

## PATHOPHYSIOLOGY OF FATAL FALCIPARUM MALARIA IN AFRICAN CHILDREN

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**Abstract.** Children living in sub-Saharan Africa bear the brunt of the mortality from falciparum malaria, yet there is a dearth of relevant post-mortem data. Clinical studies from centers in Africa suggest that the pathophysiology of severe malaria is different in children and adults. Three overlapping clinical syndromes, metabolic acidosis manifesting as hyperpnea, cerebral malaria, and severe anemia, are responsible for nearly all the deaths in African children. Despite improvements in antimalarial treatment, there has not been a significant reduction in mortality. We review the pathology and pathophysiology of fatal falciparum malaria in African children. Many questions remain, the answers to which would facilitate the development and evaluation of new approaches to the management of this disease.

Severe and complicated *Plasmodium falciparum* infections continue to threaten the survival of young children in sub-Saharan Africa: one in 15 children on the Kenyan coast will have experienced an episode of severe malaria before the age of five,<sup>1</sup> and 1% of Gambian children less than five years of age will die of malaria.<sup>2</sup> The annual death toll is estimated to be one million children across the continent; 90% of the annual worldwide malaria mortality.<sup>3</sup> Although most children die in the community, on the Kenyan coast it is estimated that more than 40% die in a hospital (Marsh K, unpublished data) and one-third of pediatric hospital admissions and one-third of pediatric hospital deaths in Malawi can be attributed to malaria.<sup>4</sup>

Until recently, most studies of severe malaria were conducted on nonimmune adults and little was known about the pathophysiology in African children. Hypoglycemia was first noted as a feature of pediatric malaria just 11 years ago,<sup>5</sup> prognostic indicators were first described by Molyneux and others in a comprehensive account of cerebral malaria (CM) in African children in 1989,<sup>6</sup> and more recently, Marsh and others described an entirely new syndrome characterized by deep, rapid acidotic breathing.<sup>7</sup> Mortality rates have not been affected by improved delivery of quinine,<sup>8</sup> the standard treatment for severe and complicated malaria, or by the use of artemether, a qinghaosu derivative capable of clearing parasitemia twice as rapidly as quinine.<sup>9,10</sup> Identifying the underlying pathophysiologic mechanisms may help to improve survival. Model animal systems for fatal malaria exist, but none is sufficiently similar to the human disease to permit useful comparisons.<sup>11</sup> The clinical aspects of severe malaria in adults, in whom multiorgan system failure is common, differ markedly from those in children,<sup>11</sup> and so extrapolating from one to the other could be misleading.

We have reviewed studies on the pathophysiology of severe falciparum malaria in African children, concentrating on features associated with fatal outcomes. Research focused on developing new therapeutic interventions may well help to decrease mortality associated with *P. falciparum* infections in African children.

### CLINICAL DESCRIPTION

Severe malaria in African children encompasses three clinical syndromes: severe anemia, CM, and malaria-asso-

ciated hyperpnea (increased rate and depth of breathing) (Figure 1). Although data from Kenya,<sup>12</sup> Malawi,<sup>13</sup> and Nigeria<sup>14</sup> suggest that there are distinct age ranges associated with these syndromes in sub-Saharan Africa, there is extensive overlap.<sup>7</sup>

All three syndromes, whether occurring alone or in combination, can kill quickly or resolve rapidly. Parents accompanying their children to a hospital typically report fever beginning 1–3 days prior to admission. Neurologic manifestations, when present, usually start within 12 hr of admission.<sup>6,15–18</sup> Most deaths occur within 24 hr of starting treatment<sup>6,7,16,19–21</sup> and most of those who survive recover fully within 48 hr of starting treatment.<sup>6,15,16,20,21</sup> To be credible, theories of fatal malaria pathogenesis must explain the rapidity with which these syndromes develop and then either kill the patient or resolve, leaving only 9–12% of survivors with neurologic sequelae.<sup>6,17,22</sup>

**Malaria hyperpneic syndrome.** Metabolic acidosis (pH < 7.30) has been described in pediatric patients with CM,<sup>21,23,24</sup> but its impact on patients with normal levels of consciousness has only recently been appreciated.<sup>7</sup> Marsh and others<sup>7</sup> defined their Kenyan patients as having respiratory distress if they had any of the following signs: alar flaring, chest recession (intercostal or subcostal), the use of accessory muscles of respiration, or abnormally deep (acidotic) breathing. A more limited definition of severe respiratory distress was used to describe children with chest recession or abnormally deep breathing. These comprised 7% of all malaria admissions; half of these children had normal levels of consciousness and thus would not necessarily have been recognized as being at risk of a malaria death. Overall, 19% of children with respiratory distress died; the mortality rate was 13.9% in those with any respiratory distress and 24.8% in the group with severe respiratory distress. Eighty-one percent of children with respiratory distress in whom plasma bicarbonate levels could be measured were severely acidotic (plasma bicarbonate concentration < 15 mmol/L). There was no association between the presence of pulmonary auscultatory findings and outcome, thus it is reasonable to assume that the respiratory distress is a manifestation of a systemic acidosis. As such, the term respiratory distress, which suggests a primary pulmonary pathology, is slightly misleading; another designation, such as malaria hyperpneic syndrome, might be more appropriate. It is important that

