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

Idro, R.I.

Citation for published version (APA):
High plasma levels of erythropoietin are associated with protection against neurological sequelae in African children with cerebral malaria

Climent Casals-Pascual, Richard Idro, Nimmo Gicheru, Samson Gwer, Barnes Kitsao, Evelyn Gitau, Robert Mwakesi, David J Roberts, Charles RJC Newton

Nuffield Department of Clinical Laboratory Sciences and National Blood Service Oxford Centre, Oxford, UK; Medical Research Council Laboratories, Fajara, PO Box 273, The Gambia; Centre for Geographical Medicine (Coast), Kenya Medical Research Institute, Kilifi, Kenya; Department of Paediatrics, Mulago Hospital/Makere University, Kampala, Uganda; Neurosciences Unit, The Wolfson Centre, University College London, Institute of Child Health, UK

Submitted
ABSTRACT

Background
Cerebral malaria in children is associated with a high mortality and long-term neurological and cognitive sequelae. Both erythropoietin and vascular endothelial growth factor (VEGF) have been shown to be neuroprotective. We hypothesised that high plasma and cerebrospinal fluid (CSF) levels of these cytokines would prevent neurological sequelae in children with cerebral malaria.

Methods
We measured erythropoietin, VEGF and tumour necrosis factor (TNF) in paired samples of plasma and CSF of Kenyan children admitted with cerebral malaria. Logistic regression models were used to identify risk and protective factors associated with the development of neurological sequelae.

Results
Children with cerebral malaria (N=124) were categorized into 3 groups: 76 without sequelae, 32 with sequelae and 16 who died. The median (IQR) plasma concentrations of erythropoietin were 278 (96-1,852) U/L, 184 (23-694) U/L and 123 (29-1,726) U/L, respectively. Conditional logistic regression analysis matching the 32 patients with cerebral malaria and neurological sequelae to 64 patients with cerebral malaria without sequelae stratified for haemoglobin level estimated that plasma erythropoietin (>200 U/L) was associated with greater than 80% reduction in the risk of developing neurological sequelae (adjusted OR 0.18, 95%CI 0.05-0.93, \( P=0.041 \)). Both the level of erythropoietin in relation to haemoglobin level and the protective effect of erythropoietin for a given erythropoietin concentration were greater in younger children. Admission with profound coma (adjusted OR 5.47, 95%CI 1.45-20.67, \( P=0.012 \)) and convulsions after admission (adjusted OR 16.35, 95%CI 2.94-90.79, \( P=0.001 \)) were also independently associated with neurological sequelae. Plasma VEGF and TNF were not associated with sequelae.

Conclusions
High levels of erythropoietin were associated with reduced risk of neurological sequelae in children with CM. The age-dependent erythropoietin response to anaemia and the age-dependent protective effect may influence the clinical epidemiology of CM. These data support further study of erythropoietin as an adjuvant therapy in CM.
INTRODUCTION

Cerebral malaria is the most severe neurological complication of infection with *Plasmodium falciparum*. Even with appropriate anti-malarial treatment, 18.6% of children with CM die and 11% have neurological deficits detected on discharge and 24% children have neurological and cognitive impairments or epilepsy when assessed many years later.

Over a wide range of endemic areas, severe malarial anaemia is the most common manifestation of severe malaria in younger children whereas the peak incidence of cerebral malaria occurs in the fourth year of life. The pathogenesis of cerebral malaria is not completely understood and the factors involved in the development of neurological sequelae remain unclear. A number of studies have consistently identified deep and prolonged coma, recurrent seizures and hypoglycaemia as independent risk factors associated with the development of neurological sequelae (reviewed in). Protective factors are less well defined. Low levels of haemoglobin (Hb) were associated with neurological sequelae in the Gambia, but not in other African studies.

