Seizures in children with acute falciparum malaria : risk factors, mechanisms of neuronal damage and neuro-protection

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Publication date
2008

Chapter 7

Pathogenesis, clinical features and neurological outcome of cerebral malaria

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Lancet Neurology, 2005; 4(12): 827 – 40

ABSTRACT

Cerebral malaria is the most severe neurological complication of falciparum malaria. Although most cases occur in children living in sub-Saharan Africa, it should be considered in anybody with impaired consciousness who has recently travelled in a malaria endemic area. It has few specific features, but there are differences in clinical presentation between African children and non-immune adults. Subsequent neurological impairments are also more common and severe in children. Sequestration of infected erythrocytes appears to be an essential component of the pathogenesis. However, other factors such as convulsions, acidosis or hypoglycaemia may impair consciousness. In this review, we describe the clinical features and epidemiology of cerebral malaria. We highlight recent insights provided by *ex-vivo* work on sequestration and the examination of pathological specimens. We also summarise the recent studies of persisting neurocognitive impairments in children who survive and suggest areas for further research.

INTRODUCTION

Cerebral malaria (CM) is the most severe neurological complication of infection with *Plasmodium falciparum* and is a major cause of acute non-traumatic encephalopathy in tropical countries (Panel I).

Mortality is high and over the last two decades, the extent of persistent neuro-cognitive deficits following survival has become apparent. In this paper, we review work that has provided further understanding of the pathogenesis, describe the long-term neuro-cognitive outcomes of CM and suggest areas for future studies.

EPIDEMIOLOGY AND IMMUNITY

It is estimated that in 2002 there were 515 million clinical episodes of malaria in the world; 25% in South East (S.E) Asia and 70% in Africa, mostly in sub-Saharan Africa.[1] In most Western countries, malaria is seen in immigrants or people returning from travels in malaria endemic areas. Thus, in the U.K, 1722 cases of malaria were seen in 2003.[2] In sub-Saharan Africa, children are most commonly affected, such that malaria may account for 40% of paediatric admissions to some hospitals in malaria endemic areas, 10% of which may be due to CM.[3] The annual burden of CM in malaria endemic areas of sub Saharan Africa is 1.12/1000 children per year[4], with a mortality of 18.6%.[5] Falciparum malaria may cause other complications such as severe anaemia, acidosis, or hypoglycaemia and, multiple complications can occur in a single patient.

The manifestations of severe malaria in young children in malaria endemic areas are dependent on age and level of transmission (i.e. number of infected mosquito bites per person per year). In areas of intense transmission, up until the age of 6 months, infection and clinical disease are rare, producing only mild symptoms as a result of passive immunity from maternal antibodies. The burden of disease falls within the first two years and by the age of four years children experience few clinical episodes which are usually mild.[6] In areas with less intense transmission, the peak incidence of severe disease falls at a later age. Severe anaemia occurs much more commonly in infants less than two years of age and the peak incidence of CM is later, - the cause of this age related differences is unclear. Repeated infections over several years provide protection against disease. Immunity is effective but partial and declines in the absence of continuous exposure, although partial protective immunity was observed in Africans who had been resident in France for at least 4 years.[7]

Table I: Clinical features of cerebral malaria in African children and Southeast Asian adults

Clinical features	African children	Adults
Coma	Develops rapidly often after a seizure9.	Develops gradually following drowsiness, disorientation, delirium, and agitation over 2-3 days or may follow a generalized seizure10.
Seizures	Over 80% present with a history of seizures and 60% have seizures during hospital admission. Recurrent seizures are focal motor in >50%, generalized tonic clonic in 34%, partial with secondary generalization in 14%, and subtle or electrographic in 15%. Status epilepticus is common.9,11	Occurs in up to 20%, mostly generalized tonic-clonic seizures. Status epilepticus is rare.10,12
Other signs	Pallor, respiratory distress, dehydration and rarely jaundice.	Jaundice (40-70%), Kussmaul's breathing, shock and spontaneous bleeding.8,13,14
Neurological signs	Brainstem signs are present in >30% and are associated with raised ICP. 15,16 Retinal abnormalities are present in >60%.17 Brain swelling on CT scan is seen in 40%.18	Patients typically have symmetrical upper motor neuron signs. Brainstem signs and retinal abnormalities are less common.10,19
Major Complications and involvement of other organs	Severe anaemia in 20-50%, of whom over 30% require a blood transfusion.8 Severe metabolic acidosis (presents as respiratory distress), often associated with hyperlacteamia. Others are hyponatraemia (>50%), hypoglycaemia(30%) and changes in potassium. Renal failure and pulmonary oedema are rare.8,9,20-25	Part of a multi-system and organ (circulatory, hepatic, coagulation, renal and pulmonary) dysfunction. Pulmonary oedema, renal failure, lactic acidosis, haemoglobinuria may be observed.26-28 Hypoglycaemia is present in only 8%.29
Outcome		
Recovery of consciousness	Rapid, within 24-48 hrs8,30	Slower, occurs within 48 hours.31
Mortality	18.6%5,8, up to 75% of deaths occur within 24 hours of admission	20%8 about 50% occur within 24 hours13
Neurological sequelae	Occurs in 11%5. Common sequelae are ataxia (2.5%), hemiparesis (4.4%), quadriparesis (3.5%), hearing (1.9%), visual (2.3%) and speech (2.1%) impairments, behavioural difficulties (1.3%) and epilepsy.8,32,33	Few, occurs in <5%. Isolated cranial nerve palsies, mononeuritis multiplex, polyneuropathy, extrapyramidal tremor and other cerebellar signs.10

The World Health Organization (WHO) proposed a definition of CM as a clinical syndrome characterized by coma (inability to localise a painful stimulus) at least 1 hour after termination of a seizure or correction of hypoglycaemia, with asexual forms of *P. falciparum* malaria parasites on peripheral blood smears and exclusion of other causes of encephalopathy.[8] This definition is particularly useful for comparisons between different areas and research studies. It is used in children and adults, however there are significant clinical differences (table I). It is not entirely clear if these differences are related to immunity or are dependent on age.

Clinical features of cerebral malaria in African children

Children who are admitted with CM present with 1-3 day history of fever, anorexia, vomiting and sometimes cough. Coma, seizures and brainstem signs are the main neurological features.[9,23,30]

Coma

Cerebral malaria is a diffuse encephalopathy characterized by coma and bilateral slowing on the electroencephalogram[30,34] (figure 1a).

Figure 1 Electroencephalography recordings in children with cerebral malaria

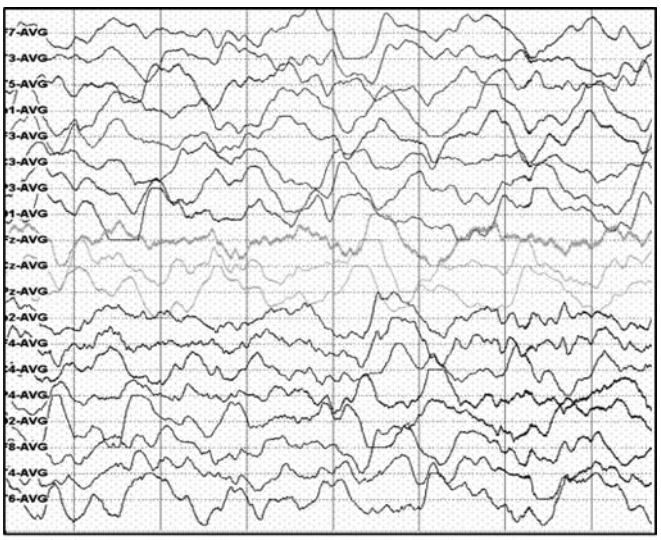


Figure 1a EEG recording in a Kenyan child with cerebral malaria showing diffuse high amplitude-slow wave activity more marked over the left hemisphere.

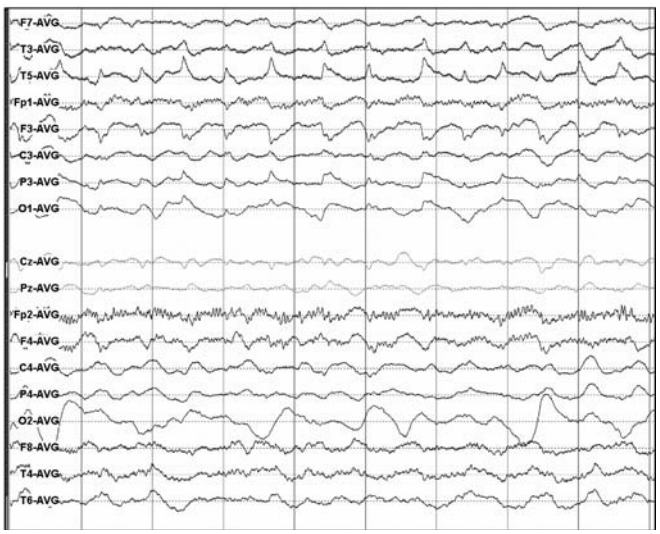


Figure 1b EEG recording in a Kenyan child with cerebral malaria showing electrical seizure activity most prominent over the left temporal region

It has many features similar to a metabolic encephalopathy such as presenting with abnormal pupillary signs and the coma being potentially reversible. The cause of impaired consciousness is unclear but is likely to be the result of a number of different interacting mechanisms. The depth of coma is an important prognostic factor.[8,30]

Seizures

Seizures are commonly reported in African children with CM and occur in over 60% after admission[11,23,34,35] (table I). Many patients with seizures are hypoxic and hypercarbic from hypoventilation and are at risk of aspiration.[11,35-37] In 65 Kenyan children, 62% had seizures following admission; while subtle seizures occurred in 15%, manifesting as nystagmoid eye movements, irregular breathing, excessive salivation, and conjugate eye deviation.[11] Seizures are often repetitive and prolonged, and 28% had an episode of status epilepticus. Multiple and prolonged seizures are associated with increased mortality[33,38,39] and neuro-cognitive deficits.[35,40]

The causes of seizures are unclear. Most are not associated with fever at the time of the seizure.[35] They do not appear to due to electrolyte disorders[41] or antimalarial drugs in children.[34] Electroencephalography show that many originate over the temporo-parietal regions (which is a watershed area) (figure 1b), suggesting that ischaemia and hypoxia may play a role.[34] The seizures may be caused by sequestration of infected erythrocytes or parasite-derived toxins. Furthermore immune mechanisms may be important, since antibodies to voltage gated calcium channels are elevated in children with severe malaria and seizures.[42]

Brainstem signs

Brainstem signs are common and are associated with other features of raised intracranial pressure (ICP) and with brain swelling (figure 2), but may occur after seizures.[15,16] They do not appear to be associated with hypoglycaemia or electrolyte disorders.[15,16] Common signs include changes in pupillary size and reaction, disorders of conjugate gaze and eye movements. Absence of corneal and oculocephalic reflexes are associated with increased mortality.[9] Other signs include abnormal respiratory patterns (such as hyperventilation, ataxic and periodic breathing)[36], posture (decerebrate, decorticate or opisthotonic posturing), and motor abnormalities of tone and reflexes.[9,23] Abnormal motor posturing appears to be related to raised ICP rather than seizures.[43]