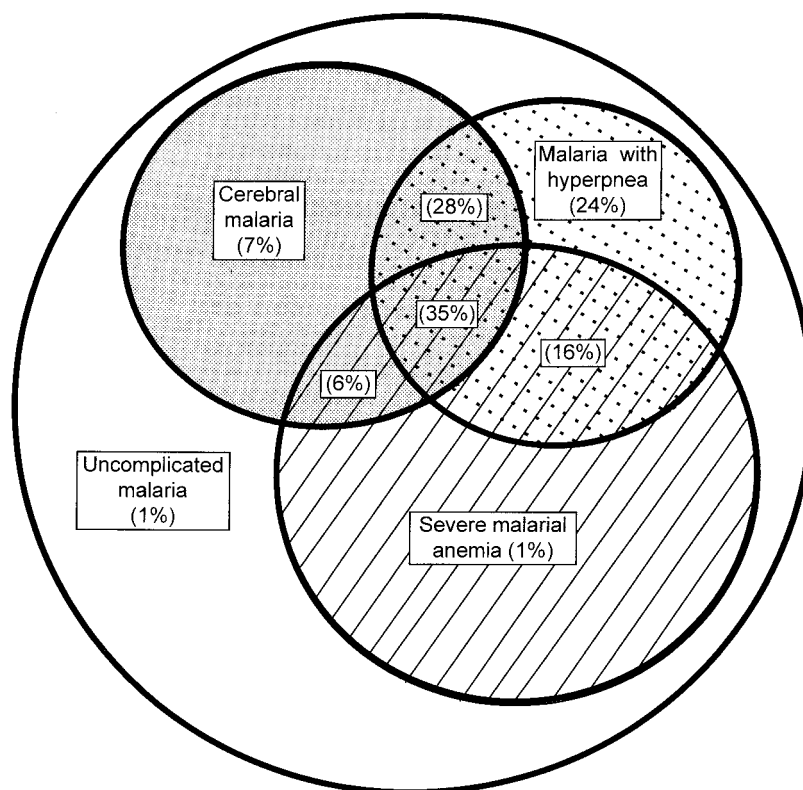


FIGURE 1. Overlapping clinical syndromes in fatal malaria (adapted from Marsh K, Forster D, Waruiru C, Mwangi I, Winstanley M, Marsh V, Newton C, Winstanley P, Warn P, Peshu N, Pasvol G, Snow RW, 1995. Indicators of life-threatening malaria in African children. *N Engl J Med* 332: 1399–1404. Reprinted with the permission of the *New England Journal of Medicine*.)

these findings be confirmed in other settings and that the clinical spectrum and response to treatment of this acidotic breathing of malaria be described in more detail.<sup>25</sup>

**Cerebral malaria.** There are many definitions of CM, and circumstances will dictate which is most appropriate. In research settings, where comparability between sites is important, and where known mortality rates are necessary for sample size calculations, a child has CM if he or she is unconscious (unable to localize pain or a Blantyre Coma Score  $\leq 2$ , Table 1) with an asexual parasitemia of any density and no other obvious cause of the clinical syndrome (e.g., meningitis).<sup>26</sup> Patients are excluded if they improve within 1 hr of a convulsion, or of being restored to a normoglycemic state.<sup>6, 7, 15, 16</sup> A less stringent definition, where any impairment of consciousness is sufficient justification for intensifying the clinical management (parenteral antimalarials, assiduous nursing care) is more appropriate in usual clinical settings.<sup>26</sup>

The neurologic manifestations of CM span the spectrum from diffuse cortical involvement to specific brainstem ab-

normalities.<sup>6, 16–20</sup> Patients can present with, or develop, hypertonic posturing (decorticate rigidity, decerebrate rigidity, opisthotonos [Figure 2]), pupillary changes, absent corneal reflexes, abnormal respiratory pattern (Kussmaul, Cheyne-Stokes, periodic apnea), and gaze abnormalities (eyes wide open, conjugate gaze deviation, nystagmus). Seizures are an important presenting feature of CM, being either reported or witnessed in 60–80% of African children with CM.<sup>6, 16–20, 22</sup> Seizures that occur after antimalarial treatment is initiated,<sup>6, 22</sup> particularly if they are prolonged or resistant to anticonvulsants, are associated with the development of neurologic sequelae<sup>27</sup> and death.<sup>28</sup> In African research settings, the mortality rate for strictly defined CM is between 15% and 30%.<sup>6, 7, 16, 20, 23, 29</sup> A consistent minority (9–12%) of survivors are discharged with neurologic sequelae;<sup>6, 17, 22</sup> half of these recover fully within 4–6 weeks.

There is considerable variation between observers in eliciting the clinical signs that are used to define the above syndromes,<sup>30</sup> and thus it is imperative that clinicians define the criteria used and agree about the definitions.

TABLE 1  
Blantyre coma scale<sup>6\*</sup>

Best motor response		Best verbal response		Eye movements	
Localizes painful stimulus†	2	Normal cry	2		
Withdraws from a painful stimulus	1	Abnormal cry	1	Follows a moving object	1
Extends, or has no response to a painful stimulus	0	No verbal response	0	Does not follow a moving object	0

\* The total score is the sum of the individual scores from the three categories.

