Singh *et al.* 2008, 2010; Daneshvar *et al.* 2009; Lee *et al.* 2010a; William *et al.* 2011; Barber *et al.* 2012, 2011; Fatih *et al.* 2012; Rajahram *et al.* 2012).

Transmission and epidemiology

The natural monkey hosts (long-tailed and pig-tailed macaques) and mosquito vectors (*Anopheles leucosphyrus* group) for *P. knowlesi* extend across South-East Asia from eastern India and southern China to Indonesia (Singh & Daneshvar 2013). While this area has a population of approximately 500 million people potentially at risk of *P. knowlesi* infection, and thousands of cases have been reported from Malaysia, the true burden of disease is not known. Human *P. knowlesi* infections have also been reported from China–Myanmar (Jiang *et al.* 2010), Thailand (Jongwutiwes *et al.* 2004; Putaporntip *et al.* 2009; Jongwutiwes *et al.* 2011; Sermwittayawong *et al.* 2012), Vietnam (van den Eede *et al.* 2009), Cambodia (Khim *et al.* 2011), Philippines (Luchavez *et al.* 2008), peninsular Malaysia (Fong *et al.* 1971; Cox-Singh *et al.* 2008; Lee *et al.* 2010a), Indonesia (Figtree *et al.* 2010; Sulistyaningsih *et al.* 2010) and Singapore (Ng *et al.* 2008), although concerns have been raised regarding specificity of the PCR assays used in the studies of archival samples (Imwong *et al.* 2009).

Clinical spectrum

Plasmodium knowlesi causes disease ranging from uncomplicated to severe and fatal malaria (Cox-Singh *et al.* 2008, 2010; Daneshvar *et al.* 2009; Lee *et al.* 2010a; William *et al.* 2011; Barber *et al.* 2012; Fatih *et al.* 2012; Rajahram *et al.* 2012). Most cases of *P. knowlesi* have been reported in adults, although uncomplicated malaria and severe anaemia have also been described in children (Barber *et al.* 2011). Clinical features of uncomplicated knowlesi malaria are indistinguishable from other species (Daneshvar *et al.* 2009; Barber *et al.* 2012).

Severe malaria in adults

Of the 86 cases of naturally acquired severe human *P. knowlesi* infection reported between 2008 and 2012, all were from Malaysia of which 19 were fatal (Cox-Singh *et al.* 2008, 2010; Daneshvar *et al.* 2009; Lee *et al.* 2010a; William *et al.* 2011; Barber *et al.* 2012; Fatih *et al.* 2012; Rajahram *et al.* 2012), with progression to death occurring in as little as 3 days after symptom onset (Cox-Singh *et al.* 2008). Knowlesi malaria is associated with a high risk of severe disease. In the largest prospective series, from a referral hospital in Sabah, 29% of knowlesi infections were severe and *P. knowlesi* infection was associated with a threefold greater risk of severe malaria than *P. falciparum* (Barber *et al.* 2012). In a prospective district hospital-based study in Sarawak, 9% of patients with *P. knowlesi* developed severe malaria, with a case-fatality rate of 2% (95% CI 0.2–6.6%) overall and 20% in severe disease (Daneshvar *et al.* 2009). In a retrospective tertiary referral centre study in Sabah, 39% of *P. knowlesi* admissions had severe malaria, with a case-fatality rate in severe disease of 27% (William *et al.* 2011). Reported case-fatality rates in quinine-treated severe disease of 20–27% are comparable to those reported in severe falciparum malaria (South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) Group 2005). While age, female gender, parasitaemia, schizontaemia and severity of thrombocytopenia have been associated with a higher risk of both severe disease and death (Daneshvar *et al.* 2009; Lee *et al.* 2010a; Cox-Singh *et al.* 2011; William *et al.* 2011; World Health Organization 2011a,b; Barber *et al.* 2012; Rajahram *et al.* 2012); on multivariate analysis, only parasitaemia and schizontaemia >10% were independent predictors of severe disease (Barber *et al.* 2012). Risk of severe disease from *P. knowlesi* occurs at lower parasitaemias than with *P. falciparum*, increasing 11-fold with parasitaemia >20 000/μl and 28-fold with parasitaemia >100 000/μl (Barber *et al.* 2012).

Multiorgan failure is common in severe knowlesi malaria (Cox-Singh *et al.* 2008, 2010; Daneshvar *et al.* 2009; William *et al.* 2011; Barber *et al.* 2012; Rajahram *et al.* 2012). In one series, 59% had respiratory distress; 55%, acute renal failure; and 55% shock (William *et al.*

2011). In non-artemisinin-treated patients, the proportions with hypoxaemia-associated respiratory distress and ARDS (59–70%) (Daneshvar *et al.* 2009; William *et al.* 2011) and shock (William *et al.* 2011) are higher than that reported in larger series of adult severe falciparum malaria (Lichtman *et al.* 1990; Aursudkij *et al.* 1998; Bruneel *et al.* 2003; Krishnan & Karnad 2003; Yeo *et al.* 2007; Dondorp *et al.* 2008a). However, ARDS is significantly less common in knowlesi malaria series treated with artemisinins (Barber *et al.* 2012). Severe anaemia is seen in both adults (Barber *et al.* 2012) and children (Barber *et al.* 2011). Metabolic acidosis, 'blackwater fever', jaundice and hypoglycaemia have also been reported (William *et al.* 2011; Barber *et al.* 2012).

Clinical spectrum in children

A single small series to date describes mostly uncomplicated disease in older children (Barber *et al.* 2011). As in adults, thrombocytopenia is nearly universal, with 94% having thrombocytopenia at diagnosis and 100% by day one of treatment. Anaemia is common, with 71% having haemoglobin <10 g/dl. Severe anaemia has also been reported (Barber *et al.* 2011).

Pregnancy

Severe disease has been reported in pregnancy, associated with intrauterine death (William *et al.* 2011). In one small series, two (18%) of women requiring hospital admission with knowlesi malaria were pregnant (William *et al.* 2011). Pregnancy may be a risk factor for severity as in falciparum malaria, but larger studies are needed.

Pathophysiology

Little is known about the pathophysiology of human knowlesi malaria. *Plasmodium knowlesi* has important differences from *P. falciparum*, including the 24-h blood-stage cycle (Knowles & Das Gupta 1932), and consequent potential for more rapid multiplication and progression to death (Cox-Singh *et al.* 2008). In simian studies, *P. knowlesi* infection in the long-tailed macaque, a natural host, is associated with mild clinical disease or asymptomatic carriage, while infections in the non-natural simian hosts such as the rhesus macaques and olive baboons result rapidly in death (Coatney *et al.* 1971; Ibiwoye *et al.* 1993; Praba-Egge *et al.* 2002). Experimental inoculation of *P. knowlesi* into olive baboons results in increased pro-inflammatory cytokine concentrations with widespread 'vascular congestion' of the capillaries with cellular necrosis in multiple organs demonstrated on histological examination (Praba-Egge *et al.* 2002). A similar histopathological appearance of widespread microvascular accumulation of parasitised and non-parasitised erythrocytes without platelet aggregation has been reported in multiple organs, including the brain, in a single autopsy report of an adult human who died from *P. knowlesi* multiorgan failure without coma (Cox-Singh *et al.* 2010). Although *P. knowlesi*-infected erythrocytes have been shown to bind to ICAM-1 *in vitro* (Fatih *et al.* 2012), ICAM-1 was not detected on brain endothelium in this autopsy, and no electron microscopy studies of endothelial cytoadherence have yet been reported. Early microvascular imaging studies of severe malaria in rhesus monkeys showed progressive intravascular agglutination of knowlesi-infected red cells, with microvascular endothelium becoming 'sticky to and solidly coated with, leucocytes' (Knisely & Stratman-Thomas 1945, 1948). Microvascular sludging of blood flow was associated with 'greatly slowed capillary circulation rates' before death (Knisely & Stratman-Thomas 1945, 1948). The absence of coma in severe knowlesi malaria suggests different mechanisms and/or consequences of parasite accumulation in brain microvasculature with *P. knowlesi* compared to the cytoadherence-mediated sequestration occurring in severe *P. falciparum* malaria.

Non-severe knowlesi malaria has a lower median parasitaemia than non-severe *P. falciparum*, indicating a lower fever threshold (pyrogenic density) with *P. knowlesi* than with *P. falciparum* (Barber *et al.* 2012), which may suggest a greater inflammatory response per parasitised red cell, as seen with *P. vivax* (Ross & Thomson 1910; Yeo *et al.* 2010a). In keeping with this, the neutrophil count is positively associated with both *P. knowlesi* parasitaemia (Cox-Singh *et al.* 2008; William *et al.* 2011; Barber *et al.* 2012) and disease severity (Cox-Singh *et al.* 2008; William *et al.* 2011; Barber *et al.* 2012). Consistent with a greater inflammatory response to *P. knowlesi* is a greater frequency of acute respiratory distress syndrome in non-artemisinin-treated severe knowlesi than in severe falciparum malaria (William *et al.* 2011). Plasma concentrations of the pro-inflammatory cytokine, TNFα, are associated with knowlesi disease severity, but levels of the anti-inflammatory cytokine IL-10 are not (Cox-Singh *et al.* 2011). Peptic ulceration has been reported in severe knowlesi malaria (Cox-Singh *et al.* 2008; Lee *et al.* 2010a; Barber *et al.* 2012) and could indicate gut ischaemia (White 2008). Concurrent Gram-negative bacteraemia has also been reported (William *et al.* 2011; Rajahram *et al.* 2012), but the true incidence of co-infection and its contribution to severe disease is not known.

Thrombocytopenia is associated with the severity of both falciparum and knowlesi malaria (Daneshvar *et al.*

2009; William *et al.* 2011; Barber *et al.* 2012), but patients infected with non-severe *P. knowlesi* have a more profound thrombocytopenia than patients with non-severe falciparum malaria (Daneshvar *et al.* 2009; William *et al.* 2011; Barber *et al.* 2012). Whether this reflects platelet activation contributing to pathophysiology (William *et al.* 2011) or consumption as part of a beneficial antiparasitic response (Cox-Singh *et al.* 2011) has not been determined. In one series, the only knowlesi-infected patients who did not develop thrombocytopenia had had previous splenectomy (Barber *et al.* 2012), suggesting a role for the spleen in platelet clearance.

Diagnosis and definition of severe knowlesi malaria

Mature trophozoites and schizonts of *P. knowlesi* are indistinguishable on thick film microscopy from *P. malariae* and subtle differences on thin film microscopy cannot distinguish the species reliably (Lee *et al.* 2009b; World Health Organization 2011a,b). Ring forms resemble *P. falciparum* leading to further misdiagnosis (Cox-Singh *et al.* 2008; Lee *et al.* 2009b; World Health Organization 2011a,b). Misdiagnosis as *P. vivax* also occurs (Cox-Singh *et al.* 2008; Barber *et al.* 2013b). All cases of malaria in knowlesi-endemic areas diagnosed as *P. malariae* should be treated immediately as *P. knowlesi* pending PCR-based diagnosis at a reference laboratory. Parasite LDH-based rapid antigen tests are not specific and insufficiently sensitive overall in *P. knowlesi* infections, although high sensitivity has been reported in severe knowlesi malaria (Barber *et al.* 2013a).

The *research definition of severe malaria* (Box), based on those used for severe *P. falciparum* malaria, is derived from severe knowlesi malaria series to date (Daneshvar *et al.* 2009; William *et al.* 2011; Barber *et al.* 2012), with relatively high specificity. The parasitaemia threshold defining hyperparasitaemia in *P. knowlesi* is >100 000/μl (compared to >10% with *P. falciparum*) with a parasitaemia threshold of 20 000/μl for jaundice (compared to >100 000/μl with *P. falciparum*). In clinical settings where laboratory assessment of severity criteria is not readily available, *a practical clinical definition for severity* requiring treatment with intravenous artesunate is inability to tolerate oral therapy, any clinical or laboratory severity criteria used for falciparum malaria or any parasitaemia >20 000/μl. The latter parasitaemia cut-off is more sensitive but less specific for severe disease, but nevertheless predicted an 11-fold greater risk of severe disease in the largest series to date (Barber *et al.* 2012).

Treatment of severe knowlesi malaria

Both quinine (Cox-Singh *et al.* 2008; Daneshvar *et al.* 2009; William *et al.* 2011) and more recently, artesunate (William *et al.* 2011; Barber *et al.* 2012) have been used. Chloroquine usage following misdiagnosis as *P. malariae* and delayed parenteral therapy have both been associated with fatal outcomes (Cox-Singh *et al.* 2008; Rajahram *et al.* 2012). In a retrospective review of treatment responses in severe knowlesi malaria, artesunate-treated patients had faster parasite clearance times, and the case-fatality rate (17%) was lower than in those who received quinine (31%) (William *et al.* 2011), although the study was not powered to demonstrate a statistically significant mortality advantage. In the largest prospective study of severe knowlesi malaria, early referral protocols and standardised (including pre-referral) use of intravenous artesunate were associated with zero mortality (Barber *et al.* 2012). Given the clear mortality advantage in severe falciparum malaria, faster parasite clearance time than quinine in *P. knowlesi* (William *et al.* 2011), demonstrated efficacy in severe knowlesi malaria (William *et al.* 2011; Barber *et al.* 2012), safety and ease of use (South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) Group 2005), parenteral artesunate is the treatment of choice for severe *P. knowlesi* infection (Barber *et al.* 2012). Other supportive care, including transfusion, intravenous antibiotics, vasopressor support, dialysis and invasive ventilation as needed, should be given as per recommendations for severe falciparum malaria.

Definition of severe malaria in *P. knowlesi* infection

Research definition

As for severe falciparum malaria (see Table 4) but with modified parasitaemia cut-offs:

- Parasite density >100 000/μl
- Jaundice and parasitaemia >20 000/μl

Clinical definition requiring treatment with intravenous artesunate:

- Inability to tolerate oral therapy
- Warning signs or clinical severity criteria as per severe falciparum malaria
- Parasitaemia >20 000/μl

*In settings where clinical or laboratory criteria of severity cannot be assessed.

Section 15: Pharmacology of antimalarials in severe malaria

Severe malaria is a medical emergency requiring immediate administration of rapidly effective antimalarial drugs. In the largest randomised trials ever conducted in severe malaria, the artemisinin derivative artesunate proved to be markedly superior to quinine in the parenteral treatment of severe malaria and is therefore now the treatment of choice for all patients (Dondorp *et al.* 2005a, 2010) (Figure 23). Artesunate has also considerably simplified the treatment of severe malaria, as it can be given by intravenous or intramuscular injection, has a simple dose regimen and requires no dose adjustments. If parenteral artesunate is not available, then intramuscular artemether is second choice and quinine third. For pre-referral treatment in the community, a rectal formulation of artesunate has been developed with satisfactory absorption kinetics. Given in the community before referral to hospital, this reduced the mortality of severe malaria in children by 25% (Gomes *et al.* 2009). As the malaria parasites causing illness are confined to the blood, it is the concentrations of antimalarial drugs in blood that determine the therapeutic response. Severe malaria affects the absorption, disposition, metabolism and elimination of many drugs. Reduced visceral blood flow (Molyneux *et al.* 1987; Pukrittayakamee *et al.* 1992, 1994) and microvascular sequestration may reduce oral and intramuscular drug absorption, respectively. Fortunately, the effects on parenteral artesunate pharmacokinetics are relatively small.

Mechanism of action

The exact mechanism of antimalarial action of artemisinin drugs is still unknown, but a reduction of the reactive peroxide bridge and the production of free radicals or other reactive intermediates are thought to be essential for antimalarial activity (Jansen & Soomro 2007; Haynes *et al.* 2010, 2012, 2011). The ferrous ion (Fe^{++}) in haem appears to be important for bioactivation as activity is reduced in the presence of iron chelators (desferrioxamine). Quinine is thought to interfere with haem detoxification (as do other quinolines and structurally related compounds).

Drug measurement

Antimalarial drug measurement has improved substantially in recent years. Pharmacokinetic studies in severe malaria need to be interpreted in the light of these advances. *Artemisinins* have posed particular measurement challenges. The peroxide bridge needed for antimalarial activity renders the artemisinins unstable and, in particular, susceptible to degradation mediated by iron (and other reactive cations) (Lindegardh *et al.* 2011, 2008). Thus, inherent properties of these drugs necessary for their potent antimalarial activity also create serious problems for quantification in biological samples. Early methods using UV detection following post-column online derivatisation reached limits of detection at 30 ng/ml (Edlund *et al.* 1984; Batty *et al.* 1996). Electrochemical detection (ECD) methods, which are notoriously difficult, were used more widely and reached detection limits of about 10 ng/ml (Navaratnam *et al.* 1997; Na-Bangchang *et al.* 1998). An indirect approach using a sensitive but non-specific bioassay was also used in early pharmacokinetic studies (Teja-Isavadharm *et al.* 1996; Bethell *et al.* 1997; Newton *et al.* 2000). None of these methods provide sufficient sensitivity and specificity for accurate characterisation of the pharmacokinetic properties of these drugs. The preferred method for quantification of the artemisinins [artesunate, artemether, artemotil and dihydroartemisinin (DHA)] is liquid chromatography tandem mass spectrometry (LC-MS/MS), which reaches quantification limits of about 1 ng/ml using only 50 μl plasma (Naik *et al.* 2005; Gu *et al.* 2008; Lindegardh *et al.* 2008, 2011; Hanpithakpong *et al.* 2009; Hodel *et al.* 2009). Artesunate, the most widely used of the artemisinin derivatives, acts as an ester pro-drug and is hydrolysed rapidly *in vivo* to the active metabolite DHA. This reaction can also occur *ex vivo* (i.e. after collection) both through chemical hydrolysis and esterase mediated hydrolysis and thus bias the analytical results (Lindegardh *et al.* 2008). Anticoagulants containing fluoride inhibit esterase hydrolysis but not chemical hydrolysis. All artemisinin derivatives are thermolabile with improved stability at lower temperatures (e.g. 4 °C or ice). Stability of these drugs in plasma varies with source. Some methods have carried out the bioanalytical work on ice to minimise degradation, while others have conducted analysis at ambient temperature. Artemisinins are relatively stable in haemolysed plasma but are degraded rapidly when they come into contact with organic solvents during sample processing (Lindegardh *et al.* 2011). The result is that analysis of these drugs in haemolysed samples or samples containing active haemolytic products are biased (concentrations usually underestimated) unless the method uses a suitable internal standard (i.e. stable isotope labelled artesunate, artemether or DHA) that compensates fully for this. In general, these confounders result in underestimation of the drug concentrations. In summary, multiple pitfalls in sample separation, storage, preparation and analysis have undoubtedly contributed to the large intersubject variation in plasma concentration profiles reported, and the derived pharmacokinetic parameters quoted for these drugs.

Quinine has been measured using increasingly sensitive and precise methods over the past 70 years. The initial spectrophotometric methods developed over sixty years ago could not distinguish the parent compound from the metabolites and substantially overestimated the correct values. The extraction fluorescence method introduced in 1963 was a significant improvement but still overestimated concentrations, as the hydroxylated metabolites were not distinguished (this was more of a problem in uncomplicated than in severe malaria) (White *et al.* 1982; Edstein *et al.* 1983). Then, in the early 1980s, specific HPLC methods with fluorescence detection were introduced, and finally in recent years, LC-MS methods have been used.

Antimalarial drug absorption

In severe malaria, the oral route is unreliable and in unconscious patients may be dangerous as aspiration of gastric contents may occur. Fortunately, the absorption of artesunate or quinine after intramuscular injection is good even in severe malaria (Waller *et al.* 1990; van Hensbroek *et al.* 1996; Krishna *et al.* 2001a; Nealon *et al.* 2002; Hien *et al.* 2004; Hendriksen *et al.* 2013c), and this is an acceptable alternative route to intravenous administration for these drugs. Parenteral artemether and artemotil are oil-based injectates which can be given only by intramuscular injection. Absorption is slow and erratic and may be dangerously slow in some patients with severe malaria (Murphy *et al.* 1997; Hien *et al.* 2004; Li *et al.* 2004). This explains why they are inferior to intravenous artesunate in severe malaria (Phu *et al.* 2010). Suppository formulations of artesunate, artemether and artemisinin are available in some countries. In a very large community-based trial, intrarectal artesunate (rectocap®) given at a community level before referral to hospital reduced malaria mortality in children by 25%, particularly in patients who took many hours to reach hospital (Gomes *et al.* 2009). Rectal bioavailability of artesunate given as a gel-filled capsule (artesunate rectocap®) averages approximately 50% although there is considerable interindividual variability (see below) (Krishna *et al.* 2001b; Simpson *et al.* 2006). Intrarectal administration of artesunate in a gel-filled capsule is simple, safe and well tolerated. Quinine and Quinimax® (a buffered mixture of *Cinchona* alkaloids comprising 96.1% quinine, 2.5% quinidine, 0.68% cinchonine and 0.67% cinchonidine) have been also administered intrarectally. Unbuffered quinine is irritant when instilled rectally, whereas Quinimax® is generally well tolerated; rectal quinine gluconate as a cream and the commercial mixture of *Cinchona* alkaloids, Quinimax®, in solution, was reportedly well tolerated and had estimated bioavailabilities of 36% and 40%, and a mean (SD) time to peak concentration of 4.1 (2.4) and 2.7 (0.4) h, respectively, in children with moderately severe malaria from Niger (Barennes *et al.* 1995). In a later study, rectal bioavailability of quinine in moderate severity malaria was shown to be dose-dependent falling from 96% with a dose of 8 mg/kg to 52% at 16 mg/kg (Pussard *et al.* 2004).

Antimalarial drug disposition

The apparent volume of distribution of the artemisinin derivatives is not greatly affected by malaria, whereas for quinine, there is a marked contraction in proportion to disease severity (White *et al.* 1982). This is explained in part by increased plasma protein binding of the basic quinine to the acute phase reactant α1-acid glycoprotein. Binding increases from 85 to 90% to approximately 93% in severe malaria (Silamut *et al.* 1985; Mansor *et al.* 1991). Thus, the free fraction in severe malaria is often less than half that in healthy subjects. Concentrations of quinine and DHA in cerebrospinal fluid in cerebral malaria are both <10% of corresponding plasma concentrations (White *et al.* 1982; Davis *et al.* 2003). Drug exposure in young children with severe malaria is lower than that in older children and adults. Young children with severe malaria have lower exposures to both artesunate and DHA given a standard weight adjusted dose of parenteral artesunate than older children and adults (Hendriksen *et al.* 2013b) (Figures 28 and 29). In a recent large population, pharmacokinetic study of artesunate from Tanzania children weighing between 6 and 10 kg body weight had 20% lower DHA exposure than children between 21 to 25 kg body weight, suggesting that a higher dose is needed for young children (Hendriksen *et al.* 2013b). In contrast, in children with severe malaria less than 2 years old, quinine concentrations were higher than in older children and adults (van Hensbroek *et al.* 1996).

Antimalarial drug clearance

Artesunate, artemether, artemotil are all converted *in vivo* to the active metabolite dihydroartemisinin, which is then inactivated by glucuronidation (principally via UGT1A9 and UGT2B7) (Ilett *et al.* 2002a). The principal route of artemisinin clearance is by biotransformation to inactive metabolites. Cytochrome P450 3A4 activity is impaired in malaria, which reduces conversion of artemether and artemotil to DHA and also reduces metabolic clearance of quinine (Pukrittayakamee *et al.* 1997). Renal impairment does not affect clearance of the artemisinins

but reduces elimination of quinine and its main biologically active metabolite 3-OH quinine. In renal failure, 3-OH quinine concentrations reach 45% of those of the parent compound, thereby contributing 12% of the antimalarial activity (Newton *et al.* 1999).

Antimalarial pharmacodynamics

The primary objective of antimalarial treatment in severe malaria is to inhibit the development of, or to kill, a sufficient number of parasites to prevent death. After antimalarial drug treatment, the reduction in parasite numbers is fractional – thus provided minimum parasiticidal concentrations are exceeded, a fixed fraction of the parasite biomass is killed per asexual cycle; a first-order process (White 1997, 2011). However, in severe malaria, it is the drug effects on the *current* asexual cycle that are paramount, and drug effects on subsequent cycles are of less importance. The pharmacodynamic properties of the antimalarial drugs can be described in terms of the stage specificity of their antiparasitic action during the asexual life cycle. The concentration–effect relationships and their maximal effects differ between the drugs. All antimalarial drugs kill the mature trophozoite stages of susceptible plasmodia. Once schizonts are formed, their effects are much less. Only the artemisinins kill the young circulating ring stages and thereby prevent their sequestration (ter Kuile *et al.* 1993; Watkins *et al.* 1993; Udomsangpetch *et al.* 1996). Evidence from the recent large SEAQUAMAT and AQUAMAT trials indicates that the substantial life-saving advantage of artesunate over quinine is explained by its parasiticidal effects on circulating ring-stage parasites, preventing their development and sequestration (Dondorp *et al.* 2005a, 2010; Caramello *et al.* 2012). The action of both drugs on the sequestered parasites is similar, although artesunate may also have a greater effect on mature schizonts. The critical advantage of artesunate is reflected in the shape of the parasite clearance curve. Following artesunate, the decline in parasitaemia is more rapid compared with quinine and any increase or plateau phase before the log-linear decline in parasite numbers is attenuated (White 1997, 2011). This advantage is lost in artemisinin resistant parasites (Dondorp *et al.* 2009). In addition, the artemisinin derivatives generally have a wide therapeutic index, whereas the quinoline antimalarial drugs have a narrow therapeutic index.

Antimalarial drug resistance

Chloroquine resistance fatally compromised the efficacy of this previously highly effective drug in severe malaria. Aside from widespread resistance, the very late-stage activity of antifols (affecting the formation of the early schizont) means that this class of drug is not appropriate for severe malaria management. Although quinine resistance has been discussed for over a century, and there is undoubtedly reduced susceptibility to quinine in *P. falciparum* in parts of South-East Asia and South America, there is no hard evidence that this has translated into an increase in quinine-treated mortality in severe malaria. In Thailand, where multidrug-resistant *P. falciparum* is prevalent, coma recovery times and parasite clearance times were noted to lengthen between 1981 and 1992, but mortality did not increase (Pukrittayakamee *et al.* 1994). Occasional early treatment failures do occur, but usually can be explained by unusual pharmacokinetics resulting in low drug concentrations (Looareesuwan *et al.* 1990; Newton *et al.* 2005b). There is no convincing evidence for high-grade resistance anywhere in the world, so quinine can still be relied upon everywhere in the treatment of severe malaria. The susceptibility of *P. falciparum* in Western Cambodia and along the Thailand–Myanmar border to artemisinins has declined, and this is manifested by reduced parasiticidal effects against younger circulating parasites (Saralamba *et al.* 2011; Phyo *et al.* 2012), which suggests that the life-saving benefit of artesunate could be compromised. However, the extent to which this compromises artesunate in severe malaria has not been determined. Fortunately, resistance is still confined to a relatively small geographical area at present, but if artemisinin resistance is suspected, then both artesunate and quinine should be given together in full doses (Newton *et al.* 2001a).

Pharmacology of antimalarial drugs

Artemisinin, also known as qinghaosu, is a sesquiterpene lactone extracted from the leaves of *Artemisia annua* (sweet wormwood). It has been used in China for the treatment of fever for more than 2000 years. It is a potent and rapidly acting blood schizontocide and is active against all *Plasmodium* species. It has an unusually broad activity against asexual malaria parasites (ter Kuile *et al.* 1993), killing all stages from young rings to schizonts. In *P. falciparum* malaria, artemisinin also kills the gametocytes – including the stage 4 and early stage 5 gametocytes, which are otherwise sensitive only to primaquine. The peroxide bridge is essential for antimalarial activity, but the exact mechanism of action remains unknown. Artemisinin is the parent compound, and it is still available in some areas in a suppository formulation and is still used orally in some ACTs, but it has now largely given way to the more potent dihydroartemisinin (DHA) and its derivatives, artemether, artemotil and artesunate. All three are formulated for parenteral administration in severe malaria. The DHA derivatives are all

converted back *in vivo* to DHA. For artesunate, this back conversion is very rapid and DHA accounts for the majority of antimalarial activity in severe malaria.