We have hypothesized that the outcome of cerebral malaria is modified by the cytokine response to hypoxia. Erythropoietin, principally produced in the kidney in response to hypoxia, is crucial for sustained proliferation and differentiation of erythroid cells. However, erythropoietin and erythropoietin receptor are also expressed in neurons and astrocytes. Recombinant human erythropoietin (rhEpo) is protective in animal models of brain injury and reduces vasoconstriction and neuronal apoptosis. Indeed, preclinical studies in patients with stroke have supported a neuroprotective role for erythropoietin. High levels of erythropoietin have been found in African children with malaria anaemia. Furthermore, the administration of rhEpo in a murine model of cerebral malaria reduced mortality by 90%.

Vascular endothelial Growth Factor (VEGF) is also upregulated by hypoxia and is both neurotrophic and neuroprotective. It improves functional outcome in cerebral ischaemia in rats, reducing motor and cognitive defects. However, VEGF can also induce intercellular adhesion molecule-1 (ICAM-1) and macrophage inflammatory protein 1α (MIP1α) in endothelial and brain parenchymal cells and increase the permeability of the brain-blood barrier (BBB). Other studies show the levels of the pro-inflammatory cytokine, TNF, increase during acute stroke, and in an animal models, intraventricular administration of TNF enlarges infarct volume. In children with malaria, high TNF levels have been associated with poor outcome.
We hypothesised that high levels of erythropoietin and VEGF protect children with cerebral malaria from developing neurological sequelae or death. We studied a well-defined group of children admitted with cerebral malaria who were assessed for neurological damage during admission and on discharge from hospital. We compared the levels of erythropoietin, VEGF and TNF in children who died and in those who survived with and without neurological deficits.

METHODS

Study design
The study was conducted in children admitted to Kilifi District Hospital (KDH), in which all children are seen by research clinicians from the Kenya Medical Research Institute/Wellcome Trust collaborative programme. This was a retrospective study of children admitted with cerebral malaria classified by outcome: children who survived without neurological deficits, children who survived with neurological deficits and children who died.

Study participants were identified from the hospital database admitted between 1999 and 2005. Cerebral malaria was defined as admission to hospital with coma (unable to localise a painful stimulus or Blantyre coma score [BCS] \(\leq 2\), at least 1 hour after termination of a seizure if present or correction of hypoglycaemia), with asexual forms of Plasmodium falciparum malaria parasites on a Giemsa stained blood smears, and no evidence of pyogenic meningitis on examination of the CSF.\(^3^3\) All patients had a detailed neurological examination on admission which included a fundoscopic examination of the retina for features of raised intracranial pressure. Lumbar punctures were performed to exclude pyogenic meningitis when the level of consciousness improved (BCS >2) or if the child did not have brainstem signs (within 48 hours of admission in almost all cases)\.\(^3^3,^3^4\) A portion of the CSF was stored at -20°C and later frozen at -80°C together with the corresponding specimen of plasma obtained at the time of the lumbar puncture. Patients with epilepsy, cerebral palsy, sickle cell disease, those without stored paired samples or samples collected after a blood transfusion were excluded. The scientific and ethical committees of Kenya Medical Research Institute approved the study.

Procedures
All patients had been treated with standard protocols developed for the management of severe falciparum malaria\(^3^5\) and according to the WHO.\(^3^6\) All children were assessed for neurological sequelae at discharge from hospital. Neurological sequelae were classified as motor sequelae (cranial nerve palsies, spasticity and hypotonia), ataxia, movement
disorders (tremors, dystonia and choreoathetoid movements), speech (speech difficulties or aphasia), visual (blindness) and/or hearing impairments, epileptic seizures, and behavioural abnormalities (aggressive behaviour and hyperactivity).\textsuperscript{10}

**Laboratory procedures**

Plasma and CSF levels of erythropoietin, VEGF and TNF were measured by ELISA following the manufacturer’s instructions (R&D Systems, Abingdon, UK). The lower limit of detection for erythropoietin was 2.5 UI/L, VEGF was 15.6 pg/mL and TNF was 15.6 pg/mL. Ten percent of samples were tested in duplicate (the correlation of values was always $r > 0.90$). Values above the standard detection range were diluted and re-assayed.