Malarial retinopathy

Retinal abnormalities are common in children with CM and may reflect the pathology in the brain.[17,44,45] Characteristic features include whitening of the macula (that spares

Figure 2: Radiological features of the brain in cerebral malaria

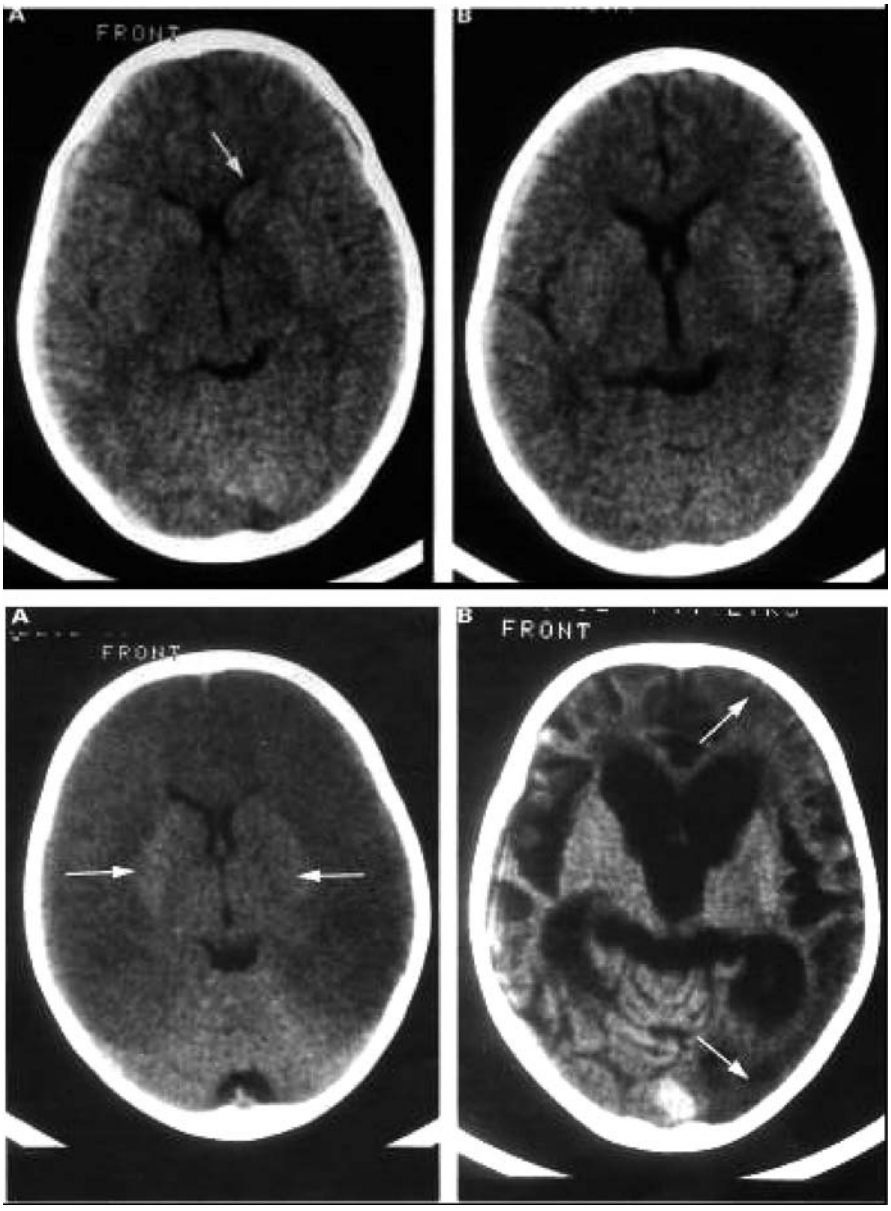


Figure 2a A CT scan of the brain in a Kenyan child with cerebral malaria showing (A) swelling of the brain with compressed ventricles (arrow) and loss of sulci and (B) Resolution of the brain swelling

Figure 2b A CT scan of the brain of a Kenyan child with cerebral malaria showing (A) brain swelling with diffuse hypodensity sparing the basal ganglia (arrows) and (B) Convalescent scan showing cerebral atrophy with infarction (arrows) of the right frontal and parietal regions. (Newton et al 1994, with permission from the BMJ Publishing Group).31

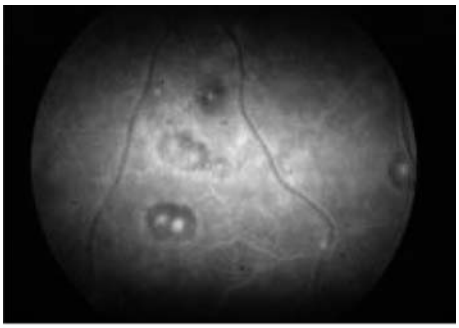


Figure 3 Retinal changes in children with cerebral malaria (retinopathy of malaria)
Figure 3a White-centred retinal haemorrhages and orange vessels in a Malawian child with cerebral malaria

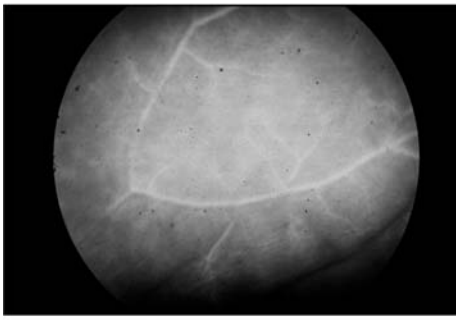


Figure 3b Macular retinal whitening around the foveola (central dark disc) in a child with cerebral malaria. Cotton wool spots are also visible superio-temporal to the optic disc.

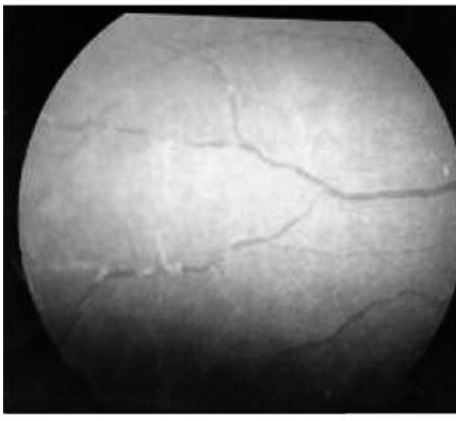


Figure 3c Vessel changes in a Malawian child with cerebral malaria - from red to pale orange

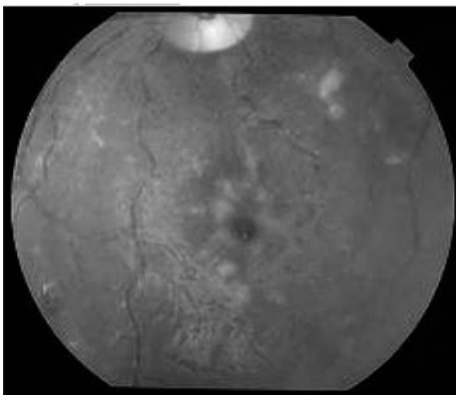


Figure 3d Vessel changes in a Malawian child with cerebral malaria - from red to white

(Photos Courtesy of Dr Nicholas Beare, Malawi-Liverpool-Wellcome Trust Clinical Research Programme College of Medicine, Malawi)

the central fovea), peripheral retina and retinal vessels; multiple retinal haemorrhages (often with pale centres) and papilloedema (figure 3). These signs are best seen by indirect ophthalmoscopy and have been found in over 60% of children with CM.[45] Their specificity may help in the diagnosis of CM. In Malawian children, presence of retinopathy, particularly papilloedema, was associated with prolonged coma and death.[17] In patients who recover, these features resolve over 1-4 weeks.

Concomitant complications

Metabolic perturbations are common in African children with CM. Hypoglycaemia is present in up to one third of patients on admission and often recurs even after initial correction. Causes include depletion of glycogen stores, inadequate intake, impaired hepatic gluconeogenesis and quinine-induced hyperinsulinaemia.[9,20,46] Metabolic acidosis manifests as deep breathing and is often associated with hyperlactaemia. It may be caused by hypovolaemia and inadequate tissue perfusion, anaemia, lactate production by parasites and cytokine-induced failure of oxygen utilization.[3,36,37,47] Resuscitation with fluids or blood transfusion may improve outcome.[48] Children with dehydration often have transient impairment of renal function but unlike adults, overt renal failure is rare. Hyponatraemia occurs in over 50% of patients[21], but the cause is unclear.[21,49] Concomitant bacterial infections occur in 5-8% of children with CM[50,51] and leucocyte counts above 15,000/μl are associated with poor prognosis.[9] Other features include hepatomegaly, splenomegaly and occasionally, jaundice.

Clinical features of cerebral malaria in adults

Cerebral malaria in adults is part of a multi-organ disease.[30] Patients usually present after a few days illness with fever, malaise, headache, joint and body aches, anorexia, delirium, and then develop coma. Seizures are less common than in African children and the incidence appears to be declining.[30] The encephalopathy in adults is characterized by symmetrical upper neuron lesion signs. Dysconjugate eye deviation, extrapyramidal rigidity, trismus, decorticate and decerebrate rigidity may be observed.[10] Papilloedema and retinal exudates are rare, but retinal haemorrhages occur in 15% and are associated with increased mortality.[52] Recovery from coma is slower than in children.[31] It has been suggested that thiamine deficiency may contribute to some of these neurological signs.[53] In a few patients, abnormalities such as cortical infarcts, cerebral venous or dural sinus thrombosis (figures 4a[54] and 4b[55]) may occur as a consequence of the hypercoagulable state.