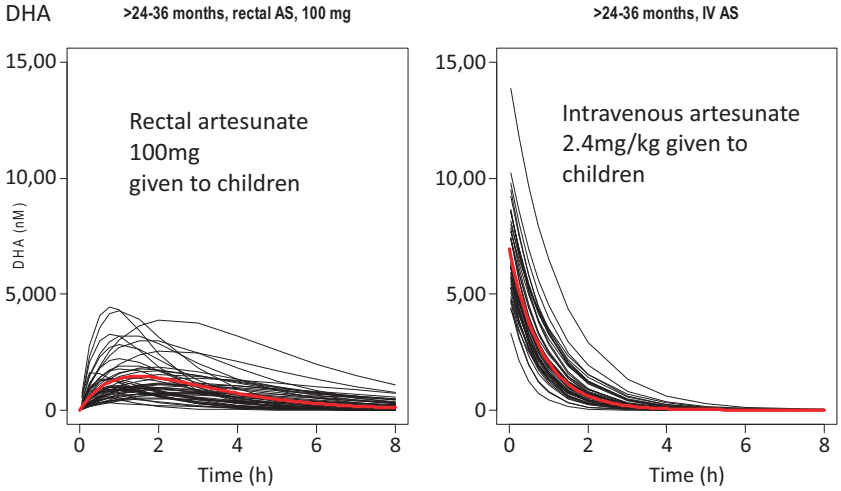
Artesunate

Artesunate is the treatment of choice for severe malaria; it is the sodium salt of the hemisuccinate ester of dihydroartemisinin. Artesunate is soluble in water but has poor stability in aqueous solutions at neutral or acid pH. In the most widely used injectable formulation, artesunate is formed by the addition of sodium bicarbonate solution to freeze-dried artesunic acid immediately before dilution in isotonic dextrose or saline and given by intravenous or intramuscular injection. Other formulations are in development. Artesunate can be given orally, rectally or by the intramuscular or intravenous routes.

Formulations.

- Ampoules for intramuscular or intravenous injection containing 30, 60 or 120 mg of anhydrous artesunic acid with a separate ampoule of 5% sodium bicarbonate solution.
- Rectal capsules containing 100 or 400 mg of sodium artesunate.

Pharmacokinetics and pharmacodynamics. Early methods of assay for artesunate, artemether and dihydroartemisinin were difficult and techniques for sample preparation did not satisfactorily limit haem-mediated degradation. As a result, concentration measurements were sometimes inaccurate. More recent studies employing LC-MS/MS-based assay methods have generally given more reliable assays and therefore more reliable pharmacokinetic estimates. Artesunate is rapidly absorbed, with peak plasma levels occurring 0.5 h after intramuscular and 2 h after rectal administration (Nealon *et al.* 2002; Hendriksen *et al.* 2013a). Intramuscular bioavailability in Gabonese children with severe malaria was estimated at a median of 86%. Following rectal administration, bioavailability is variable but averages approximately 50%. Artesunate is rapidly and almost entirely converted by



PK parameter	DHA following rectal artesunate	DHA following intravenous artesunate
Ka (/hr)	0.63†	-
CL (L/hr)	58.11†	14.9†
V (L)	87.91†	13.4†
Between-subject variability (expressed as SD*100)		
ηka	50%‡	-
ηCL	62%‡	43%*
ηV	75%‡	24%*

Figure 28 Simulated pharmacokinetic profiles of the active metabolite, dihydroartemisinin (DHA), following rectal or intravenous administration of artesunate (Simpson *et al.* 2006). Artesunate (ARS) was assumed to be converted immediately and completely to DHA. Weight distributions for each age group were derived from age-weight data from 88, 054 individuals (Taylor *et al.* 2006b).

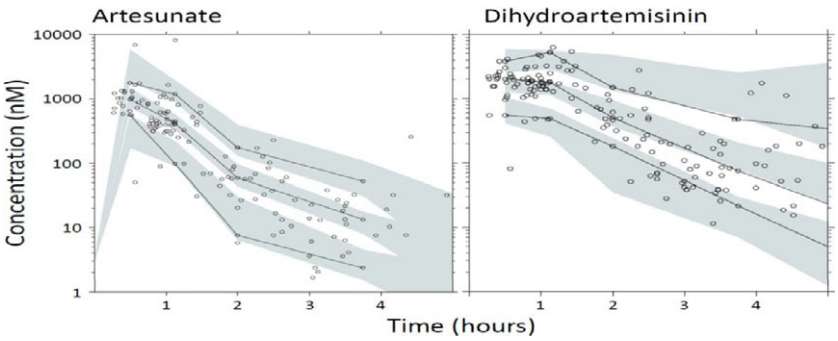


Figure 29 Prediction-corrected venous log plasma concentrations based on a study of the population pharmacokinetics of artesunate and dihydroartemisinin in 70 Tanzanian children with severe malaria (Hendriksen *et al.* 2013a). Open circles, observed data points; solid lines, 5th, 50th and 95th percentiles of the observed data; shaded area, 95% confidence interval of simulated (n = 2000) 5th, 50th and 95th percentiles.

blood esterases to DHA, the active metabolite. Thus, DHA accounts for nearly all the antimalarial effect. DHA plasma protein binding is estimated at approximately 75%. Red cell concentrations are lower than corresponding plasma concentrations (Lindegardh *et al.* 2011). DHA is eliminated mainly by conversion to inactive glucuronides. Elimination of artesunate is very rapid, and antimalarial activity is determined by dihydroartemisinin elimination (half-life approximately 45 min) (Simpson *et al.* 2006). This rapid rate of elimination means that concentrations may fall below the minimum parasitical concentration for the infecting parasites during the dose interval. This does not matter for sensitive parasites, as maximum killing effects are obtained with approximately 4 h exposure, but in the presence of artemisinin resistance may lead to submaximal effects if the majority of parasites are circulating young rings with reduced susceptibility (Saralamba *et al.* 2011). This emphasises the importance of the second dose given at 12 h as an 'insurance policy' in case the parasites were relatively refractory 12 h earlier when the first dose was given. Evidence suggests that severe malaria has relatively little effect on the disposition of parenteral artesunate. Artesunate does not penetrate to the CSF whereas DHA concentrations averaged 8% of those in plasma (Davis *et al.* 2003).

Summarising the literature after intravenous artesunate doses of between 1.2 and 4 mg/kg, the observed median (or mean) maximum plasma concentrations of artesunate ranged from 13 685 to 29 677 ng/ml and for DHA ranged from 1280 to 3277 ng/ml. After intramuscular doses of 1. to 2.4 mg/kg, plasma artesunate ranged from 615 to 2195 ng/ml and plasma DHA ranged from 341 to 1166 ng/ml, and following rectal doses of 2 to 3 mg/kg, 10 mg/kg and 20 mg/kg plasma artesunate ranged from 90 to 561 ng/ml and plasma DHA ranged from 180 to 1535 ng/ml (Batty *et al.* 1998a,b; Halpaap *et al.* 1998; Navaratnam *et al.* 1998; Sabchareon *et al.* 1998; Davis *et al.* 2001; Krishna *et al.* 2001b; Ilett *et al.* 2002b; Nealon *et al.* 2002; Awad *et al.* 2004; Hien *et al.* 2004; Sirivichayakul *et al.* 2007; McGready *et al.* 2012c; Hendriksen *et al.* 2013a,b). The median times to achieve maximum artesunate concentrations were within a few minutes, 7–10 min and 0.5–1 h (Halpaap *et al.* 1998; Sirivichayakul *et al.* 2007) and to reach maximum plasma, DHA concentrations were within 12 min, 25–40 min and 1–3 h for intravenous, intramuscular and rectal administration, respectively. The median artesunate $AUC_{0\text{-inf}}$ was similar for all three routes of administration; 555–1269 ng × h/ml for intravenous, 535–999 ng × h/ml for intramuscular and 548–1076 ng × h/ml for rectal administration. The median $AUC_{0\text{-inf}}$ values for DHA varied widely (partly because of different dosing). The ranges of median DHA $AUC_{0\text{-inf}}$ values were 737 to 3298 ng × h/ml for intravenous, 396–2474 ng × h/ml for intramuscular and 726 to 9576 ng × h/ml for rectal artesunate. The median volume of distribution for artesunate ranged from 5 to 53 l, the median clearance from 35 to 213 l/h and the elimination half-life from 2 to 8 min across the different study populations of healthy volunteers, adults with vivax malaria, adults with falciparum malaria, children with severe malaria and pregnant and post-partum women. For DHA concentrations following intravenous administration of artesunate, the median volume of distribution ranged from 9 to 108 l, the median clearance from 9 to 62 l/h and the elimination half-life from 18 to 69 min across the different study populations.

Implications for dosing. No dose modifications are necessary in renal or hepatic impairment. The pharmacokinetic properties of intravenous artesunate are also relatively unaffected by pregnancy. In a study of 70 Tanzanian children aged between 6 months and 11 years who presented with severe falciparum malaria, body weight significantly affected clearance and apparent volume of distribution ($P < 0.001$), resulting in lower

artesunate and dihydroartemisinin exposure in smaller children (Hendriksen *et al.* 2013a) (Figures 26 and 29). This suggests that weight-based dosing should be adjusted and doses increased in young children (Table 10).

Toxicity. Artemisinin and its derivatives are safe and remarkably well tolerated. There have been reports of mild gastrointestinal disturbances, dizziness, tinnitus, reticulocytopenia, neutropenia, elevated liver enzyme values, allergic reactions, and electrocardiographic abnormalities, including bradycardia and prolongation of the QT interval, although most studies have not found any electrocardiographic abnormalities (Price *et al.* 1999; Maude *et al.* 2009a). The only potentially serious adverse effect reported with this class of drugs is type 1 hypersensitivity reactions in approximately 1 in 3000 patients (Leonardi *et al.* 2001). Neurotoxicity has been reported in animal studies (Genovese & Newman 2008), particularly with very high doses of intramuscular artemotil and artemether, but has not been substantiated in humans (Kissinger *et al.* 2000; Van Vugt *et al.* 2000; Hien *et al.* 2003). Similarly, evidence of death of embryos and morphological abnormalities in early pregnancy has been demonstrated in animal studies (Longo *et al.* 2006; Clark 2009). Embryolethality occurs within a narrow time window in early pregnancy and is related to specific toxicity to primitive red blood cell precursors. In experimental studies, limb developmental abnormalities were observed in rodents, but not in primates (Clark 2009). There is no evidence these drugs cause abortion, stillbirth or developmental abnormalities in humans (McGready *et al.* 2012b).

At doses higher than currently recommended the artemisinins (6 mg/kg/day for 7 days) may cause temporary neutropenia (Bethell *et al.* 2010). Following the treatment of severe malaria with artesunate, particularly in hyperparasitaemic patients, there may be a delayed but severe anaemia (Zoller *et al.* 2011; Kreeftmeijer-Vegter *et al.* 2012; Rolling *et al.* 2012). This is attributed, at least partly, to the accelerated destruction of 'once parasitised' erythrocytes – red cells which contained a parasite which was killed and then removed by the spleen ('pitting') (Angus *et al.* 1997; Newton *et al.* 2001b). Thus, this apparent adverse effect is a direct function of the life-saving effect of artesunate in killing young parasites in circulating red cells before they can cytoadhere (Udomsangpetch *et al.* 1996).

Drug interactions. Artemisinin and, to a lesser extent, artemether induce their own metabolism. There are no significant interactions with other drugs. The antimalarial action of these drugs is antagonised by desferrioxamine.

Artemether

Artemether is the methyl ether of DHA. It is more lipid soluble than artemisinin or artesunate. It can be given as an oil-based intramuscular injection or orally. It is also co-formulated with lumefantrine (previously called benflumetol) for combination therapy.

Formulations.

- Ampoules of injectable oil for intramuscular injection containing 80 mg of artemether in 1 ml for adults or 40 mg of artemether in 1 ml for paediatric use.

Pharmacokinetics and pharmacodynamics. Following intramuscular injection in severe malaria, absorption is very variable, especially in children with poor peripheral perfusion: peak plasma concentrations generally occur after around 6 h, but absorption is slow and erratic and times to peak can be 18 h or longer in some cases (Murphy *et al.* 1997; Hien *et al.* 2004; Mithwani *et al.* 2004) (Figure 30). The intrinsic activity of artemether is less than that of DHA, which combined with the very variable absorption in severe malaria makes it an inferior treatment to artesunate, but probably still better than quinine in severe malaria (Dondorp *et al.* 2010; Phu *et al.* 2010). Artemether is metabolised to DHA, the active metabolite. Biotransformation is mediated predominantly via the cytochrome P450 enzyme CYP3A4. Autoinduction of metabolism is less than with artemisinin. After intramuscular administra-

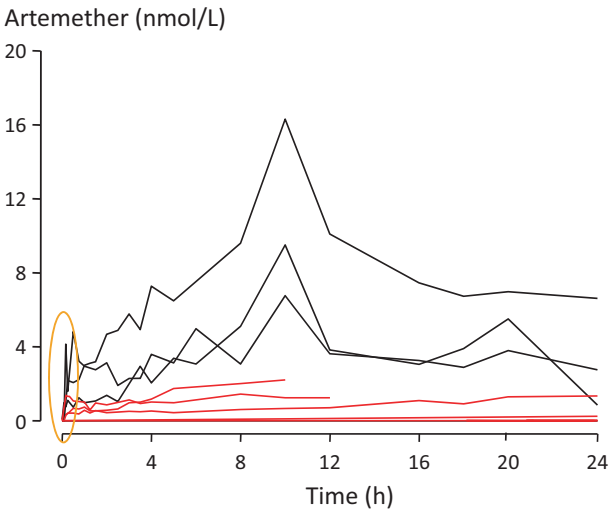


Figure 30 Individual concentration–time profiles for artemether after the first intramuscular dose of 10.7 μmol (3.2 mg) of ARM/kg to 10 patients with severe falciparum malaria (Hien *et al.* 1996). Oval highlights critical early period in which some patients may absorb very little drug.

tion, artemether predominates in the blood (Teja-Isavadharm *et al.* 1996; Hien *et al.* 2004), whereas after oral administration DHA predominates. Artemether is 95% bound to plasma proteins. The elimination half-life is approximately 1 h, but following intramuscular administration, the elimination phase is prolonged considerably because of continued absorption. No dose modifications are necessary in renal or hepatic impairment.

Toxicity. In all species of animals tested, intramuscular artemether and artemotil cause an unusual selective pattern of neuronal damage to certain brain stem nuclei (Brewer *et al.* 1994). Neurotoxicity in experimental animals is related to the sustained blood concentrations that follow intramuscular administration, because it is much less frequent when the same doses are given orally or with similar doses of water-soluble drugs such as artesunate. Clinical, neurophysiological and pathological studies in humans have not shown similar findings with therapeutic use of these compounds (Kissinger *et al.* 2000; Van Vugt *et al.* 2000 Hien *et al.* 2003). Toxicity is otherwise similar to that of artemisinin.

Artemotil

Artemotil is the beta ethyl ether of dihydro artemisinin (arteether) and is very closely related to the more widely used artemether. It is oil-based so is also water insoluble. A mixture of α and β ethers is also available in India for intramuscular administration. Artemotil and α β arteether are given by intramuscular injection only.

Formulations.

- Ampoules containing 150 mg of artemotil in 2 ml of injectable solution.

Pharmacokinetics. There is substantially less published information on artemotil than for artemether. Absorption is slower and more erratic, with some patients having undetectable plasma artemotil until more than 24 h after administration (Afolabi & Okoromah 2004; Li *et al.* 2004; Pareek *et al.* 2006; Mukim *et al.* 2011).

Toxicity. As for artemisinin.

Drug interactions. None known.

Artemisinin

Formulations. A wide variety of formulations for oral, parenteral and rectal use is available.

- These include: Suppositories containing 100, 200, 300, 400 or 500 mg of artemisinin.

Pharmacokinetics. Peak plasma concentrations occur around 3 h after oral and around 11 h after rectal administration. Artemisinin is converted to inactive metabolites via the cytochrome P450 mixed function oxidases notably CYP2B6 and several other enzymes. Artemisinin is a potent inducer of its own metabolism. The elimination half-life is approximately 1 h (Simonsson *et al.* 2003; Asimus *et al.* 2007).

Toxicity. As for artesunate.

Drug interactions. None known apart from autoinduction.

Quinine

Quinine is an alkaloid derived from the bark of the *Cinchona* tree. Four antimalarial alkaloids can be derived from the bark: quinine (the main alkaloid by weight), quinidine, cinchonine and cinchonidine. Quinine is the L-stereoisomer of quinidine, which was used as an alternative treatment of severe malaria in temperate countries where quinine was not readily available. Quinine has been the mainstay of antimalarial treatment of severe malaria for 350 years but has now been overtaken by the artemisinin derivatives. Nevertheless, quinine is still widely available and extensively used, and it needs to be kept available in case artemisinin resistance worsens. The mechanisms of quinine's antimalarial actions are thought to involve inhibition of parasite haem detoxification in the food vacuole, but are not well understood. Quinine can be given orally, rectally (if buffered), and by intramuscular injection (to the anterior thigh, preferably diluted to a concentration of 60–100 mg/ml)) or by constant rate intravenous infusion. Quinine should not be given subcutaneously because this can result in skin necrosis.

Formulations.

- Injectable solutions of quinine hydrochloride, quinine dihydrochloride and quinine sulphate containing 82%, 82% and 82.6% quinine base, respectively.

Pharmacokinetics and pharmacodynamics. The pharmacokinetic properties of quinine are altered significantly by malaria infection, with reductions in apparent volume of distribution and clearance in proportion to disease severity. In children under 2 years of age with severe malaria,

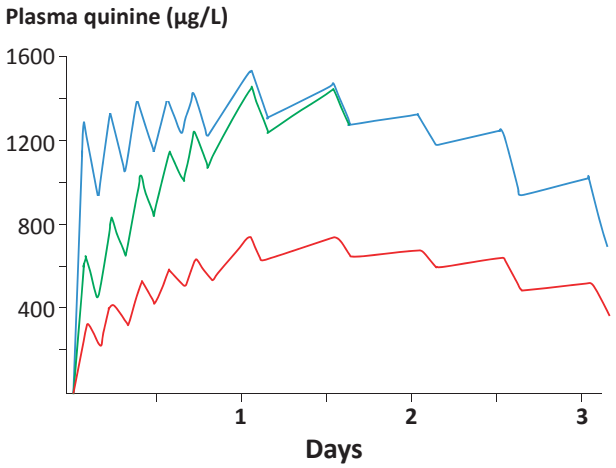


Figure 31 Mean plasma quinine concentration profiles in adult patients with severe falciparum malaria receiving a loading dose of quinine (20 mg salt/kg over 4 h) in the upper panel (blue), the predicted plasma concentrations based on derived pharmacokinetic parameters without a loading dose (10 mg/kg/8 h) (green) and the predicted levels in the once advocated 5 mg/kg/8 h regimen (red). Note the decline in plasma concentrations after the first days of treatment as systemic clearance improves, and the volume of distribution expands (White *et al.* 1983c).

concentrations are slightly higher than in older children and adults (van Hensbroek *et al.* 1996). There is no evidence for dose-dependent kinetics.

Absorption. Quinine is preferably given by rate controlled intravenous infusion to patients with severe malaria (Figure 31) (White *et al.* 1982). Quinine is also well absorbed after intramuscular injection in severe malaria. Intramuscular quinine has good bioavailability even in very young children (<2 years) with severe malaria (Shann *et al.* 1985; van Hensbroek *et al.* 1996; Hendriksen *et al.* 2013c). Overall, there is good agreement between recent studies in African children with moderately severe to severe falciparum malaria. The overall mean (SD) peak plasma quinine concentration after the 20 mg/kg loading dose is 15.6 (5.2) mg/l (N = 57) (White 1995b). This compares with an overall mean (SD) peak quinine concentration after intravenous administration of 15.2 (3.6) mg/l (N = 74). Absorption is more rapid and the intramuscular injection is less painful if quinine dihydrochloride (usual concentration 300 mg/ml) is diluted 1:2 to 1:5 before injection. The plasma concentration profile after intramuscular administration may be biphasic in some patients. Rectal quinine gluconate as a cream and the commercial mixture of *Cinchona* alkaloids, Quinimax®, in solution, had estimated bioavailabilities of 36% and 40%, and a mean (SD) time to peak concentration of 4.1 (2.4) and 2.7 (0.4) h, respectively, in children with moderately severe malaria from Niger (Barennes *et al.* 1995). These solutions were reportedly well tolerated. Rectal absorption is dose-dependent (Pussard *et al.* 1999, 2004). Rectal quinine proved effective in childhood cerebral malaria in a recent trial although much larger trials would be needed for definitive information, and before this could be recommended widely. (Achan *et al.* 2007).

Disposition. A single compartment model is adequate to define the disposition of quinine after intravenous infusion. The total apparent volume of distribution (Vd_{ss}) in healthy adult subjects ranges from 1.5 to 3.5 l/kg (White *et al.* 1982). This is contracted in malaria proportional to the severity of disease (to approximately 1.7 l/kg in Thai adults with uncomplicated falciparum malaria and 1.2 l/kg in adults with cerebral malaria). Similar changes are seen in global malnutrition (Pussard *et al.* 1999). Plasma concentrations for a given dose are therefore highest in severe malaria. In Kenyan children with severe malaria, estimated mean Vd_{ss} values were lower; 1.22 l/kg and 0.87 l/kg in severe and 0.45 l/kg (Winstanley *et al.* 1993), and 0.75 l/kg (Winstanley *et al.* 1994) in cerebral malaria. Quinine pharmacokinetics can be described by a two compartment model. The mean (SD) volume of the central compartment (V_c) is also contracted from 0.7 (0.3) in healthy Thai adults to 0.17 (0.1) l/kg in cerebral malaria (Davis *et al.* 1988). In Kenyan children with cerebral malaria, the estimated volume of the central compartment was even smaller; 0.27 (0.1) l/kg or 40% lower than the value in adults (Winstanley *et al.* 1993). This explains why intravenous injections (as opposed to infusions) of quinine are potentially so dangerous in severe infections. Quinine is distributed throughout most of the body fluids. The mean concentration in erythrocytes is approximately one-third of that in plasma, rising to one half (presumably because of concentration within malaria parasites) in severe malaria (White *et al.* 1983a). Concentrations in saliva and in breast milk are about 30% of those in plasma (Salako & Sowunmi 1992, Phillips *et al.* 1986b). Cerebrospinal fluid concentrations are 7 ± 3% of those in plasma in cerebral malaria (White *et al.* 1982). These values are approximately half those of free (unbound) quinine in plasma, which suggests that quinine does not freely traverse the blood–brain barrier (Silamut *et al.* 1985). Quinine is a base and the principal plasma protein to which it binds is the acute phase protein, α1-acid glycoprotein (Mihaly *et al.* 1987; Silamut *et al.* 1991). Plasma protein binding is therefore increased from approximately 85% in healthy subjects to 90–93%

in severe malaria (i.e. the free fraction is reduced by one-third to one half). Thus, although total plasma concentrations are higher in severe malaria, free concentrations may be no different from those in patients with uncomplicated infections or healthy subjects. Increased protein binding may explain why relatively high total plasma quinine concentrations (10–20 mg/l) do not cause major toxicity in the treatment of severe malaria. The relationship between quinine binding (expressed as the association constant) and pH is hyperbolic such that binding falls with pH to a nadir around pH 7 (Winstanley *et al.* 1993). There is no clinical evidence for increased toxicity of quinine in acidosis.

Elimination. Extensive metabolism via the cytochrome P450 enzyme CYP3A4 occurs in the liver (Zhao *et al.* 1996), and elimination of more polar metabolites is mainly renal. The initial metabolite 3-hydroxyquinine may accumulate in renal failure. In healthy subjects and patients with malaria, quinine is predominantly (80%) biotransformed (White *et al.* 1982) first to 3 and 2 hydroxyquinine and then to a series of more polar water-soluble metabolites. Plasma concentrations of 3-hydroxyquinine are approximately one-third to one-fifth of those of the parent compound, with a lower ratio in malaria indicating impairment of hepatic biotransformation. 3-hydroxyquinine contributes approximately 5% of antimalarial activity in acute malaria rising to 10% in convalescence (Nontprasert *et al.* 1996; Pukrittayakamee *et al.* 1997) and 12% in renal failure (Newton *et al.* 1999).

Approximately 20% of the quinine dose is eliminated by the kidneys and the remaining 80% by hepatic biotransformation. Small amounts appear in the bile and saliva. Total systemic clearance is reduced in uncomplicated malaria, and further reduced in severe malaria (White *et al.* 1982; White 1987). Renal clearance and hepatic clearance are reduced in parallel. The observation that plasma quinine concentrations are high in patients with acute renal failure probably relates more to the overall severity of disease and the consequent pharmacokinetic changes, rather than to a reduction in glomerular filtration rate *per se*. The mean terminal elimination half-life in healthy adult subjects is 11 h, compared with 16 h in uncomplicated malaria, and 18 h in cerebral malaria. The clearance of quinine is reduced, and the elimination half-life prolonged from 11 to 18 h in the elderly (age 65–74 were studied) (Wanwimolruk *et al.* 1991). Quinine elimination is more rapid in smokers (Wanwimolruk *et al.* 1993), although the relevance of this to severe malaria is uncertain. Quinine clearance is increased by drugs which induce CYP 3A4 such as phenobarbitone and rifampicin (Pukrittayakamee *et al.* 2003).

Children and pregnant women. Absorption of quinine is similar in children and adults. The total apparent volume of distribution and the volume of the central compartment are smaller in children (Waller *et al.* 1990; Pasvol *et al.* 1991; Winstanley *et al.* 1993), and pregnant women (Looareesuwan *et al.* 1985; Phillips *et al.* 1985) than in adults with disease of comparable severity, but elimination is more rapid in both groups. Systemic clearance values are therefore similar. Cord blood concentrations and breast milk concentrations are approximately one-third of those in simultaneously sampled maternal plasma (Phillips *et al.* 1986b).

Areas of uncertainty. It has been suggested that there be a ceiling dose above which quinine should not be given, but there is no evidence to support this. In obese patients, dosing should be based on ideal rather than observed body weight (Viriyayudhakorn *et al.* 2000). Studies in malnutrition have given variable findings, but overall suggest that no dose alterations should be made (Salako *et al.* 1989; Treluyer *et al.* 1996; Pussard *et al.* 1999). The relationship between plasma concentrations and parasiticidal effects in severe malaria is unclear, but available evidence suggests a therapeutic range of free quinine concentrations between 0.5 and 2.0 mg/l (corresponding roughly to total plasma concentrations of 5–20 mg/l (White 1995b). Modelling suggested an *in vivo* minimum parasiticidal concentration for Thai *P. falciparum* isolates of 3.5 mg/l (Pukrittayakamee *et al.* 2003). Total plasma concentrations between 8 and 20 mg/l in severe malaria are usually safe and effective (White 1987, 1995b, White *et al.* 1983c).

Pharmacodynamics. Quinine acts principally on the mature trophozoite stage of parasite development and does not prevent sequestration or further development of circulating ring stages of *P. falciparum*. Like other structurally similar antimalarials, quinine also kills the sexual stages of *P. vivax, P. malariae* and *P. ovale*, but not mature gametocytes of *P. falciparum*. It does not kill the pre-erythrocytic stages of malaria parasites.