† The standard painful stimulus is firm pressure on a nail bed, the sternum, or the supraorbital ridge.



FIGURE 2. A Malawian child with cerebral malaria and marked opisthotonos (photograph by T. E. Taylor).

**Severe malarial anemia.** Severe malarial anemia is defined as a hemoglobin concentration  $\leq 5$  g/dL (or a hematocrit  $\leq 15\%$ ) in a patient with a *P. falciparum* parasitemia in excess of 10,000 trophozoites/mm<sup>3</sup> of blood.<sup>26</sup> However, children who have adjusted physiologically to low hemoglobin concentrations may decompensate rapidly when challenged with a febrile illness, and in this setting, the development of a life-threatening anemia can occur at lower parasitemias.<sup>31</sup> Overall, mortality rates for children hospitalized with severe malarial anemia range from 4.7%<sup>7</sup> to 14–16%;<sup>13</sup> higher rates occur in conjunction with the other syndromes.

The incidence of severe malarial anemia has increased with the spread of chloroquine-resistant *P. falciparum* parasites across Africa.<sup>32</sup> Patients may improve symptomatically following chloroquine treatment, but unless the parasites are cleared completely, the expected reticulocytosis does not ensue and over time, with repeated infections, patients can develop life-threatening anemias.<sup>33,34</sup>

The characteristic physical findings of severe malarial anemia are respiratory distress and a hyperdynamic circulation. These findings are likely to be caused by a combination of inadequate oxygenation and cardiac compromise, although the relative contribution of each is difficult to determine.<sup>35</sup> Blood transfusions improve the clinical findings in most cases. The transfusions can be administered rapidly in children with metabolic acidosis, since most of these children have depleted intravascular volumes,<sup>36</sup> unlike in non-immune adults in whom rapid transfusions can precipitate pulmonary edema.<sup>26</sup> However, many malaria-endemic areas are also challenged by a high proportion of human immunodeficiency virus-seropositive blood donors, and blood transfusions carry a certain risk. Most clinicians prefer to restrict blood transfusions to individuals with signs of cardiorespiratory compromise, hyperparasitemia, or CM.<sup>33,36,37</sup>

#### PATHOPHYSIOLOGY OF FATAL MALARIA

In studies of African children admitted to a hospital with falciparum malaria, a variety of clinical and laboratory fea-

tures are associated with death. Logistic regression analysis identified impaired consciousness, respiratory distress, and hypoglycemia as independent indicators of a fatal outcome in two studies.<sup>7,16</sup> Other studies of children with CM identified these as well as the following factors: acidemia (capillary blood pH  $< 7.30$ ), young ( $< 3$  years) age, leukocytosis ( $\geq 15,000/\text{mm}^3$ ), hyperlactatemia ( $> 5$  mmol/L), hyperparasitemia ( $\geq 1 \times 10^6/\text{mm}^3$ ), profound coma (Blantyre Coma Score = 0), absent corneal reflexes, signs of decerebration, and witnessed convulsions.<sup>6,21,23</sup> Despite these associations, the actual mechanisms of death remain unclear.

**Metabolic acidosis.** Various manifestations of systemic metabolic acidosis have been described in children with *P. falciparum* infections. Acidemia in Malawian children,<sup>23</sup> hyperlactatemia in Gambian children,<sup>16,38</sup> and hyperpnea in Kenyan children<sup>7,35</sup> are all strongly associated with disease severity and are predictors of a poor outcome. Metabolic acidosis may be the sole indicator of a complicated falciparum infection, in which case the predominant clinical symptoms are respiratory (deep breathing, intercostal/subcostal recession). Metabolic acidosis in patients with CM and/or severe malarial anemia is the strongest single predictor of a fatal outcome.<sup>23,38</sup>

There are several possible etiologies for the metabolic acidosis in pediatric malaria. Those commonly associated with shock states are unlikely as these patients rarely present with or develop signs and/or symptoms of hypotension, renal failure, or sepsis.<sup>6,7,16,23,38</sup> Acidosis and hypoglycemia are strongly associated in most studies,<sup>7,16,23,38</sup> suggesting that parasite and/or host metabolism may contribute to the derangements. *Plasmodium falciparum* derives most of its energy from anaerobic glycolysis, and the latter stages (late trophozoites and meronts) are the most metabolically active.<sup>39</sup> Sequestration of mature *P. falciparum* parasites in relatively inaccessible areas is unmeasurable and thus, at present, it is difficult to predict the degree of metabolic acidosis on the basis of the peripheral parasitemia.<sup>21,23</sup> There is a

theoretical risk of fueling an acidosis by administering 50% dextrose to hypoglycemic acidotic patients, but this has not been demonstrated.<sup>23, 40, 41</sup> The sequestration of mature parasites in various tissues may, by compromising local blood flow and by enhancing local glucose consumption, force the host tissues into an anaerobic metabolic state. Although 6–7% of the parasite-derived lactate is D-lactate,<sup>42</sup> and the host produces only L-lactate, only L-lactate is detectable in the peripheral blood of humans (Krishna S, unpublished data).