In some patients, CM is complicated by pulmonary oedema or adult respiratory distress syndrome.[13,56] Kussmaul's breathing occurs with acute renal failure and

Figure 4: Cerebral infarcts in adults with cerebral malaria

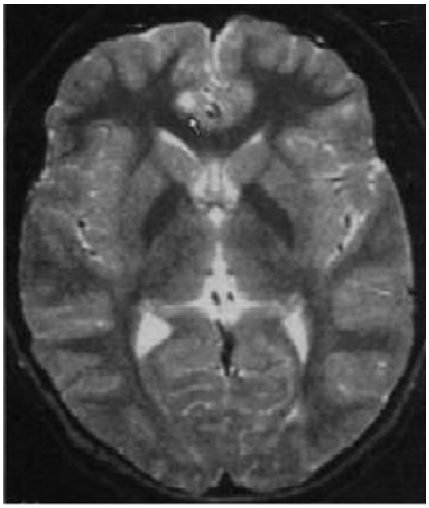


Figure 4a Infarcts in a 36-year old man with cerebral malaria Hyperintense cortical areas (infarcts) seen on a fast spin-echo T2 weighted MR image (arrow) (Cordoliani et al 2004, with permission © American Society of Neuroradiology).54

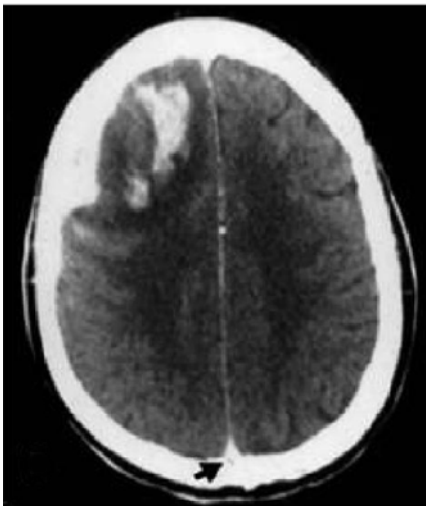


Figure 4b Contrast enhanced brain CT scan of a 48-year old man who presented with left focal becoming generalised seizures and left hemiparesis. A large area of hemorrhagic infarction is seen in the right frontal cortexwith surrounding oedema. Absence of contrast is seen as a hypodense area in the posterior aspect of the superior sagittal sinus. (Krishan et al. 2004, with permission © the British Infection Society).55

severe lactic acidosis.[10,19] Other complications of falciparum malaria such as anaemia, haemoglobinuria, jaundice, shock and coagulation disorders may be observed.[57-60] A higher incidence of multi-organ failure is observed among those admitted to intensive care units since mostly very ill patients who did not respond to earlier treatment are admitted to such units[26]. Bacterial co-infection is common, particularly in those with shock, and accounts for the majority of late deaths. Respiratory failure has the worst prognosis and develops late in the course of the illness.[26] Chronic hepatitis B infection may be a risk factor for severe malaria, including CM in adults.[61]

DIAGNOSIS

Cerebral malaria should be considered in the differential diagnosis of any patient who has a febrile illness with impaired consciousness in a malaria endemic area or one with recent travel to such areas. At least 3 negative blood smears (on microscopy) at 8-12 hourly intervals are required before the diagnosis can be excluded. Rapid tests such as the immunochromatographic test for *P. falciparum* histidine-rich protein 2 and lactate dehydrogenase may be helpful in the absence of positive blood smear although they do not estimate the parasite load and their sensitivity and specificity decreases at low parasitaemia.[62] Polymerase chain reaction tests are more sensitive than microscopy but expensive and do not estimate the parasite load.[63]

In malaria endemic areas, CM is a diagnosis of exclusion. The high prevalence of asymptomatic parasitaemia in such populations makes accurate diagnosis less certain – almost any viral encephalopathy with incidental parasitaemia fulfils the diagnostic criteria for CM. Thus in a recent study, 24% of Malawian children who fulfilled the criteria for CM prior to death had evidence at post-mortem of an alternative cause for coma, including Reyes syndrome, hepatic necrosis and ruptured arterio-venous malformation.[64] The presence of malarial retinopathy was the only clinical feature to distinguish patients with typical histopathological features of CM from those with alternative pathologies. A lumbar puncture must be performed to exclude other causes for the encephalopathy, although there are differences of opinion about the timing of this procedure[16,65]. There may be a mild pleocytosis and an increase in protein.[66] Plasma and CSF lactate levels is of prognostic value since elevated levels are associated with increased mortality.[9,46] Neuroimaging shows a swollen brain in over 40% of African children[18](figure 2) although this finding is less common in adults.[67]

PATHOGENESIS

In falciparum infections, consciousness can be impaired by a variety of mechanisms which may interact with each other[30] (table II). The relative contributions of these mechanisms may differ in children and adults. Thus unlike adults, seizures appear to be an important cause of impairment of consciousness in African children.

Research strategies

The main research strategies for studying pathogenesis have been clinical case series and case control studies, post-mortem surveys, *in vitro* or animal models. There is not a reliable animal model of CM. Many primates naturally suffer *Plasmodium* infections

Table II Postulated mechanisms for coma in cerebral malaria

Obstruction of cerebral microvascular flow
Parasite sequestration mediated through cytoadherence[71], rosette formation[72], autoagglutination[72,73] and reduced red cell deformability.[74]
Seizures
Overt seizures[11,35,37]
Subtle and electrographic seizures[11,37]
Post-ictal state[37]
Impaired delivery of substrate
Hypoglycaemia[9,23]
Anaemia[75]
Hypoxia[76]
Impaired perfusion
Hypovolaemia[47,77]
Shock[78]
Acidosis[78]
Raised intracranial pressure and brain swelling
Disruption of the blood-brain barrier[79,80]
Raised intracranial pressure[15,16,18]
Cerebral oedema[81-83]
Cytotoxic odema[18,81]
Toxins
Nitric oxide[84]
Reactive oxygen species[85,86]
Excitotoxins[30,87-89]
Malaria toxin[90]
Clotting
Intravascular coagulation as a minor mechanism[91]

but rarely develop clinical features similar to human CM. *P. falciparum* will infect the new world monkeys but severe symptoms are common only in splenectomized animals. Some species do develop cerebral dysfunction associated with adherence of infected erythrocytes to cerebral endothelial cells.[68,69] Although coma is not a typical finding, adherence of infected erythrocytes to cerebral endothelial cells has contributed to the understanding of parasite sequestration.

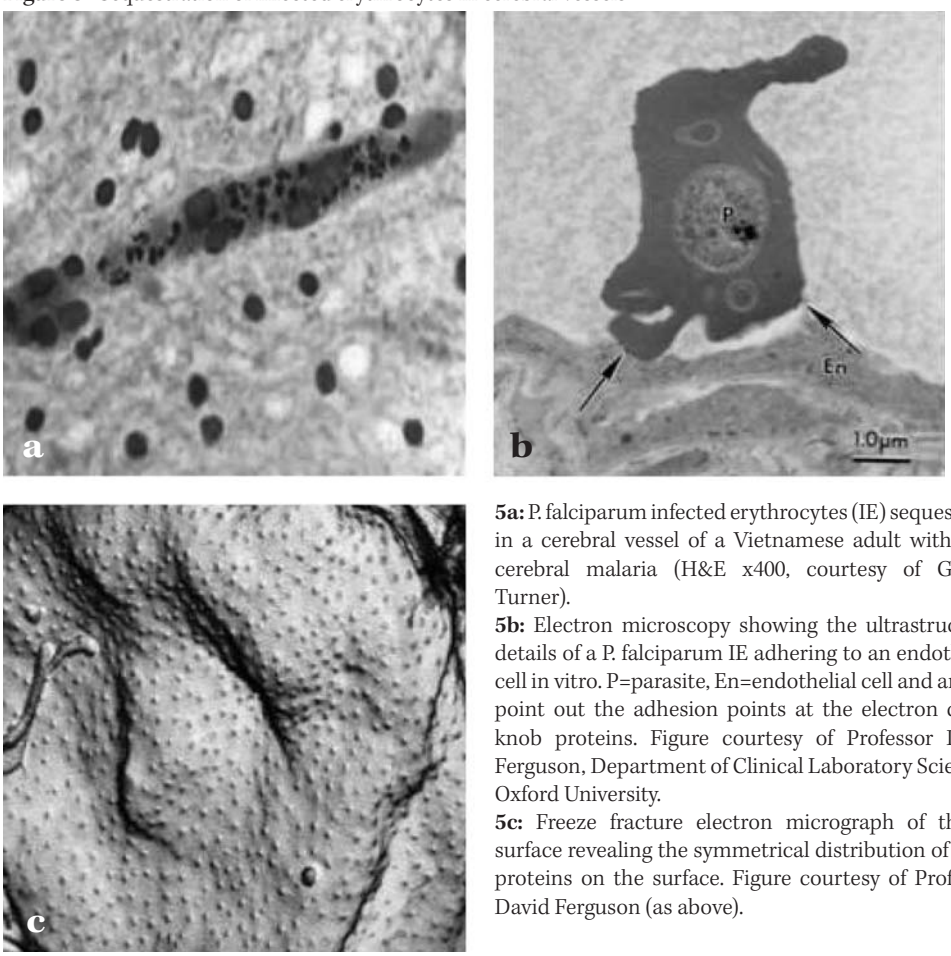
Considerable research on CM has been carried out in mice. The characteristics of the infection are dependent on strains of mouse and Plasmodium. The most popular model is CBA-mice infected with *P. bergei* ANKA[70]. Coma, seizures and death occur, but unlike human CM, this cannot be reversed with treatment. The pathology is different in mice, in that infected erythrocytes do not commonly sequester; instead, monocytes occur in cerebral vessels, and inflammatory cytokines are essential for the pathogenesis.

However, monocytes are also seen in the cerebral vessels of some African children[64], but the significance of this finding is still unclear. The use of murine models, particularly using gene 'knock-out' strains, has provided much information on the immune and inflammatory responses to Plasmodium infections.

Sequestration

The consistent histological finding in CM in both children and adults is the presence of infected and non-infected erythrocytes packed within cerebral vessels (figure 5). It is thought that sequestration occurs as a consequence of cytoadherence of infected erythrocytes to endothelial cells, via *P. falciparum* derived proteins on the infected erythrocyte surface attaching to ligands upregulated in the venules. Sequestration may be increased when adherent infected erythrocytes bind other infected erythrocytes

Figure 5 Sequestration of infected erythrocytes in cerebral vessels



5a: P. falciparum infected erythrocytes (IE) sequestered in a cerebral vessel of a Vietnamese adult with fatal cerebral malaria (H&E x400, courtesy of Gareth Turner).
5b: Electron microscopy showing the ultrastructural details of a P. falciparum IE adhering to an endothelial cell in vitro. P=parasite, En=endothelial cell and arrows point out the adhesion points at the electron dense knob proteins. Figure courtesy of Professor David Ferguson, Department of Clinical Laboratory Sciences, Oxford University.
5c: Freeze fracture electron micrograph of the IE surface revealing the symmetrical distribution of knob proteins on the surface. Figure courtesy of Professor David Ferguson (as above).

(auto-agglutination), non-infected erythrocytes (rosetting) or use platelets to bind other infected erythrocytes (platelet-mediated clumping). Not all parasites display these adhesive properties, but these phenotypes are more commonly present in infected erythrocytes taken from children and adults with severe malaria.

Parasite binding is mediated by a group of variant surface antigens expressed at the red cell surface during development. The best described is *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) which is encoded by a family of approximately 60 *var* genes which are associated with different binding phenotypes. Each parasite expresses the transcript of only one *var* gene but, can switch to express a different *var* gene (about 2% per generation *in vitro*)[92], and therefore display both a change in binding phenotype and antigen. Although the trigger for *var* gene switching is unknown this rapid switching in non immune volunteers does not support the role of immune pressure.[93] Some variant surface antigens appear to be more common in young children with severe disease, and thus may be more capable of causing CM than others[94] but whether this is due to adhesion phenotype or host response is unclear.