Toxicity. Administration of quinine or its salts regularly causes a complex of symptoms known as cinchonism, which is characterised in its mild form by tinnitus, impaired high-tone hearing, headache, nausea, dizziness and dysphoria, and sometimes disturbed vision. More severe manifestations include vomiting, abdominal pain, diarrhoea and severe vertigo. Hypersensitivity reactions to quinine range

from urticaria, bronchospasm, flushing of the skin and fever, through antibody-mediated thrombocytopenia and haemolytic anaemia, to life-threatening haemolytic–uraemic syndrome (which is very rare). Massive haemolysis with renal failure ('black water fever') has been linked epidemiologically and historically to quinine, but its aetiology remains uncertain. Severe concentration-related toxicity, including hypotension, myocardial conduction and repolarisation disturbances, blindness, deafness and coma, is very unusual in the treatment of malaria with plasma concentrations under 20 mg/l (Pukrittayakamee *et al.* 1994; White 1995b). Hypoglycaemia is a more commonly encountered problem. Quinine is a potent stimulus to pancreatic insulin secretion (White *et al.* 1983b), and hyperinsulinaemic hypoglycaemia is particularly likely in pregnant women (Looareesuwan *et al.* 1985) who have amplified responses to islet cell stimulation, or patients who remain severely ill for several days. Half of quinine-treated women with severe malaria in late pregnancy develop hypoglycaemia during treatment. Intravenous injections may cause acute cardiovascular toxicity, especially after rapid intravenous injection, presumably because transiently toxic blood concentrations occur before adequate distribution (Davis *et al.* 1988). Quinine causes an approximately 10% prolongation of the electrocardiograph QT interval – mainly as a result of slight QRS widening. The effect on ventricular repolarisation is much less than that with quinidine. Intravenous quinine should be given only by infusion, never injection. Overdosage of quinine may cause oculotoxicity, including blindness from direct retinal toxicity, and cardiotoxicity, and can be fatal. Postural hypotension is common in acute malaria, and this is exacerbated by quinine. Thrombocytopenia, Coomb's positive haemolytic anaemia, haemolytic–uraemic syndrome and other allergic manifestations are all rare in malaria treatment.

Intramuscular quinine is certainly painful if concentrated acidic solutions are administered (undiluted quinine dihydrochloride 300 mg/ml has a pH of 2), and in the past, these have been associated with sterile abscess formation. Intramuscular injections of undiluted quinine dihydrochloride cause pain, focal necrosis and in some cases abscess formation and in endemic areas are a common cause of sciatic nerve palsy. Tetanus associated with intramuscular quinine injections is usually fatal (Yen *et al.* 1994). In animals, toxic concentrations of quinine are oxytocic and induce premature labour. Quinine was once widely used as an abortifacient. This has led to concern over the use of quinine in pregnancy, but prospective studies (Looareesuwan *et al.* 1985) show no evidence of an oxytocic effect. Indeed administration of quinine was associated with a reduction in uterine irritability. Despite an apparently long list of potential adverse effects, quinine is actually generally well tolerated in the treatment of malaria.

Drug interactions. There is a theoretical concern that drugs that may prolong the QT interval should not be given with quinine, although whether or not quinine increases the risk of iatrogenic ventricular tachyarrhythmia has not been established. Antiarrhythmics, such as flecainide and amiodarone, should probably be avoided. Quinine increases the plasma concentrations of digoxin (Doering 1981; Wilkerson 1981). Cimetidine inhibits quinine metabolism, causing increased quinine levels and rifampicin, phenobarbitone, ritonavir and other enzyme inducers increase metabolic clearance leading to low plasma concentrations and an increased therapeutic failure rate. Combination with other antimalarials, such as the artemisinins, lumefantrine, mefloquine and tetracyclines, appears to be safe.

Doxycycline

Doxycycline is a tetracycline derivative with uses similar to those of tetracycline. It may be preferred to tetracycline because of its longer half-life, more reliable absorption and better safety profile in patients with renal insufficiency, where it may be used with caution. It is relatively water insoluble but very lipid soluble. It may be given orally or intravenously and is often given as a 'follow-on' treatment of severe malaria to complete a 7-day course with artesunate, artemether or quinine. It should never be used alone to treat malaria. It is available as the hydrochloride salt or phosphate complex, or as a complex prepared from the hydrochloride and calcium chloride.

Formulations.

- Capsules and tablets containing 100 mg of doxycycline salt as hydrochloride.

Pharmacokinetics. Doxycycline is readily and almost completely absorbed from the gastrointestinal tract, and absorption is not affected significantly by the presence of food. Peak plasma concentrations occur 2 h after administration. Some 80–95% is protein-bound and the elimination half-life is 10–24 h. It is widely distributed in body tissues and fluids. In patients with normal renal function, 40% of doxycycline is excreted in the urine, although more if the urine is alkalinised. It may accumulate in renal failure. However, the majority of the dose is excreted in the faeces. Pharmacokinetic studies in severe malaria renal failure indicate that twice daily dosing would be preferable to the current once-a-day regimen (Newton *et al.* 2005b).

Toxicity. Doxycycline's gastrointestinal effects are fewer than with tetracycline, although oesophageal ulceration can still be a problem if insufficient water is taken with tablets or capsules. There is less accumulation in patients with renal impairment. Doxycycline should not be given to pregnant or lactating women, or children younger than 8 years.

Drug interactions. Doxycycline has a lower affinity for binding with calcium than other tetracyclines, so may be taken with food or milk. However, antacids and iron may still affect absorption. Metabolism may be accelerated by drugs that induce hepatic enzymes, such as carbamazepine, phenytoin, phenobarbital and rifampicin, and by chronic alcohol use.

References

Abdulla MN, Sokrab TE, Zaidan ZA, Siddig HE & Ali ME (1997) Post-malarial cerebellar ataxia in adult Sudanese patients. *East African Medical Journal* **74**, 570–572.

Abrams ET, Brown H, Chensue SW, *et al.* (2003) Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. *Journal of Immunology* **170**, 2759–2764.

Abu Sayeed A, Maude RJ, Hasan MU, *et al.* (2011) Malarial retinopathy in Bangladeshi adults. *American Journal of Tropical Medicine and Hygiene* **84**, 141–147.

Abubakar A, Van De Vijver FJ, Mithwani S, *et al.* (2007) Assessing developmental outcomes in children from Kilifi, Kenya, following prophylaxis for seizures in cerebral malaria. *Journal of Health Psychology* **12**, 417–430.

Abu-Raddad LJ, Patnaik P & Kublin JG (2006) Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. *Science* **314**, 1603–1606.

Achan J, Byarugaba J, Barennes H & Tumwine JK (2007) Rectal versus intravenous quinine for the treatment of childhood cerebral malaria in Kampala, Uganda: a randomized, double-blind clinical trial. *Clinical Infectious Diseases* **45**, 1446–1452.

Afolabi B & Okoromah C (2004) Intramuscular arteether for treating severe malaria. *The Cochrane Database of Systematic Reviews* **18**, CD004391.

Agarwal R, Nath A & Gupta D (2007) Noninvasive ventilation in *Plasmodium vivax* related ALI/ARDS. *Internal Medicine (Tokyo, Japan)* **46**, 2007–2011.

Agarwal B, Kovari F, Saha R, Shaw S & Davenport A (2011) Do bicarbonate-based solutions for continuous renal replacement therapy offer better control of metabolic acidosis than lactate-containing fluids? *Nephron Clinical Practice* **118**, c392–c398.

Aikawa M, Iseki M, Barnwell JW, Taylor D, Oo MM & Howard RJ (1990) The pathology of human cerebral malaria. *American Journal of Tropical Medicine and Hygiene* **43**, 30–37.

Akachi Y & Atun R (2011) Effect of investment in malaria control on child mortality in sub-Saharan Africa in 2002-2008. *PLoS ONE* **6**, e21309.

Akech S, Ledermann H & Maitland K (2010) Choice of fluids for resuscitation in children with severe infection and shock: systematic review. *British Medical Journal* **341**, c4416.

Akhtar S & Mukherjee S (1993) Chloroquine induced mania. *International Journal of Psychiatry in Medicine* **23**, 349–356.

Alexandre MA, Ferreira CO, Siqueira AM, *et al.* (2010) Severe *Plasmodium vivax* malaria, Brazilian Amazon. *Emerging Infectious Diseases* **16**, 1611–1614.

Alkizim FO, Matheka D & Mwanda OW (2011) Case report - malaria complicated by gangrene: a case presentation and review. *Pan African Medical Journal* **10**, e46.

Allegranzi B, Sax H, Bengaly L, *et al.* (2010) Successful implementation of the World Health Organization hand hygiene improvement strategy in a referral hospital in Mali, Africa. *Infection Control and Hospital Epidemiology* **31**, 133–141.

Allegranzi B, Bagheri Nejad S, Combescure C, *et al.* (2011) Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *Lancet* **377**, 228–241.

Allen SJ, O'Donnell A, Alexander ND, *et al.* (1997) alpha+-Thalassemia protects children against disease caused by other infections as well as malaria. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 14736–14741.

Alonso PL, Lindsay SW, Schellenberg JRMA, *et al.* (1993) A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa: 2 Mortality and morbidity from malaria in the study area. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**(Suppl 2), 13–17.

Alonso A, Lau J & Jaber BL (2008) Biocompatible hemodialysis membranes for acute renal failure. *The Cochrane Database of Systematic Reviews*, CD005283.

Amaragiri SV & Lees TA (2000) Elastic compression stockings for prevention of deep vein thrombosis. *The Cochrane Database of Systematic Reviews*, CD001484.

Anand AC & Puri P (2005) Jaundice in malaria. *Journal of Gastroenterology and Hepatology* **20**, 1322–1332.

Anand AC, Ramji C, Narula AS & Singh W (1992) Malarial hepatitis: a heterogeneous syndrome? *The National Medical Journal of India* **5**, 59–62.

Anderson WK (1927) Malaria Psychoses and Neuroses Their Medical, Sociological and Legal Aspects. *London: Oxford, Medical Publications* 251–254.

Anderson RJ, Linas SL, Berns AS, *et al.* (1977) Nonoliguric acute renal failure. *New England Journal of Medicine* **296**, 1134–1138.

Andrade BB, Reis-Filho A, Souza-Neto SM, *et al.* (2010) Severe *Plasmodium vivax* malaria exhibits marked inflammatory imbalance. *Malaria Journal* **9**, 13.

Angus BJ, Chotivanich K, Udomsangpetch R & White NJ (1997) In vivo removal of malaria parasites from red blood cells without their destruction in acute falciparum malaria. *Blood* **90**, 2037–2040.

Anochie IC & Eke FU (2005) Acute renal failure in Nigerian children: Port Harcourt experience. *Pediatric Nephrology* **20**, 1610–1614.

Anstey NM & Price RN (2007) Improving case definitions for severe malaria. *PLoS Medicine* **4**, e267.

Anstey NM, Weinberg JB, Hassanali MY, *et al.* (1996) Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. *Journal of Experimental Medicine* **184**, 557–567.

Anstey NM, Jacups SP, Cain T, *et al.* (2002) Pulmonary manifestations of uncomplicated falciparum and vivax malaria: cough, small airways obstruction, impaired gas transfer, and increased pulmonary phagocytic activity. *Journal of Infectious Diseases* **185**, 1326–1334.

Anstey NM, Handojo T, Pain MC, *et al.* (2007) Lung injury in vivax malaria: pathophysiological evidence for pulmonary vascular sequestration and posttreatment alveolar-capillary inflammation. *Journal of Infectious Diseases* **195**, 589–596.

Anstey NM, Russell B, Yeo TW & Price RN (2009) The pathophysiology of vivax malaria. *Trends in Parasitology* **25**, 220–227.

Anstey NM, Douglas NM, Poespoprodjo JR & Price RN (2012) *Plasmodium vivax*: clinical spectrum, risk factors and pathogenesis. *Advances in Parasitology* **80**, 151–201.

Antia R, Yates A & De Roode JC (2008) The dynamics of acute malaria infections. I. Effect of the parasite's red blood cell preference. *Proceedings. Biological Sciences* **275**, 1449–1458.

Apisarnthanarak A, Thongphubeth K, Sirinvaravong S, *et al.* (2007) Effectiveness of multifaceted hospitalwide quality improvement programs featuring an intervention to remove unnecessary urinary catheters at a tertiary care center in Thailand. *Infection Control and Hospital Epidemiology* **28**, 791–798.

Armah HB, Wilson NO, Sarfo BY, Powell MD, Bond VC, Anderson W, Adjei AA, Gyasi RK, Tettey Y, Wiredu EK, Tongren JE, Udhayakumar V & Stiles JK (2007) Cerebrospinal fluid and serum biomarkers of cerebral malaria mortality in Ghanaian children. *Malaria Journal* **6**, 147.

Asimus S, Elsherbiny D, Hai TN, *et al.* (2007) Artemisinin antimalarials moderately affect cytochrome P450 enzyme activity in healthy subjects. *Fundamental & Clinical Pharmacology* **21**, 307–316.

Asindi AA, Ekanem EE, Ibia EO & Nwangwa MA (1993) Upsurge of malaria-related convulsions in a paediatric emergency room in Nigeria. Consequence of emergence of chloroquine-resistant *Plasmodium falciparum*. *Tropical and Geographical Medicine* **45**, 110–113.

Aursudkij B, Wilairatana P, Vannaphan S, Walsh DS, Gordeux VR & Looareesuwan S (1998) Pulmonary edema in cerebral malaria patients in Thailand. *The South East Asian Journal of Tropical Medicine and Public Health* **29**, 541–545.

Avril M, Brazier AJ, Melcher M, Sampath S & Smith JD (2013) DC8 and DC13 var genes associated with severe malaria bind avidly to diverse endothelial cells. *PLoS Pathogens* **9**, e1003430.

Awad MI, Eltayeb IB, Baraka OZ, Behrens RH & Alkadru AM (2004) Pharmacokinetics of artesunate following oral and rectal administration in healthy Sudanese volunteers. *Tropical Doctor* **34**, 132–135.

Axton JH & Siebert SL (1982) Aetiology of convulsions in Zimbabwe children three months to eight years old. *The Central African Journal of Medicine* **28**, 246–249.

Bagshaw SM, Uchino S, Bellomo R, *et al.* (2009) Timing of renal replacement therapy and clinical outcomes in critically ill patients with severe acute kidney injury. *Journal of Critical Care* **24**, 129–140.

Baird JK (2007) Neglect of *Plasmodium vivax* malaria. *Trends in Parasitology* **23**, 533–539.

Baird JK (2009) Severe and fatal vivax malaria challenges 'benign tertian malaria' dogma. *Annals of Tropical Paediatrics* **29**, 251–252.

Baird JK (2013) Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. *Clinical Microbiology Reviews* **26**, 36–57.

Baird JK & Surjadjaja C (2010) Consideration of ethics in primaquine therapy against malaria transmission. *Trends in Parasitology* **27**, 11–16.

Barber BE, William T, Jikal M, *et al.* (2011) *Plasmodium knowlesi* malaria in children. *Emerging Infectious Diseases* **17**, 814–820.

Barber BE, William T, Grigg M, *et al.* (2012) A prospective comparative study of knowlesi, falciparum and vivax malaria in Sabah, Malaysia: high proportion with severe disease from *Plasmodium knowlesi* and *P. vivax* but no mortality with early referral and artesunate therapy. *Clinical Infectious Diseases* **56**, 383–397.

Barber BE, William T, Grigg MJ, Piera K, Yeo TW & Anstey NM (2013a) Evaluation of the sensitivity of a pLDH-based and an aldolase-based rapid diagnostic test for diagnosis of uncomplicated and severe malaria caused by PCR-confirmed *Plasmodium knowlesi, Plasmodium falciparum*, and *Plasmodium vivax*. *Journal of Clinical Microbiology* **51**, 1118–1123.

Barber BE, William T, Grigg MJ, Yeo TW & Anstey NM (2013b) Limitations of microscopy to differentiate *Plasmodium* species in a region co-endemic for *Plasmodium falciparum, Plasmodium vivax* and *Plasmodium knowlesi*. *Malaria Journal* **12**, 8.

Barcus MJ, Basri H, Picarima H, *et al.* (2007) Demographic risk factors for severe and fatal vivax and falciparum malaria among hospital admissions in northeastern Indonesian Papua. *American Journal of Tropical Medicine and Hygiene* **77**, 984–991.

Barennes H, Kahiatani F, Pussard E, *et al.* (1995) Intrarectal Quinimax (an association of *Cinchona* alkaloids) for the treatment of *Plasmodium falciparum* malaria in children in Niger: efficacy and pharmacokinetics. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 418–421.

Barnes KI, Durrheim DN, Little F, *et al.* (2005) Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal. *South Africa. PLoS Medicine* **2**, e330.

Barsoum RS (2000) Malarial acute renal failure. *Journal of the American Society of Nephrology* **11**, 2147–2154.

Bassat Q & Alonso PL (2011) Defying malaria: Fathoming severe *Plasmodium vivax* disease. *Nature Medicine* **17**, 48–49.

Bassat Q, Guinovart C, Sigauque B, *et al.* (2008) Malaria in rural Mozambique. Part II: children admitted to hospital. *Malaria Journal* **7**, 37.

Batty KT, Davis TM, Thu LT, Binh TQ, Anh TK & Ilett KF (1996) Selective high-performance liquid chromatographic determination of artesunate and alpha- and beta-dihydroartemisinin in patients with falciparum malaria. *Journal of Chromatography B, Biomedical Applications* **677**, 345–350.

Batty KT, Le AT, Ilett KF, *et al.* (1998a) A pharmacokinetic and pharmacodynamic study of artesunate for vivax malaria. *American Journal of Tropical Medicine and Hygiene* **59**, 823–827.

Batty KT, Thu LT, Davis TM, *et al.* (1998b) A pharmacokinetic and pharmacodynamic study of intravenous vs oral artesunate in uncomplicated falciparum malaria. *British Journal of Clinical Pharmacology* **45**, 123–129.

Beare NA, Southern C, Lochhead J, Molyneux ME, Lewallen S & Harding SP (2002) Inter-observer concordance in grading retinopathy in cerebral malaria. *Annals of Tropical Medicine and Parasitology* **96**, 105–108.

Beare NA, Southern C, Chalira C, Taylor TE, Molyneux ME & Harding SP (2004) Prognostic significance and course of retinopathy in children with severe malaria. *Archives of Ophthalmology* **122**, 1141–1147.

Beare NA, Taylor TE, Harding SP, Lewallen S & Molyneux ME (2006) Malarial retinopathy: a newly established diagnostic sign in severe malaria. *American Journal of Tropical Medicine and Hygiene* **75**, 790–797.

Beare NA, Lewallen S, Taylor TE & Molyneux ME (2011) Redefining cerebral malaria by including malaria retinopathy. *Future Microbiology* **6**, 349–355.

Beg M, Khan R, Baig S, Gulzar Z, Hussain R & Smego R (2002) Cerebral involvement in benign tertian malaria. *American Journal of Tropical Medicine and Hygiene* **67**, 230–232.

Bejon P, Berkley JA, Mwangi T, *et al.* (2007) Defining childhood severe falciparum malaria for intervention studies. *PLoS Medicine* **4**, e251.

Bellomo R & Ronco C (1998) Indications and criteria for initiating renal replacement therapy in the intensive care unit. *Kidney International Supplements* **66**, S106–S109.

Bellomo R, Chapman M, Finfer S, Hickling K & Myburgh J (2000) Low-dose dopamine in patients with early renal dysfunction: a placebo-controlled randomised trial. Australian and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group. *Lancet* **356**, 2139–2143.

Berg A, Patel S, Langeland N & Blomberg B (2008) Falciparum malaria and HIV-1 in hospitalized adults in Maputo, Mozambique: does HIV-infection obscure the malaria diagnosis? *Malaria Journal* **7**, 252.

Berkley JA, Mwarumba S, Bramham K, Lowe B & Marsh K (1999) Bacteraemia complicating severe malaria in children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 283–286.

Berkley JA, Lowe BS, Mwangi I, *et al.* (2005) Bacteremia among children admitted to a rural hospital in Kenya. *New England Journal of Medicine* **352**, 39–47.

Berkley JA, Bejon P, Mwangi T, *et al.* (2009) HIV infection, malnutrition, and invasive bacterial infection among children with severe malaria. *Clinical Infectious Diseases* **49**, 336–343.

Bertin GI, Lavstsen T, Guillonneau F, *et al.* (2013) Expression of the domain cassette 8 *Plasmodium falciparum* erythrocyte membrane protein 1 is associated with cerebral malaria in Benin. *PLoS ONE* **8**, e68368.

Bethell DB, Phuong PT, Phuong CX, *et al.* (1996) Electrocardiographic monitoring in severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 266–269.

Bethell DB, Teja-Isavadharm P, Cao XT, *et al.* (1997) Pharmacokinetics of oral artesunate in children with moderately severe *Plasmodium falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 195–198.

Bethell D, Se Y, Lon C, *et al.* (2010) Dose-dependent risk of neutropenia after 7-day courses of artesunate monotherapy in Cambodian patients with acute *Plasmodium falciparum* malaria. *Clinical Infectious Diseases* **51**, e105–e114.

Bhattacharya SK, Sur D, Dutta S, *et al.* (2013) Vivax malaria and bacteraemia: a prospective study in Kolkata, India. *Malaria Journal* **12**, 176.

Bhattarai A, Ali AS, Kachur SP, *et al.* (2007) Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Medicine* **4**, e309.

Biemba G, Dolmans D, Thuma PE, Weiss G & Gordeuk VR (2000) Severe anaemia in Zambian children with *Plasmodium falciparum* malaria. *Tropical Medicine and International Health* **5**, 9–16.

Billings F & Post W (1915) Fatal malaria due to the tertian parasite. *Transactions. Chicago Pathological Society* **9**, 209–215.

Birbeck GL, Beare N, Lewallen S, *et al.* (2010a) Identification of malaria retinopathy improves the specificity of the clinical diagnosis of cerebral malaria: findings from a prospective cohort study. *American Journal of Tropical Medicine and Hygiene* **82**, 231–234.

Birbeck GL, Molyneux ME, Kaplan PW, *et al.* (2010b) Blantyre Malaria Project Epilepsy Study (BMPES) of neurological outcomes in retinopathy-positive paediatric cerebral malaria survivors: a prospective cohort study. *Lancet Neurology* **9**, 1173–1181.

Bircan Z, Kervancioglu M, Soran M, Gonlusen G & Tuncer I (1997) Two cases of nephrotic syndrome and tertian malaria in south-eastern Anatolia. *Pediatric Nephrology* **11**, 78–79.

Black RE, Cousens S, Johnson HL, *et al.* (2010) Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet* **375**, 1969–1987.

Bloland PB, Redd SC, Kazembe P, Tembenu R, Wirima JJ & Campbell CC (1991) Co-trimoxazole for childhood febrile illness in malaria-endemic regions. *Lancet* **337**, 518–520.

Boivin MJ (2002) Effects of early cerebral malaria on cognitive ability in Senegalese children. *Journal of Developmental and Behavioral Pediatrics* **23**, 353–364.

Boivin MJ, Bangirana P, Byarugaba J, *et al.* (2007) Cognitive impairment after cerebral malaria in children: a prospective study. *Pediatrics* **119**, e360–e366.

Boivin MJ, Gladstone MJ, Vokhiwa M, *et al.* (2011) Developmental outcomes in Malawian children with retinopathy-positive cerebral malaria. *Tropical Medicine and International Health* **16**, 263–271.

Bojang KA, van Hensbroek MB, Palmer A, Banya WA, Jaffar S & Greenwood BM (1997) Predictors of mortality in Gambian children with severe malaria anaemia. *Annals of Tropical Paediatrics* **17**, 355–359.

Bondi FS (1992) The incidence and outcome of neurological abnormalities in childhood cerebral malaria: a long-term follow-up of 62 survivors. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 17–19.

Boonpucknavig V & Sitprija V (1979) Renal disease in acute *Plasmodium falciparum* infection in man. *Kidney International* **16**, 44–52.

Boonpucknavig V & Soontornniyomkij V (2003) Pathology of renal diseases in the tropics. *Seminars in Nephrology* **23**, 88–106.

Bouyou-Akotet MK, Dzeing-Ella A, Kendjo E, *et al.* (2009) Impact of *Plasmodium falciparum* infection on the frequency of moderate to severe anaemia in children below 10 years of age in Gabon. *Malaria Journal* **8**, 166.

Brabin BJ (2007) Congenital malaria–a recurrent problem. *Annals of Tropical Paediatrics* **27**, 95–98.

Brewer TG, Peggins JO, Grate SJ, *et al.* (1994) Neurotoxicity in animals due to arteether and artemether. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**(Suppl. 1), S33–S36.

Brewster DR, Kwiatkowski D & White NJ (1990) Neurological sequelae of cerebral malaria in children. *Lancet* **336**, 1039–1043.

Bridges DJ, Bunn J, Van Mourik JA, *et al.* (2010) Rapid activation of endothelial cells enables *Plasmodium falciparum* adhesion to platelet-decorated von Willebrand factor strings. *Blood* **115**, 1472–1474.

Bronzan RN, Taylor TE, Mwenechanya J, *et al.* (2007) Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. *Journal of Infectious Diseases* **195**, 895–904.

Brooker S, Akhwale W, Pullan R, *et al.* (2007) Epidemiology of *Plasmodium*-helminth co-infection in Africa: populations at risk, potential impact on anemia, and prospects for combining control. *American Journal of Tropical Medicine and Hygiene* **77**, 88–98.

Brooks MH, Kiel FW, Sheehy TW & Barry KG (1968) Acute pulmonary edema in falciparum malaria: a clinicopathological correlation. *New England Journal of Medicine* **279**, 732–737.

Brooks MH, Kiel FW, Sheehy TW & Barry KG (1969) Detection of hypervolemia. *New England Journal of Medicine* **280**, 110.

Brower RG (2009) Consequences of bed rest. *Critical Care Medicine* **37**, S422–S428.

Brown H, Hien TT, Day N, *et al.* (1999) Evidence of blood-brain barrier dysfunction in human cerebral malaria. *Neuropathology and Applied Neurobiology* **25**, 331–340.

Brown HC, Chau TT, Mai NT, *et al.* (2000) Blood-brain barrier function in cerebral malaria and CNS infections in Vietnam. *Neurology* **55**, 104–111.

Bruce-Chwatt LJ (1987) Quinine and the mystery of blackwater fever. *Acta Leidensia* **55**, 181–196.

Bruetsch W (1932) The histopathology of therapeutic (tertian) malaria. *American Journal of Psychiatry* **12**, 19–65.

Bruneel F, Hocqueloux L, Alberti C, *et al.* (2003) The clinical spectrum of severe imported falciparum malaria in the intensive care unit: report of 188 cases in adults. *American Journal of Respiratory and Critical Care Medicine* **167**, 684–689.

Bruneel F, Tubach F, Corne P, *et al.* (2010) Severe imported falciparum malaria: a cohort study in 400 critically ill adults. *PLoS ONE* **5**, e13236.

Brunkhorst FM, Engel C, Bloos F, *et al.* (2008) Intensive insulin therapy and pentastarch resuscitation in severe sepsis. *New England Journal of Medicine* **358**, 125–139.

Buck RL, Alcantara AK, Uylangco CV & Cross JH (1983) Malaria at San Lazaro Hospital, Manila, Philippines, 1979–1981. *American Journal of Tropical Medicine and Hygiene* **32**, 212–216.

Buffet PA, Safeukui I, Deplaine G, *et al.* (2011) The pathogenesis of *Plasmodium falciparum* malaria in humans: insights from splenic physiology. *Blood* **117**, 381–392.

Bulmer JN, Rasheed FN, Francis N, Morrison L & Greenwood BM (1993) Placental malaria. I. Pathological classification. *Histopathology* **22**, 211–218.

Calis JC, Phiri KS, Faragher EB, *et al.* (2008) Severe anemia in Malawian children. *New England Journal of Medicine* **358**, 888–899.

Camara B, Diagne-Gueye NR, Faye PM, *et al.* (2011) Malaria severity criteria and prognostic factors among children in Dakar. *Medecine et Maladies Infectieuses* **41**, 63–67.

Caniza MA, Duenas L, Lopez B, *et al.* (2009) A practical guide to alcohol-based hand hygiene infrastructure in a resource-poor pediatric hospital. *American Journal of Infection Control* **37**, 851–854.

Cao XT, Bethell DB, Pham TP, *et al.* (1997) Comparison of artemisinin suppositories, intramuscular artesunate and intravenous quinine for the treatment of severe childhood malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 335–342.

Caramello P, Balbiano R, De Blasi T, Chiriotto M, Deagostini M & Calleri G (2012) Severe malaria, artesunate and haemolysis. *Journal of Antimicrobial Chemotherapy* **67**, 2053–2054.