Acidosis is also associated with seizures; in the Malawian study, 28% of acidemic patients had convulsions observed on admission compared with 6% of patients with normal pH values.<sup>23</sup> Gambian children with recent convulsions were associated with a greater percentage decrease in lactate concentrations within 4 hr of admission (56%) than those admitted without a history of convulsions (38%).<sup>38</sup> Overdose of salicylates to treat fever has been suggested as another cause,<sup>43</sup> although these drugs are not used in many areas where acidosis occurs (Krishna S, unpublished data).

Altered respiratory rates and rhythms are commonly observed in acidotic pediatric malaria patients. Kussmaul respirations are a compensatory response to metabolic acidosis, and a proportion of malaria patients use this mechanism<sup>23</sup> to achieve a state of compensated metabolic acidosis (pH 7.301–7.400,  $p\text{CO}_2 < 30$  mm of Hg). The mortality rate in this group is significantly less than among patients with uncompensated metabolic acidosis (pH  $\leq 7.300$ ,  $p\text{CO}_2 = 31$ –50 mm of Hg). Thus, the capacity of the central respiratory center to respond appropriately to a low plasma pH may be another factor in the metabolic acidosis of malaria.

Whatever the cause of metabolic acidosis, a consistent finding in published studies is that acidosis usually improves rapidly once treatment with intravenous fluids and parenteral antimalarials are instituted. Both acidemia (in Malawian patients)<sup>23</sup> and lactic acidosis (in Gambian patients)<sup>38</sup> resolved within 8 hr of admission in survivors. Among fatal cases, only one of five Malawian patients who were acidemic on admission and survived more than 8 hr remained acidemic. In contrast, sustained lactic acidosis was a common finding among fatalities in the Gambian study,<sup>38</sup> and elevated plasma lactate concentrations between 4 and 24 hr following the start of treatment were the best single predictor of fatal outcomes.

Should malaria-associated acidosis be specifically targeted for treatment? In severely anemic patients who are acidotic, blood transfusions can be administered rapidly without precipitating cardiac failure and are associated with decreased plasma lactate concentrations.<sup>36</sup> A focused intervention, targeted at a specific cause of acidosis, might be more useful than merely normalizing acid-base balance by administering alkali, for instance. Hyperlactatemia can be treated with sodium dichloroacetate (DCA), which stimulates the aerobic consumption of lactate. Following of increased survival in *P. berghei*-infected rats with lactic acidosis treated with DCA<sup>44</sup> and a pharmacodynamic/pharmacokinetic assessment in adults with severe malaria,<sup>21</sup> Krishna and others conducted a randomized, double-blind study of DCA (single dose, 50 mg/kg) as adjunct treatment for lactic acidosis in children with severe malaria.<sup>45</sup> Throughout the first 4 hr after treatment, the mean plasma lactate concentrations in DCA-treated patients were significantly less than that in controls, but

thereafter, the mean plasma lactate concentrations decreased in both groups. This intervention may have limited applicability since hyperlactatemia may be only one of several contributors to malaria acidosis. A large clinical trial to evaluate the impact of DCA on mortality is being conducted (Krishna S, unpublished data).

**Hypoglycemia.** Hypoglycemia (blood glucose concentration  $\leq 2.2$  mmol/L or 40 mg%) is a complication of many different pediatric illnesses and is usually associated with a poor outcome.<sup>46, 47</sup> In adults with malaria, hypoglycemia is generally associated with quinine infusions, but in African children, pretreatment hypoglycemia is more important; 10–20% of children with CM are hypoglycemic at the time of admission to the hospital.<sup>5, 7, 16, 21, 40, 48</sup>

Quinine is a potent insulin secretagogue,<sup>49</sup> and insulin levels in quinine-induced hypoglycemia are inappropriately high. In African children, rapid infusions of quinine ( $\geq 10$  mg/kg over a 1-hr period) can also precipitate hypoglycemia,<sup>50</sup> but slower infusion rates and the use of intravenous solutions with dextrose concentrations  $\geq 5\%$  are safe and well-tolerated.<sup>51</sup> In pretreatment hypoglycemia, in contrast, plasma insulin levels are appropriately low,<sup>40, 49, 52</sup> and possible mechanisms include impaired production,<sup>53</sup> accelerated metabolism, and/or parasite glucose consumption.<sup>54</sup> Gluconeogenic precursors are present in sufficient concentrations to exclude starvation as an etiology; hepatic glycogen stores may be depleted, and gluconeogenesis may be impaired.<sup>40, 44, 52</sup>

Regardless of the etiology, pretreatment hypoglycemia is consistently associated with a poor prognosis (22–37% mortality) in children with CM,<sup>7, 40, 48</sup> and children with recurrent hypoglycemia fare even worse (71% mortality).<sup>40</sup> The emergency treatment of hypoglycemia is an intravenous infusion of 50% dextrose (1 ml/kg). Some hypoglycemic malaria patients regain consciousness with this treatment, and their prognosis is excellent (Taylor T, unpublished data), but the majority do not, indicating that impaired consciousness in malaria may have multiple etiologies.