PfEMP-1 is able to bind to many host receptors on endothelial cells chief among which are CD36 and the intercellular adhesion molecule-1 (ICAM-1).[95,96] The binding of infected erythrocytes to ICAM-1 has been implicated in the pathogenesis of CM.[97] Postmortem studies have revealed upregulation of ICAM-1 expression on the cerebral vascular endothelium in CM[98,79] which, in adults, was co-localised to areas of parasite sequestration.[99] A common ICAM-1 polymorphism (ICAM-1^{Kilifi}) that alters protein binding to infected erythrocytes[100] was associated with susceptibility to CM in Kenyan children[101] but not in the Gambia.[102] In a study describing the binding affinities of parasites taken from Kenyan children with malaria, ICAM-1 binding was highest in CM cases.[96] The inconsistency in the polymorphisms associated with CM in different sites illustrates a methodological problem of this *ex-vivo* work. However, we do not know how representative circulating parasites are of those sequestered within cerebral vessels and although ICAM-1 appears important, it is likely that a number of host receptors are involved in concert in the process resulting in CM.

Reduction in microvascular flow

Sequestration of infected and non-infected erythrocytes within the cerebral vessels reduces the microvascular flow. In addition, the presence of parasites inside the erythrocyte decreases the ability of the erythrocyte to deform (reduced red cell deformability, RCD) so that erythrocytes have more difficulty in passing through the microvasculature. Studies of Thai adults[103] and Kenyan children[104] have found strong associations between reduced RCD and severe disease and in adults with outcome. The

rapid reversibility of clinical symptoms suggests that tissue necrosis is unlikely to occur. However, there may be a critical reduction in the supply of metabolic substrate to the brain. This will be exacerbated by increased demand during seizures and fever, and may be worse in patients with severe anaemia or hypoglycaemia.[23,75] Cerebral blood flow may also be reduced by raised ICP. Inflammatory cytokines may result in inefficient use of substrates.

The inflammatory response

P. falciparum infection results in increases in both pro- and anti-inflammatory cytokines. The balance of inflammatory mediators appears critical to parasite control, but their role in the pathogenesis of severe malaria is unclear. Thus in Malian children, both IL-6 (pro-inflammatory) and IL-10 (anti-inflammatory) were increased in CM compared to non-CM, but not IL-1β, IL-8, IL-12 or TNF.[105] In Gambian and Ghanaian children, TNF and TNF receptor levels were higher in CM compared to non-severe malaria.[106,107] Several polymorphisms in the TNF promoter region have also been associated with increased risk of CM and death (reviewed in Gimenez et al 2003)[108] or neurological sequelae.[109] In Vietnamese adults, levels of IL-6, IL-10 and TNF were raised in patients with multi-organ severe disease but were lower in patients with CM alone, suggesting their involvement in the process leading to severe malaria but not coma itself.[110] Postmortem analysis of brains from Malawian children with CM suggest increased local production of TNF and IL-1β.[111] However, there was no correlation between production or staining for these cytokines and parasite sequestration.

Nitric oxide (NO) has been suggested as a key effector for TNF in the pathogenesis of malaria. It is involved in host defence by killing intracellular organisms, maintaining vascular status and in neurotransmission. Cytokines may upregulate inducible NO synthase (iNOS) in brain endothelial cells, increasing NO production which diffuses into brain tissue and interferes with neuronal function.[84] It may rapidly and reversibly reduce the level of consciousness[84] since it is short-lived and can easily diffuse across the blood-brain barrier to interfere with neuronal function.

The associations between NO activity, iNOS or genetic polymorphisms in the iNOS promoter gene have not been consistent. Results have varied with age, endemicity and geographical location. Post-mortem staining of brain specimens in African children and SE Asian adults have revealed increased iNOS in vessel walls associated with sequestered parasites in CM[112] while in other studies, NO is associated with protection.[113,114] It is thought that TNF upregulation of iNOS sets off a negative feedback mechanism through NO to control the stimulatory action on iNOS. However, in some individuals, generation of NO occurs too slowly to downregulate the primary wave of TNF induction, so that a

relatively slow build up of iNOS-induced NO allows iNOS and NO to reach the harmful levels seen in CM.[115]

The blood-brain barrier function

Since parasites are largely confined to the intravascular space, one major question regarding the pathogenesis of CM is how these parasites cause neuronal dysfunction.[116] There is accumulating evidence that parasite sequestration changes blood brain barrier (BBB) function. In Thai adults, there was no increase in transfer of radioactively labelled albumin into CSF during unconsciousness when compared to convalescence.[117] No significant changes were also observed in the albumin index (ratio of CSF to blood albumin levels) in Vietnamese adults.[80] However, in Malawian children, albumin indices were significantly higher when compared to UK controls[79] although there were no differences between children who died and those who survived.

Post mortem analysis has shown widespread cerebral vascular endothelial cell activation (increased ICAM-1 endothelial staining, reduction in cell junction staining and disruption of junctional proteins), particularly in vessels containing infected erythrocytes.[118] Perivascular macrophages in these areas expressed scavenger receptor and sialoadhesin –normally only expressed after contact with plasma proteins. However, such disruption of intercellular junctions was not associated with evidence of significant leakage of plasma proteins (fibrinogen, IgG or C5b-9) into perivascular areas or CSF.[79] Adams et al[119] suggested that ICAM-1 binding by infected erythrocytes results in a cascade of intracellular signalling events that disrupt the cytoskeletal-cell junction structure and cause focal disruption to BBB. Focal disruptions in the barrier at sites of sequestration could result in the exposure of sensitive perivascular neurons to plasma proteins and the increased levels of cytokines and metabolites produced by abnormalities in the microcirculation. This may contribute to reduced consciousness and/or seizure activity.

Brain swelling

In Kenyan children with deep coma, 40% had evidence of brain swelling on computerised tomography (CT) scan (figure 2), however during recovery, some children with severe encephalopathy had evidence of cytotoxic oedema which may contribute to the severe intracranial hypertension.[18] Severe intracranial hypertension was associated with death or neurological sequelae.[15] In a study of 21 Indian adults, abnormalities on CT scans were related to Glasgow Coma Score and mortality.[120] Cerebral oedema was seen in eight patients, two of whom died. Other studies of Thai adults have found little evidence of cerebral oedema on CT scan[121] or magnetic resonance imagining[67] but documented

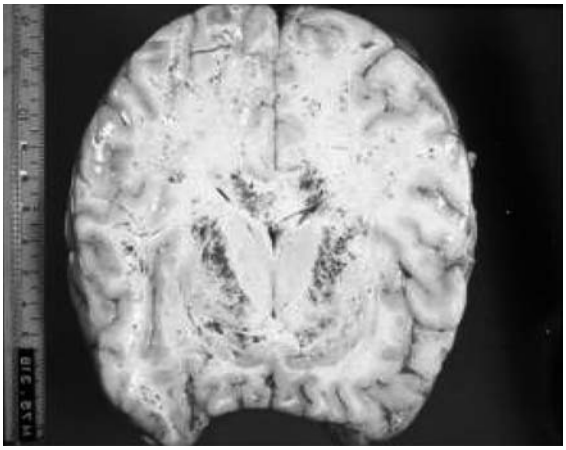
brain swelling. Although no significant leakage of plasma proteins has been observed[79], the disruption of the BBB may contribute to the raised ICP found in African children. However the most likely cause of raised ICP is increased cerebral blood volume due to sequestration of infected erythrocytes and increased cerebral blood flow from seizures, hyperthermia or anaemia[5,30].

Pathological findings

Postmortem studies have provided a wealth of detailed information but they reflect, at best, pathology at a single point after death in the most severely ill patients. The recent surveys from Malawi and SE Asia have found a significant relationship between the amount of sequestration and antemortem diagnosis of cerebral malaria. Sequestration is extensive, occurring in all parts of the brain to a similar extent, but with considerable variability between individuals and between vessels in an individual.[122] The brain is often swollen but evidence for frank herniation is unusual in adults, although more common in African children.[83] The cut surface shows petechial haemorrhages (figure 6).[123]

Electron microscopy show knob-like protrusions on the surface of infected erythrocytes and at sites of attachment to vascular endothelium (figures 5).[81] Studies in Malawian children show intravascular and perivascular pathologies (haemorrhages, accumulation of pigmented white blood cells and thrombi) in 75% of cases. These are associated with an increased extraerythrocytic haemazoin (a product haemoglobin metabolism by malaria parasites) inside cerebral vessels. Thus, rupture of infected erythrocytes may lead to an inflammatory process within and around brain capillaries.[64] These findings have not been seen consistently in adults[122,124] and may reflect a difference between adults and children.

Figure 6 Gross pathological appearance of the cut surface of the brain in fatal cerebral malaria



Macroscopic section of the brain in a fatal case of CM showing petechial haemorrhages in the white matter, particularly in the subcortical rim and corpus callosum (Courtesy of Dr Peter King, South African Institute of Medical Research, Johannesburg, South Africa).

β-amyloid precursor protein staining (a marker of axonal injury) was found on postmortem brain specimens of adults with CM.[125] Two patterns were observed; a diffuse increase or a predominance of axonal injury in one brain region, typically the internal capsule or pons. Axonal injury correlated with plasma lactate, CSF protein and Glasgow coma score. The increased levels of the microtubule-associated protein tau (from degenerated axons) but not neural cell body or astrocyte proteins in CSF suggested that most of the brain parenchymal damage is with axons.[126] High levels of quinolinic acid was found in CSF[87] but in Vietnamese adults, this was related to impaired renal function.[88]

OUTCOME OF CEREBRAL MALARIA

The majority of patients with CM appear to make a full recovery, but neuro-cognitive sequelae have been increasingly recognised particularly in African children, in the last 20 years.