Cariou A, Vinsonneau C & Dhainaut JF (2004) Adjunctive therapies in sepsis: an evidence-based review. *Critical Care Medicine* **32**, S562–S570.

Carlson J, Helmby H, Hill AV, Brewster D, Greenwood BM & Wahlgren M (1990) Human cerebral malaria: association with

erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet* **336**, 1457–1460.
Carneiro I, Roca-Feltrer A, Griffin JT, *et al.* (2010) Age-patterns of malaria vary with severity, transmission intensity and seasonality in Sub-Saharan Africa: a systematic review and pooled analysis. *PLoS ONE* **5**, e8988.
Carrara VI, Sirilak S, Thonglairuam J, *et al.* (2006) Deployment of early diagnosis and mefloquine-artesunate treatment of falciparum malaria in Thailand: the Tak Malaria Initiative. *PLoS Medicine* **3**, e183.
Carter JA, Neville BG, White S, *et al.* (2004) Increased prevalence of epilepsy associated with severe falciparum malaria in children. *Epilepsia* **45**, 978–981.
Carter JA, Mung'ala-Odera V, Neville BG, *et al.* (2005a) Persistent neurocognitive impairments associated with severe falciparum malaria in Kenyan children. *Journal of Neurology, Neurosurgery, and Psychiatry* **76**, 476–481.
Carter JA, Ross AJ, Neville BG, *et al.* (2005b) Developmental impairments following severe falciparum malaria in children. *Tropical Medicine and International Health* **10**, 3–10.
Carter JA, Lees JA, Gona JK, *et al.* (2006) Severe falciparum malaria and acquired childhood language disorder. *Developmental Medicine and Child Neurology* **48**, 51–57.
Carvalho BO, Lopes SC, Nogueira PA, *et al.* (2010) On the cytoadhesion of *Plasmodium vivax*-infected erythrocytes. *Journal of Infectious Diseases* **202**, 638–647.
Centers for Disease Control and Prevention (2013) Published reports of delayed hemolytic anemia after treatment with artesunate for severe malaria-worldwide, 2010-2012. *Morbidity and Mortality Weekly Report* **62**, 5–8.
Chakravarty A, Ghosh B, Bhattacharyya R, Sengupta S & Mukherjee S (2004) Acute inflammatory demyelinating polyneuropathy following *Plasmodium vivax* malaria. *Neurology India* **52**, 130–131.
Chalwe V, Van Geertruyden JP, Mukwamataba D, *et al.* (2009) Increased risk for severe malaria in HIV-1-infected adults, Zambia. *Emerging Infectious Disease*, **15**, 749; quiz 858.
Chau TTH, Day NP, Van Chuong L, *et al.* (1996) Blackwater fever in southern Vietnam: a prospective descriptive study of 50 cases. *Clinical Infectious Diseases* **23**, 1274–1281.
Chimalizeni Y, Kawaza K, Taylor T & Molyneux M (2010) The platelet count in cerebral malaria, is it useful to the clinician? *American Journal of Tropical Medicine and Hygiene* **83**, 48–50.
Chin W, Contacos PG, Collins WE, Jeter MH & Alpert E (1968) Experimental mosquito-transmission of *Plasmodium knowlesi* to man and monkey. *American Journal of Tropical Medicine and Hygiene* **17**, 355–358.
Choi HJ, Lee SY, Yang H & Bang JK (2004) Retinal haemorrhage in vivax malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **98**, 387–389.
Cholera R, Brittain NJ, Gillrie MR, *et al.* (2008) Impaired cytoadherence of *Plasmodium falciparum*-infected erythrocytes containing sickle hemoglobin. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 991–996.
Chotivanich KT, Dondorp AM, White NJ, *et al.* (2000) The resistance to physiological shear stresses of the erythrocytic rosettes formed by cells infected with *Plasmodium falciparum*. *Annals of Tropical Medicine and Parasitology* **94**, 219–226.
Chotivanich K, Udomsangpetch R, Suwanarusk R, *et al.* (2012) *Plasmodium vivax* adherence to placental glycosaminoglycans. *PLoS ONE* **7**, e34509.
Christophers S (1911). *Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India*. Government of India Press, Delhi.
Chung BH, Lee SW, Lee SE, Hwang TJ & Shin HS (2008) Predictors of *Plasmodium vivax* malaria-induced nephropathy in young Korean men. *Nephron. Clinical Practice* **110**, c172–c177.
Ciuca M, Chelarescu M, Sofletea A, *et al.* (1955) Contribution experimentale a l'etude de l'immunite dans le paludisme. *Editions Acad Rep Pop Roumaine*, 1–108.
Ciuca M, Lupasco G, Negulici E & Constaninesco P (1964) Research on the experimental transmission of *Plasmodium malariae* to man [in French]. *Archives Roumaines de Pathologie Expérimentales et de Microbiologie* **23**, 763–776.
Claessens A, Adams Y, Ghumra A, *et al.* (2012) A subset of group A-like var genes encodes the malaria parasite ligands for binding to human brain endothelial cells. *Proceedings of the National Academy of Sciences of the United States of America* **109**, E1772–E1781.
Clark RL (2009) Embryotoxicity of the artemisinin antimalarials and potential consequences for use in women in the first trimester. *Reproductive Toxicology* **28**, 285–296.
Clemens R, Pramoolsinsap C, Lorenz R, Pukrittayakamee S, Bock HL & White NJ (1994) Activation of the coagulation cascade in severe falciparum malaria through the intrinsic pathway. *British Journal of Haematology* **87**, 100–105.
Coatney GR, Collins WE, Warren M & Contacos PG (1971). *The Primate Malarias*. National Institutes of Health, Bethesda, MD.
Colin JF, Rottcher KH & Dhali DP (1975) Letter: venous thrombosis in Africans. *Lancet* **1**, 408.
Collins WE, Jeffery GM & Roberts JM (2003) A retrospective examination of anemia during infection of humans with *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene* **68**, 410–412.
Collomb H (1977) Putting the family to death. *La Psychiatrie de l'enfant* **20**, 245–299.
Commey JOO, Mills-Tetteh D & Phillips BJ (1980) Cerebral malaria in Accra, Ghana. *Ghana Medical Journal* **19**, 68–72.
Conroy AL, Glover SJ, Hawkes M, *et al.* (2012) Angiopoietin-2 levels are associated with retinopathy and predict mortality in Malawian children with cerebral malaria: a retrospective case-control study. *Critical Care Medicine* **40**, 952–959.
Cooke BM, Berendt AR, Craig AG, MacGregor J, Newbold CI & Nash GB (1994) Rolling and stationary cytoadhesion of red blood cells parasitized by *Plasmodium falciparum:* separate roles for ICAM-1, CD36 and thrombospondin. *British Journal of Haematology* **87**, 162–170.

Cordoliani YS, Sarrazin JL, Felten D, Caumes E, Leveque C & Fisch A (1998) MRT of cerebral malaria. *American Journal of Neuroradiology* **19**, 871–874.

Cox-Singh J, Davis TM, Lee KS, *et al.* (2008) *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clinical Infectious Diseases* **46**, 165–171.

Cox-Singh J, Hiu J, Lucas SB, *et al.* (2010) Severe malaria - a case of fatal *Plasmodium knowlesi* infection with post-mortem findings: a case report. *Malaria Journal* **9**, 10.

Cox-Singh J, Singh B, Daneshvar C, Planche T, Parker-Williams J & Krishna S (2011) Anti-inflammatory cytokines predominate in acute human *Plasmodium knowlesi* infections. *PLoS ONE* **6**, e20541.

Craig A & Scherf A (2001) Molecules on the surface of the *Plasmodium falciparum* infected erythrocyte and their role in malaria pathogenesis and immune evasion. *Molecular and Biochemical Parasitology* **115**, 129–143.

Crawley J, Smith S, Kirkham F, Muthinji P, Waruiru C & Marsh K (1996) Seizures and status epilepticus in childhood cerebral malaria. *Quarterly Journal of Medicine* **89**, 591–597.

Crawley J, Waruiru C, Mithwani S, *et al.* (2000) Effect of phenobarbital on seizure frequency and mortality in childhood cerebral malaria: a randomised, controlled intervention study. *Lancet* **355**, 701–706.

Cunnington AJ, De Souza JB, Walther M & Riley EM (2012a) Malaria impairs resistance to *Salmonella* through heme- and heme oxygenase-dependent dysfunctional granulocyte mobilization. *Nature Medicine* **18**, 120–127.

Cunnington AJ, Njie M, Correa S, Takem EN, Riley EM & Walther M (2012b) Prolonged neutrophil dysfunction after *Plasmodium falciparum* malaria is related to hemolysis and heme oxygenase-1 induction. *Journal of Immunology* **189**, 5336–5346.

Curley JM, Mody RM & Gasser RA (2011) Malaria caused by *Plasmodium vivax* complicated by acalculous cholecystitis. *American Journal of Tropical Medicine and Hygiene* **85**, 42–49.

Cyrklaff M, Sanchez CP, Kilian N, *et al.* (2011) Hemoglobins S and C interfere with actin remodeling in *Plasmodium falciparum*-infected erythrocytes. *Science* **334**, 1283–1286.

D'Alessandro U, Ubben D, Hamed K, *et al.* (2012) Malaria in infants aged less than six months - is it an area of unmet medical need? *Malaria Journal* **11**, 400.

Daneshvar C, Davis TM, Cox-Singh J, *et al.* (2009) Clinical and laboratory features of human *Plasmodium knowlesi* infection. *Clinical Infectious Diseases* **49**, 852–860.

Darouiche RO, Wall MJ, Itani KM, *et al.* (2010) Chlorhexidine-alcohol versus povidone-iodine for surgical-site antisepsis. *New England Journal of Medicine* **362**, 18–26.

Das BS, Satpathy SK, Mohanty D, *et al.* (1988) Hypoglycaemia in severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 197–201.

Das SN, Mohapatra B, Mohanty R, Dash PC, Kar K & Dash PK (2007) Malarial hepatitis as a component of multiorgan failure–a bad prognostic sign. *Journal of the Indian Medical Association* **105**, 247–250.

Dasari P, Reiss K, Lingelbach K, *et al.* (2011) Digestive vacuoles of *Plasmodium falciparum* are selectively phagocytosed by and impair killing function of polymorphonuclear leukocytes. *Blood* **118**, 4946–4956.

David J, Shanbag P & More V (2009) *Plasmodium vivax* malaria presenting as the nephrotic syndrome in an infant. *Tropical Doctor* **39**, 127–128.

Davis TM, White NJ, Looareesuwan S, Silamut K & Warrell DA (1988) Quinine pharmacokinetics in cerebral malaria: predicted plasma concentrations after rapid intravenous loading using a two-compartment model. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 542–547.

Davis TM, Pukrittayakamee S, Woodhead JS, Holloway P, Chaivisuth B & White NJ (1991) Calcium and phosphate metabolism in acute falciparum malaria. *Clinical Science* **81**, 297–304.

Davis TM, Phuong HL, Ilett KF, *et al.* (2001) Pharmacokinetics and pharmacodynamics of intravenous artesunate in severe falciparum malaria. *Antimicrobial Agents and Chemotherapy* **45**, 181–186.

Davis TM, Binh TQ, Ilett KF, *et al.* (2003) Penetration of dihydroartemisinin into cerebrospinal fluid after administration of intravenous artesunate in severe falciparum malaria. *Antimicrobial Agents and Chemotherapy* **47**, 368–370.

Davis JS, Cheng AC, McMillan M, Humphrey AB, Stephens DP & Anstey NM (2011) Sepsis in the tropical Top End of Australia's Northern Territory: disease burden and impact on Indigenous Australians. *The Medical Journal of Australia* **194**, 519–524.

Day NP, Pham TD, Phan TL, *et al.* (1996a) Clearance kinetics of parasites and pigment-containing leukocytes in severe malaria. *Blood* **88**, 4694–4700.

Day NP, Phu NH, Bethell DP, *et al.* (1996b) The effects of dopamine and adrenaline infusions on acid-base balance and systemic haemodynamics in severe infection. *Lancet* **348**, 219–223.

Day NP, Phu NH, Mai NT, *et al.* (2000a) Effects of dopamine and epinephrine infusions on renal hemodynamics in severe malaria and severe sepsis. *Critical Care Medicine* **28**, 1353–1362.

Day NP, Phu NH, Mai NT, *et al.* (2000b) The pathophysiologic and prognostic significance of acidosis in severe adult malaria. *Critical Care Medicine* **28**, 1833–1840.

De Mast Q, Groot E, Asih PB, *et al.* (2009) ADAMTS13 deficiency with elevated levels of ultra-large and active von Willebrand factor in *P. falciparum* and *P. vivax* malaria. *American Journal of Tropical Medicine and Hygiene* **80**, 492–498.

De Silva HJ, Goonetilleke AK, Senaratna N, *et al.* (1988) Skeletal muscle necrosis in severe falciparum malaria. *British Medical Journal (Clinical Research ed.)* **296**, 1039.

Del Punta V, Gulletta M, Matteelli A, Spinoni V, Regazzoli A & Castelli F (2010) Congenital *Plasmodium vivax* malaria mimicking neonatal sepsis: a case report. *Malaria Journal* **9**, 63.

Dellinger RP, Levy MM, Carlet JM, *et al.* (2008) Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Intensive Care Medicine* **34**, 17–60.

Deplaine G, Safeukui I, Jeddi F, *et al.* (2011) The sensing of poorly deformable red blood cells by the human spleen can be mimicked in vitro. *Blood* **117**, e88–e95.

Desai M, Ter Kuile FO, Nosten F, *et al.* (2007) Epidemiology and burden of malaria in pregnancy. *Lancet Infectious Diseases* **7**, 93–104.

Desakorn V, Dondorp AM, Silamut K, *et al.* (2005) Stage-dependent production and release of histidine-rich protein 2 by *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99**, 517–524.

Devakul K, Harinasuta T & Reid HA (1966) 125-I-labelled fibrinogen in cerebral malaria. *Lancet* **2**, 886–888.

Dhingra N, Jha P, Sharma VP, *et al.* (2010) Adult and child malaria mortality in India: a nationally representative mortality survey. *Lancet* **376**, 1768–1774.

Doering W (1981) Is there a clinically relevant interaction between quinine and digoxin in human beings? *American Journal of Cardiology* **48**, 975–976.

Dondorp AM, Angus BJ, Hardeman MR, *et al.* (1997) Prognostic significance of reduced red blood cell deformability in severe falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **57**, 507–511.

Dondorp AM, Angus BJ, Chotivanich K, *et al.* (1999) Red blood cell deformability as a predictor of anemia in severe falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **60**, 733–737.

Dondorp AM, Nyanoti M, Kager PA, Mithwani S, Vreeken J & Marsh K (2002) The role of reduced red cell deformability in the pathogenesis of severe falciparum malaria and its restoration by blood transfusion. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96**, 282–286.

Dondorp AM, Chau TT, Phu NH, *et al.* (2004a) Unidentified acids of strong prognostic significance in severe malaria. *Critical Care Medicine* **32**, 1683–1688.

Dondorp AM, Pongponratn E & White NJ (2004b) Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology. *Acta Tropica* **89**, 309–317.

Dondorp A, Nosten F, Stepniewska K, Day N & White N (2005a) Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* **366**, 717–725.

Dondorp AM, Desakorn V, Pongtavornpinyo W, *et al.* (2005b) Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. *PLoS Medicine* **2**, e204.

Dondorp AM, Ince C, Charunwatthana P, *et al.* (2008a) Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. *Journal of Infectious Diseases* **197**, 79–84.

Dondorp AM, Lee SJ, Faiz MA, *et al.* (2008b) The relationship between age and the manifestations of and mortality associated with severe malaria. *Clinical Infectious Diseases* **47**, 151–157.

Dondorp AM, Nosten F, Yi P, *et al.* (2009) Artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine* **361**, 455–467.

Dondorp AM, Fanello CI, Hendriksen IC, *et al.* (2010) Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet* **376**, 1647–1657.

Dorovini-Zis K, Schmidt K, Huynh H, *et al.* (2011) The neuropathology of fatal cerebral malaria in Malawian children. *American Journal of Pathology* **178**, 2146–2158.

Douglas NM, Anstey NM, Angus BJ, Nosten F & Price RN (2010) Artemisinin combination therapy for vivax malaria. *Lancet Infectious Diseases* **10**, 405–416.

Douglas NM, Anstey NM, Buffet PA, *et al.* (2012) The anaemia of *Plasmodium vivax* malaria. *Malaria Journal* **11**, 135.

Douglas NM, Lampah DA, Kenangalem E, *et al.* (2013) Major burden of severe anemia from non-falciparum species: a hospital-based study. *PLoS Medicine* **10**, e1001575.

Duarte MIS, Corbett CEP, Boulos M & Amato Neto V (1985) Ultrastructure of the lung in falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **34**, 31–35.

Dugbartey AT, Dugbartey MT & Apedo MY (1998) Delayed neuropsychiatric effects of malaria in Ghana. *Journal of Nervous and Mental Disease* **186**, 183–186.

Duggan ST & Scott LJ (2009) Intravenous paracetamol (acetaminophen). *Drugs* **69**, 101–113.

Dumas M, Léger J-M, Pestre-Alexandre M & Malauzat D (1986) *Manifestations Neurologiques et Psychiatriques Des Parasitoses*. Masson, Presented at Congrès de Psychiatrie et de Neurologie de Langue Française, session LXXXIV, Le Mans, France,1986.

Dunser MW, Festic E, Dondorp A, *et al.* (2012) Recommendations for sepsis management in resource-limited settings. *Intensive Care Medicine* **38**, 557–574.

Edlund PO, Westerlund D, Carlqvist J, Wu BL & Jin YH (1984) Determination of artesunate and dihydroartemisinine in plasma by liquid chromatography with post-column derivatization and UV-detection. *Acta Pharmaceutica Suecica* **21**, 223–234.

Edstein M, Stace J & Shann F (1983) Quantification of quinine in human serum by high-performance liquid chromatography. *Journal of Chromatography* **278**, 445–451.

Eiam-Ong S (2003) Malarial nephropathy. *Seminars in Nephrology* **23**, 21–33.

Ekanem AD, Anah MU & Udo JJ (2008) The prevalence of congenital malaria among neonates with suspected sepsis in Calabar, Nigeria. *Tropical Doctor* **38**, 73–76.

Elesha SO, Adepoju FB & Banjo AA (1993) Rising incidence of cerebral malaria in Lagos, Nigeria: a postmoterm study. *East African Medical Journal* **70**, 302–306.

Elhassan E & Schrier R (2011) Acute Kidney Injury. In *Textbook of Critical Care Medicine*, (eds Moore F, Vincent J-L, Abraham E, Kochanek P & Fink M) Elsevier, Philadelphia.

Eltahir HG, Omer AA, Mohamed AA & Adam I (2010) Comparison of artesunate and quinine in the treatment of Sudanese children with severe *Plasmodium falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **104**, 684–686.

English M, Punt J, Mwangi I, McHugh K & Marsh K (1996a) Clinical overlap between malaria and severe pneumonia in Africa children in hospital. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 658–662.

English M, Waruiru C, Amukoye E, *et al.* (1996b) Deep breathing in children with severe malaria: indicator of metabolic aci-

dosis and poor outcome. *American Journal of Tropical Medicine and Hygiene* **55**, 521–524.
English M, Waruiru C & Marsh K (1996c) Transfusion for respiratory distress in life-threatening childhood malaria. *American Journal of Tropical Medicine and Hygiene* **55**, 525–530.
English MC, Waruiru C, Lightowler C, Murphy SA, Kirigha G & Marsh K (1996d) Hyponatraemia and dehydration in severe malaria. *Archives of Disease in Childhood* **74**, 201–205.
English M, Muambi B, Mithwani S & Marsh K (1997) Lactic acidosis and oxygen debt in African children with severe anaemia. *Quarterly Journal of Medicine* **90**, 563–569.
English M, Ahmed M, Ngando C, Berkley J & Ross A (2002) Blood transfusion for severe anaemia in children in a Kenyan hospital. *Lancet* **359**, 494–495.
Erdman LK, Dhabangi A, Musoke C, *et al.* (2011) Combinations of host biomarkers predict mortality among Ugandan children with severe malaria: a retrospective case-control study. *PLoS ONE* **6**, e17440.
Ernest SK, Anunobi NE & Adeniyi A (2002) Correlates of emergency response interval and mortality from severe anaemia in childhood. *West African Journal of Medicine* **21**, 177–179.
Esamai F, Ernerudh J, Janols H, *et al.* (2003) Cerebral malaria in children: serum and cerebrospinal fluid TNF-alpha and TGF-beta levels and their relationship to clinical outcome. *Journal of Tropical Pediatrics* **49**, 216–223.
Evans JA, Adusei A, Timmann C, *et al.* (2004) High mortality of infant bacteraemia clinically indistinguishable from severe malaria. *QJM: Monthly Journal of the Association of Physicians* **97**, 591–597.
Ewing J (1901) Malarial parasitology. *Journal of Experimental Medicine* **5**, 429–491.
Falade C, Mokuolu O, Okafor H, *et al.* (2007) Epidemiology of congenital malaria in Nigeria: a multi-centre study. *Tropical Medicine and International Health* **12**, 1279–1287.
Falade CO, Tongo OO, Ogunkunle OO & Orimadegun AE (2010) Effects of malaria in pregnancy on newborn anthropometry. *Journal of Infection in Developing Countries* **4**, 448–453.
Falchook GS, Malone CM, Upton S & Shandera WX (2003) Postmalaria neurological syndrome after treatment of *Plasmodium falciparum* malaria in the United States. *Clinical Infectious Diseases* **37**, e22–e24.
Familusi JB & Sinnette CH (1971) Febrile convulsions in Ibadan children. *African Journal of Medicine and Medical Sciences* **2**, 135–150.
Fatih FA, Siner A, Ahmed A, *et al.* (2012) Cytoadherence and virulence - the case of *Plasmodium knowlesi* malaria. *Malaria Journal* **11**, 33.
Field JW (1949) Blood examination and prognosis in acute falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **43**, 33–48.
Field JW & Niven IC (1937) A note on prognosis in relation to parasite counts in acute subtertian malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **30**, 569–574.
Field J & Shute P (1956). *Plasmodium vivax* in the Microscopic Diagnosis of Human Malaria. Vol 2. A Morphological Study of the Erythrocytic Parasites. Institute of Medical Research.
Figtree M, Lee R, Bain L, *et al.* (2010) *Plasmodium knowlesi* in Human, Indonesian Borneo. *Emerging Infectious Diseases* **16**, 672–674.
Fleming AF (1989) Tropical obstetrics and gynaecology. 1. Anaemia in pregnancy in tropical Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 441–448.
Flowe B, Armstrong-Jame D, Danc C, Bremne F & Doherty T (2011) Blind, breathless, and paralysed from benign malaria. *Lancet* **377**, 438.
Fong Y, Cadigan F & Coatneyi G (1971) A presumptive case of naturally occurring *Plasmodium knowlesi* malaria in man in Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **65**, 839–840.
Fowkes FJ, Allen SJ, Allen A, Alpers MP, Weatherall DJ & Day KP (2008) Increased microerythrocyte count in homozygous alpha(+)-thalassaemia contributes to protection against severe malarial anaemia. *PLoS Medicine* **5**, e56.
Fox LL, Taylor TE, Pensulo P, *et al.* (2013) Histidine-rich protein 2 plasma levels predict progression to cerebral malaria in Malawian children with *Plasmodium falciparum* infection. *Journal of Infectious Diseases* **208**, 500–503.
Franklin BS, Parroche P, Ataide MA, *et al.* (2009) Malaria primes the innate immune response due to interferon-gamma induced enhancement of toll-like receptor expression and function. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 5789–5794.
Fried M & Duffy PE (1996) Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. *Science* **272**, 1502–1504.
Fried M, Nosten F, Brockman A, Brabin BJ & Duffy PE (1998) Maternal antibodies block malaria. *Nature* **395**, 851–852.
Friedrich JO, Adhikari N, Herridge MS & Beyene J (2005) Meta-analysis: low-dose dopamine increases urine output but does not prevent renal dysfunction or death. *Annals of Internal Medicine* **142**, 510–524.
Gabriel DP, Caramori JT, Martin LC, Barretti P & Balbi AL (2009) Continuous peritoneal dialysis compared with daily hemodialysis in patients with acute kidney injury. *Peritoneal Dialysis International* **29**(Suppl 2), S62–S71.
Gambaro G, Bertaglia G, Puma G & D'Angelo A (2002) Diuretics and dopamine for the prevention and treatment of acute renal failure: a critical reappraisal. *Journal of Nephrology* **15**, 213–219.
Genovese RF & Newman DB (2008) Understanding artemisinin-induced brainstem neurotoxicity. *Archives of Toxicology* **82**, 379–385.
Genton B, D'Acremont V, Rare L, *et al.* (2008) *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. *PLoS Medicine* **5**, e127.
Gerardin P, Rogier C, Ka AS, Jouvencel P, Brousse V & Imbert P (2002) Prognostic value of thrombocytopenia in African children with falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **66**, 686–691.

Gilles HM & Hendrickse RG (1960) Possible aetiological role of *Plasmodium malariae* in "nephrotic syndrome" in Nigerian children. *Lancet* **1**, 806–807.

Gilles HM & Hendrickse RG (1963) Nephrosis in Nigerian children. Role of *Plasmodium malariae*, and effect of antimalarial treatment. *British Medical Journal* **2**, 27–31.

Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P & Hornick RB (1975) Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of *Salmonella typhi* in typhoid fever. *Lancet* **1**, 1211–1213.

Goller JL, Jolley D, Ringwald P & Biggs BA (2007) Regional differences in the response of *Plasmodium vivax* malaria to primaquine as anti-relapse therapy. *American Journal of Tropical Medicine and Hygiene* **76**, 203–207.

Gomes MF, Faiz MA, Gyapong JO, *et al.* (2009) Pre-referral rectal artesunate to prevent death and disability in severe malaria: a placebo-controlled trial. *Lancet* **373**, 557–566.

Goncalves RM, Scopel KK, Bastos MS & Ferreira MU (2012) Cytokine balance in human malaria: does *Plasmodium vivax* elicit more inflammatory responses than *Plasmodium falciparum*? *PLoS ONE* **7**, e44394.

Gopinathan VP & Subramanian AR (1986) Vivax and falciparum malaria seen at an Indian service hospital. *Journal of Tropical Medicine and Hygiene* **89**, 51–55.

Graham SM, Walsh AL, Molyneux EM, Phiri AJ & Molyneux ME (2000) Clinical presentation of non-typhoidal Salmonella bacteraemia in Malawian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 310–314.

Grams ME, Estrella MM, Coresh J, Brower RG & Liu KD (2011) Fluid balance, diuretic use, and mortality in acute kidney injury. *Clinical Journal of the American Society of Nephrology* **6**, 966–973.

Grau GE, Piguet PF, Vassalli P & Lambert PH (1989) Tumor-necrosis factor and other cytokines in cerebral malaria: experimental and clinical data. *Immunological Reviews* **112**, 49–70.

Grau GE, Mackenzie CD, Carr RA, *et al.* (2003) Platelet accumulation in brain microvessels in fatal pediatric cerebral malaria. *Journal of Infectious Diseases* **187**, 461–466.

Graves PM, Osgood DE, Thomson MC, *et al.* (2008) Effectiveness of malaria control during changing climate conditions in Eritrea, 1998-2003. *Tropical Medicine and International Health* **13**, 218–228.

Greenwood BM, Bradley AK, Greenwood AM, *et al.* (1987) Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 478–486.

Greenwood B, Marsh K & Snow R (1991) Why do some African children develop severe malaria? *Parasitology Today (Personal ed.)* **7**, 277–281.

Grimwade K, French N, Mbatha DD, Zungu DD, Dedicoat M & Gilks CF (2004) HIV infection as a cofactor for severe falciparum malaria in adults living in a region of unstable malaria transmission in South Africa. *AIDS* **18**, 547–554.