**STATISTICAL ANALYSIS**

Data were analysed using STATA 9 (Stata Corporation, Texas, USA). Binary logistic regression analysis was used to identify risk/protective factors independently associated with the development of neurological sequelae in children with cerebral malaria. Goodness-of-fit was assessed by the Hosmer-Lemeshow test. Variables detected from previous studies in the unit and elsewhere,\textsuperscript{10,37} known to be associated with the dependent variable (neurological sequelae) were included in the analysis, namely, hypoglycaemia, depth of coma and seizures. The independent variables were checked for interaction. Conditional logistic regression was used to match 32 children with neurological sequelae to 64 children with good outcome. The subgroup of children not used in the matching (N=12) had similar age and Hb concentrations to those used in the regression. Matching was by age (within 18 months) and Hb level (within groups Hb<5, 5.1 to 7, 7.1 to 9 and >9.1 g/dL). Matching criteria were based on biologically meaningful cut-off points, but also on a value range that allowed inclusion of all children with sequelae. Ordinal logistic regression was used to identify risk factors associated with outcome (no sequelae at discharge, neurological sequelae and death). For ordinal logistic regression analysis deviance (chi-square test) was used to assess goodness-of-fit.

**RESULTS**

Four hundred and twenty six children were admitted to Kilifi District Hospital with cerebral malaria from January 1999 through December 2001. Nine had incomplete admission data and were excluded. Of the remaining 417, paired plasma and CSF samples were available for 171 cases. Out of those, 65 received a blood transfusion. In 55 cases the samples had been obtained after a blood transfusion and were excluded from the study.
In addition, 8 other children were identified from among admissions of 2002 to 2005. Thus we included in this study 124 children with paired CSF and plasma samples. Table 1 is a description of the children by outcome.