Mortality

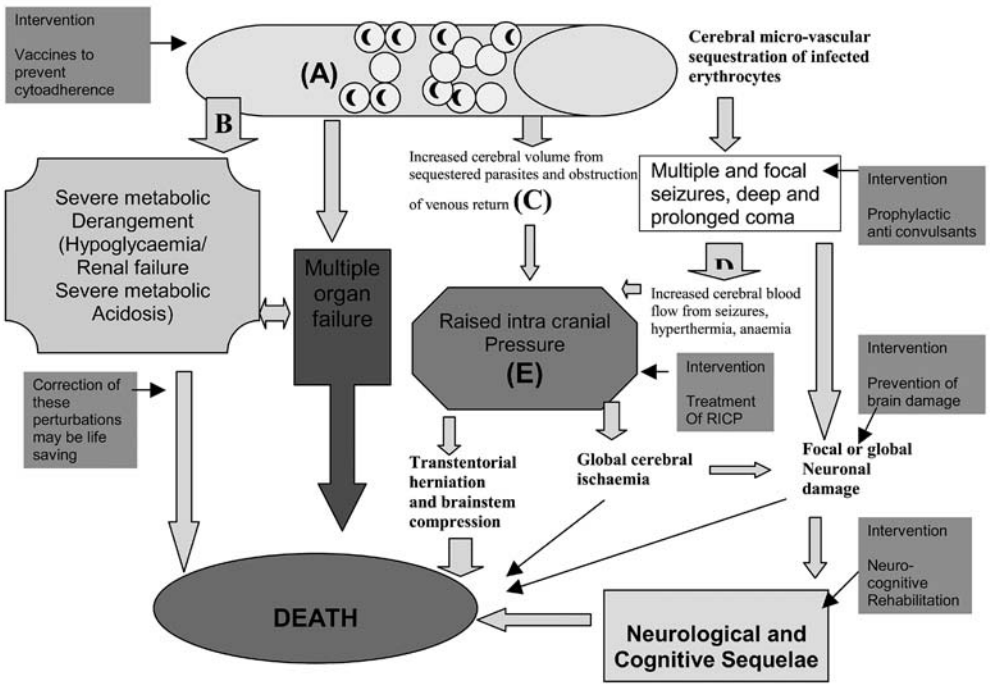
The mortality rate in adults and children is approximately 20%, and most deaths occur within 24 hours of admission before the anti-malarial drugs may have had time to work. The mechanisms of death appear to be different (figure 7). In African children, most deaths occur with brainstem signs following a respiratory arrest (initially with a good cardiac output) suggestive of transtentorial herniation or cardio-respiratory arrest in association with severe metabolic acidosis. Four out of seven children in Nigeria had cerebral oedema and/or features of herniation at autopsy.[83] Mortality is high among children with shock, hypoglycaemia, multiple and prolonged seizures, deep coma, or severe acidosis.[9,23,127]

Many adults die with renal failure of pulmonary oedema. Mortality is particularly increased in pregnant patients or those with vital organ dysfunction.[28,128] Patients may die with an acute respiratory arrest, often following a period of respiratory irregularity, but with a normal blood pressure. Others die with shock or hypoxia secondary to acute pulmonary oedema.

Neuro-cognitive deficits

In African children, a higher incidence of neurological deficits (10.9%) was reported in a meta-analysis which used studies with a similar definition of CM.[5] Some deficits are transient (*e.g.* ataxia), whilst others *e.g.* hemiparesis, improve over months, but may not resolve completely. Children living in Africa with severe neurological sequelae (spastic

Figure 7 Possible mechanisms for death and neuro-cognitive impairment in cerebral malaria and some areas for possible intervention



P. falciparum IE adhere to vascular endothelium sequestering in large numbers in the brain (A). Local and systemic changes produce significant vital organ dysfunction leading to severe metabolic derangement (B) which may quickly result in death unless urgent correction (e.g correction of blood glucose, dialysis or ventilation) is initiated. Sequestration of IE within the cerebral vessels increases the cerebral volume (C) which together with the increase in cerebral blood flow (from seizures, anaemia and hyperthermia - D), altered BBB function lead to brain swelling and raised ICP (E). This may cause death (through transtentorial herniation, brainstem compression or global cerebral ischaemia) or result in neuronal damage with consequent neuro-cognitive impairments. Sequestered parasites may also produce local toxins and ischaemia or influence the production of inflammatory products such as cytokines and result in multiple seizures and neuronal damage. Metabolic derangement is more common in adults while raised ICP and seizures are commoner in children. Possible areas for intervention are highlighted.

quadriparesis and vegetative states) often die within a few months of discharge.[129] More recently, it has been demonstrated that epilepsy is associated with exposure to CM.[130]

Cognitive impairments have been described in some studies[39], but not others.[131] Impairment has been reported in a wide range of cognitive functions; memory, attention, executive functions and language.[39,129,132-134] Neuro-cognitive impairments are associated with protracted seizures[11,32,38], deep and prolonged coma[33], hypoglycaemia and severe anaemia in some studies[38] but not in others.[32] The consistent association found between prolonged seizures or hypoglycaemia and neuro-cognitive impairment suggests hippocampal damage, which may manifest as memory impairment and complex

partial seizures at a later date. Other smaller studies have shown an association between the development of impairments and pathophysiological processes such as raised ICP.[15] Most of these factors are also associated with death, and may simply reflect the severity of the underlying insult, rather than a specific neuropathogenic process. The fact that up to 24% of children have evidence of some impairment following CM, represents a significant burden in malaria endemic areas, suggesting that at least 250,000 children will develop neuro-cognitive impairments from malaria in sub Saharan Africa per year.[134]

In non-immune adults, the incidence (<5%) and severity of subsequent neurological impairments is less than in children. Impairments are not confined to CM, but may follow non-cerebral malaria.[135] They include cranial nerve lesions, neuropathies and extrapyramidal disorders.[10,136] Some patients develop a transient psychosis or delirium during recovery, whilst others develop focal epilepsy sometimes associated with transient tomographic opacities in the brain. In Vietnam, a self-limiting "post-malaria neurological syndrome" consisting of acute confusional state, acute psychosis, generalised convulsions or tremor occurred in 0.12% of patients with falciparum malaria.[135]

Cognitive deficits following malaria in adults are less well documented. There are case reports of impairment of memory and naming ability. Psychological tests did not detect any residual defects in a small group of American soldiers following CM[137], although a recent retrospective study suggests that CM results in multiple, neuropsychiatric symptoms, including poor dichotic listening, "personality change," depression, and in some cases, partial seizure-like symptoms.[138] A study of Ghanaian adults suggested that subclinical, mixed anxiety-depression syndrome may occur after falciparum malaria.[139]

MANAGEMENT OF CEREBRAL MALARIA

The World Health Organization has developed guidelines for the management of patients with CM[8] and a new guidelines were recently proposed for the UK.[140] Emergency management aims at rapidly correcting the severely deranged metabolic states, restoration of vital physiological functions (Panel II) and, the administration of an effective and rapidly active parasiticidal drug.

Resuscitation on admission

Since most patients die within 24 hours of admission before the therapeutic benefits of anti-malaria drugs[5], supportive therapy may improve outcome. Correction of hypoxaemia, hypoglycaemia, shock, severe metabolic acidosis and control of seizures are important. Urgent resuscitation with fluids may be required for those with hypovolaemia[22,47,48,140], although fluids should be administered carefully. The administration of albumin reduced mortality in a small trial of children with CM [78], but confirmatory trials are still awaited. Whole blood or packed cell transfusions should be administered for severe anaemia. Recurrences of hypoglycaemia may be prevented by continuous infusion of glucose containing fluids until consciousness is regained.

Antimalarial therapy

The cinchona alkaloids (quinine, quinidine and cinchonin) and artemisinin derivatives (artesunate, artemether and arteether) are recommended for CM (table III). The cinchona alkaloids act during the later stages of parasite development, whilst the artemisinins have a broader stage-spectrum of action. A loading dose of either drug should be given, to rapidly achieve antiparasiticidal concentrations.

Quinine is still used extensively and can be given intravenously or intramuscularly. A loading dose is associated with faster clearance of parasitaemia, resolution of fever and coma.[141] A 12 hourly dose regimen may be used in younger children.[142] Quinidine is more

Table III: Antimalarial treatment of cerebral malaria

Drug	Route	Indicated for	Loading dose	Maintenance dose
Quinine dihydrochloride	IV	Children and adults	20 mg salt/kg over 2-4 hrs (max 600 mg)	10 mg salt/kg every 8 hrs, until able to take orally[8]
Quinine dihydrochloride	IV	Children	15-20 mg salt/kg over 2-4 hrs	10 mg salt/kg every 12 hrs, until able to take orally[8,153]
Quinine dihydrochloride	IM	Children and adults	20 mg salt/kg (dilute iv formulation to 60mg/ml) given in 2 injection sites (anterior thigh)	10 mg salt/kg every 8–12 hrs until able to take orally[142,154]
Quinidine gluconate	IV	Children and adults	10 mg salt/kg in normal saline over 1-2 hrs	0.02 mg salt/kg/min continuous infusion with ECG monitoring up to 72 hrs OR 10 mg salt/kg every 8-12 hrs[143]
Artemether	IM	Children and adults	3.2 mg/kg	1.6 mg/kg/day for a minimum of 5 days[5]
Artesunate	IV/IM	Children and adults	2.4 mg/kg	1.2 mg/kg after 12 and 24 hrs, then 1.2 mg/kg/day for 7 days. Change to oral route when possible[5]

toxic (especially cardiotoxicity) and expensive than quinine and, a dose reduction may be necessary if the corrected QT interval is prolonged.[143] In some parts of Francophone Africa, Quinimax (a combination of quinine, quinidine, cinchonine and cinchonidine) is commonly used.[144] The main side effects of the cinchona alkaloids are hyperinsulinaemic hypoglycaemia and cinchonism (giddiness, tinnitus, high tone deafness and colour aberrations). Although high doses of quinine may induce uterine contractions, normal therapeutic doses can be used safely in pregnancy.[145] Doses of the cinchona alkaloids should be reduced by 30-50% if intravenous therapy is required beyond 3 days to avoid accumulation.

The artemisinin derivatives clear circulating parasites faster than other antimalarial drugs[146] and adults treated with artesunate have a lower mortality than those treated with quinine.[147] The artemisinin derivatives should be used in combination with other antimalarial drugs to prevent resistance. Side effects are infrequent[148] and they are easier to administer than the cinchoniods. Animal studies show that parenteral artemether and artheeter are associated with damage to brainstem nuclei[149] but no evidence of such neurotoxic effects has been detected in humans.[150] Rectal preparations may be useful in rural health facilities.[151]

Supportive therapy

Ventilation and dialysis may be life saving in adults with pulmonary oedema or renal failure respectively. Children should receive antimicrobials to cover the possibility of bacterial infections until these can confidently be excluded by examination of CSF, blood and urine.[8] Exchange transfusion has been recommended for non-immune adult patients with parasite densities >30% as it lowers parasitaemia and improves red cell rheology but there is no conclusive evidence that it reduces mortality.[152]

Therapies with deleterious or unproven value

A number of other adjunct therapies have been tried, but as yet remain unproven.[8] Steroids are deleterious, while acetyl salicylic acid, sodium bicarbonate and heparin may be harmful. Desferoxamine and dextran have unclear roles. Hyperimmune serum confers no benefit, while monoclonal antibodies to TNF were associated with a worse neurological outcome. Although pentoxifylline, was associated with earlier resolution of coma and lower mortality in Burundian children, no benefit was observed in other studies. Mannitol reduces intracranial hypertension but such decreases are neither sustained nor does it prevent the development of severe intracranial hypertension. Prophylactic Phenobarbital, 10 mg/kg did not control seizures[155], 20 mg/Kg was associated with increased mortality in unventilated Kenyan children[156] but in Thai adults a single intramuscular injection of 3.5 mg/kg prevented convulsions.[157] Dicloroacetate,

an activator of pyruvate dehydrogenase, has been shown to reduce blood lactic acid levels, but clinical trials are awaited to evaluate its effects on outcome[158].