Gu Y, Li Q, Melendez V & Weina P (2008) Comparison of HPLC with electrochemical detection and LC-MS/MS for the separation and validation of artesunate and dihydroartemisinin in animal and human plasma. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences* **867**, 213–218.

Guerin C, Reignier J, Richard JC, *et al.* (2013) Prone positioning in severe acute respiratory distress syndrome. *New England Journal of Medicine* **368**, 2159–2168.

Gwer S, Newton CR & Berkley JA (2007) Over-diagnosis and co-morbidity of severe malaria in African children: a guide for clinicians. *American Journal of Tropical Medicine and Hygiene* **77**, 6–13.

Gwer SA, Idro RI, Fegan G, *et al.* (2013) Fosphenytoin for seizure prevention in childhood coma in Africa: a randomized clinical trial. *Journal of Critical Care* **28**, 1086–1092.

Gyr K, Speck B, Ritz R, Cornu P & Buckner CD (1974) Cerebral tropical malaria with blackwater fever. A current diagnostic and therapeutic problem]. *Schweizerische Medizinische Wochenschrift* **104**, 1628–1630.

Halpaap B, Ndjave M, Paris M, Benakis A & Kremsner PG (1998) Plasma levels of artesunate and dihydroartemisinin in children with *Plasmodium falciparum* malaria in Gabon after administration of 50-milligram artesunate suppositories. *American Journal of Tropical Medicine and Hygiene* **58**, 365–368.

Hamel MJ, Adazu K, Obor D, *et al.* (2011) A reversal in reductions of child mortality in western Kenya, 2003-2009. *American Journal of Tropical Medicine and Hygiene* **85**, 597–605.

Handayani S, Chiu DT, Tjitra E, *et al.* (2009) High deformability of *Plasmodium vivax*-infected red blood cells under microfluidic conditions. *Journal of Infectious Diseases* **199**, 445–450.

Hanpithakpong W, Kamanikom B, Singhasivanon P, White NJ, Day NP & Lindegardh N (2009) A liquid chromatographic-tandem mass spectrometric method for determination of artemether and its metabolite dihydroartemisinin in human plasma. *Bioanalysis* **1**, 37–46.

Hanson J, Hossain A, Charunwatthana P, *et al.* (2009) Hyponatremia in severe malaria: evidence for an appropriate antidiuretic hormone response to hypovolemia. *American Journal of Tropical Medicine and Hygiene* **80**, 141–145.

Hanson J, Lee SJ, Mohanty S, *et al.* (2010) A simple score to predict the outcome of severe malaria in adults. *Clinical Infectious Diseases* **50**, 679–685.

Hanson J, Hasan MM, Royakkers AA, *et al.* (2011a) Laboratory prediction of the requirement for renal replacement in acute falciparum malaria. *Malaria Journal* **10**, 217.

Hanson J, Lam SW, Mohanty S, *et al.* (2011b) Central venous catheter use in severe malaria: time to reconsider the World Health Organization guidelines? *Malaria Journal* **10**, 342.

Hanson J, Lam SW, Mahanta KC, *et al.* (2012) Relative contributions of macrovascular and microvascular dysfunction to disease severity in falciparum malaria. *Journal of Infectious Diseases* **206**, 571–579.

Hanson JP, Lam SW, Mohanty S, *et al.* (2013) Fluid resuscitation of adults with severe falciparum malaria: effects on Acid-

base status, renal function, and extravascular lung water. *Critical Care Medicine* **41**, 972–981.

Harding SP, Lewallen S, Beare NA, Smith A, Taylor TE & Molyneux ME (2006) Classifying and grading retinal signs in severe malaria. *Tropical Doctor* **36**(Suppl 1), 1–13.

Harish R & Gupta S (2009) *Plasmodium vivax* malaria presenting with severe thrombocytopenia, cerebral complications and hydrocephalus. *Indian Journal of Pediatrics* **76**, 551–552.

Haynes RK, Chan WC, Wong HN, *et al.* (2010) Facile oxidation of leucomethylene blue and dihydroflavins by artemisinins: relationship with flavoenzyme function and antimalarial mechanism of action. *ChemMedChem* **5**, 1282–1299.

Haynes RK, Cheu KW, Tang MM, *et al.* (2011) Reactions of antimalarial peroxides with each of leucomethylene blue and dihydroflavins: flavin reductase and the cofactor model exemplified. *ChemMedChem* **6**, 279–291.

Haynes RK, Cheu KW, Chan HW, *et al.* (2012) Interactions between artemisinins and other antimalarial drugs in relation to the cofactor model–a unifying proposal for drug action. *ChemMedChem* **7**, 2204–2226.

Hebert PC, Wells G, Blajchman MA, *et al.* (1999) A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. *New England Journal of Medicine* **340**, 409–417.

Helbok R, Kendjo E, Issifou S, *et al.* (2009) The Lambarene Organ Dysfunction Score (LODS) is a simple clinical predictor of fatal malaria in African children. *Journal of Infectious Diseases* **200**, 1834–1841.

Hemmer CJ, Holst FG, Kern P, Chiwakata CB, Dietrich M & Reisinger EC (2006) Stronger host response per parasitized erythrocyte in *Plasmodium vivax* or *ovale* than in *Plasmodium falciparum* malaria. *Tropical Medicine and International Health* **11**, 817–823.

Hendriksen IC, Mtove G, Pedro AJ, *et al.* (2011) Evaluation of a PfHRP2 and a pLDH-based rapid diagnostic test for the diagnosis of severe malaria in 2 populations of African children. *Clinical Infectious Diseases* **52**, 1100–1107.

Hendriksen IC, Ferr J, Montoya P, *et al.* (2012a) Diagnosis, clinical presentation, and in-hospital mortality of severe malaria in HIV-coinfected children and adults in Mozambique. *Clinical Infectious Diseases* **55**, 1144–1153.

Hendriksen IC, Mwanga-Amumpaire J, von Seidlein L, *et al.* (2012b) Diagnosing severe falciparum malaria in parasitaemic African children: a prospective evaluation of plasma PfHRP2 measurement. *PLoS Medicine* **9**, e1001297.

Hendriksen IC, Maiga D, Lemnge MM, *et al.* (2013a) Population pharmacokinetic and pharmacodynamic properties of intramuscular quinine in Tanzanian children with severe Falciparum malaria. *Antimicrobial Agents and Chemotherapy* **57**, 775–783.

Hendriksen IC, Mtove G, Kent A, *et al.* (2013b) Population pharmacokinetics of intramuscular artesunate in African children with severe malaria: implications for a practical dosing regimen. *Clinical Pharmacology & Therapeutics* **93**, 443–450.

Hendriksen IC, White LJ, Veenemans J, *et al.* (2013c) Defining falciparum-malaria-attributable severe febrile illness in moderate-to-high transmission settings on the basis of plasma PfHRP2 concentration. *Journal of Infectious Diseases* **207**, 351–361.

Hero M, Harding SP, Riva CE, Winstanley PA, Peshu N & Marsh K (1997) Photographic and angiographic characterization of the retina of Kenyan children with severe malaria. *Archives of Ophthalmology* **115**, 997–1003.

Hien TT, Vinh H & Cuong B (1990) Experience in the management of severe falciparum malaria. The Malaria Symposium & Workshop on Complications, Management and Prevention Feb 21-24, 199 Ho Chi Minh City, Vietnam, 23–24.

Hien TT, Arnold K, Vinh H, *et al.* (1992) Comparison of artemisinin suppositories with intravenous artesunate and intravenous quinine in the treatment of cerebral malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 582–583.

Hien TT, Phu NH, Mai N, *et al.* (1996) Controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. *New England Journal of Medicine* **335**, 76–83.

Hien TT, Turner GD, Mai NT, *et al.* (2003) Neuropathological assessment of artemether-treated severe malaria. *Lancet* **362**, 295–296.

Hien TT, Davis TM, Chuong LV, *et al.* (2004) Comparative pharmacokinetics of intramuscular artesunate and artemether in patients with severe falciparum malaria. *Antimicrobial Agents and Chemotherapy* **48**, 4234–4239.

Ho M, Davis TM, Silamut K, Bunnag D & White NJ (1991) Rosette formation of *Plasmodium falciparum*-infected erythrocytes from patients with acute malaria. *Infection and Immunity* **59**, 2135–2139.

Hodel EM, Zanolari B, Mercier T, *et al.* (2009) A single LC-tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma. *Journal of Chromatography B* **877**, 867–886.

Hoffman SL, Rustama D, Punjabi NH, *et al.* (1988) High-dose dexamethasone in quinine-treated patients with cerebral malaria: a double-blind, placebo-controlled trial. *Journal of Infectious Diseases* **158**, 325–331.

Holding PA, Stevenson J, Peshu N & Marsh K (1999) Cognitive sequelae of severe malaria with impaired consciousness. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 529–534.

Holst FG, Hemmer CJ, Kern P & Dietrich M (1994) Inappropriate secretion of antidiuretic hormone and hyponatremia in severe falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **50**, 602–607.

Horstmann RD, Dietrich M, Bienzle U & Rasche H (1981) Malaria-induced thrombocytopenia. *Blut* **42**, 157–164.

Ibiwoye MO, Howard CV, Sibbons P, Hasan M & Van Velzen D (1993) Cerebral malaria in the rhesus monkey (*Macaca mulatta*): observations on host pathology. *Journal of Comparative Pathology* **108**, 303–310.

Idro R, Karamagi C & Tumwine J (2004) Immediate outcome and prognostic factors for cerebral malaria among children

admitted to Mulago Hospital, Uganda. *Annals of Tropical Paediatrics* **24**, 17–24.

Idro R, Jenkins NE & Newton CR (2005a) Pathogenesis, clinical features, and neurological outcome of cerebral malaria. *Lancet Neurology* **4**, 827–840.

Idro R, Otieno G, White S, *et al.* (2005b) Decorticate, decerebrate and opisthotonic posturing and seizures in Kenyan children with cerebral malaria. *Malaria Journal* **4**, 57.

Idro R, Aloyo J, Mayende L, Bitarakwate E, John CC & Kivumbi GW (2006a) Severe malaria in children in areas with low, moderate and high transmission intensity in Uganda. *Tropical Medicine and International Health* **11**, 115–124.

Idro R, Carter JA, Fegan G, Neville BG & Newton CR (2006b) Risk factors for persisting neurological and cognitive impairments following cerebral malaria. *Archives of Disease in Childhood* **91**, 142–148.

Idro R, Ndiritu M, Ogutu B, *et al.* (2007) Burden, features, and outcome of neurological involvement in acute falciparum malaria in Kenyan children. *Journal of The American Medical Association* **297**, 2232–2240.

Idro R, Kakooza-Mwesige A, Balyejjussa S, *et al.* (2010) Severe neurological sequelae and behaviour problems after cerebral malaria in Ugandan children. *BMC Research Notes* **3**, 104.

Ilett KF, Batty KT, Powell SM, *et al.* (2002a) The pharmacokinetic properties of intramuscular artesunate and rectal dihydroartemisinin in uncomplicated falciparum malaria. *British Journal of Clinical Pharmacology* **53**, 23–30.

Ilett KF, Ethell BT, Maggs JL, *et al.* (2002b) Glucuronidation of dihydroartemisinin in vivo and by human liver microsomes and expressed UDP-glucuronosyltransferases. *Drug Metabolism and Disposition* **30**, 1005–1012.

Imani PD, Musoke P, Byarugaba J & Tumwine JK (2011) Human immunodeficiency virus infection and cerebral malaria in children in Uganda: a case-control study. *BMC Pediatrics* **11**, 5.

Imbert P, Rapp C & Buffet PA (2009) Pathological rupture of the spleen in malaria: analysis of 55 cases (1958-2008). *Travel Medicine and Infectious Disease* **7**, 147–159.

Imwong M, Tanomsing N, Pukrittayakamee S, Day NP, White NJ & Snounou G (2009) Spurious amplification of a *Plasmodium vivax* small-subunit RNA gene by use of primers currently used to detect *P. knowlesi*. *Journal of Clinical Microbiology* **47**, 4173–4175.

Iqbal J, Siddique A, Jameel M & Hira PR (2004) Persistent histidine-rich protein 2, parasite lactate dehydrogenase, and panmalarial antigen reactivity after clearance of *Plasmodium falciparum* monoinfection. *Journal of Clinical Microbiology* **42**, 4237–4241.

Ismail S, Na Bangchang K, Karbwang J, Back DJ & Edwards G (1995) Paracetamol disposition in Thai patients during and after treatment of falciparum malaria. *European Journal of Clinical Pharmacology* **48**, 65–69.

Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, Hirt R, Cardesa A & Alonso PL (2000) Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. *Human Pathology* **31**, 85–93.

Issifou S, Kendjo E, Missinou MA, *et al.* (2007) Differences in presentation of severe malaria in urban and rural Gabon. *American Journal of Tropical Medicine and Hygiene* **77**, 1015–1019.

Jain V, Lucchi NW, Wilson NO, *et al.* (2011) Plasma levels of angiopoietin-1 and -2 predict cerebral malaria outcome in Central India. *Malaria Journal* **10**, 383.

Jakeman GN, Saul A, Hogarth WL & Collins WE (1999) Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. *Parasitology* **119**(Pt 2), 127–133.

Jakobsen PH, Morris-Jones S, Ronn A, *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.

Jallow M, Casals-Pascual C, Ackerman H, *et al.* (2012) Clinical features of severe malaria associated with death: a 13-year observational study in the Gambia. *PLoS ONE* **7**, e45645.

James S (1925) The disappearance of malaria from England. *Proceedings of the Royal Society of Medicine* **23**, 71–85.

James S (1933) Third general report of the malaria commission of the health organization of the League of Nations. *Quarterly Bulletin Health Organisation of League of Nations* **2**, 217.

Jansen FH & Soomro SA (2007) Chemical instability determines the biological action of the artemisinins. *Current Medicinal Chemistry* **14**, 3243–3259.

Jarvis JN, Planche T, Bicanic T, *et al.* (2006) Lactic acidosis in Gabonese children with severe malaria is unrelated to dehydration. *Clinical Infectious Diseases* **42**, 1719–1725.

Jat KR, Guglani V & Khairwa A (2012) Severe and complicated *Plasmodium vivax* malaria in children. *Tropical Doctor* **42**, 85–87.

Jenkins N, Wu Y, Chakravorty S, Kai O, Marsh K & Craig A (2007) *Plasmodium falciparum* intercellular adhesion molecule-1-based cytoadherence-related signaling in human endothelial cells. *Journal of Infectious Diseases* **196**, 321–327.

Jiang N, Chang Q, Sun X, *et al.* (2010) Co-infections with *Plasmodium knowlesi* and other malaria parasites, Myanmar. *Emerging Infectious Diseases* **16**, 1476–1478.

John CC, Bangirana P, Byarugaba J, *et al.* (2008) Cerebral malaria in children is associated with long-term cognitive impairment. *Pediatrics* **122**, e92–e99.

Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T & Kanbara H (2004) Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. *Emerging Infectious Diseases* **10**, 2211–2213.

Jongwutiwes S, Buppan P, Kosuvin R, *et al.* (2011) *Plasmodium knowlesi* malaria in humans and Macaques, Thailand. *Emerging Infectious Diseases* **17**, 1799–1806.

Kapland LI, Read HS & Mullins DF (1946) Spontaneous rupture of the spleen during malaria therapy; report of a case. *Annals of Internal Medicine* **24**, 707–714.

Karunaweera ND, Carter R, Grau GE, Kwiatkowski D, Del Giudice G & Mendis KN (1992) Tumour necrosis factor-depen-

dent parasite-killing effects during paroxysms in non-immune *Plasmodium vivax* malaria patients. *Clinical and Experimental Immunology* **88**, 499–505.
Karunaweera ND, Wijesekera SK, Wanasekera D, Mendis KN & Carter R (2003) The paroxysm of *Plasmodium vivax* malaria. *Trends in Parasitology* **19**, 188–193.
Karunaweera N, Wanasekara D, Chandrasekharan V, Mendis K & Carter R (2007) *Plasmodium vivax:* paroxysm-associated lipids mediate leukocyte aggregation. *Malaria Journal* **6**, 62.
Kasliwal P, Rao MS & Kujur R (2009) *Plasmodium vivax* malaria: an unusual presentation. *Indian Journal of Critical Care Medicine* **13**, 103–105.
Kastl AJ, Daroff RB & Blocker WW (1968) Psychological testing of cerebral malaria patients. *Journal of Nervous and Mental Disease* **147**, 553–561.
Kaushik JS, Gomber S & Dewan P (2012) Clinical and epidemiological profiles of severe malaria in children from Delhi, India. *Journal of Health, Population, and Nutrition* **30**, 113–116.
Khim N, Siv S, Kim S, *et al.* (2011) *Plasmodium knowlesi* infection in humans, Cambodia, 2007–2010. *Emerging Infectious Diseases* **17**, 1900–1902.
Kibukamusoke JW & Hutt MS (1967) Histological features of the nephrotic syndrome associated with quartan malaria. *Journal of Clinical Pathology* **20**, 117–123.
Kihara M, Carter JA, Holding PA, *et al.* (2009) Impaired everyday memory associated with encephalopathy of severe malaria: the role of seizures and hippocampal damage. *Malaria Journal* **8**, 273.
Kim EM, Cho HJ, Cho CR, Kwak YG, Kim MY & Cho YK (2010) Abdominal computed tomography findings of malaria infection with *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene* **83**, 1202–1205.
Kissinger E, Hien TT, Hung NT, *et al.* (2000) Clinical and neurophysiological study of the effects of multiple doses of artemisinin on brain-stem function in Vietnamese patients. *American Journal of Tropical Medicine and Hygiene* **6**, 48–55.
Kitchen SF (1938) The infection of reticulocytes by *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene* **s1-18**, 347–359.
Kitchen SF (1949a). Symptomatology: General Considerations. In: *Malariology*. (ed MF Boyd) W.B. Saunders, Philadelphia.
Kitchen SF (1949b). Vivax malaria. In: *Malariology*. (ed MF Boyd) W.B. Saunders, Philadelphia.
Klahr S & Miller SB (1998) Acute oliguria. *New England Journal of Medicine* **338**, 671–675.
Knisely M & Stratman-Thomas W (1945) Knowlesi malaria in monkeys: microscopic pathological circulatory physiology of rhesus monkeys during acute *Plasmodium knowlesi* malaria. *Journal of the National Malaria Society (U.S.)* **4**, 285–300.
Knisely M & Stratman-Thomas WK (1948) Microscopic observations of intravascular agglutination of red cells and consequent sludging of blood in rhesus monkeys infected with knowlesi malaria. *The Anatomical Record* **101**, 701.
Knowles R & Das Gupta B (1932) A study of monkey-malaria and its experimental transmission to man. *Indian Medical Gazette* **67**, 301–321.
Kochar DK, Shubhakaran Kumawat BL, *et al.* (2002) Cerebral malaria in Indian adults: a prospective study of 441 patients from Bikaner, north-west India. *Journal of the Association of Physicians of India* **50**, 234–241.
Kochar DK, Singh P, Agarwal P, Kochar SK, Pokharna R & Sareen PK (2003) Malarial hepatitis. *Journal of the Association of Physicians of India* **51**, 1069–1072.
Kochar DK, Saxena V, Singh N, Kochar SK, Kumar SV & Das A (2005) *Plasmodium vivax* malaria. *Emerging Infectious Diseases* **11**, 132–134.
Kochar DK, Sirohi P, Kochar SK, *et al.* (2007) Post-malaria neurological syndrome–a case of bilateral facial palsy after *Plasmodium vivax* malaria. *Journal of Vector Borne Diseases* **44**, 227–229.
Kochar DK, Das A, Kochar SK, *et al.* (2009) Severe *Plasmodium vivax* malaria: a report on serial cases from Bikaner in northwestern India. *American Journal of Tropical Medicine and Hygiene* **80**, 194–198.
Kochar DK, Tanwar GS, Khatri PC, *et al.* (2010) Clinical features of children hospitalized with malaria–a study from Bikaner, northwest India. *American Journal of Tropical Medicine and Hygiene* **83**, 981–989.
Koibuchi T, Nakamura T, Miura T, *et al.* (2003) Acute disseminated encephalomyelitis following *Plasmodium vivax* malaria. *Journal of Infection and Chemotherapy* **9**, 254–256.
Kolloffel WJ, Driessen FG & Goldhoorn PB (1996) Rectal administration of paracetamol: a comparison of a solution and suppositories in adult volunteers. *Pharmacy World & Science* **18**, 26–29.
Koram KA & Molyneux ME (2007) When is "malaria" malaria? The different burdens of malaria infection, malaria disease, and malaria-like illnesses. *American Journal of Tropical Medicine and Hygiene* **77**, 1–5.
Koram KA, Owusu-Agyei S, Utz G, *et al.* (2000) Severe anemia in young children after high and low malaria transmission seasons in the Kassena-Nankana district of northern Ghana. *American Journal of Tropical Medicine and Hygiene* **62**, 670–674.
Kreeftmeijer-Vegter AR, Van Genderen PJ, Visser LG, *et al.* (2012) Treatment outcome of intravenous artesunate in patients with severe malaria in the Netherlands and Belgium. *Malaria Journal* **11**, 102.
Kremsner PG, Valim C, Missinou MA, *et al.* (2009) Prognostic value of circulating pigmented cells in African children with malaria. *Journal of Infectious Diseases* **199**, 142–150.
Krishna S, Waller DW, Ter Kuile F, *et al.* (1994) Lactic acidosis and hypoglycaemia in children with severe malaria: pathophysiological and prognostic significance. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 67–73.
Krishna S, Nagaraja NV, Planche T, *et al.* (2001a) Population pharmacokinetics of intramuscular quinine in children with severe malaria. *Antimicrobial Agents and Chemotherapy* **45**, 1803–1809.
Krishna S, Planche T, Agbenyega T, *et al.* (2001b) Bioavailability and preliminary clinical efficacy of intrarectal artesunate in Ghanaian children with moderate malaria. *Antimicrobial Agents and Chemotherapy* **45**, 509–516.

Krishnan A & Karnad DR (2003) Severe falciparum malaria: an important cause of multiple organ failure in Indian intensive care unit patients. *Critical Care Medicine* **31**, 2278–2284.

Kublin JG, Patnaik P, Jere CS, *et al.* (2005) Effect of *Plasmodium falciparum* malaria on concentration of HIV-1-RNA in the blood of adults in rural Malawi: a prospective cohort study. *Lancet* **365**, 233–240.

Kumar N & Zheng H (1990) Stage-specific gametocytocidal effect in vitro of the antimalaria drug qinghaosu on *Plasmodium falciparum*. *Parasitology Research* **76**, 214–218.

Kute VB, Shah PR, Munjappa BC, *et al.* (2012a) Outcome and prognostic factors of malaria-associated acute kidney injury requiring hemodialysis: a single center experience. *Indian Journal of Nephrology* **22**, 33–38.

Kute VB, Trivedi HL, Vanikar AV, *et al.* (2012b) *Plasmodium vivax* malaria-associated acute kidney injury, India, 2010–2011. *Emerging Infectious Diseases* **18**, 842–845.

Kwiatkowski D, Hill AV, Sambou I, *et al.* (1990) TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. *Lancet* **336**, 1201–1204.

Lacerda MV, Fragoso SC, Alecrim MG, *et al.* (2012) Postmortem characterization of patients with clinical diagnosis of *Plasmodium vivax* malaria: to what extent does this parasite kill? *Clinical Infectious Diseases* **55**, e67–e74.

Lackritz EM, Campbell CC, Ruebush TK, *et al.* (1992) Effect of blood transfusion on survival among children in a Kenyan hospital. *Lancet* **340**, 524–528.

Lackritz EM, Ruebush TK, Zucker JR, Adungosi JE, Were JB & Campbell CC (1993) Blood transfusion practices and blood-banking services in a Kenyan hospital. *AIDS* **7**, 995–999.

Lacroix J, Hebert PC, Hutchison JS, *et al.* (2007) Transfusion strategies for patients in pediatric intensive care units. *New England Journal of Medicine* **356**, 1609–1619.

Ladeia-Andrade S, Ferreira MU, De Carvalho ME, Curado I & Coura JR (2009) Age-dependent acquisition of protective immunity to malaria in riverine populations of the Amazon Basin of Brazil. *American Journal of Tropical Medicine and Hygiene* **80**, 452–459.

Ladhani S, Lowe B, Cole AO, Kowuondo K & Newton CR (2002) Changes in white blood cells and platelets in children with falciparum malaria: relationship to disease outcome. *British Journal of Haematology* **119**, 839–847.

Lalloo DG, Trevett AJ, Paul M, *et al.* (1996) Severe and complicated falciparum malaria in Melanesian adults in Papua New Guinea. *American Journal of Tropical Medicine and Hygiene* **55**, 119–124.

Lampah DA, Yeo TW, Hardianto SO, *et al.* (2011) Coma associated with microscopy-diagnosed *Plasmodium vivax:* a prospective study in Papua, Indonesia. *PLoS Neglected Tropical Diseases* **5**, e1032.

Lanca EF, Magalhaes BM, Vitor-Silva S, *et al.* (2012) Risk factors and characterization of *Plasmodium vivax*-associated admissions to pediatric intensive care units in the Brazilian Amazon. *PLoS ONE* **7**, e35406.

Larkin GL & Thuma PE (1991) Congenital malaria in a hyperendemic area. *American Journal of Tropical Medicine and Hygiene* **45**, 587–592.

Larkin D, De Laat B, Jenkins PV, *et al.* (2009) Severe *Plasmodium falciparum* malaria is associated with circulating ultralarge von Willebrand multimers and ADAMTS13 inhibition. *PLoS Pathogens* **5**, e1000349.

Lee AD, Stephen E, Agarwal S & Premkumar P (2009a) Venous thrombo-embolism in India. *European Journal of Vascular and Endovascular Surgery* **37**, 482–485.

Lee KS, Cox-Singh J & Singh B (2009b) Morphological features and differential counts of *Plasmodium knowlesi* parasites in naturally acquired human infections. *Malaria Journal* **8**, 73.

Lee C, Adeeba K & Freigang G (2010a) Human *Plasmodium knowlesi* infections in Klang Valley, Malaysia: case series. *The Medical Journal of Malaysia* **65**, 63–65.

Lee JH, Chin HS, Chung MH & Moon YS (2010b) Retinal hemorrhage in *Plasmodium vivax* malaria. *American Journal of Tropical Medicine and Hygiene* **82**, 219–222.

Leonardi E, Gilvary G, White NJ & Nosten F (2001) Severe allergic reactions to oral artesunate: a report of two cases. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 182–183.

Leslie T, Briceno M, Mayan I, *et al.* (2010) The impact of phenotypic and genotypic G6PD deficiency on risk of *Plasmodium vivax* infection: a case-control study amongst Afghan refugees in Pakistan. *PLoS Medicine* **7**, e1000283.

Levy MJ, Seelig CB, Robinson NJ & Ranney JE (1997) Comparison of omeprazole and ranitidine for stress ulcer prophylaxis. *Digestive Diseases and Sciences* **42**, 1255–1259.

Lewallen S, Taylor TE, Molyneux ME, Wills BA & Courtright P (1993) Ocular fundus findings in Malawian children with cerebral malaria. *Ophthalmology* **100**, 857–861.

Lewallen S, Bakker H, Taylor TE, Wills BA, Courtright P & Molyneux ME (1996) Retinal findings predictive of outcome in cerebral malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 144–146.

Lewallen S, Harding SP, Ajewole J, *et al.* (1999) A review of the spectrum of clinical ocular fundus findings in *P. falciparum* malaria in African children with a proposed classification and grading system. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 619–623.

Lewallen S, Bronzan RN, Beare NA, Harding SP, Molyneux ME & Taylor TE (2008) Using malarial retinopathy to improve the classification of children with cerebral malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**, 1089–1094.

Li Q, Lugt CB, Looareesuwan S, *et al.* (2004) Pharmacokinetic investigation on the therapeutic potential of artemotil (beta-arteether) in Thai patients with severe *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene* **71**, 723–731.