**Increased intracranial pressure.** Increased intracranial pressure (ICP) is associated with poor outcome in pediatric encephalopathies, particularly in central nervous system infections.<sup>55</sup> It can cause death by transtentorial herniation or a reduction in cerebral perfusion pressure (CPP), where  $\text{CPP} = \text{mean arterial pressure} - \text{ICP}$ . Increased ICP was thought not to be important in adults with CM, since more than 80% of opening cerebrospinal fluid (CSF) pressures were in the normal range, there was no difference in the CSF pressures between those who died and those who survived,<sup>56</sup> and cerebral edema was thought to be an agonal event.<sup>57</sup> However, opening CSF pressures do not predict maximum ICP<sup>55</sup> and increased ICP may be caused by an increase in cerebral blood volume.<sup>19</sup>

Opening CSF pressures are increased in most African children with CM<sup>19, 58, 59</sup> above the normal range for children. Most Kenyan children dying of CM have clinical signs compatible with transtentorial herniation,<sup>19</sup> although these signs may be caused by other mechanisms. There is limited evidence of herniation at post-mortem, but ICP monitoring confirmed that Kenyan children deeply unconscious from CM, all had increased ICP (with ICP higher than the upper limit of adults) and that opening ICP did not predict maximum

ICP.<sup>60</sup> Furthermore, all children who developed severe intracranial hypertension either died or survived with severe neurologic sequelae. One child who developed an ICP of 158 mm of Hg died with signs of transtentorial herniation. Transcranial Doppler studies showed that about half of the children dying of CM had sonographic features of progressive intracranial hypertension, while the remainder had sonographic features similar to children who died of non-CM.<sup>61</sup> The most likely cause of increased ICP in CM is an increase in cerebral blood volume<sup>19</sup> aggravated by cytotoxic edema in those who develop severe neurologic sequelae.<sup>62</sup> Computer tomography was normal in most children recovering from CM, but transient brain swelling occurred in children with intermediate intracranial hypertension and tomographic features of cytotoxic edema were seen in children who developed severe intracranial hypertension. There was no evidence of vasogenic edema or acute hydrocephalus.<sup>62</sup> Increased cerebral blood volume could be caused by sequestration of parasitized red blood cells (PRBCs), either acting as a diffuse space occupying lesion or obstructing venous outflow. Other features of CM, such as seizures, anemia, and hyperthermia would also increase cerebral blood flow.<sup>61</sup>

Thus, increased ICP appears to be a feature of CM, and severe intracranial hypertension is associated with a poor outcome. Whether intracranial hypertension is a primary pathophysiologic process remains to be established. Mannitol was effective in lowering the ICP and may have prevented children with mild degrees of intracranial hypertension from dying or developing neurologic sequelae, but it did not prevent the development of intractable intracranial hypertension in those children with a poor outcome.<sup>60</sup> Further studies on the incidence of herniation at post-mortem, the relationship between clinical signs and herniation, and the effectiveness of substances to reduce ICP are needed before treatment recommendations can be made.

**Seizures.** Seizures, particularly those witnessed after admission in children with CM, are associated with death.<sup>6, 16</sup> They aggravate intracranial hypertension, cause neuronal loss and may precipitate aspiration. The cause of the seizures in CM is unclear. Intracranial sequestration of metabolically active parasites is a potential mechanism, but is difficult to investigate. Seizures are unlikely to be simply febrile convulsions, since 54% of seizures occurred when the rectal temperatures were less than 38.0°C, 47% were partial, and more than 70% were repetitive.<sup>63</sup> Other potential causes of seizures are hypoglycemia and hyponatremia; studies of Malawian and Kenyan children, however, indicate that these mechanisms are probably not important.<sup>6, 64</sup>

Although seizures are associated with a poor outcome, it has not been shown that preventing seizures improves outcome. Phenobarbitone (3.5 mg/kg) reduced the incidence of seizures in Thai adults,<sup>65</sup> but higher doses (10-15 mg/kg) in Kenyan children had no effect.<sup>66</sup> The efficacy of even higher doses is under evaluation (Crawley J, unpublished data).

**Cytokines and nitric oxide.** Plasma concentrations of the cytokine tumor necrosis factor (TNF) are elevated in children with severe *P. falciparum* infections, particularly those with fatal infections<sup>67-69</sup> who often exhibit more sustained elevations of TNF than do survivors.<sup>70</sup> Both host and parasite may contribute: strains of *P. falciparum* vary in their abilities to stimulate TNF production<sup>71</sup> and Gambian children ho-

mozygous for a particular TNF gene promoter are at increased risk of dying of CM.<sup>72</sup> This cytokine may also have beneficial effects: it exerts an antiparasitic effect in animal models,<sup>73-75</sup> and recent data suggest that Gabonese patients with severe malaria who had high TNF production capacities enjoyed more rapid clinical and parasitologic recoveries.<sup>76</sup>

A recent trial of an anti-TNF monoclonal antibody as adjunct treatment in Gambian children with falciparum malaria demonstrated a dose-dependent effect on fever reduction,<sup>29</sup> suggesting a causal relationship between TNF and at least one clinical manifestation of malaria. In CM patients, TNF receptors circulate in much higher concentrations than TNF, and bioactive TNF is seldom detectable.<sup>77-80</sup> The TNF concentrations in uncomplicated *P. vivax* infections are as high as those measured in CM patients,<sup>81</sup> but this infection rarely causes severe disease. Therefore, if cytokines play an important pathogenic role, it may be at the local tissue level, where merogony in *P. falciparum* occurs and the parasite products that stimulate TNF production are released. For instance, TNF- $\alpha$  up-regulates the expression of endothelial ligands capable of binding PRBCs (intracellular adhesion molecule-1, CD-36, thrombospondin)<sup>82</sup> and may thus enhance the cytoadherence of these cells in various tissues. Plasma concentrations of TNF are likely to be imprecise indicators of TNF effects in patients with CM; identifying sites of TNF production (immunohistochemically or through *in situ* hybridization) might permit a more detailed analysis of the relationship between TNF and clinical illness.