AREAS FOR RESEARCH

Prevention of malaria is clearly a priority and the widespread use of preventative measures such as insecticide-treated materials can reduce all childhood deaths by 20%.[159] Together with the prompt treatment of fever with effective anti malarial drugs, these interventions may reverse the rising mortality due to malaria in Africa. Basic malaria research continues to explore vaccines as an ideal preventive tool. A vaccine that produces protection against infection has remained elusive, due to the complexity of parasite biology. Insights into the processes leading to CM might identify targets for a vaccine that allows infection and the acquisition of immunity, but prevents CM.

Further definition of the phenotype of CM would help provide insights into the pathogenesis, in particular the associations with genetic polymorphisms. A more robust exclusion of other causes of encephalopathies in patients presenting with coma and a peripheral parasitaemia in endemic areas would reduce the contamination effect of these conditions on the pathogenesis and outcome of studies of CM. More careful documentation of the retinal findings may be particularly important.
There are technical difficulties in the study of subtle cerebral processes in comatose patients. The development of a reliable animal or *in vitro* model may provide further insights. The technology exists to refine the murine model by inserting human genes (transgenics) into the mouse genome to allow the replacement of murine proteins with human ones. Infection of these models with *P. falciparum* would recreate the key clinical and pathological processes.
Most patients die before antimalarials have had time to kill the parasites. In addition to addressing the public health problems resulting in delayed presentation to hospital and ensuring children receive prompt and appropriate resuscitation, novel interventions that address the pathophysiological processes causing these early deaths is a priority.

The scale of neuro-cognitive impairment reflects an enormous socio-economic burden in resource-poor countries. Research is needed to more clearly define the patients at risk and identify risk factors for persistent impairments. Magnetic resonance imaging, particularly of African children during the acute illness and on recovery may provide insights into the pathogenesis of the neuro-cognitive damage. Interventions to prevent brain damage and rehabilitation programmes for those with neuro-cognitive impairments are needed. Development of neuro-protective agents, improvement in

prophylactic anticonvulsant regimes, treatment of raised ICP in children, or addressing alterations in brain biochemistry may be options (figure 7).

ACKNOWLEDGEMENTS

Professor Charles Newton (070114) and Dr Neil Jenkins (GR066684MF) are supported by The Wellcome Trust, UK.

REFERENCES

1. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature 2005;434(7030):214-7.
2. Health-Protection-Agency. Malaria imported into UK, 2003: implications for those advising travellers. CDR Weekly, 2004: 18-23.
3. Marsh K, Forster D, Waruiru C, et al. Indicators of life-threatening malaria in African children. N Engl J Med 1995;332(21):1399-404.
4. Snow RW CM, Newton CRJC, Steketee RW. The Public Health Burden of Plasmodium falciparum Malaria in Africa: Deriving the Numbers. Bethesda, MD: Fogarty International Center, National Institutes of Health, 2003: Working Paper No. 11. Disease Control Priorities Project, Bethesda, MD: Fogarty International Center, National Institutes of Health, August 2003.
5. Newton CR, Krishna S. Severe falciparum malaria in children: current understanding of pathophysiology and supportive treatment. Pharmacol Ther 1998;79(1):1-53.
6. Reyburn H, Mbatia R, Drakeley C, et al. Association of transmission intensity and age with clinical manifestations and case fatality of severe Plasmodium falciparum malaria. Jama 2005;293(12):1461-70.
7. Bouchaud O, Cot M, Kony S, et al. Do African Immigrants Living in France Have Long-Term Malarial Immunity? Am J Trop Med Hyg 2005;72(1):21-25.
8. WHO. Severe falciparum malaria. Trans R Soc Trop Med Hyg 2000;94 Suppl 1:S1-90.
9. Molyneux ME, Taylor TE, Wirima JJ, Borgstein A. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. Q J Med 1989;71(265):441-59.
10. Kochar DK, Shubhakaran, Kumawat BL, et al. Cerebral malaria in Indian adults: a prospective study of 441 patients from Bikaner, north-west India. J Assoc Physicians India 2002;50:234-41.
11 Crawley J, Smith S, Kirkham F, Muthinji P, Waruiru C, Marsh K. Seizures and status epilepticus in childhood cerebral malaria. Q J Med 1996;89(8):591-7.
12. Warrell DA. Cerebral malaria: clinical features, pathophysiology and treatment. Ann Trop Med Parasitol 1997;91(7):875-84.
13. Aursudkij B, Wilairatana P, Vannaphan S, Walsh DS, Gordeux VR, Looareesuwan S. Pulmonary edema in cerebral malaria patients in Thailand. Southeast Asian J Trop Med Public Health 1998;29(3):541-5.
14. Nacher M, Treeprasertsuk S, Singhasivanon P, et al. Association of hepatomegaly and jaundice with acute renal failure but not with cerebral malaria in severe falciparum malaria in Thailand. Am J Trop Med Hyg 2001;65(6):828-33.
15. Newton CR, Crawley J, Sowumni A, et al. Intracranial hypertension in Africans with cerebral malaria. Arch Dis Child 1997;76(3):219-26.

16. Newton CR, Kirkham FJ, Winstanley PA, et al. Intracranial pressure in African children with cerebral malaria. Lancet 1991;337(8741):573-6.
17. Beare NA, Southern C, Chalira C, Taylor TE, Molyneux ME, Harding SP. Prognostic significance and course of retinopathy in children with severe malaria. Arch Ophthalmol 2004;122(8):1141-7.
18. Newton CR, Peshu N, Kendall B, et al. Brain swelling and ischaemia in Kenyans with cerebral malaria. Arch Dis Child 1994;70(4):281-7.
19. Garg RK, Karak B, Misra S. Neurological manifestations of malaria : an update. Neurol India 1999;47(2):85-91.
20. English M, Wale S, Binns G, Mwangi I, Sauerwein H, Marsh K. Hypoglycaemia on and after admission in Kenyan children with severe malaria. Q J Med 1998;91(3):191-7.
21. English MC, Waruiru C, Lightowler C, Murphy SA, Kirigha G, Marsh K. Hyponatraemia and dehydration in severe malaria. Arch Dis Child 1996;74(3):201-5.
22. English M, Sauerwein R, Waruiru C, et al. Acidosis in severe childhood malaria. Q J Med 1997;90(4):263-70.
23. Idro R, Karamagi C, Tumwine J. Immediate outcome and prognostic factors for cerebral malaria among children admitted to Mulago Hospital, Uganda. Ann Trop Paediatr 2004;24(1):17-24.
24. Waller D, Krishna S, Crawley J, et al. Clinical features and outcome of severe malaria in Gambian children. Clin Infect Dis 1995;21(3):577-87.
25. Maitland K, Pamba A, Newton CR, Lowe B, Levin M. Hypokalemia in children with severe falciparum malaria. Pediatr Crit Care Med 2004;5(1):81-5.
26. Krishnan A, Karnad DR. Severe falciparum malaria: an important cause of multiple organ failure in Indian intensive care unit patients. Crit Care Med 2003;31(9):2278-84.
27. Faiz MA, Rahman MR, Hossain MA, Rashid HA. Cerebral malaria--a study of 104 cases. Bangladesh Med Res Counc Bull 1998;24(2):35-42.
28. Day NP, Phu NH, Mai NT, et al. The pathophysiologic and prognostic significance of acidosis in severe adult malaria. Crit Care Med 2000;28(6):1833-40.
29. White NJ, Warrell DA, Chanthavanich P, et al. Severe hypoglycemia and hyperinsulinemia in falciparum malaria. N Engl J Med 1983;309(2):61-6.
30. Newton CR, Hien TT, White N. Cerebral malaria. J Neurol Neurosurg Psychiatry 2000;69(4):433-41.
31. Mohanty S, Mishra SK, Pati SS, Pattnaik J, Das BS. Complications and mortality patterns due to Plasmodium falciparum malaria in hospitalized adults and children, Rourkela, Orissa, India. Trans R Soc Trop Med Hyg 2003;97(1):69-70.
32. Bondi FS. The incidence and outcome of neurological abnormalities in childhood cerebral malaria: a long-term follow-up of 62 survivors. Trans R Soc Trop Med Hyg 1992;86(1):17-9.
33. van Hensbroek MB, Palmer A, Jaffar S, Schneider G, Kwiatkowski D. Residual neurologic sequelae after childhood cerebral malaria. J Pediatr 1997;131(1 Pt 1):125-9.
34. Crawley J, Smith S, Muthinji P, Marsh K, Kirkham F. Electroencephalographic and clinical features of cerebral malaria. Arch Dis Child 2001;84(3):247-53.
35. Waruiru CM, Newton CR, Forster D, et al. Epileptic seizures and malaria in Kenyan children. Trans R Soc Trop Med Hyg 1996;90(2):152-5.
36. Crawley J, English M, Waruiru C, Mwangi I, Marsh K. Abnormal respiratory patterns in childhood cerebral malaria. Trans R Soc Trop Med Hyg 1998;92(3):305-8.
37. Marsh K, English M, Crawley J, Peshu N. The pathogenesis of severe malaria in African children. Ann Trop Med Parasitol 1996;90(4):395-402.
38. Brewster DR, Kwiatkowski D, White NJ. Neurological sequelae of cerebral malaria in children. Lancet 1990;336(8722):1039-43.