Lichtman AR, Mohrcken S, Engelbrecht M & Bigalke M (1990) Pathophysiology of severe forms of falciparum malaria. *Critical Care Medicine* **18**, 666–668.

Lin E, Kiniboro B, Gray L, *et al.* (2010) Differential patterns of infection and disease with *P. falciparum* and *P. vivax* in young Papua New Guinean children. *PLoS ONE* **5**, e9047.

Lindegardh N, Hanpithakpong W, Kamanikom B, *et al.* (2008) Major pitfalls in the measurement of artemisinin derivatives in plasma in clinical studies. *Journal of Chromatography B* **876**, 54–60.

Lindegardh N, Hanpithakpong W, Kamanikom B, *et al.* (2011) Quantification of dihydroartemisinin, artesunate and artemisinin in human blood: overcoming the technical challenge of protecting the peroxide bridge. *Bioanalysis* **3**, 1613–1624.

Lins RL, Elseviers MM, Van Der Niepen P, *et al.* (2009) Intermittent versus continuous renal replacement therapy for acute kidney injury patients admitted to the intensive care unit: results of a randomized clinical trial. *Nephrology, Dialysis, Transplantation* **24**, 512–518.

Liu X, Tao ZY, Fang Q, *et al.* (2012) A case of congenital *Plasmodium vivax* malaria from a temperate region in central China. *Malaria Journal* **11**, 182.

Lomar AV, Vidal JE, Lomar FP, Barbas CV, De Matos GJ & Boulos M (2005) Acute respiratory distress syndrome due to vivax malaria: case report and literature review. *The Brazilian Journal of Infectious Diseases* **9**, 425–430.

Longo M, Zanoncelli S, Torre PD, *et al.* (2006) In vivo and in vitro investigations of the effects of the antimalarial drug dihydroartemisinin (DHA) on rat embryos. *Reproductive Toxicology* **22**, 797–810.

Looareesuwan S, Warrell DA, White NJ, *et al.* (1983a) Retinal hemorrhage, a common sign of prognostic significance in cerebral malaria. *American Journal of Tropical Medicine and Hygiene* **32**, 911–915.

Looareesuwan S, Warrell DA, White NJ, *et al.* (1983b) Do patients with cerebral malaria have cerebral oedema? A computed tomography study. *Lancet* **1**, 434–437.

Looareesuwan S, Phillips RE, White NJ, *et al.* (1985) Quinine and severe falciparum malaria in late pregnancy. *Lancet* **2**, 4–8.

Looareesuwan S, Merry AH, Phillips RE, *et al.* (1987) Reduced erythrocyte survival following clearance of malarial parasitaemia in Thai patients. *British Journal of Haematology* **67**, 473–478.

Looareesuwan S, Charoenpan P, Ho M, *et al.* (1990) Fatal *Plasmodium falciparum* malaria after an inadequate response to quinine treatment. *Journal of Infectious Diseases* **161**, 577–580.

Looareesuwan S, Wilairatana P, Krishna S, *et al.* (1995) Magnetic resonance imaging of the brain in patients with cerebral malaria. *Clinical Infectious Diseases* **21**, 300–309.

Lopansri BK, Anstey NM, Weinberg JB, *et al.* (2003) Low plasma arginine concentrations in children with cerebral malaria and decreased nitric oxide production. *Lancet* **361**, 676–678.

Lovegrove FE, Tangpukdee N, Opoka RO, *et al.* (2009) Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria and predict clinical outcome in African children. *PLoS ONE* **4**, e4912.

Lowenberg EC, Charunwatthana P, Cohen S, *et al.* (2010) Severe malaria is associated with a deficiency of von Willebrand factor cleaving protease, ADAMTS13. *Thrombosis and Haemostasis* **103**, 181–187.

Lubitz J (1949) Pathology of ruptured spleen in acute vivax malaria. *Blood* **4**, 1168–1176.

Luchavez J, Espino F, Curameng P, *et al.* (2008) Human Infections with *Plasmodium knowlesi*, The Philippines. *Emerging Infectious Diseases* **14**, 811–813.

Lumlertgul D, Keoplung M, Sitprija V, Moollaor P & Suwangool P (1989) Furosemide and dopamine in malarial acute renal failure. *Nephron* **52**, 40–44.

Luxemburger C, Ter Kuile FO, Nosten F, *et al.* (1994) Single day mefloquine-artesunate combination in the treatment of multi-drug resistant falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 213–217.

Luxemburger C, Nosten F, Raimond SD, Chongsuphajaisiddhi T & White NJ (1995) Oral artesunate in the treatment of uncomplicated hyperparasitemic falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **61**, 53–55.

Luxemburger C, Ricci F, Nosten F, Raimond D, Bathet S & White NJ (1997) The epidemiology of severe malaria in an area of low transmission in Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 256–262.

Luxemburger C, McGready R, Kham A, *et al.* (2001) Effects of malaria during pregnancy on infant mortality in an area of low malaria transmission. *American Journal of Epidemiology* **154**, 459–465.

Lyke KE, Diallo DA, Dicko A, *et al.* (2003) Association of intraleukocytic *Plasmodium falciparum* malaria pigment with disease severity, clinical manifestations, and prognosis in severe malaria. *American Journal of Tropical Medicine and Hygiene* **69**, 253–259.

Lynch M, Korenromp E, Eisele T, *et al.* (2012) New global estimates of malaria deaths. *Lancet* **380**, 559.

Mabeza GF, Moyo VM, Thuma PE, *et al.* (1995) Predictors of severity of illness on presentation in children with cerebral malaria. *Annals of Tropical Medicine and Parasitology* **89**, 221–228.

Macallan DC, Pocock M, Robinson GT, Parker-Williams J & Bevan DH (2000) Red cell exchange, erythrocytapheresis, in the treatment of malaria with high parasitaemia in returning travellers. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 353–356.

Machado Siqueira A, Lopes Magalhaes BM, Cardoso Melo G, *et al.* (2012) Spleen rupture in a case of untreated *Plasmodium vivax* infection. *PLoS Neglected Tropical Diseases* **6**, e1934.

MacPherson GG, Warrell MJ, White NJ, Looareesuwan S & Warrell DA (1985) Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. *American Journal of Pathology* **119**, 385–401.

Maguire GP, Handojo T, Pain MC, *et al.* (2005) Lung injury in uncomplicated and severe falciparum malaria: a longitudinal study in Papua, Indonesia. *Journal of Infectious Diseases* **192**, 1966–1974.

Mahgoub H, Gasim GI, Musa IR & Adam I (2012) Severe *Plasmodium vivax* malaria among Sudanese children at New Halfa Hospital, Eastern Sudan. *Parasites & Vectors* **5**, 154.

Maitland K & Newton CR (2005) Acidosis of severe falciparum malaria: heading for a shock? *Trends in Parasitology* **21**, 11–16.

Maitland K, Williams TN, Peto TE, *et al.* (1997) Absence of malaria-specific mortality in children in an area of hyperendemic malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 562–566.

Maitland K, Levin M, English M, *et al.* (2003a) Severe *P. falciparum* malaria in Kenyan children: evidence for hypovolaemia. *QJM: Monthly Journal of the Association of Physicians* **96**, 427–434.

Maitland K, Pamba A, Newton CR & Levin M (2003b) Response to volume resuscitation in children with severe malaria. *Pediatric Critical Care Medicine* **4**, 426–431.

Maitland K, Pamba A, Newton CR, Lowe B & Levin M (2004) Hypokalemia in children with severe falciparum malaria. *Pediatric Critical Care Medicine* **5**, 81–85.

Maitland K, Kiguli S, Opoka RO, *et al.* (2011) Mortality after fluid bolus in African children with severe infection. *New England Journal of Medicine* **364**, 2483–2495.

Maitland K, George EC, Evans JA, *et al.* (2013) Exploring mechanisms of excess mortality with early fluid resuscitation: insights from the FEAST trial. *BMC Medicine* **11**, 68.

Malamba S, Hladik W, Reingold A, *et al.* (2007) The effect of HIV on morbidity and mortality in children with severe malarial anaemia. *Malaria Journal* **6**, 143.

Mallewa M, Vallely P, Faragher B, *et al.* (2013) Viral CNS infections in children from a malaria-endemic area of Malawi: a prospective cohort study. *Lancet Global Health* **1**, e153–e160.

Manning L, Laman M, Law I, *et al.* (2011) Features and prognosis of severe malaria caused by *Plasmodium falciparum, Plasmodium vivax* and mixed *Plasmodium* species in Papua New Guinean children. *PLoS ONE* **6**, e29203.

Manning L, Rosanas-Urgell A, Laman M, *et al.* (2012) A histopathologic study of fatal paediatric cerebral malaria caused by mixed *Plasmodium falciparum/Plasmodium vivax* infections. *Malaria Journal* **11**, 107.

Mansor SM, Molyneux ME, Taylor TE, Ward SA, Wirima JJ & Edwards G (1991) Effect of *Plasmodium falciparum* malaria infection on the plasma concentration of alpha 1-acid glycoprotein and the binding of quinine in Malawian children. *British Journal of Clinical Pharmacology* **32**, 317–321.

Marchiafava F & Bignami A (1892) Sulle febbri malariche estivo-autunnali. *Bull del R Acad Med di Roma* **297**

Markley JD & Edmond MB (2009) Post-malaria neurological syndrome: a case report and review of the literature. *Journal of Travel Medicine* **16**, 424–430.

Marsh K, Forster D, Waruiru C, *et al.* (1995) Indicators of life-threatening malaria in African children. *New England Journal of Medicine* **332**, 1399–1404.

Maude RJ, Beare NA, Abu Sayeed A, *et al.* (2009a) The spectrum of retinopathy in adults with *Plasmodium falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **103**, 665–671.

Maude RJ, Plewes K, Faiz MA, *et al.* (2009b) Does artesunate prolong the electrocardiograph QT interval in patients with severe malaria? *American Journal of Tropical Medicine and Hygiene* **80**, 126–132.

Maude RJ, Hoque G, Hasan MU, *et al.* (2011) Timing of enteral feeding in cerebral malaria in resource-poor settings: a randomized trial. *PLoS ONE* **6**, e27273.

Mayor A, Bardaji A, Felger I, *et al.* (2012) Placental infection with *Plasmodium vivax*: a histopathological and molecular study. *Journal of Infectious Diseases* **206**, 1904–1910.

Mayxay M, Pukrittayakamee S, Chotivanich K, Looareesuwan S & White NJ (2001) Persistence of *Plasmodium falciparum* HRP-2 in successfully treated acute falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 179–182.

Mayxay M, Pukrittayakamee S, Newton PN & White NJ (2004) Mixed-species malaria infections in humans. *Trends in Parasitology* **20**, 233–240.

McGready R, Davison BB, Stepniewska K, *et al.* (2004) The effects of *Plasmodium falciparum* and *P. vivax* infections on placental histopathology in an area of low malaria transmission. *American Journal of Tropical Medicine and Hygiene* **70**, 398–407.

McGready R, Boel M, Rijken MJ, *et al.* (2012a) Effect of early detection and treatment on malaria related maternal mortality on the north-Western border of Thailand 1986-2010. *PLoS ONE* **7**, e40244.

McGready R, Lee SJ, Wiladphaingern J, *et al.* (2012b) Adverse effects of falciparum and vivax malaria and the safety of antimalarial treatment in early pregnancy: a population-based study. *Lancet Infectious Diseases* **12**, 388–396.

McGready R, Phyo AP, Rijken MJ, *et al.* (2012c) Artesunate/dihydroartemisinin pharmacokinetics in acute falciparum malaria in pregnancy: absorption, bioavailability, disposition and disease effects. *British Journal of Clinical Pharmacology* **73**, 467–477.

McGregor IA, Gilles HM, Walters JH, Davies AH & Pearson FA (1956) Effects of heavy and repeated malarial infections on Gambian infants and children; effects of erythrocytic parasitization. *British Medical Journal* **2**, 686–692.

McMorrow ML, Masanja MI, Abdulla SM, Kahigwa E & Kachur SP (2008) Challenges in routine implementation and quality control of rapid diagnostic tests for malaria–Rufiji District, Tanzania. *American Journal of Tropical Medicine and Hygiene* **79**, 385–390.

McQueen PG & McKenzie FE (2004) Age-structured red blood cell susceptibility and the dynamics of malaria infections. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 9161–9166.

Medana IM & Turner GD (2006) Human cerebral malaria and the blood-brain barrier. *International Journal for Parasitology* **36**, 555–568.

Medana IM, Day NP, Hien TT, *et al.* (2002) Axonal injury in cerebral malaria. *American Journal of Pathology* **160**, 655–666.

Medana IM, Day NP, Sachanonta N, *et al.* (2011) Coma in fatal adult human malaria is not caused by cerebral oedema. *Malaria Journal* **10**, 267.

Mehta KS, Halankar AR, Makwana PD, Torane PP, Satija PS & Shah VB (2001) Severe acute renal failure in malaria. *Journal of Postgraduate Medicine* **47**, 24–26.

Melo GC, Reyes-Lecca RC, Vitor-Silva S, *et al.* (2010) Concurrent helminthic infection protects schoolchildren with *Plasmodium vivax* from anemia. *PLoS ONE* **5**, e11206.

Menendez C, Romagosa C, Ismail MR, *et al.* (2008) An autopsy study of maternal mortality in Mozambique: the contribution of infectious diseases. *PLoS Medicine* **5**, e44.

Mengistu G, Diro E & Kassu A (2006) Outcomes of pregnancy in severe malaria wth emphasis on neurological manifestations in Gondar Hospital northwest Ethiopia. *Ethiopian Medical Journal* **44**, 321–330.

Michon P, Cole-Tobian JL, Dabod E, *et al.* (2007) The risk of malarial infections and disease in Papua New Guinean children. *American Journal of Tropical Medicine and Hygiene* **76**, 997–1008.

Mihaly GW, Ching MS, Klejn MB, Paull J & Smallwood RA (1987) Differences in the binding of quinine and quinidine to plasma proteins. *British Journal of Clinical Pharmacology* **24**, 769–774.

Millan JM, San Millan JM, Munoz M, Navas E & Lopez-Velez R (1993) CNS complications in acute malaria: MR findings. *American Journal of Neuroradiology* **14**, 493–494.

Miller LH, Mason SJ, Clyde DF & McGinniss MH (1976) The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype. *FyFy. New England Journal of Medicine* **295**, 302–304.

Miller KD, White NJ, Lott JA, Roberts JM & Greenwood BM (1989) Biochemical evidence of muscle injury in African children with severe malaria. *Journal of Infectious Diseases* **159**, 139–142.

Miller L, Baruch D, Marsh K & Doumbo O (2002) The pathogenic basis of malaria. *Nature* **415**, 673–679.

Milner DA & Taylor TE (2006) The pathology of cerebral malaria: autopsy studies from Blantyre, Malawi (1996–2006). 11th International Congress of Parasitology, **2006**, 147–154.

Milner D, Valim C, Luo R, *et al.* (2012a) Supraorbital post-mortem brain sampling for definitive quantitative confirmation of cerebral sequestration of *Plasmodium falciparum* parasites. *Journal of Infectious Diseases* **205**, 1601–1606.

Milner D, Vareta J, Valim C, *et al.* (2012b) Human cerebral malaria and *Plasmodium falciparum* genotypes in Malawi. *Malaria Journal* **11**, 35.

Mishra SK & Das BS (2008) Malaria and acute kidney injury. *Seminars in Nephrology* **28**, 395–408.

Mishra SK & Mahanta KC (2012) Peritoneal dialysis in patients with malaria and acute kidney injury. *Peritoneal Dialysis International* **32**, 656–659.

Mishra SK, Dietz K, Mohanty S & Pati SS (2007a) Influence of acute renal failure in patients with cerebral malaria - a hospital-based study from India. *Tropical Doctor* **37**, 103–104.

Mishra SK, Mohanty S, Satpathy SK & Mohapatra DN (2007b) Cerebral malaria in adults – a description of 526 cases admitted to Ispat General Hospital in Rourkela, India. *Annals of Tropical Medicine and Parasitology* **101**, 187–193.

Mishra SK, Panigrahi P, Mishra R & Mohanty S (2007c) Prediction of outcome in adults with severe falciparum malaria: a new scoring system. *Malaria Journal* **6**, 24.

Mithwani S, Aarons L, Kokwaro GO, *et al.* (2004) Population pharmacokinetics of artemether and dihydroartemisinin following single intramuscular dosing of artemether in African children with severe falciparum malaria. *British Journal of Clinical Pharmacology* **57**, 146–152.

Modiano D, Sawadogo A & Pagnoni F (1995) Indicators of life-threatening malaria. *New England Journal of Medicine* **333**, 1011.

Modiano D, Sirima BS, Sawadogo A, *et al.* (1998) Severe malaria in Burkina Faso: influence of age and transmission level on clinical presentation. *American Journal of Tropical Medicine and Hygiene* **59**, 539–542.

Mohanty S, Mishra SK, Patnaik R, Dutt AK, Pradhan S, Das B, Patnai J, Mohanty AK, Lee SJ & Dondorp AM (2011) Brain swelling and mannitol therapy in adult cerebral malaria: a randomized trial. *Clinical Infectious Diseases* **53**, 349–355.

Mohammed I, Ibrahim UY, Mukhtar M, *et al.* (2011) Brain swelling and mannitol therapy in adult cerebral malaria: a randomized trial. *Clinical Infectious Diseases* **53**, 349–355.

Mojon M, Wallon M, Gravey A, Peaud PY, Sartre J & Peyron F (1994) Intravascular haemolysis following halofantrine intake. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 91.

Mokuolu OA, Falade CO, Orogade AA, *et al.* (2009) Malaria at parturition in Nigeria: current status and delivery outcome. *Infectious Diseases in Obstetrics and Gynecology* **2009**, 473971.

Molyneux ME, Looareesuwan S, Menzies IS, *et al.* (1989a) Reduced hepatic blood flow and intestinal malabsorption in severe falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **40**, 470–476.

Molyneux ME, Taylor TE, Wirima JJ & Borgstein A (1989b) Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *Quarterly Journal of Medicine* **71**, 441–459.

Molyneux E, Walsh A, Phiri A & Molyneux M (1998) Acute bacterial meningitis in children admitted to the Queen Elizabeth Central Hospital, Blantyre, Malawi in 1996-97. *Tropical Medicine and International Health* **3**, 610–618.

Montgomery J, Milner DA, Tse MT, *et al.* (2006) Genetic analysis of circulating and sequestered populations of *Plasmodium falciparum* in fatal pediatric malaria. *Journal of Infectious Diseases* **194**, 115–122.

Montgomery J, Mphande FA, Berriman M, *et al.* (2007) Differential var gene expression in the organs of patients dying of falciparum malaria. *Molecular Microbiology* **65**, 959–967.

Moses WR (1945) Splenic rupture in therapeutic malaria. *Southern Medical Journal* **38**, 745.

Most H & Hayman JM (1946) Uncommon clinical manifestations of vivax malaria. *Journal of the American Medical Association* **130**, 480–485.

Moxon CA, Wassmer SC, Milner DA, *et al.* (2013) Loss of endothelial protein C receptors links coagulation and inflammation to parasite sequestration in cerebral malaria in African children. *Blood* **122**, 842–851.

Mpimbaza A, Ndeezi G, Staedke S, Rosenthal PJ & Byarugaba J (2008) Comparison of buccal midazolam with rectal diazepam in the treatment of prolonged seizures in Ugandan children: a randomized clinical trial. *Pediatrics* **121**, e58–e64.

Mudenda SS, Kamocha S, Mswia R, *et al.* (2011) Feasibility of using a World Health Organization-standard methodology for Sample Vital Registration with Verbal Autopsy (SAVVY) to report leading causes of death in Zambia: results of a pilot in four provinces, 2010. *Population Health Metrics* **9**, 40.

Muehlenbachs A, Fried M, Mcgready R, Harrington WE, Mutabingwa TK, Nosten F & Duffy PE (2010) A novel histological grading scheme for placental malaria applied in areas of high and low malaria transmission. *The Journal of Infectious Diseases* **202**, 1608–1616.

Mujuzi G, Magambo B, Okech B & Egwang TG (2006) Pigmented monocytes are negative correlates of protection against severe and complicated malaria in Ugandan children. *American Journal of Tropical Medicine and Hygiene* **74**, 724–729.

Mukim A, Shukla M, Patel J, *et al.* (2011) A phase III clinical trial of alpha, beta-arteether injection 150 mg/ml in patients of P falciparum malaria. *Journal of the Indian Medical Association* **109**, 597–599.

Murphy SA, Mberu E, Muhia D, *et al.* (1997) The disposition of intramuscular artemether in children with cerebral malaria: a preliminary study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 331–334.

Murray CJ, Rosenfeld LC, Lim SS, *et al.* (2012) Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet* **379**, 413–431.

Mwaniki MK, Talbert AW, Mturi FN, *et al.* (2010) Congenital and neonatal malaria in a rural Kenyan district hospital: an eight-year analysis. *Malaria Journal* **9**, 313.

Na-Bangchang K, Congpuong K, Hung LN, Molunto P & Karbwang J (1998) Simple high-performance liquid chromatographic method with electrochemical detection for the simultaneous determination of artesunate and dihydroartemisinin in biological fluids. *Journal of Chromatography B* **708**, 201–207.

Nadjm B, Mtove G, Amos B, *et al.* (2013) Blood glucose as a predictor of mortality in children admitted to the hospital with febrile illness in Tanzania. *American Journal of Tropical Medicine and Hygiene* **89**, 232–237.

Naik H, Murry DJ, Kirsch LE & Fleckenstein L (2005) Development and validation of a high-performance liquid chromatography-mass spectroscopy assay for determination of artesunate and dihydroartemisinin in human plasma. *Journal of Chromatography B* **816**, 233–242.

Namutangula B, Ndeezi G, Byarugaba JS & Tumwine JK (2007) Mannitol as adjunct therapy for childhood cerebral malaria in Uganda: a randomized clinical trial. *Malaria Journal* **6**, 138.

Nanda NC, Rath P, Acharya J, Mishra P & Mishra SK (2011) Falciparum malaria in children-a brief report of 305 patients from rourkela, eastern India. *Indian Journal of Pediatrics* **78**, 475–477.

Nankabirwa J, Zurovac D, Njogu JN, *et al.* (2009) Malaria misdiagnosis in Uganda–implications for policy change. *Malaria Journal* **8**, 66.

Navaratnam V, Mordi MN & Mansor SM (1997) Simultaneous determination of artesunic acid and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. *Journal of Chromatography B* **692**, 157–162.

Navaratnam V, Mansor SM, Mordi MN, Akbar A & Abdullah MN (1998) Comparative pharmacokinetic study of oral and rectal formulations of artesunic acid in healthy volunteers. *European Journal of Clinical Pharmacology* **54**, 411–414.

Nayak KC, Khatri MP, Gupta BK, *et al.* (2009) Spectrum of vivax malaria in pregnancy and its outcome: a hospital-based study. *Journal of Vector Borne Diseases* **46**, 299–302.

Nealon C, Dzeing A, Muller-Romer U, *et al.* (2002) Intramuscular bioavailability and clinical efficacy of artesunate in gabonese children with severe malaria. *Antimicrobial Agents and Chemotherapy* **46**, 3933–3939.

Nevill CG, Some ES, Mung'ala VO, *et al.* (1996) Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Tropical Medicine and International Health* **1**, 139–146.

Newbold C, Warn P, Black G, *et al.* (1997) Receptor-specific adhesion and clinical disease in *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene* **57**, 389–398.

Newton CR, Kirkham FJ, Winstanley PA, *et al.* (1991) Intracranial pressure in African children with cerebral malaria. *Lancet* **337**, 573–576.

Newton CR, Peshu N, Kendall B, *et al.* (1994) Brain swelling and ischaemia in Kenyans with cerebral malaria. *Archives of Disease in Childhood* **70**, 281–287.

Newton CR, Chokwe T, Schellenberg JA, *et al.* (1997a) Coma scales for children with severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **91**, 161–165.

Newton CR, Crawley J, Sowumni A, *et al.* (1997b) Intracranial hypertension in Africans with cerebral malaria. *Archives of Disease in Childhood* **76**, 219–226.

Newton P, Keeratithakul D, Teja-Isavadharm P, Pukrittayakamee S, Kyle D & White N (1999) Pharmacokinetics of quinine and 3-hydroxyquinine in severe falciparum malaria with acute renal failure. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 69–72.

Newton P, Suputtamongkol Y, Teja-Isavadharm P, *et al.* (2000) Antimalarial bioavailability and disposition of artesunate in acute falciparum malaria. *Antimicrobial Agents and Chemotherapy* **44**, 972–977.

Newton PN, Chierakul W, Ruangveerayuth R, *et al.* (2001a) A comparison of artesunate alone with combined artesunate and

quinine in the parenteral treatment of acute falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 519–523.

Newton PN, Chotivanich K, Chierakul W, *et al.* (2001b) A comparison of the in vivo kinetics of *Plasmodium falciparum* ring-infected erythrocyte surface antigen-positive and -negative erythrocytes. *Blood* **98**, 450–457.

Newton PN, Angus BJ, Chierakul W, *et al.* (2003) Randomized comparison of artesunate and quinine in the treatment of severe falciparum malaria. *Clinical Infectious Diseases* **37**, 7–16.

Newton CR, Valim C, Krishna S, *et al.* (2005a) The prognostic value of measures of acid/base balance in pediatric falciparum malaria, compared with other clinical and laboratory parameters. *Clinical Infectious Diseases* **41**, 948–957.

Newton PN, Chaulet JF, Brockman A, *et al.* (2005b) Pharmacokinetics of oral doxycycline during combination treatment of severe falciparum malaria. *Antimicrobial Agents and Chemotherapy* **49**, 1622–1625.

Newton PN, Stepniewska K, Dondorp A, *et al.* (2013) Prognostic indicators in adults hospitalized with falciparum malaria in Western Thailand. *Malaria Journal* **12**, 229.

Ng OT, Ooi EE, Lee CC, *et al.* (2008) Naturally acquired human *Plasmodium knowlesi* infection, Singapore. *Emerging Infectious Diseases* **14**, 814–816.

Ngoungou EB, Dulac O, Poudiougou B, *et al.* (2006a) Epilepsy as a consequence of cerebral malaria in area in which malaria is endemic in Mali, West Africa. *Epilepsia* **47**, 873–879.

Ngoungou EB, Koko J, Druet-Cabanac M, *et al.* (2006b) Cerebral malaria and sequelar epilepsy: first matched case-control study in Gabon. *Epilepsia* **47**, 2147–2153.

Nguansangiam S, Day NP, Hien TT, *et al.* (2007) A quantitative ultrastructural study of renal pathology in fatal *Plasmodium falciparum* malaria. *Tropical Medicine and International Health* **12**, 1037–1050.

Nguyen PH, Day N, Pram TD, Ferguson DJ & White NJ (1995) Intraleucocytic malaria pigment and prognosis in severe malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 200–204.

Nguyen TH, Day NP, Ly VC, *et al.* (1996) Post-malaria neurological syndrome. *Lancet* **348**, 917–921.

Nontprasert A, Pukrittayakamee S, Kyle DE, Vanijanonta S & White NJ (1996) Antimalarial activity and interactions between quinine, dihydroquinine and 3-hydroxyquinine against *Plasmodium falciparum* in vitro. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**, 553–555.

Nosten F, Mcgready R, Simpson JA, *et al.* (1999) Effects of *Plasmodium vivax* malaria in pregnancy. *Lancet* **354**, 546–549.

Nurleila S, Syafruddin D, Elyazar IR & Baird JK (2012) Serious and fatal illness associated with falciparum and vivax malaria among patients admitted to hospital at West Sumba in eastern Indonesia. *American Journal of Tropical Medicine and Hygiene* **87**, 41–49.

Obonyo CO, Vulule J, Akhwale WS & Grobbee DE (2007) In-hospital morbidity and mortality due to severe malarial anemia in western Kenya. *American Journal of Tropical Medicine and Hygiene* **77**, 23–28.

Ochola LB, Siddondo BR, Ocholla H, *et al.* (2011) Specific receptor usage in *Plasmodium falciparum* cytoadherence is associated with disease outcome. *PLoS ONE* **6**, e14741.