How might a TNF- $\alpha$  effect be mediated in patients with malaria infection? One possible explanation<sup>83, 84</sup> is that TNF- $\alpha$  stimulates the release of nitric oxide (NO)<sup>85</sup> from endothelial cells.<sup>86</sup> Nitric oxide is a short-lived, freely diffusible, free radical capable of functioning as a neurotransmitter.<sup>87</sup> Clark and others suggested that NO diffuses into the central nervous system and disrupts normal neurotransmission, thereby creating a severe neurologic disturbance that could be completely reversible.<sup>84</sup> Plasma concentrations of nitrate and nitrite have been used as surrogate measures for nitric oxide, but early data on these more stable end products in malaria patients were contradictory.<sup>88-90</sup> More recent data, which combined measures of urinary and plasma nitrate and nitrite (NO<sub>x</sub>), leukocyte-inducible nitric oxide synthase (NOS), TNF- $\alpha$ , and interleukin-10 (an anti-inflammatory cytokine) in healthy control children, and in children with asymptomatic falciparum parasitemia, uncomplicated malaria, and CM describe an inverse association between fasting NO<sub>x</sub> levels and leukocyte NOS and disease severity.<sup>91</sup> Cerebrospinal fluid levels of NO<sub>x</sub> were not increased in Ghanaian children with CM, and there is no difference in those who die compared with the survivors.<sup>92</sup> At this point, detecting tissue expression of NO synthase immunohistochemically, or via *in situ* hybridization, may provide the most reliable measures of NO effects in fatal malaria.

**Anemia.** The causes of severe, life-threatening anemia are difficult to define since other causes of anemia (e.g., iron deficiency and hemoglobinopathies) are common in malaria-endemic areas. Severe anemia often develops as the parasites are being cleared or in association with a chronic, low-grade infection. Destruction of the PRBCs is an inevitable consequence of malaria either at merogony or by erythrophagocytosis in the spleen.<sup>93</sup> Markers of hemolysis (e.g., hypohapto-

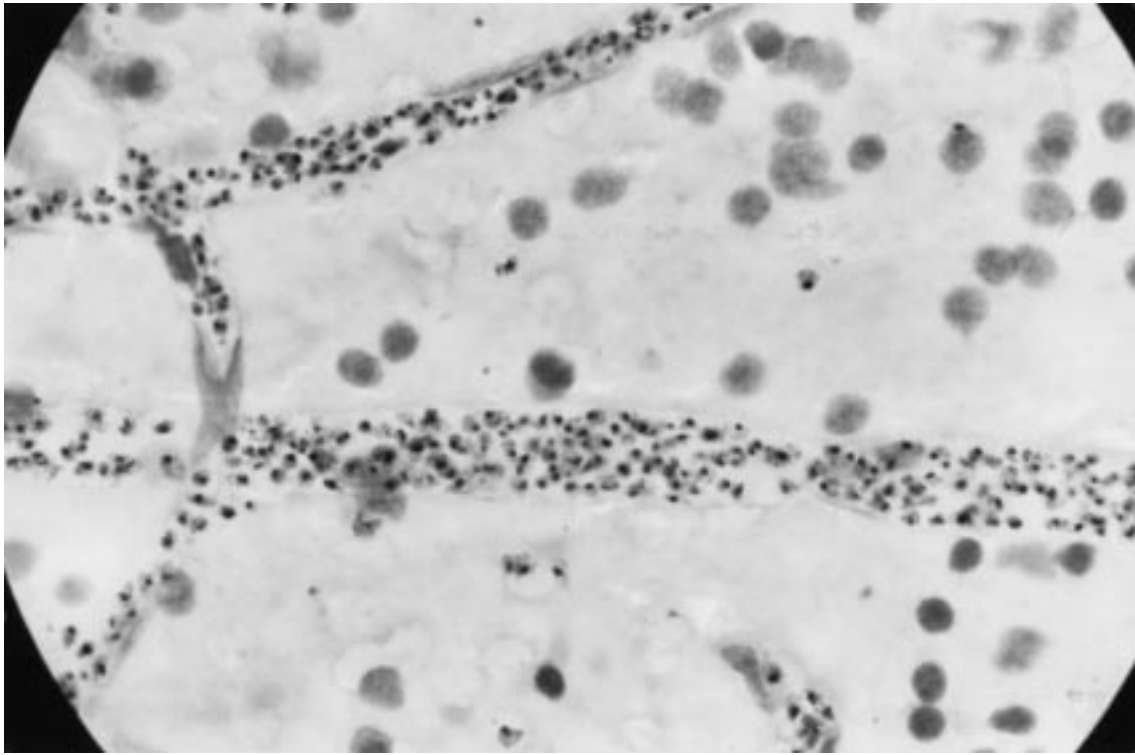


FIGURE 3. Brain smear from a Malawian child dying of cerebral malaria, stained with Field's A and B (photograph by R. O. Whitten) (magnification  $\times 1,000$ ).