39. Holding PA, Stevenson J, Peshu N, Marsh K. Cognitive sequelae of severe malaria with impaired consciousness. Trans R Soc Trop Med Hyg 1999;93(5):529-34.
40. Akpede GO, Sykes RM, Abiodun PO. Convulsions with malaria: febrile or indicative of cerebral involvement? J Trop Pediatr 1993;39(6):350-5.
41. Crawley J, Kokwaro G, Ouma D, Watkins W, Marsh K. Chloroquine is not a risk factor for seizures in childhood cerebral malaria. Trop Med Int Health 2000;5(12):860-4.
42. Lang B, Newbold CI, Williams G, Peshu N, Marsh K, Newton CR. Antibodies to voltage-gated calcium channels in children with falciparum malaria. J Infect Dis 2005;191(1):117-21.
43. Idro R, Otieno G, White S, et al. Decorticate, decerebrate and opisthotonic posturing and seizures in Kenyan children with cerebral malaria. Malar J 2005;4(1):57.
44. Lewallen S, Bakker H, Taylor TE, Wills BA, Courtright P, Molyneux ME. Retinal findings predictive of outcome in cerebral malaria. Trans R Soc Trop Med Hyg 1996;90(2):144-6.
45. Lewallen S, White VA, Whitten RO, et al. Clinical-histopathological correlation of the abnormal retinal vessels in cerebral malaria. Arch Ophthalmol 2000;118(7):924-8.
46. Krishna S, Waller DW, ter Kuile F, et al. Lactic acidosis and hypoglycaemia in children with severe malaria: pathophysiological and prognostic significance. Trans R Soc Trop Med Hyg 1994;88(1):67-73.
47. Maitland K, Levin M, English M, et al. Severe P. falciparum malaria in Kenyan children: evidence for hypovolaemia. Q J Med 2003;96(6):427-34.
48. Maitland K, Pamba A, Newton CR, Levin M. Response to volume resuscitation in children with severe malaria. Pediatr Crit Care Med 2003;4(4):426-31.
49. Sowunmi A, Newton CR, Waruiru C, Lightman S, Dunger DB. Arginine vasopressin secretion in Kenyan children with severe malaria. J Trop Pediatr 2000;46(4):195-9.
50. Berkley J, Mwarumba S, Bramham K, Lowe B, Marsh K. Bacteraemia complicating severe malaria in children. Trans R Soc Trop Med Hyg 1999;93(3):283-6.
51. Enwere G, Van Hensbroek MB, Adegbola R, et al. Bacteraemia in cerebral malaria. Ann Trop Paediatr 1998;18(4):275-8.
52. Looareesuwan S, Warrell DA, White NJ, et al. Retinal hemorrhage, a common sign of prognostic significance in cerebral malaria. Am J Trop Med Hyg 1983;32(5):911-5.
53. Krishna S, Taylor AM, Supanaranond W, et al. Thiamine deficiency and malaria in adults from southeast Asia. Lancet 1999;353(9152):546-9.
54. Cordoliani YS, Sarrazin JL, Felten D, Caumes E, Leveque C, Fisch A. MR of cerebral malaria. AJNR Am J Neuroradiol 1998;19(5):871-4.
55. Krishnan A, Karnad DR, Limaye U, Siddharth W. Cerebral venous and dural sinus thrombosis in severe falciparum malaria. J Infect 2004;48(1):86-90.
56. Tran TH, Day NP, Nguyen HP, et al. A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. N Engl J Med 1996;335(2):76-83.
57. Lalloo DG, Trevett AJ, Paul M, et al. Severe and complicated falciparum malaria in Melanesian adults in Papua New Guinea. Am J Trop Med Hyg 1996;55(2):119-24.
58. Trang TT, Phu NH, Vinh H, et al. Acute renal failure in patients with severe falciparum malaria. Clin Infect Dis 1992;15(5):874-80.
59. Bruneel F, Hocqueloux L, Alberti C, et al. The clinical spectrum of severe imported falciparum malaria in the intensive care unit: report of 188 cases in adults. Am J Respir Crit Care Med 2003;167(5):684-9.
60. Ha V, Nguyen NH, Tran TB, et al. Severe and complicated malaria treated with artemisinin, artesunate or artemether in Viet Nam. Trans R Soc Trop Med Hyg 1997;91(4):465-7.

61. Barcus MJ, Hien TT, White NJ, et al. Short report: Hepatitis B infection and severe Plasmodium falciparum malaria in Vietnamese adults. Am J Trop Med Hyg 2002;66(2):140-2.

62. Rubio JM, Buhigas I, Subirats M, Baquero M, Puente S, Benito A. Limited level of accuracy provided by available rapid diagnosis tests for malaria enhances the need for PCR-based reference laboratories. J Clin Microbiol 2001;39(7):2736-7.

63. Farnert A, Arez AP, Babiker HA, et al. Genotyping of Plasmodium falciparum infections by PCR: a comparative multicentre study. Trans R Soc Trop Med Hyg 2001;95(2):225-32.

64. Taylor TE, Fu WJ, Carr RA, et al. Differentiating the pathologies of cerebral malaria by postmortem parasite counts. Nat Med 2004;10(2):143-5.

65. White NJ. Lumbar puncture in cerebral malaria. Lancet 1991;338(8767):640-1.

66. Das BS, Mohanty S, Mishra SK, et al. Increased cerebrospinal fluid protein and lipid peroxidation products in patients with cerebral malaria. Trans R Soc Trop Med Hyg 1991;85(6):733-4.

67. Looareesuwan S, Wilairatana P, Krishna S, et al. Magnetic resonance imaging of the brain in patients with cerebral malaria. Clin Infect Dis 1995;21(2):300-9.

68. Aikawa M, Brown A, Smith CD, et al. A primate model for human cerebral malaria: Plasmodium coatneyi-infected rhesus monkeys. Am J Trop Med Hyg 1992;46(4):391-7.

69. Kawai S, Aikawa M, Kano S, Suzuki M. A primate model for severe human malaria with cerebral involvement: Plasmodium coatneyi-infected Macaca fuscata. Am J Trop Med Hyg 1993;48(5):630-6.

70. Neill AL, Hunt NH. Effects of endotoxin and dexamethasone on cerebral malaria in mice. Parasitology 1995;111 (Pt 4):443-54.

71. Newbold C, Craig A, Kyes S, Rowe A, Fernandez-Reyes D, Fagan T. Cytoadherence, pathogenesis and the infected red cell surface in Plasmodium falciparum. Int J Parasitol 1999;29(6):927-37.

72. Fernandez V, Wahlgren M. Rosetting and autoagglutination in Plasmodium falciparum. Chem Immunol 2002;80:163-87.

73. Pain A, Ferguson DJ, Kai O, et al. Platelet-mediated clumping of Plasmodium falciparum-infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. Proc Natl Acad Sci U S A 2001;98(4):1805-10.

74. Dondorp AM, Pongponratn E, White NJ. Reduced microcirculatory flow in severe falciparum malaria: pathophysiology and electron-microscopic pathology. Acta Trop 2004;89(3):309-17.

75. Idro R. Severe anaemia in childhood cerebral malaria is associated with profound coma. Afr Health Sci 2003;3(1):15-8.

76. Sanni LA. The role of cerebral oedema in the pathogenesis of cerebral malaria. Redox Rep 2001;6(3):137-42.

77. Newton CR, Marsh K, Peshu N, Kirkham FJ. Perturbations of cerebral hemodynamics in Kenyans with cerebral malaria. Pediatr Neurol 1996;15(1):41-9.

78. Maitland K, Pamba A, English M, et al. Randomized Trial of Volume Expansion with Albumin or Saline in Children with Severe Malaria: Preliminary Evidence of Albumin Benefit. Clinical Infectious Diseases 2005;40:538-545.

79. Brown H, Rogerson S, Taylor T, et al. Blood-brain barrier function in cerebral malaria in Malawian children. Am J Trop Med Hyg 2001;64(3-4):207-13.

80. Brown HC, Chau TT, Mai NT, et al. Blood-brain barrier function in cerebral malaria and CNS infections in Vietnam. Neurology 2000;55(1):104-11.

81. Pongponratn E, Riganti M, Harinasuta T, Bunnag D. Electron microscopy of the human brain in cerebral malaria. Southeast Asian J Trop Med Public Health 1985;16(2):219-27.

82. SenGupta SK, Naraqi S. The brain in cerebral malaria: a pathological study of 24 fatal cases in Papua New Guinea. P N G Med J 1992;35(4):270-4.

83. Walker O, Salako LA, Sowunmi A, Thomas JO, Sodeine O, Bondi FS. Prognostic risk factors and post mortem findings in cerebral malaria in children. Trans R Soc Trop Med Hyg 1992;86(5):491-3.

84. Clark IA, Rockett KA, Cowden WB. Possible central role of nitric oxide in conditions clinically similar to cerebral malaria. Lancet 1992;340(8824):894-6.

85. Sanni LA, Fu S, Dean RT, et al. Are reactive oxygen species involved in the pathogenesis of murine cerebral malaria? J Infect Dis 1999;179(1):217-22.

86. Griffiths MJ, Ndungu F, Baird KL, Muller DP, Marsh K, Newton CR. Oxidative stress and erythrocyte damage in Kenyan children with severe Plasmodium falciparum malaria. Br J Haematol 2001;113(2):486-91.

87. Dobbie M, Crawley J, Waruiru C, Marsh K, Surtees R. Cerebrospinal fluid studies in children with cerebral malaria: an excitotoxic mechanism? Am J Trop Med Hyg 2000;62(2):284-90.

88. Medana IM, Hien TT, Day NP, et al. The clinical significance of cerebrospinal fluid levels of kynurenine pathway metabolites and lactate in severe malaria. J Infect Dis 2002;185(5):650-6.

89. Medana IM, Day NP, Salahifar-Sabet H, et al. Metabolites of the kynurenine pathway of tryptophan metabolism in the cerebrospinal fluid of Malawian children with malaria. J Infect Dis 2003;188(6):844-9.

90. Bate CA, Kwiatkowski D. Inhibitory immunoglobulin M antibodies to tumor necrosis factor-inducing toxins in patients with malaria. Infect Immun 1994;62(8):3086-91.

91. Meyer-Breiting E, Zimmermann H. [A contribution to the intravascular coagulation in cerebral malaria (author's transl)]. Zentralbl Allg Pathol 1975;119(4):286-93.

92. Roberts DJ, Craig AG, Berendt AR, et al. Rapid switching to multiple antigenic and adhesive phenotypes in malaria. Nature 1992;357(6380):689-92.

93. Bull PC, Kortok M, Kai O, et al. Plasmodium falciparum-infected erythrocytes: agglutination by diverse Kenyan plasma is associated with severe disease and young host age. J Infect Dis 2000;182(1):252-9.

94. Lindenthal C, Kremsner PG, Klinkert MQ. Commonly recognised Plasmodium falciparum parasites cause cerebral malaria. Parasitol Res 2003;91(5):363-8.

95. Craig A, Scherf A. Molecules on the surface of the Plasmodium falciparum infected erythrocyte and their role in malaria pathogenesis and immune evasion. Mol Biochem Parasitol 2001;115(2):129-43.

96. Newbold C, Warn P, Black G, et al. Receptor-specific adhesion and clinical disease in Plasmodium falciparum. Am J Trop Med Hyg 1997;57(4):389-98.

97. Berendt AR, Simmons DL, Tansey J, Newbold CI, Marsh K. Intercellular adhesion molecule-1 is an endothelial cell adhesion receptor for Plasmodium falciparum. Nature 1989;341(6237):57-9.

98. Silamut K, Phu NH, Whitty C, et al. A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. Am J Pathol 1999;155(2):395-410.

99. Turner GD, Morrison H, Jones M, et al. 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. Am J Pathol 1994;145(5):1057-69.

100. Craig A, Fernandez-Reyes D, Mesri M, et al. A functional analysis of a natural variant of intercellular adhesion molecule-1 (ICAM-1Kilifi). Hum Mol Genet 2000;9(4):525-30.

101. Fernandez-Reyes D, Craig AG, Kyes SA, et al. A high frequency African coding polymorphism in the N-terminal domain of ICAM-1 predisposing to cerebral malaria in Kenya. Hum Mol Genet 1997;6(8):1357-60.

102. Bellamy R, Kwiatkowski D, Hill AV. Absence of an association between intercellular adhesion molecule 1, complement receptor 1 and interleukin 1 receptor antagonist gene polymorphisms and severe malaria in a West African population. Trans R Soc Trop Med Hyg 1998;92(3):312-6.