O'Dempsey TJ, McArdle TF, Laurence BE, Lamont AC, Todd JE & Greenwood BM (1993) Overlap in the clinical features of pneumonia and malaria in African children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 662–665.

O'Donnell A, Premawardhena A, Arambepola M, *et al.* (2009) Interaction of malaria with a common form of severe thalassemia in an Asian population. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 18716–18721.

Oduro AR, Koram KA, Rogers W, *et al.* (2007) Severe falciparum malaria in young children of the Kassena-Nankana district of northern Ghana. *Malaria Journal* **6**, 96.

Ogetii GN, Akech S, Jemutai J, *et al.* (2010) Hypoglycaemia in severe malaria, clinical associations and relationship to quinine dosage. *BMC Infectious Diseases* **10**, 334.

Ogutu BR, Newton CR, Crawley J, *et al.* (2002) Pharmacokinetics and anticonvulsant effects of diazepam in children with severe falciparum malaria and convulsions. *British Journal of Clinical Pharmacology* **53**, 49–57.

Okiro EA, Al-Taiar A, Reyburn H, Idro R, Berkley JA & Snow RW (2009) Age patterns of severe paediatric malaria and their relationship to *Plasmodium falciparum* transmission intensity. *Malaria Journal* **8**, 4.

Omanga U, Ntihinyurwa M, Shako D & Mashako M (1983) Hemiplegia in pernicious attacks of *Plasmodium falciparum* in children. *Annales de Pediatrie* **30**, 294–296.

O'Meara WP, Bejon P, Mwangi TW, *et al.* (2008) Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. *Lancet* **372**, 1555–1562.

Opoka RO, Bangirana P, Boivin MJ, John CC & Byarugaba J (2009) Seizure activity and neurological sequelae in Ugandan children who have survived an episode of cerebral malaria. *African Health Sciences* **9**, 75–81.

Orimadegun AE, Fawole O, Okereke JO, Akinbami FO & Sodeinde O (2007) Increasing burden of childhood severe malaria in a Nigerian tertiary hospital: implication for control. *Journal of Tropical Pediatrics* **53**, 185–189.

Orogade AA, Falade CO, Okafor HU, *et al.* (2008) Clinical and laboratory features of congenital malaria in Nigeria. *Journal of Pediatric Infectious Diseases* **3**, 181–187.

Osime U, Lawrie J & Lawrie H (1976) Post-operative deep vein thrombosis incidence in Nigerians. *Nigerian Medical Journal* **6**, 26–28.

Otieno RO, Ouma C, Ong'echa JM, *et al.* (2006) Increased severe anemia in HIV-1-exposed and HIV-1-positive infants and children during acute malaria. *AIDS* **20**, 275–280.

Pain A, Ferguson DJ, Kai O, *et al.* (2001) Platelet-mediated clumping of *Plasmodium falciparum*-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 1805–1810.

Parakh A, Agarwal N, Aggarwal A & Aneja A (2009) *Plasmodium vivax* malaria in children: uncommon manifestations. *Annals of Tropical Paediatrics* **29**, 253–256.

Pareek A, Nandy A, Kochar D, Patel KH, Mishra SK & Mathur PC (2006) Efficacy and safety of beta-arteether and alpha/beta-arteether for treatment of acute *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene* **75**, 139–142.

Pasvol G, Newton CR, Winstanley PA, *et al.* (1991) Quinine treatment of severe falciparum malaria in African children: a randomized comparison of three regimens. *American Journal of Tropical Medicine and Hygiene* **45**, 702–713.

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.

Patel CC (1971) Acute febrile encephalopathy in Ugandan children. *African Journal of Medicine and Medical Sciences* **2**, 127–134.

Patel MP, Kute VB, Gumber MR, *et al.* (2012) An unusual case of *Plasmodium vivax* malaria monoinfection associated with crescentic glomerulonephritis: a need for vigilance. *Parasitology Research* **112**, 427–430.

Patnaik JK, Das BS, Mishra SK, Mohanty S, Satpathy SK & Mohanty D (1994) Vascular clogging, mononuclear cell margination, and enhanced vascular permeability in the pathogenesis of human cerebral malaria. *American Journal of Tropical Medicine and Hygiene* **51**, 642–647.

Patnaik P, Jere CS, Miller WC, *et al.* (2005) Effects of HIV-1 serostatus, HIV-1 RNA concentration, and CD4 cell count on the incidence of malaria infection in a cohort of adults in rural Malawi. *Journal of Infectious Diseases* **192**, 984–991.

Pedro R, Akech S, Fegan G & Maitland K (2010) Changing trends in blood transfusion in children and neonates admitted in Kilifi District Hospital, Kenya. *Malaria Journal* **9**, 307.

Perel P, Roberts I & Pearson M (2007) Colloids versus crystalloids for fluid resuscitation in critically ill patients. *Cochrane Database of Systematic Reviews* **4**, CD000567.

Petithory JC, Lebeau G, Galeazzi G & Chauty A (1983) Hypocalcemia in malaria. Study of correlations with other parameters. *Bulletin de la Societe de Pathologie Exotique et de ses Filiales* **76**, 455–462.

Pfeiffer K, Some F, Muller O, *et al.* (2008) Clinical diagnosis of malaria and the risk of chloroquine self-medication in rural health centres in Burkina Faso. *Tropical Medicine and International Health* **13**, 418–426.

Pham-Hung G, Truffert A, Delvallee G, Michel G, Laporte JP & Duval G (1990) Cerebral infarction in pernicious malaria. Diagnostic value of computed tomography. *Annales Francaises d'Anesthesie et de Reanimation* **9**, 185–187.

Phillips RE & Warrell DA (1986) The pathophysiology of severe falciparum malaria. *Parasitology Today* **2**, 271–282.

Phillips RE, Warrell DA, White NJ, Looareesuwan S & Karbwang J (1985) Intravenous quinidine for the treatment of severe falciparum malaria. Clinical and pharmacokinetic studies. *New England Journal of Medicine* **312**, 1273–1278.

Phillips RE, Looareesuwan S, Warrell DA, *et al.* (1986a) The importance of anaemia in cerebral and uncomplicated falciparum malaria: role of complications, dyserythropoiesis and iron sequestration. *Quarterly Journal of Medicine* **58**, 305–323.

Phillips RE, Looareesuwan S, White NJ, Silamut K, Kietinun S & Warrell DA (1986b) Quinine pharmacokinetics and toxicity in pregnant and lactating women with falciparum malaria. *British Journal of Clinical Pharmacology* **21**, 677–683.

Phiri HT, Bridges DJ, Glover SJ, *et al.* (2011) Elevated plasma von Willebrand factor and propeptide levels in Malawian children with malaria. *PLoS ONE* **6**, e25626.

Phu NH, Hien TT, Mai NT, *et al.* (2002) Hemofiltration and peritoneal dialysis in infection-associated acute renal failure in Vietnam. *New England Journal of Medicine* **347**, 895–902.

Phu NH, Tuan PQ, Day N, *et al.* (2010) Randomized controlled trial of artesunate or artemether in Vietnamese adults with severe falciparum malaria. *Malaria Journal* **9**, 97.

Phyo AP, Nkhoma S, Stepniewska K, *et al.* (2012) Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. *Lancet* **379**, 1960–1966.

Piñeros-Jiménez JG, Arboleda M, Jaramillo JC & Blair S (2008) [Report of five cases of severe neonatal *Plasmodium vivax* malaria in Urabá, Colombia]. *Biomédica: Revista Del Instituto Nacional De Salud* **28**, 471–479.

Pinhas-Hamiel O, Paret G & Barzilay Z (1993) Is lumbar puncture in bacterial meningitis necessary? *Harefuah* **124**(546–8), 599.

Pittet D, Hugonnet S, Harbarth S, *et al.* (2000) Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Infection Control Programme. *Lancet* **356**, 1307–1312.

Pittet D, Allegranzi B & Boyce J (2009) The World Health Organization Guidelines on Hand Hygiene in Health Care and their consensus recommendations. *Infection Control and Hospital Epidemiology* **30**, 611–622.

Piyaphanee W, Issarachaikul R, Soontarach P & Silachamroon U (2007) Concurrent salmonella bacteremia in *P. vivax* infection–a report of 2 cases at the Hospital for Tropical Diseases, Thailand. *The South East Asian Journal of Tropical Medicine and Public Health* **38**, 616–618.

Plum F & Posner JB (1972) The diagnosis of stupor and coma. *Contemporary Neurology Series* **10**, 1–286.

Poespoprodjo JR, Fobia W, Kenangalem E, *et al.* (2008) Adverse pregnancy outcomes in an area where multidrug-resistant *Plasmodium vivax* and *Plasmodium falciparum* infections are endemic. *Clinical Infectious Diseases* **46**, 1374–1381.

Poespoprodjo JR, Fobia W, Kenangalem E, *et al.* (2009) Vivax malaria: a major cause of morbidity in early infancy. *Clinical Infectious Diseases* **48**, 1704–1712.

Poespoprodjo JR, Hasanuddin A, Fobia W, *et al.* (2010) Severe congenital malaria acquired in utero. *American Journal of Tropical Medicine and Hygiene* **82**, 563–565.

Poespoprodjo JR, Fobia W, Kenangalem E, *et al.* (2011) Highly effective therapy for maternal malaria associated with a lower risk of vertical transmission. *Journal of Infectious Diseases* **204**, 1613–1619.

Pongponratn E, Riganti M, Punpoowong B & Aikawa M (1991) Microvascular sequestration of parasitized erythrocytes in human falciparum malaria: a pathological study. *American Journal of Tropical Medicine and Hygiene* **44**, 168–175.

Pongponratn E, Turner GD, Day NP, *et al.* (2003) An ultrastructural study of the brain in fatal *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene* **69**, 345–359.

Ponsford M, Medana I, Prapansilp P, *et al.* (2012) Sequestration and microvascular congestion are associated with coma in human cerebral malaria. *Journal of Infectious Diseases* **205**, 663–671.

Poschl B, Waneesorn J, Thekisoe O, Chutipongvivate S & Karanis P (2010) Comparative diagnosis of malaria infections by microscopy, nested PCR, and LAMP in northern Thailand. *American Journal of Tropical Medicine and Hygiene* **83**, 56–60.

Potchen MJ, Birbeck GL, Demarco JK, *et al.* (2010) Neuroimaging findings in children with retinopathy-confirmed cerebral malaria. *European Journal of Radiology* **74**, 262–268.

Potchen MJ, Kampondeni SD, Seydel KB, *et al.* (2012) Acute brain MRI findings in 120 Malawian children with cerebral malaria: new insights into an ancient disease. *American Journal of Neuroradiology* **33**, 1740–1746.

Praba-Egge AD, Montenegro S, Cogswell FB, Hopper T & James MA (2002) Cytokine responses during acute simian *Plasmodium cynomolgi* and *Plasmodium knowlesi* infections. *American Journal of Tropical Medicine and Hygiene* **67**, 586–596.

Prakash MV & Stein G (1990) Malaria presenting as atypical depression. *The British Journal of Psychiatry* **156**, 594–595.

Prakash J, Singh AK, Kumar NS & Saxena RK (2003) Acute renal failure in *Plasmodium vivax* malaria. *Journal of the Association of Physicians of India* **51**, 265–267.

Prendki V, Elziere C, Durand R, *et al.* (2008) Post-malaria neurological syndrome–two cases in patients of African origin. *American Journal of Tropical Medicine and Hygiene* **78**, 699–701.

Price R, Van Vugt M, Phaipun L, *et al.* (1999) Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *American Journal of Tropical Medicine and Hygiene* **60**, 547–555.

Price RN, Simpson JA, Nosten F, *et al.* (2001) Factors contributing to anemia after uncomplicated falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **65**, 614–622.

Price RN, Tjitra E, Guerra CA, Yeung S, White NJ & Anstey NM (2007) Vivax malaria: neglected and not benign. *American Journal of Tropical Medicine and Hygiene* **77**, 79–87.

Price RN, Douglas NM & Anstey NM (2009) New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. *Current Opinion in Infectious Diseases* **22**, 430–435.

Price R, Douglas NM, Anstey NM & von Seidlein L (2011) *Plasmodium vivax* treatments: what are we looking for? *Current Opinion Infectious Diseases* **24**, 578–585.

Prommano O, Chaisri U, Turner GD, *et al.* (2005) A quantitative ultrastructural study of the liver and the spleen in fatal falciparum malaria. *The South East Asian Journal of Tropical Medicine and Public Health* **36**, 1359–1370.

Pronovost P, Berenholtz S, Dorman T, Lipsett PA, Simmonds T & Haraden C (2003) Improving communication in the ICU using daily goals. *Journal of Critical Care* **18**, 71–75.

Pronovost P, Needham D, Berenholtz S, *et al.* (2006) An intervention to decrease catheter-related bloodstream infections in the ICU. *New England Journal of Medicine* **355**, 2725–2732.

Pukrittayakamee S, White NJ, Davis TM, *et al.* (1992) Hepatic blood flow and metabolism in severe falciparum malaria: clearance of intravenously administered galactose. *Clinical Science* **82**, 63–70.

Pukrittayakamee S, Supanaranond W, Looareesuwan S, Vanijanonta S & White NJ (1994) Quinine in severe falciparum malaria: evidence of declining efficacy in Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 324–327.

Pukrittayakamee S, Looareesuwan S, Keeratithakul D, *et al.* (1997) A study of the factors affecting the metabolic clearance of quinine in malaria. *European Journal of Clinical Pharmacology* **52**, 487–493.

Pukrittayakamee S, Pitisuttithum P, Zhang H, Jantra A, Wanwimolruk S & White NJ (2002) Effects of cigarette smoking on quinine pharmacokinetics in malaria. *European Journal of Clinical Pharmacology* **58**, 315–319.

Pukrittayakamee S, Wanwimolruk S, Stepniewska K, *et al.* (2003) Quinine pharmacokinetic-pharmacodynamic relationships in uncomplicated falciparum malaria. *Antimicrobial Agents and Chemotherapy* **47**, 3458–3463.

Punyagupta S, Srichaikul T, Nitiyanant P & Petchclai B (1974) Acute pulmonary insufficiency in falciparum malaria: summary of 12 cases with evidence of disseminated intravascular coagulation. *American Journal of Tropical Medicine and Hygiene* **23**, 551–559.

Pussard E, Barennes H, Daouda H, *et al.* (1999) Quinine disposition in globally malnourished children with cerebral malaria. *Clinical Pharmacology and Therapeutics* **65**, 500–510.

Pussard E, Straczek C, Kabore I, *et al.* (2004) Dose-dependent resorption of quinine after intrarectal administration to children with moderate *Plasmodium falciparum* malaria. *Antimicrobial Agents and Chemotherapy* **48**, 4422–4426.

Putaporntip C, Hongsrimuang T, Seethamchai S, *et al.* (2009) Differential prevalence of *Plasmodium* infections and cryptic *Plasmodium knowlesi* malaria in humans in Thailand. *Journal of Infectious Diseases* **199**, 1143–1150.

Putensen C, Theuerkauf N, Zinserling J, Wrigge H & Pelosi P (2009) Meta-analysis: ventilation strategies and outcomes of the acute respiratory distress syndrome and acute lung injury. *Annals of Internal Medicine* **151**, 566–576.

Raghunandan J, Rajeshwari K, Dubey AP & Singh T (2012) Peripheral gangrene in an 18-month-old boy with *Plasmodium vivax* malaria. *Paediatrics and International Child Health* **32**, 164–166.

Rajahram GS, Barber BE, William T, Menon J, Anstey NM & Yeo TW (2012) Deaths due to *Plasmodium knowlesi* malaria

in Sabah, Malaysia: association with reporting as *Plasmodium malariae* and delayed parenteral artesunate. *Malaria Journal* **11**, 284.

Rajaratnam JK, Marcus JR, Flaxman AD, *et al.* (2010) Neonatal, postneonatal, childhood, and under-5 mortality for 187 countries, 1970-2010: a systematic analysis of progress towards Millennium Development Goal 4. *Lancet* **375**, 1988–2008.

Ramutton T, Hendriksen IC, Mwanga-Amumpaire J, *et al.* (2012) Sequence variation does not confound the measurement of plasma PfHRP2 concentration in African children presenting with severe malaria. *Malaria Journal* **11**, 276.

Ranque S, Poudiougou B, Traore A, *et al.* (2008) Life-threatening malaria in African children: a prospective study in a mesoendemic urban setting. *Pediatric Infectious Disease Journal* **27**, 130–135.

Ratcliff A, Siswantoro H, Kenangalem E, *et al.* (2007) Therapeutic response of multidrug-resistant *Plasmodium falciparum* and *P. vivax* to chloroquine and sulfadoxine-pyrimethamine in southern Papua, Indonesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **101**, 351–359.

Reader TW, Flin R, Mearns K & Cuthbertson BH (2009) Developing a team performance framework for the intensive care unit. *Critical Care Medicine* **37**, 1787–1793.

Recht J, Ashley EA & White NJ (2013). *The Safety of 8-Aminoquinoline Antimalarials*. World Health Organization, Geneva.

Redd SC, Bloland PB, Kazembe PN, Patrick E, Tembenu R & Campbell CC (1992) Usefulness of clinical case-definitions in guiding therapy for African children with malaria or pneumonia. *Lance* **340**, 1140–1143.

Reid HA & Nkrumah FK (1972) Fibrin-degradation products in cerebral malaria. *Lancet* **1**, 218–221.

Rey M, Nouhouayi A & Mar ID (1966) Clinical manifestations of *Plasmodium falciparum* malaria in African Negro children (according to experiences in a Dakar hospital). *Bulletin de la Societe de Pathologie Exotique et de ses Filiales* **59**, 683–704.

Reyburn H, Mbatia R, Drakeley C, *et al.* (2004) Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *British Medical Journal* **329**, 1212.

Reyburn H, Mbatia R, Drakeley C, *et al.* (2005) Association of transmission intensity and age with clinical manifestations and case fatality of severe *Plasmodium falciparum* malaria. *Journal of The American Medical Association* **293**, 1461–1470.

Riddle MS, Jackson JL, Sanders JW & Blazes DL (2002) Exchange transfusion as an adjunct therapy in severe *Plasmodium falciparum* malaria: a meta-analysis. *Clinical Infectious Diseases* **34**, 1192–1198.

Rijken MJ, McGready R, Boel ME, *et al.* (2012a) Malaria in pregnancy in the Asia-Pacific region. *Lancet Infectious Diseases* **12**, 75–88.

Rijken MJ, Papageorghiou AT, Thiptharakun S, *et al.* (2012b) Ultrasound evidence of early fetal growth restriction after maternal malaria infection. *PLoS ONE* **7**, e31411.

Roca-Feltrer A, Carneiro I & Armstrong Schellenberg JR (2008) Estimates of the burden of malaria morbidity in Africa in children under the age of 5 years. *Tropical Medicine and International Health* **13**, 771–783.

Rodriguez-Morales AJ, Benitez JA & Arria M (2008) Malaria mortality in Venezuela: focus on deaths due to *Plasmodium vivax* in children. *Journal of Tropical Pediatrics* **54**, 94–101.

Rogerson SJ, Pollina E, Getachew A, Tadesse E, Lema VM & Molyneux ME (2003) Placental monocyte infiltrates in response to *Plasmodium falciparum* malaria infection and their association with adverse pregnancy outcomes. *American Journal of Tropical Medicine and Hygiene* **68**, 115–119.

Rogerson SJ, Hviid L, Duffy PE, Leke RF & Taylor DW (2007) Malaria in pregnancy: pathogenesis and immunity. *Lancet Infectious Diseases* **7**, 105–117.

Rolling T, Schmiedel S, Wichmann D, Wittkopf D, Burchard GD & Cramer JP (2012) Post-treatment haemolysis in severe imported malaria after intravenous artesunate: case report of three patients with hyperparasitaemia. *Malaria Journal* **11**, 169.

Rosanas-Urgell A, Lin E, Manning L, *et al.* (2012) Reduced risk of *Plasmodium vivax* malaria in Papua New Guinean children with South East Asian ovalocytosis in two cohorts and a case-control study. *PLoS Medicine* **9**, e1001305.

Rosenthal VD, Maki DG, Salomao R, *et al.* (2006) Device-associated nosocomial infections in 55 intensive care units of 8 developing countries. *Annals of Internal Medicine* **145**, 582–591.

Ross R & Thomson D (1910) Some enumerative studies on malaria fever. *Proceedings of the Royal Society of London. Series B* **83**, 159–173.

Ru YX, Mao BY, Zhang FK, *et al.* (2009) Invasion of erythroblasts by *Plasmodium vivax*: a new mechanism contributing to malarial anemia. *Ultrastructural Pathology* **33**, 236–242.

Rudolf G & Ramsay J (1927) Enumeration of parasites in therapeutic malaria. *Journal of Tropical Medicine and Hygiene* **30**, 1–8.

Runsewe-Abiodun IT, Ogunfowora OB & Fetuga BM (2006) Neonatal malaria in Nigeria–a 2 year review. *BMC Pediatrics* **6**, 19.

Rupani AB & Amarapurkar AD (2009) Hepatic changes in fatal malaria: an emerging problem. *Annals of Tropical Medicine and Parasitology* **103**, 119–127.

Sabchareon A, Attanath P, Chanthavanich P, *et al.* (1998) Comparative clinical trial of artesunate suppositories and oral artesunate in combination with mefloquine in the treatment of children with acute falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **58**, 11–16.

Safeukui I, Correas JM, Brousse V, *et al.* (2008) Retention of *Plasmodium falciparum* ring-infected erythrocytes in the slow, open microcirculation of the human spleen. *Blood* **112**, 2520–2528.

Saharan S, Kohli U, Lodha R, Sharma A & Bagga A (2008) Thrombotic microangiopathy associated with *Plasmodium vivax* malaria. *Pediatric Nephrology* **24**, 623–624.

Salako LA & Sowunmi A (1992) Disposition of quinine in plasma, red blood cells and saliva after oral and intravenous administration to healthy adult Africans. *European Journal of Clinical Pharmacology* **42**, 171–174.

Salako LA, Sowunmi A & Akinbami FO (1989) Pharmacokinetics of quinine in African children suffering from kwashiorkor. *British Journal of Clinical Pharmacology* **28**, 197–201.

Saralamba S, Pan-Ngum W, Maude RJ, *et al.* (2011) Intrahost modeling of artemisinin resistance in *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 397–402.

Sarkar S & Bhattacharya P (2008) Cerebral malaria caused by *Plasmodium vivax* in adult subjects. *Indian Journal of Critical Care Medicine* **12**, 204–205.

Sasi P, Burns SP, Waruiru C, *et al.* (2007) Metabolic acidosis and other determinants of hemoglobin-oxygen dissociation in severe childhood *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene* **77**, 256–260.

Schellenberg D, Schellenberg J, Mushi A, *et al.* (2003) The silent burden of anaemia in Tanzanian children: a community-based study. *Bulletin of the World Health Organization* **81**, 581–590.

Schmutzhard E & Gerstenbrand F (1984) Cerebral malaria in Tanzania. Its epidemiology, clinical symptoms and neurological long term sequelae in the light of 66 cases. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78**, 351–353.

Schweickert WD, Pohlman MC, Pohlman AS, *et al.* (2009) Early physical and occupational therapy in mechanically ventilated, critically ill patients: a randomised controlled trial. *Lancet* **373**, 1874–1882.

Scott JA, Berkley JA, Mwangi I, *et al.* (2011) Relation between falciparum malaria and bacteraemia in Kenyan children: a population-based, case-control study and a longitudinal study. *Lancet* **378**, 1316–1323.

Senanayake N (1987) Delayed cerebellar ataxia: a new complication of falciparum malaria? *British Medical Journal* **294**, 1253–1254.

Sermwittayawong N, Singh B, Nishibuchi M, Sawangjaroen N & Vuddhakul V (2012) Human *Plasmodium knowlesi* infection in Ranong province, southwestern border of Thailand. *Malaria Journal* **11**, 36.

Seydel K, Kampondeni SD, Potchen MJ, Birbeck GL, Molyneux M & Taylor T (2011) Clinical correlates of magnetic resonance imaging in pediatric cerebral malaria. *American Society of Tropical Medicine and Hygiene*, 60th Annual Meeting.

Seydel KB, Fox LL, Glover SJ, *et al.* (2012) Plasma concentrations of parasite histidine-rich protein 2 distinguish between retinopathy-positive and retinopathy-negative cerebral malaria in Malawian children. *Journal of Infectious Diseases* **206**, 309–318.

Shann F, Stace J & Edstein M (1985) Pharmacokinetics of quinine in children. *Journal of Pediatrics* **106**, 506–510.

Sharma J, Bharadawa K, Shah K & Dave S (1993) *Plasmodium vivax* malaria presenting as hemolytic–uremic syndrome. *Indian Pediatrics* **30**, 369–371.

Silamut K & White NJ (1993) Relation of the stage of parasite development in the peripheral blood to prognosis in severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 436–443.

Silamut K, White NJ, Looareesuwan S & Warrell DA (1985) Binding of quinine to plasma proteins in falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **34**, 681–686.

Silamut K, Molunto P, Ho M, Davis TM & White NJ (1991) Alpha 1-acid glycoprotein (orosomucoid) and plasma protein binding of quinine in falciparum malaria. *British Journal of Clinical Pharmacology* **32**, 311–315.

Silamut K, Phu NH, Whitty C, *et al.* (1999) A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. *American Journal of Pathology* **155**, 395–410.

Sim JE, Choi YC & Kim WJ (2010) Facial diplegia in *Plasmodium vivax* malaria. *Journal of Clinical Neurology* **6**, 102–103.

Simonsson US, Jansson B, Hai TN, Huong DX, Tybring G & Ashton M (2003) Artemisinin autoinduction is caused by involvement of cytochrome P450 2B6 but not 2C9. *Clinical Pharmacology and Therapeutics* **74**, 32–43.

Simpson JA, Silamut K, Chotivanich K, Pukrittayakamee S & White NJ (1999) Red cell selectivity in malaria: a study of multiple-infected erythrocytes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, 165–168.

Simpson JA, Agbenyega T, Barnes KI, *et al.* (2006) Population pharmacokinetics of artesunate and dihydroartemisinin following intra-rectal dosing of artesunate in malaria patients. *PLoS Medicine* **3**, e444.

Singh B & Daneshvar C (2013) Human infections and detection of *Plasmodium knowlesi*. *Clinical Microbiology Reviews* **26**, 165–184.

Singh B, Lee K, Matusop A, *et al.* (2004) A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet* **363**, 1017–1024.

Sinha A, Singh G, Bhat AS, *et al.* (2012) Thrombotic microangiopathy and acute kidney injury following vivax malaria. *Clinical and Experimental Nephrology* **17**, 66–72.

Sirivichayakul C, Sabchareon A, Pengsaa K, *et al.* (2007) Comparative study of the effectiveness and pharmacokinetics of two rectal artesunate/oral mefloquine combination regimens for the treatment of uncomplicated childhood falciparum malaria. *Annals of Tropical Paediatrics* **27**, 17–24.

Sitprija V (1988) Nephropathy in falciparum malaria. *Kidney International* **34**, 867–877.

Slutsker L, Taylor TE, Wirima JJ & Steketee RW (1994) In-hospital morbidity and mortality due to malaria-associated severe anaemia in two areas of Malawi with different patterns of malaria infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 548–551.

Smith T, Schellenberg JA & Hayes R (1994) Attributable fraction estimates and case definitions for malaria in endemic areas. *Statistics in Medicine* **13**, 2345–2358.

Snounou G & White NJ (2004) The co-existence of *Plasmodium*: sidelights from falciparum and vivax malaria in Thailand. *Trends in Parasitology* **20**, 333–339.

Snow RW & Marsh K (1998) New insights into the epidemiology of malaria relevant for disease control. *British Medical Bulletin* **54**, 293–309.

Snow RW, Armstrong JR, Forster D, *et al.* (1992) Childhood deaths in Africa: uses and limitations of verbal autopsies. *Lancet* **340**, 351–355.