globinemia) are associated with parasitization in endemic areas.<sup>94,95</sup> Hemolysis may not only be caused by destruction of the PRBCs,<sup>96</sup> and there is evidence that unparasitized erythrocytes have shorter lifespans during a malaria infection.<sup>97</sup> Autoimmune hemolysis has been suggested as an important component of severe malarial anemia;<sup>98</sup> a positive direct agglutination test result was associated with parasitemia,<sup>99</sup> but its association with anemia has been variable.<sup>98-102</sup>

Chronic malaria infections in Gambian children were characterized by dyserythropoiesis with little evidence of hemolysis. Dyserythropoiesis is common in adults with CM,<sup>103</sup> but is a nonspecific finding in African children with severe anemia from a variety of causes.<sup>31</sup> It has been induced by TNF in mice,<sup>104</sup> but data from humans are lacking. Erythropoietin levels are appropriately increased,<sup>31,105</sup> eliminating this as a potential cause of poor bone marrow response, and parvovirus-induced aplasia, suggested in one study,<sup>106</sup> was not found in another.<sup>31</sup>

**Other causes of death.** Acute renal failure and pulmonary edema are common complications of falciparum malaria and a major cause of death in adults, but are rarely seen in African children.<sup>6,7,16</sup> Renal impairment, however, is common in CM<sup>107</sup> and while associated with death,<sup>16</sup> it does not progress to frank renal failure. Renal impairment is probably caused by hypovolemia<sup>35</sup> and aggressive fluid replacement may be required in children, particularly with blood, if there is evidence of a severe metabolic acidosis.<sup>108</sup> Hyperkalemia is uncommon and when it occurs it is often associated with severe hemolysis or renal impairment. Cardiac arrhythmias were not found to be responsible for deaths in Gambian children.<sup>109</sup> Superimposed bacterial infections (pneumonia, sep-

ticemia, and urinary tract infections) often cause death in nonimmune adults with malaria, and although *Salmonella* septicemia was associated with malaria infection in Gambian children,<sup>110</sup> superimposed infections only rarely cause death in children with severe and complicated malaria.<sup>6,7</sup>

#### POST-MORTEM FINDINGS

**Gross brain abnormalities.** A slate gray, leaden, or plum-gray discoloration of the cortex is often noted; this discoloration is presumed to be due to deposition of hemozoin (malaria pigment). Gross cerebral edema is not a consistent finding in autopsy studies and in the description of edema,<sup>20,111,112</sup> brain weights are not often reported. Objective measures of herniation are rarely described (i.e., forced movement of the uncus gyrus of the temporal lobe medially over the edge of the tentorium, compression of the cerebellar tonsils into the foramen magnum, mammillary body encroachment of Greenhall's line, axial pressure on the mid-brain), although one Nigerian child was noted to have frank herniation,<sup>20</sup> and uncus grooving was seen in Senegalese children with PRBCs present in cerebral capillaries.<sup>113</sup>

**Sequestration of PRBCs.** The most consistent and perhaps earliest recognized pathologic feature in fatal malaria infections is the presence of PRBCs in brain capillaries and postcapillary venules (Figure 3),<sup>14</sup> although they are found in large caliber venules.<sup>115</sup> There are reports of CM deaths in patients in whom these sequestered PRBCs could not be identified,<sup>112,116,117</sup> but since ante-mortem details are scanty, other causes of encephalopathy cannot be excluded and the effects of treatment were not assessed.<sup>115</sup> In Thai adults with

TABLE 2  
Postmortem (PM) findings from African children with fatal malaria infections\*

Investigators	PM studies*	Clinical correlation	Findings
Edington <sup>122</sup>	4	Detailed	Scanty peripheral parasitemia in the treated patients contrasted with cerebral capillaries engorged with parasitized red blood cells.
Thomas <sup>132</sup>	13	Scanty	Diagnosis of cerebral malaria was made if petechial hemorrhages were observed in the brain. Little mention of sequestered parasitized red blood cells, more emphasis on hemorrhages.
Attah and Ejeckam <sup>137</sup>	10	Scanty	“The brains were histologically positive for malaria.”
Walker and others <sup>20</sup>	7	Scanty	Capillary congestion with parasitized red blood cells, malaria pigment, perivascular hemorrhages, marked cerebral edema in four of seven brains and herniation evident in one of these.

\* Only studies in which ante-mortem data were available and in which brains were examined and commented upon have been included.