103. Dondorp AM, Angus BJ, Hardeman MR, et al. Prognostic significance of reduced red blood cell deformability in severe falciparum malaria. Am J Trop Med Hyg 1997;57(5):507-11.

104. Dondorp AM, Nyanoti M, Kager PA, Mithwani S, Vreeken J, Marsh K. The role of reduced red cell deformability in the pathogenesis of severe falciparum malaria and its restoration by blood transfusion. Trans R Soc Trop Med Hyg 2002;96(3):282-6.

105. Lyke KE, Burges R, Cissoko Y, et al. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe Plasmodium falciparum malaria and matched uncomplicated malaria or healthy controls. Infect Immun 2004;72(10):5630-7.

106. Kwiatkowski D. Tumour necrosis factor, fever and fatality in falciparum malaria. Immunol Lett 1990;25(1-3):213-6.

107. Akanmori BD, Kurtzhals JA, Goka BQ, et al. Distinct patterns of cytokine regulation in discrete clinical forms of Plasmodium falciparum malaria. Eur Cytokine Netw 2000;11(1):113-8.

108. Gimenez F, Barraud de Lagerie S, Fernandez C, Pino P, Mazier D. Tumor necrosis factor alpha in the pathogenesis of cerebral malaria. Cell Mol Life Sci 2003;60(8):1623-35.

109. McGuire W, Hill AV, Allsopp CE, Greenwood BM, Kwiatkowski D. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature 1994;371(6497):508-10.

110. Day NP, Hien TT, Schollaardt T, et al. The prognostic and pathophysiologic role of pro- and antiinflammatory cytokines in severe malaria. J Infect Dis 1999;180(4):1288-97.

111. Brown H, Turner G, Rogerson S, et al. Cytokine expression in the brain in human cerebral malaria. J Infect Dis 1999;180(5):1742-6.

112. Clark IA, Awburn MM, Whitten RO, et al. Tissue distribution of migration inhibitory factor and inducible nitric oxide synthase in falciparum malaria and sepsis in African children. Malar J 2003;2(1):6.

113. Anstey NM, Weinberg JB, Hassanali MY, et al. Nitric oxide in Tanzanian children with malaria: inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. J Exp Med 1996;184(2):557-67.

114. Cramer JP, Nussler AK, Ehrhardt S, et al. Age-dependent effect of plasma nitric oxide on parasite density in Ghanaian children with severe malaria. Tropical Medicine and International Health 2005;10(7):672-680.

115. Clark IA, Alleva LM, Mills AC, Cowden WB. Pathogenesis of Malaria and Clinically Similar Conditions. Clin. Microbiol. Rev. 2004;17(3):509-539.

116. Gitau EN, Newton CR. Review Article: Blood-brain barrier in falciparum malaria. Trop Med Int Health 2005;10(3):285-92.

117. Warrell DA, Looareesuwan S, Phillips RE, et al. Function of the blood-cerebrospinal fluid barrier in human cerebral malaria: rejection of the permeability hypothesis. Am J Trop Med Hyg 1986;35(5):882-9.

118. Brown H, Hien TT, Day N, et al. Evidence of blood-brain barrier dysfunction in human cerebral malaria. Neuropathol Appl Neurobiol 1999;25(4):331-40.

119. Adams S, Brown H, Turner G. Breaking down the blood-brain barrier: signaling a path to cerebral malaria? Trends Parasitol 2002;18(8):360-6.

120. Patankar TF, Karnad DR, Shetty PG, Desai AP, Prasad SR. Adult cerebral malaria: prognostic importance of imaging findings and correlation with postmortem findings. Radiology 2002;224(3):811-6.

121. Looareesuwan S, Warrell DA, White NJ, et al. Do patients with cerebral malaria have cerebral oedema? A computed tomography study. Lancet 1983;1(8322):434-7.

122. Pongponratn E, Turner GD, Day NP, et al. An ultrastructural study of the brain in fatal Plasmodium falciparum malaria. Am J Trop Med Hyg 2003;69(4):345-59.

123. Turner G. Cerebral malaria. Brain Pathol 1997;7(1):569-82.

124. Patnaik JK, Das BS, Mishra SK, Mohanty S, Satpathy SK, Mohanty D. Vascular clogging, mononuclear cell margination, and enhanced vascular permeability in the pathogenesis of human cerebral malaria. Am J Trop Med Hyg 1994;51(5):642-7.

125. Medana IM, Day NP, Hien TT, et al. Axonal injury in cerebral malaria. Am J Pathol 2002;160(2):655-66.

126. Medana IM, Lindert RB, Wurster U, et al. Cerebrospinal fluid levels of markers of brain parenchymal damage in Vietnamese adults with severe malaria. Trans R Soc Trop Med Hyg 2005;99(8):610-7.

127. Jaffar S, Van Hensbroek MB, Palmer A, Schneider G, Greenwood B. Predictors of a fatal outcome following childhood cerebral malaria. Am J Trop Med Hyg 1997;57(1):20-4.

128. Phu NH, Hien TT, Mai NT, et al. Hemofiltration and peritoneal dialysis in infection-associated acute renal failure in Vietnam. N Engl J Med 2002;347(12):895-902.

129. Carter JA, Mung'ala-Odera V, Neville BG, et al. Persistent neurocognitive impairments associated with severe falciparum malaria in Kenyan children. J Neurol Neurosurg Psychiatry 2005;76(4):476-81.

130. Carter JA, Neville BG, White S, et al. Increased prevalence of epilepsy associated with severe falciparum malaria in children. Epilepsia 2004;45(8):978-81.

31. Muntendam AH, Jaffar S, Bleichrodt N, van Hensbroek MB. Absence of neuropsychological sequelae following cerebral malaria in Gambian children. Trans R Soc Trop Med Hyg 1996;90(4):391-4.

132. Boivin MJ. Effects of early cerebral malaria on cognitive ability in Senegalese children. J Dev Behav Pediatr 2002;23(5):353-64.

133. Dugbartey AT, Spellacy FJ, Dugbartey MT. Somatosensory discrimination deficits following pediatric cerebral malaria. Am J Trop Med Hyg 1998;59(3):393-6.

134. Carter JA, Ross AJ, Neville BG, et al. Developmental impairments following severe falciparum malaria in children. Trop Med Int Health 2005;10(1):3-10.

135. Nguyen TH, Day NP, Ly VC, et al. Post-malaria neurological syndrome. Lancet 1996;348(9032):917-21.

136. White NJ LS. Cerebral malaria. London: Butterworths, 1987.

137. Kastl AJ, Daroff RB, Blocker WW. Psychological testing of cerebral malaria patients. J Nerv Ment Dis 1968;147(6):553-61.

138. Varney NR, Roberts RJ, Springer JA, Connell SK, Wood PS. Neuropsychiatric sequelae of cerebral malaria in Vietnam veterans. J Nerv Ment Dis 1997;185(11):695-703.

139. Dugbartey AT, Dugbartey MT, Apedo MY. Delayed neuropsychiatric effects of malaria in Ghana. J Nerv Ment Dis 1998;186(3):183-6.

140. Maitland K, Nadel S, Pollard AJ, Williams TN, Newton CR, Levin M. Management of severe malaria in children: proposed guidelines for the United Kingdom. Bmj 2005;331(7512):337-43.

141. van der Torn M, Thuma PE, Mabeza GF, et al. Loading dose of quinine in African children with cerebral malaria. Trans R Soc Trop Med Hyg 1998;92(3):325-31.

142. Pasvol G, Newton CR, Winstanley PA, et al. Quinine treatment of severe falciparum malaria in African children: a randomized comparison of three regimens. Am J Trop Med Hyg 1991;45(6):702-13.

143. Stauffer W, Fischer PR. Diagnosis and treatment of malaria in children. Clin Infect Dis 2003;37(10):1340-8.

144. Barennes H, Munjakazi J, Verdier F, Clavier F, Pussard E. An open randomized clinical study of intrarectal versus infused Quinimax for the treatment of childhood cerebral malaria in Niger. Trans R Soc Trop Med Hyg 1998;92(4):437-40.

145. Looareesuwan S, Phillips RE, White NJ, et al. Quinine and severe falciparum malaria in late pregnancy. Lancet 1985;2(8445):4-8.

146. A meta-analysis using individual patient data of trials comparing artemether with quinine in the treatment of severe falciparum malaria. Trans R Soc Trop Med Hyg 2001;95(6):637-50.

147. Dondorp A, Nosten F, Stepniewska K, Day N, White N. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. Lancet 2005;366(9487):717-25.

148. Adjuik M, Babiker A, Garner P, Olliaro P, Taylor W, White N. Artesunate combinations for treatment of malaria: meta-analysis. Lancet 2004;363(9402):9-17.

149. Petras JM, Young GD, Bauman RA, et al. Arteether-induced brain injury in Macaca mulatta. I. The precerebellar nuclei: the lateral reticular nuclei, paramedian reticular nuclei, and perihypoglossal nuclei. Anat Embryol (Berl) 2000;201(5):383-97.

150. Hien TT, Turner GD, Mai NT, et al. Neuropathological assessment of artemether-treated severe malaria. Lancet 2003;362(9380):295-6.

151. Aceng JR, Byarugaba JS, Tumwine JK. Rectal artemether versus intravenous quinine for the treatment of cerebral malaria in children in Uganda: randomised clinical trial. BMJ 2005;330(7487):334-.

152. Riddle MS, Jackson JL, Sanders JW, Blazes DL. Exchange transfusion as an adjunct therapy in severe Plasmodium falciparum malaria: a meta-analysis. Clin Infect Dis 2002;34(9):1192-8.

153. Winstanley PA, Mberu EK, Watkins WM, Murphy SA, Lowe B, Marsh K. Towards optimal regimens of parenteral quinine for young African children with cerebral malaria: unbound quinine concentrations following a simple loading dose regimen. Trans R Soc Trop Med Hyg 1994;88(5):577-80.

154. Waller D, Krishna S, Craddock C, et al. The pharmacokinetic properties of intramuscular quinine in Gambian children with severe falciparum malaria. Trans R Soc Trop Med Hyg 1990;84(4):488-91.

155. Winstanley PA, Newton CR, Pasvol G, et al. Prophylactic phenobarbitone in young children with severe falciparum malaria: pharmacokinetics and clinical effects. Br J Clin Pharmacol 1992;33(2):149-54.

156. Crawley J, Waruiru C, Mithwani S, et al. Effect of phenobarbital on seizure frequency and mortality in childhood cerebral malaria: a randomised, controlled intervention study. Lancet 2000;355(9205):701-6.

157. White NJ, Looareesuwan S, Phillips RE, Chanthavanich P, Warrell DA. Single dose phenobarbitone prevents convulsions in cerebral malaria. Lancet 1988;2(8602):64-6.

158. Agbenyega T, Planche T, Bedu-Addo G, et al. Population kinetics, efficacy, and safety of dichloroacetate for lactic acidosis due to severe malaria in children. J Clin Pharmacol 2003;43(4):386-96.

159. Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst Rev 2004(2):CD000363.