Snow RW, Bastos De Azevedo I, Lowe BS, *et al.* (1994) Severe childhood malaria in two areas of markedly different falciparum transmission in East Africa. *Acta Tropica* **57**, 289–300.

Snow RW, Guerra CA, Noor AM, Myint HY & Hay SI (2005) The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* **434**, 214–217.

Solomon T, Felix JM, Samuel M, *et al.* (1994) Hypoglycaemia in paediatric admissions in Mozambique. *Lancet* **343**, 149–150.

Song HH, O SO, Kim SH, *et al.* (2003) Clinical features of *Plasmodium vivax* malaria. *Korean Journal of Internal Medicine* **18**, 220–224.

Song JY, Park CW, Jo YM, *et al.* (2007) Two cases of *Plasmodium vivax* Malaria with the clinical picture resembling toxic shock. *American Journal of Tropical Medicine and Hygiene* **77**, 609–611.

Sotunmbi PT, Idowu AT, Akang EE & Aken'ova YA (2006) Prevalence of venous thromboembolism at post-mortem in an African population: a cause for concern. *African Journal of Medicine and Medical Sciences* **35**, 345–348.

South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) Group (2005) Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* **366**, 717–725.

Sowunmi A (1993) Psychosis after cerebral malaria in children. *Journal of the National Medical Association* **85**, 695–696.

Sowunmi A, Akindele JA, Omitowoju GO, Omigbodun AO, Oduola AMJ & Salako LA (1993) Intramuscular sulfadoxine-pyrimethamine in uncomplicated chloroquine-resistant falciparum malaria during pregnancy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 472.

Spitz S (1946) The pathology of acute falciparum malaria. *The Military Surgeon* **99**, 555–572.

Srichaikul T (1993) Hemostatic alterations in malaria. *The South East Asian Journal of Tropical Medicine and Public Health* **24** (Suppl 1), 86–91.

Stacpoole PW, Wright EC, Baumgartner TG, *et al.* (1994) Natural history and course of acquired lactic acidosis in adults. DCA-Lactic Acidosis Study Group. *American Journal of Medicine* **97**, 47–54.

Stein CM (1987) Leucocytosis in acute *Plasmodium falciparum* malaria. *South African Medical Journal* **72**, 363.

Steketee RW & Campbell CC (2010) Impact of national malaria control scale-up programmes in Africa: magnitude and attribution of effects. *Malaria Journal* **9**, 299.

Steketee RW, Wirima JJ, Slutsker L, *et al.* (1996) Malaria parasite infection during pregnancy and at delivery in mother, placenta, and newborn: efficacy of chloroquine and mefloquine in rural Malawi. *American Journal of Tropical Medicine and Hygiene* **55**, 24–32.

Stone WJ, Hanchett JE & Knepshield JH (1972) Acute renal insufficiency due to falciparum malaria. Review of 42 cases. *Archives of Internal Medicine* **129**, 620–628.

Stoppacher R & Adams SP (2003) Malaria deaths in the United States: case report and review of deaths, 1979-1998. *Journal of Forensic Sciences* **48**, 404–408.

Sulistyaningsih E, Fitri L, Loscher T & Berens-Riha N (2010) Diagnostic difficulties with *Plasmodium knowlesi* infections in humans. *Emerging Infectious Diseases* **16**, 1033–1034.

Sumawinata IW, Bernadeta Leksana B, *et al.* (2003) Very high risk of therapeutic failure with chloroquine for uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in Indonesian Papua. *American Journal of Tropical Medicine and Hygiene* **68**, 416–420.

Sur D, von Seidlein L, Manna B, *et al.* (2006) The malaria and typhoid fever burden in the slums of Kolkata, India: data from a prospective community-based study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **100**, 725–733.

Suratt BT & Parsons PE (2006) Mechanisms of acute lung injury/acute respiratory distress syndrome. *Clinics in Chest Medicine* **27**, 579–589; abstract viii.

Suwanarusk R, Cooke BM, Dondorp AM, *et al.* (2004) The deformability of red blood cells parasitized by *Plasmodium falciparum* and *P. vivax*. *Journal of Infectious Diseases* **189**, 190–194.

Swarthout TD, Counihan H, Senga RK & Van Den Broek I (2007) Paracheck-Pf accuracy and recently treated *Plasmodium falciparum* infections: is there a risk of over-diagnosis? *Malaria Journal* **6**, 58.

Swellengrebel NH & De Buck A (1938). *Malaria in The Netherlands*. Scheltema & Holkema Ltd., Amsterdam.

Tan LK, Yacoub S, Scott S, Bhagani S & Jacobs M (2008) Acute lung injury and other serious complications of *Plasmodium vivax* malaria. *Lancet Infectious Diseases* **8**, 449–454.

Tan KR, Wiegand RE & Arguin PM (2013) Exchange transfusion for severe malaria: evidence base and literature review. *Clinical Infectious Diseases* **57**, 923–928.

Tantipong H, Morkchareonpong C, Jaiyindee S & Thamlikitkul V (2008) Randomized controlled trial and meta-analysis of oral decontamination with 2% chlorhexidine solution for the prevention of ventilator-associated pneumonia. *Infection Control and Hospital Epidemiology* **29**, 131–136.

Tanwar GS, Khatri PC, Sengar GS, *et al.* (2011) Clinical profiles of 13 children with *Plasmodium vivax* cerebral malaria. *Annals of Tropical Paediatrics* **31**, 351–356.

Tapko JB, Sam O & Diarra-Nama AJ (2007). *Status of Blood Safety in the WHO African Region: Report of the 2004 Survey*. WHO Regional Office for Africa, Brazzaville.

Taylor WR & White NJ (2002) Malaria and the lung. *Clinics in Chest Medicine* **23**, 457–468.

Taylor TE, Molyneux ME, Wirima JJ, Fletcher KA & Morris K (1988) Blood glucose levels in Malawian children before and during the administration of intravenous quinine for severe falciparum malaria. *New England Journal of Medicine* **319**, 1040–1047.

Taylor TE, Borgstein A & Molyneux ME (1993) Acid-base status in paediatric *Plasmodium falciparum* malaria. *Quarterly Journal of Medicine* **86**, 99–109.

Taylor TE, Fu WJ, Carr RA, *et al.* (2004) Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nature Medicine* **10**, 143–145.

Taylor T, Olola C, Valim C, *et al.* (2006a) Standardized data collection for multi-center clinical studies of severe malaria in African children: establishing the SMAC network. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **100**, 615–622.

Taylor W, Terlouw DJ, Olliaro PL, White NJ, Brasseur P & Ter Kuile FO (2006b) Use of weight-for-age-data to optimize tablet strength and dosing regimens for a new fixed-dose artesunate-amodiaquine combination for treating falciparum malaria. *Bulletin of the World Health Organization* **84**, 956–964.

Taylor WR, Canon V & White NJ (2006c) Pulmonary manifestations of malaria: recognition and management. *Treatments in Respiratory Medicine* **5**, 419–428.

Taylor SM, Parobek CM & Fairhurst RM (2012a) Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. *Lancet Infectious Diseases* **12**, 457–468.

Taylor W, Hanson J, Turner GD, White NJ & Dondorp AM (2012b) Respiratory manifestations of malaria. *Chest* **142**, 492–505.

Teasdale G & Jennett B (1974) Assessment of coma and impaired consciousness. A practical scale. *Lancet* **2**, 81–84.

Teja-Isavadharm P, Nosten F, Kyle DE, *et al.* (1996) Comparative bioavailability of oral, rectal, and intramuscular artemether in healthy subjects: use of simultaneous measurement by high performance liquid chromatography and bioassay. *British Journal of Clinical Pharmacology* **42**, 599–604.

Teklehaimanot HD, Teklehaimanot A, Kiszewski A, Rampao HS & Sachs JD (2009) Malaria in Sao Tome and principe: on the brink of elimination after three years of effective antimalarial measures. *American Journal of Tropical Medicine and Hygiene* **80**, 133–140.

Ter Kuile F, White NJ, Holloway P, Pasvol G & Krishna S (1993) *Plasmodium falciparum*: in vitro studies of the pharmacodynamic properties of drugs used for the treatment of severe malaria. *Experimental Parasitology* **76**, 85–95.

Thapa R, Patra V & Kundu R (2007) *Plasmodium vivax* cerebral malaria. *Indian Pediatrics* **44**, 433–434.

Thapa R, Ranjan R, Patra VS & Chakrabartty S (2009) Childhood cerebral vivax malaria with pancytopenia. *Journal of Pediatric Hematology and Oncology* **31**, 116–117.

Thwing J, Eisele TP & Steketee RW (2011) Protective efficacy of malaria case management and intermittent preventive treatment for preventing malaria mortality in children: a systematic review for the Lives Saved Tool. *BMC Public Health* **11**(Suppl 3), S14.

Tjitra E, Anstey NM & Sugiarto P (2008) Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. *PLoS Medicine* **5**, e128.

Trang TT, Phu NH, Vinh H, *et al.* (1992) Acute renal failure in patients with severe falciparum malaria. *Clinical Infectious Diseases* **15**, 874–880.

Trape JF, Pison G, Preziosi MP, *et al.* (1998) Impact of chloroquine resistance on malaria mortality. *Comptes rendus de l'Academie des sciences* **321**, 689–697.

Treluyer JM, Roux A, Mugnier C, Flouvat B & Lagardere B (1996) Metabolism of quinine in children with global malnutrition. *Pediatric Research* **40**, 558–563.

Tripathi AK, Sullivan DJ & Stins MF (2007) *Plasmodium falciparum*-infected erythrocytes decrease the integrity of human blood-brain barrier endothelial cell monolayers. *Journal of Infectious Diseases* **195**, 942–950.

Tripathy R, Parida S, Das L, *et al.* (2007) Clinical manifestations and predictors of severe malaria in Indian children. *Pediatrics* **120**, e454–e460.

Turner GD, Morrison H, Jones M, *et al.* (1994) An immunohistochemical study of the pathology of fatal malaria: evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. *American Journal of Pathology* **145**, 1057.

Turner GD, Ly VC, Nguyen TH, *et al.* (1998) Systemic endothelial activation occurs in both mild and severe malaria. Correlating dermal microvascular endothelial cell phenotype and soluble cell adhesion molecules with disease severity. *American Journal of Pathology* **152**, 1477–1487.

Turner L, Lavstsen T, Berger SS, Wang CW, Petersen JE, Avril M, Brazier AJ, Freeth J, Jespersen JS, Nielsen MA, Magistrado P, Lusingu J, Smith JD, Higgins MK & Theander TG (2013) Severe malaria is associated with parasite binding to endothelial protein C receptor. *Nature* **498**, 502–505.

Udomsangpetch R, Pipitaporn B, Krishna S, *et al.* (1996) Antimalarial drugs reduce cytoadherence and rosetting *Plasmodium falciparum*. *Journal of Infectious Diseases* **173**, 691–698.

Udomsangpetch R, Pipitaporn B, Silamut K, *et al.* (2002) Febrile temperatures induce cytoadherence of ring-stage *Plasmodium falciparum*-infected erythrocytes. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 11825–11829.

Udomsanpetch R, Thanikkul K, Pukrittayakamee S & White NJ (1995) Rosette formation by *Plasmodium vivax*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 635–637.

Umbers AJ, Aitken EH & Rogerson SJ (2011) Malaria in pregnancy: small babies, big problem. *Trends in Parasitology* **27**, 168–175.

Urban BC, Hien TT, Day NP, *et al.* (2005) Fatal *Plasmodium falciparum* malaria causes specific patterns of splenic architectural disorganization. *Infection and Immunity* **73**, 1986–1994.

Vachon F, Fajac I, Gachot B, Coulaud JP & Charmot G (1992) Halofantrine and acute intravascular haemolysis. *Lancet* **340**, 909–910.

Valecha N, Bhatia S, Mehta S, Biswas S & Dash AP (2007) Congenital malaria with atypical presentation: a case report from low transmission area in India. *Malaria Journal* **6**, 43.

Valecha N, Pinto RG, Turner GD, *et al.* (2009) Histopathology of fatal respiratory distress caused by *Plasmodium vivax* malaria. *American Journal of Tropical Medicine and Hygiene* **81**, 758–762.

Van Den Eede P, Van HN, Van Overmeir C, *et al.* (2009) Human *Plasmodium knowlesi* infections in young children in central Vietnam. *Malaria Journal* **8**, 249.

Van Der Voort PH, Boerma EC, Koopmans M, *et al.* (2009) Furosemide does not improve renal recovery after hemofiltration for acute renal failure in critically ill patients: a double blind randomized controlled trial. *Critical Care Medicine* **37**, 533–538.

Van Hensbroek MB, Kwiatkowski D, Van Den Berg B, Hoek FJ, Van Boxtel CJ & Kager PA (1996) Quinine pharmacokinetics in young children with severe malaria. *American Journal of Tropical Medicine and Hygiene* **54**, 237–242.

Van Hensbroek MB, Palmer A, Jaffar S, Schneider G & Kwiatkowski D (1997) Residual neurologic sequelae after childhood cerebral malaria. *Journal of Pediatrics* **131**, 125–129.

Van Velthuysen ML & Florquin S (2000) Glomerulopathy associated with parasitic infections. *Clinical Microbiology Reviews* **13**, 55–66.

Van Vugt M, Angus BJ, Price RN, *et al.* (2000) A case-control auditory evaluation of patients treated with artemisinin derivatives for multidrug-resistant *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene* **62**, 65–69.

Vekemans J, Marsh K, Greenwood B, *et al.* (2011) Assessment of severe malaria in a multicenter, phase III, RTS, S/AS01 malaria candidate vaccine trial: case definition, standardization of data collection and patient care. *Malaria Journal* **10**, 221.

Vinsonneau C, Camus C, Combes A, *et al.* (2006) Continuous venovenous haemodiafiltration versus intermittent haemodialysis for acute renal failure in patients with multiple-organ dysfunction syndrome: a multicentre randomised trial. *Lancet* **368**, 379–385.

Viriyayudhakorn S, Thitiarchakul S, Nachaisit S, Ho PC & Wanwimolruk S (2000) Pharmacokinetics of quinine in obesity. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 425–428.

Von Seidlein L, Olaosebikan R, Hendriksen IC, *et al.* (2012) Predicting the clinical outcome of severe falciparum malaria in African children: findings from a large randomized trial. *Clinical Infectious Diseases* **54**, 1080–1090.

Vottier G, Arsac M, Farnoux C, Mariani-Kurkdjian P, Baud O & Aujard Y (2008) Congenital malaria in neonates: two case reports and review of the literature. *Acta Paediatrica* **97**, 505–508.

Walker O, Salako LA, Sowunmi A, Thomas JO, Sodeine O & Bondi FS (1992) Prognostic risk factors and post mortem findings in cerebral malaria in children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 491–493.

Walker IA, Merry AF, Wilson ICH, *et al.* (2009) Global oximetry: an international anaesthesia quality improvement project. *Anaesthesia* **64**, 1051–1060.

Waller D, Krishna S, Craddock C, *et al.* (1990) The pharmacokinetic properties of intramuscular quinine in Gambian children with severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 488–491.

Waller D, Crawley J, Nosten F, *et al.* (1991) Intracranial pressure in childhood cerebral malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 362–364.

Waller D, Krishna S, Crawley J, *et al.* (1995) Clinical features and outcome of severe malaria in Gambian children. *Clinical Infectious Diseases* **21**, 577–587.

Walter ND, Lyimo T, Skarbinski J, *et al.* (2009) Why first-level health workers fail to follow guidelines for managing severe disease in children in the Coast Region, the United Republic of Tanzania. *Bulletin of the World Health Organization* **87**, 99–107.

Wanwimolruk S, Chalcroft S, Coville PF & Campbell AJ (1991) Pharmacokinetics of quinine in young and elderly subjects. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 714–717.

Wanwimolruk S, Wong SM, Coville PF, Viriyayudhakorn S & Thitiarchakul S (1993) Cigarette smoking enhances the elimination of quinine. *British Journal of Clinical Pharmacology* **36**, 610–614.

Warrell DA, Looareesuwan S, Warrell MJ, *et al.* (1982) Dexamethasone proves deleterious in cerebral malaria. A double-blind trial in 100 comatose patients. *New England Journal of Medicine* **306**, 313–319.

Warrell DA, Looareesuwan S, Phillips RE, *et al.* (1986) Function of the blood-cerebrospinal fluid barrier in human cerebral malaria: rejection of the permeability hypothesis. *American Journal of Tropical Medicine and Hygiene* **35**, 882–889.

Watkins WM, Woodrow C & Marsh K (1993) Falciparum malaria: differential effects of antimalarial drugs on ex vivo parasite viability during the critical early phase of therapy. *American Journal of Tropical Medicine and Hygiene* **49**, 106–112.

Wattanagoon Y, Srivilairit S, Looareesuwan S & White NJ (1994) Convulsions in childhood malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 426–428.

Weber MW, Zimmermann U, Van Hensbroek MB, *et al.* (1999) Renal involvement in Gambian children with cerebral or mild malaria. *Tropical Medicine and International Health* **4**, 390–394.

Weinke T, Trautmann M, Held T, *et al.* (1991) Neuropsychiatric side effects after the use of mefloquine. *American Journal of Tropical Medicine and Hygiene* **45**, 86–91.

Were T, Davenport GC, Hittner JB, *et al.* (2011) Bacteremia in Kenyan children presenting with malaria. *Journal of Clinical Microbiology* **49**, 671–676.

White NJ (1987) The pharmacokinetics of quinine and quinidine in malaria. *Acta Leidensia* **55**, 65–76.

White NJ (1995a) Controversies in the management of severe falciparum malaria. In: *Bailliere's Clinical Infectious Diseases*. 2nd edn, (ed G Pasvol), Balliere, pp 309–330.

White NJ (1995b) Optimal regimens of parenteral quinine. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **89**, 462–464.

White N (1997) Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. *Antimicrobial Agents and Chemotherapy* **41**, 1413.

White NJ (2008) *Plasmodium knowlesi*: the fifth human malaria parasite. *Clinical Infectious Diseases* **46**, 172–173.

White NJ (2011) The parasite clearance curve. *Malaria Journal* **10**, 278.

White NJ & Krishna S (1989) Treatment of malaria: some considerations and limitations of the current methods of assessment. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 767–777.

White NJ, Looareesuwan S, Warrell DA, Warrell MJ, Bunnag D & Harinasuta T (1982) Quinine pharmacokinetics and toxicity in cerebral and uncomplicated falciparum malaria. *American Journal of Medicine* **73**, 564–572.

White NJ, Looareesuwan S & Silamut K (1983a) Red cell quinine concentrations in falciparum malaria. *American Journal of Tropical Medicine and Hygiene* **32**, 456–460.

White NJ, Warrell DA, Chanthavanich P, *et al.* (1983b) Severe hypoglycemia and hyperinsulinemia in falciparum malaria. *New England Journal of Medicine* **309**, 61–66.

White NJ, Looareesuwan S, Warrell DA, Warrell MJ, Chanthavanich P, Bunnag D & Harinasuta T (1983c) Quinine loading dose in cerebral malaria. *American Journal of Tropical Medicine and Hygiene* **32**, 1–5.

White NJ, Miller KD, Brown J, Marsh K & Greenwood B (1987a) Prognostic value of CSF lactate in cerebral malaria. *Lancet* **1**, 1261.

White NJ, Miller KD, Marsh K, *et al.* (1987b) Hypoglycaemia in African children with severe malaria. *Lancet* **1**, 708–711.

White NJ, Looareesuwan S, Phillips RE, Chanthavanich P & Warrell DA (1988) Single dose phenobarbitone prevents convulsions in cerebral malaria. *Lancet* **2**, 64–66.

White VA, Lewallen S, Beare N, Kayira K, Carr RA & Taylor TE (2001) Correlation of retinal haemorrhages with brain haemorrhages in children dying of cerebral malaria in Malawi. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **95**, 618–621.

White VA, Lewallen S, Beare NA, Molyneux ME & Taylor TE (2009) Retinal pathology of pediatric cerebral malaria in Malawi. *PLoS ONE* **4**, e4317.

White NJ, Dondorp AM, Faiz A, Mishra S & Hien TT (2012) New global estimates of malaria deaths. *Lancet* **380**, 559–560.

White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA & Dondorp AM (2013a) Malaria. *Lancet* **383**, 723–735.

White NJ, Turner GD, Day NP & Dondorp AM (2013b) Lethal malaria: Marchiafava and Bignami were right. *Journal of Infectious Diseases* **208**, 192–198.

Whitten R, Milner DA, Yeh MM, Kamiza S, Molyneux ME & Taylor TE (2011) Liver pathology in Malawian children with fatal encephalopathy. *Human Pathology* **42**, 1230–1239.

Whitworth J, Morgan D, Quigley M, *et al.* (2000) Effect of HIV-1 and increasing immunosuppression on malaria parasitaemia and clinical episodes in adults in rural Uganda: a cohort study. *Lancet* **356**, 1051–1056.

Wickramasinghe SN, Looareesuwan S, Nagachinta B & White NJ (1989) Dyserythropoiesis and ineffective erythropoiesis in *Plasmodium vivax* malaria. *British Journal of Haematology* **72**, 91–99.

Wickramasuriya GW (1937) Malaria and Ankylostomiasis in the Pregnant Woman. Their More Serious Complications and Sequelae.

Wilairatana P, Looareesuwan S & Charoenlarp P (1994) Liver profile changes and complications in jaundiced patients with falciparum malaria. *Tropical Medicine and Parasitology* **45**, 298–302.

Wilairatana P, Looareesuwan S, Kaojarern S, Vanijanonta S & Charoenlarp P (1995) Gastric emptying in acute uncomplicated falciparum malaria. *Journal of Tropical Medicine and Hygiene* **98**, 22–24.

Wilkerson RD (1981) Acute effects of intravenous furosemide administration on serum digoxin concentration. *American Heart Journal* **102**, 63–65.

Willcox ML, Forster M, Dicko MI, Graz B, Mayon-White R & Barennes H (2010) Blood glucose and prognosis in children with presumed severe malaria: is there a threshold for 'hypoglycaemia'? *Tropical Medicine and International Health* **15**, 232–240.

William T, Menon J, Rajahram G, *et al.* (2011) Severe *Plasmodium knowlesi* malaria in a tertiary care hospital, Sabah, Malaysia. *Emerging Infectious Diseases* **17**, 1248–1255.

Williams TN (2012) Balancing act: haemoglobinopathies and malaria. *Lancet Infectious Diseases* **12**, 427–428.

Williams TN, Maitland K, Bennett S, *et al.* (1996) High incidence of malaria in alpha-thalassaemic children. *Nature* **383**, 522–525.

Win K, Than M & Thwe Y (1992) Comparison of combinations of parenteral artemisinin derivatives plus oral mefloquine with intravenous quinine plus oral tetracycline for treating cerebral malaria. *Bulletin of the World Health Organization* **70**, 777–782.

Winkler AS, Salmhofer G, Helbok R, Kalokola F, Matuja W & Schmutzhard E (2008) In-hospital risk estimation in children with malaria–early predictors of morbidity and mortality. *Journal of Tropical Pediatrics* **54**, 184–191.

Winstanley PA, Newton CR, Pasvol G, *et al.* (1992) Prophylactic phenobarbitone in young children with severe falciparum malaria: pharmacokinetics and clinical effects. *British Journal of Clinical Pharmacology* **33**, 149–154.

Winstanley P, Newton C, Watkins W, *et al.* (1993) Towards optimal regimens of parenteral quinine for young African children with cerebral malaria: the importance of unbound quinine concentration. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 201–206.

Winstanley PA, Mberu EK, Watkins WM, Murphy SA, Lowe B & Marsh K (1994) Towards optimal regimens of parenteral quinine for young African children with cerebral malaria: unbound quinine concentrations following a simple loading

dose regimen. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 577–580.
Woodruff AW, Ansdell VE & Pettitt LE (1979) Cause of anaemia in malaria. *Lancet* **1**, 1055–1057.
World Health Organization (2000) Severe falciparum malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**(Suppl 1), S1–S90.
World Health Organization (2005a). *Emergency Triage and Treatment (ETAT)*. World Health Organization, Geneva.
World Health Organization (2005b). *Hospital Care for Children: Guidelines for the Management of Common Illnesses with Limited Resources*. World Health Organization, Geneva.
World Health Organization (2010a). *World Malaria Report 2010*. World Health Organization, Geneva.
World Health Organization (2010b). *Guidelines for the Treatment of Malaria*, 2nd edn.. World Health Organization, Geneva.
World Health Organization (2011a). *Meeting Report: Informal Consultation on the Public Health Importance of Plasmodium knowlesi Kota Samarahan, Sarawak*. World Health Organization, Geneva.
World Health Organization (2011b). *Achieving Universal Access to Malaria Diagnostic Testing: an Operational Manual*. World Health Organization, Geneva.
World Health Organization (2013). *World Malaria Report 2013*. World Health Organization, Geneva.
Yacoub S, Lang HJ, Shebbe M, *et al.* (2010) Cardiac function and hemodynamics in Kenyan children with severe malaria. *Critical Care Medicine* **38**, 940–945.
Yadav P, Sharma R, Kumar S & Kumar U (2008) Magnetic resonance features of cerebral malaria. *Acta Radiologica* **49**, 566–569.
Yadav D, Chandra J, Aneja S, Kumar V, Kumar P & Dutta AK (2012) Changing profile of severe malaria in north Indian children. *Indian Journal of Pediatrics* **79**, 483–487.
Yakoob MY, Zakaria A, Waqar SN, *et al.* (2005) Does malaria during pregnancy affect the newborn? *Journal of the Pakistan Medical Association* **55**, 543–546.
Yen LM, Dao LM, Day NP, *et al.* (1994) Role of quinine in the high mortality of intramuscular injection tetanus. *Lancet* **344**, 786–787.
Yeo TW, Lampah DA, Gitawati R, *et al.* (2007) Impaired nitric oxide bioavailability and L-arginine reversible endothelial dysfunction in adults with falciparum malaria. *Journal of Experimental Medicine* **204**, 2693–2704.
Yeo TW, Lampah DA, Gitawati R, *et al.* (2008a) Recovery of endothelial function in severe falciparum malaria: relationship with improvement in plasma L-arginine and blood lactate concentrations. *Journal of Infectious Diseases* **198**, 602–608.
Yeo TW, Lampah DA, Gitawati R, *et al.* (2008b) Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 17097–17102.
Yeo TW, Lampah DA, Tjitra E, *et al.* (2009) Relationship of cell-free hemoglobin to impaired endothelial nitric oxide bioavailability and perfusion in severe falciparum malaria. *Journal of Infectious Diseases* **200**, 1522–1529.
Yeo TW, Lampah DA, Tjitra E, *et al.* (2010a) Increased asymmetric dimethylarginine in severe falciparum malaria: association with impaired nitric oxide bioavailability and fatal outcome. *PLoS Pathogens* **6**, e1000868.
Yeo TW, Lampah DA, Tjitra E, *et al.* (2010b) Greater endothelial activation, Weibel-Palade body release and host inflammatory response to *Plasmodium vivax,* compared with *Plasmodium falciparum*: a prospective study in Papua, Indonesia. *Journal of Infectious Diseases* **202**, 109–112.
Yeo TW, Lampah DA, Kenangalem E, Tjitra E, Price RN & Anstey NM (2012) Impaired skeletal muscle microvascular function and increased skeletal muscle oxygen consumption in severe falciparum malaria. *Journal of Infectious Diseases* **207**, 528–536.
Zhao SZ & Mackenzie IJ (2011) Deafness: malaria as a forgotten cause. *Annals of Tropical Paediatrics* **31**, 1–10.
Zhao XJ, Yokoyama H, Chiba K, Wanwimolruk S & Ishizaki T (1996) Identification of human cytochrome P450 isoforms involved in the 3-hydroxylation of quinine by human live microsomes and nine recombinant human cytochromes P450. *Journal of Pharmacology and Experimental Therapeutics* **279**, 1327–1334.
Zoller T, Junghanss T, Kapaun A, *et al.* (2011) Intravenous artesunate for severe malaria in travelers, Europe. *Emerging Infectious Diseases* **17**, 771–777.