CM, there were more PRBCs/vessel in the brain than in heart, lungs, kidneys, liver, and small intestines and overall, cerebral vessels contained more parasites in patients with CM than in patients with noncerebral forms of malaria.<sup>118, 119</sup> All stages of *P. falciparum* have been observed in sequestered PRBCs,<sup>120</sup> but studies correlating the relative proportions of various stages with the clinical presentation have not been reported. There is no association between the density of the peripheral parasitemia and the density of sequestered PRBCs.<sup>121, 122</sup>

More recent studies using electron microscopy have identified electron dense knobs on the surface of PRBCs and have identified, using immunofluorescence or immunohistochemical techniques, malarial antigens in or associated with these knobs.<sup>118, 121, 123, 124</sup> In Vietnamese adults, the localization of PRBCs significantly correlated with putative ligands for sequestration.<sup>125</sup>

**Endothelial cell involvement.** Several investigators have described endothelial injury and endothelial cell swelling in association with PRBCs,<sup>117, 126</sup> but since the post-mortem intervals were not stated, these may reflect a post-mortem artifact. They are not present in samples from studies with short post-mortem intervals.<sup>118, 127</sup> One of the few studies that addressed this issue obtained brain samples within 90 min of death; only minor endothelial damage was apparent by electron microscopy and there was no significant difference in endothelial damage when CM patients were compared with controls.<sup>118</sup> Recent studies on Vietnamese adults provide evidence of endothelial activation.<sup>125</sup>

**Hemorrhages.** Brain hemorrhages have long been observed in CM,<sup>123, 127–132</sup> and are described as petechial, punctuate, and most specifically, as ring hemorrhages. These are hemorrhages composed of extravasated red blood cells (usually not PRBCs) surrounding an occluded, or possibly thrombosed vessel. They are more common in white matter than gray matter, particularly in the cerebellum.<sup>130, 133</sup> The hemorrhages have generally been assumed to be the result of red blood cells leaking out of damaged vessels, though the exact source of the endothelial injury is unknown.

Vascular thrombi were initially thought to be critical components of CM, but no unequivocal histologic features of thrombi (e.g., cellular organization) have ever been reported. One study showed occluded vessels containing fibrin via a nonspecific histochemical stain,<sup>111</sup> and there has been one report of giant nuclear masses in the lungs and peripheral blood, which were thought to be indicators of disseminated

intravascular coagulation.<sup>134</sup> Their origin was not identified, however, and this finding has not been reported again.

**Inflammation and granulomas.** Inflammation, characterized by infiltrates of inflammatory cells, is not generally described as a feature of human CM,<sup>118, 135</sup> but it has been noted in a few studies.<sup>117, 121, 136</sup> Inflammatory changes are common in mice with CM,<sup>66</sup> however, and this is one of the most important discrepancies between animal models and human disease.

Malarial granulomas (Durck's granulomas) are thought to be collections of macrophages (microglia) surrounding necrotic regions encircling occluded vessels. Granulomas are not seen in all cases of CM, and none was seen in one study with very short post-mortem intervals.<sup>118</sup> However, because the samples for this study were obtained by needle biopsy, sampling error is a possible explanation. The exact cellular composition of these granulomas has not been determined by electron microscopy or by immunohistochemistry.

**Clinicopathologic correlates.** The few clinicopathologic correlations of fatal malaria in African children that have been published (Table 2)<sup>20, 122, 132, 137</sup> corroborate the findings described earlier in this report, but the clinical details provided are scanty, and it is not possible to correlate specific neurologic syndromes with the distribution of sequestered parasites. There are no controlled studies involving African children dying with non-CM, so it is difficult to determine the relative contributions of sequestration (site, intensity), hemorrhage, cerebral edema, and granuloma formation to overall outcome. Furthermore, there are no post-mortem studies in children that use current analytical techniques (immunohistochemistry, *in situ* hybridization); such studies would be useful in evaluating the various theories of malaria pathogenesis.

**Cause of death.** It is not possible to determine an immediate cause of death in most cases of fatal malaria. To implicate increased ICP, either transtentorial herniation or diffuse cerebral ischemia must be present. Herniation is difficult to identify in children, and it has not been consistently sought or reported. Ischemia from vascular occlusion due to sequestration has been hypothesized, but the characteristic histologic features (e.g., coagulation necrosis of neurons) have not been described. Cerebral hemorrhages do occur, but they are small and are generally not associated with significant necrosis. Inflammatory cells are rarely observed in the brains of patients dying of CM. The contribution of hypoglycemia to cerebral injury is not represented pathologically.

The anatomic cause of death from severe anemia is often thought to be high-output heart failure with pulmonary edema, but this has not been reported in pediatric malaria patients. The role of myocardial vascular sequestration has not been carefully studied, and no other pathologic correlates have been sought.

#### CONCLUSIONS

Malaria remains a major cause of morbidity and mortality, especially among children in sub-Saharan Africa. Basic clinical studies have, over the past 11 years, provided more detailed descriptions of fatal infections, most of which involve acidosis, CM and/or severe malarial anemia. Associations between the clinical features of malaria and fatal outcomes have been described, but causality has not been established. Prospective post-mortem studies could investigate the role of PRBC sequestration in the brain, the activation of endothelial receptors, and the production of TNF and NO with new techniques. Furthermore, these studies could help to determine if the primary mechanism of death in CM is within the central nervous system (e.g., sequestration or increased ICP). Interventions can only be tested if the pathophysiological processes leading to death can be assessed during life. In this regard, sophisticated monitoring is warranted in a few centers to identify these mechanisms, and to conduct pilot studies of interventions. Since there are, as yet, no surrogate measures for fatal outcomes in malaria, intervention trials with mortality as an outcome will probably need to be multicentered to enroll a sufficiently large number of patients. Financial support: This work was supported by the Burroughs Wellcome Fund, United States (TET, ROW) and The Wellcome Trust, United Kingdom. C. R. J. C. Newton holds a Wellcome Trust Career Position in Clinical Tropical Medicine (no. 050533).

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