Table 1: Description of the study population

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Good outcome (N=76)</th>
<th>Neurological sequelae (N=32)</th>
<th>Dead (N=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months), median (IQR)</td>
<td>27 (14.5-36.5)</td>
<td>28 (21-46)</td>
<td>36.5 (27-46)</td>
</tr>
<tr>
<td>Gender, male N (%)</td>
<td>45 (59.2)</td>
<td>14 (43.7)</td>
<td>9 (56.2)</td>
</tr>
<tr>
<td>Weight for age Z-score, mean (sd)</td>
<td>-1.7 (1.1)</td>
<td>-2 (1.02)</td>
<td>-1.7 (1.6)</td>
</tr>
<tr>
<td>Fever, n (%)</td>
<td>69 (90.7)</td>
<td>29 (90.6)</td>
<td>15 (93.7)</td>
</tr>
<tr>
<td>Seizures before admission, n (%)</td>
<td>71 (93.4)</td>
<td>29 (90.6)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Seizures during admission, n (%)</td>
<td>37 (48.6)</td>
<td>26 (81.2)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Profound coma, BCS = 0, n (%)</td>
<td>14 (18.4)</td>
<td>13 (40.6)</td>
<td>6 (66.6)</td>
</tr>
<tr>
<td>Abnormal motor posturing during admission, n (%)</td>
<td>21 (27.6)</td>
<td>17 (53.1)</td>
<td>8 (50)</td>
</tr>
<tr>
<td>Features of raised intracranial pressure on fundoscopy a, n (%)</td>
<td>4 (7.8)</td>
<td>8 (34.7)</td>
<td>3 (27.2)</td>
</tr>
<tr>
<td>Coma duration (hours), median (IQR)</td>
<td>8 (4-23)</td>
<td>78 (38-125)</td>
<td>17.5 (7.5-36.5)</td>
</tr>
<tr>
<td>Deep (acidotic) breathing, n (%)</td>
<td>15 (19.7)</td>
<td>8 (25)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Hypoglycaemia, n (%)</td>
<td>8 (10.5)</td>
<td>6 (18.7)</td>
<td>5 (31.2)</td>
</tr>
<tr>
<td>Parasite density / μL, geometric mean, (95% CI of mean)</td>
<td>42,328</td>
<td>28,439</td>
<td>60,495</td>
</tr>
<tr>
<td>Hb (g/dL), mean (sd)</td>
<td>8.0 (1.8)</td>
<td>8.6 (2.0)</td>
<td>8.3 (1.9)</td>
</tr>
<tr>
<td>Severe anaemia (haemoglobin &lt; 50 g/L), n (%)</td>
<td>3 (3.9)</td>
<td>2 (6.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Transfused, n (%)</td>
<td>2 (3.8)</td>
<td>3 (6.4)</td>
<td>5 (31.2)</td>
</tr>
<tr>
<td>Platelets (10³μl⁻¹), median (IQR)</td>
<td>101 (58-195)</td>
<td>192 (76-284)</td>
<td>93 (63-219)</td>
</tr>
<tr>
<td>Plasma Epo (mU/mL), median (IQR)</td>
<td>278.6 (96.7-1,852)</td>
<td>184.2 (23.9-694.4)</td>
<td>123.5 (29.5-1,726.2)</td>
</tr>
<tr>
<td>Plasma VEGF (ng/mL), median (IQR)</td>
<td>39.6 (23.2-77.2)</td>
<td>53.1 (25.9-118.1)</td>
<td>42.9 (18.1-122.7)</td>
</tr>
<tr>
<td>Plasma TNF (pg/mL), median (IQR)</td>
<td>60.6 (24.8-137.4)</td>
<td>80.6 (13.9-187.80</td>
<td>92.1 (63.0-487.8)</td>
</tr>
<tr>
<td>CSF Epo (mU/mL), geom. mean (95% CI)</td>
<td>14.3 (3.8-52.6)</td>
<td>14.3 (1.2-165.2)</td>
<td>29.8 (1.6-534.9)</td>
</tr>
<tr>
<td>CSF VEGF (ng/mL), geom. mean (95% CI)</td>
<td>42.9 (21.1-87.2)</td>
<td>59.1(16.1-216.8)</td>
<td>37.7 (2.3-608.9)</td>
</tr>
<tr>
<td>CSF TNF (pg/mL), geom. mean (95% CI)</td>
<td>361.3 (58.3-2,236.9)</td>
<td>199.8 (2.8-1,4165.6)</td>
<td>470.6*</td>
</tr>
</tbody>
</table>

aN=85 (N=51, N=23, N=11, respectively) *TNF was detectable only in one case, hence the 95%CI of the geometric mean cannot be calculated.

The median age of the children was 28.5 (IQR 16-40) months and the mean (sd) haemoglobin concentration was 8.2 (1.91) g/dL. Fifteen out of 32 (46%) children discharged with neurological deficits had multiple neurological sequelae. The major sequelae were visual impairment (n=8), impairment of speech (n=14), motor impairment (paresis, hemiplegia, hemiparesis, quadripleasis and monoparesis) (n=9).
Erythropoietin levels and development of neurological sequelae

To test our hypothesis that high erythropoietin levels were associated with protection from neurological sequelae in children with cerebral malaria, we first examined different concentrations of erythropoietin (ranging from 100 U/L to 2000 U/L) associated with development of neurological sequelae adjusting for confounders, namely hypoglycaemia, seizures during admission and depth of coma. A protective effect (odds ratio from 0.15 to 0.43) was found for erythropoietin concentrations ranging from 200 to 1000 U/L. We chose 200 U/L as a cut-off as this was the lowest value of erythropoietin associated with a significant protective effect (figure 1).

Logistic regression analysis identified plasma erythropoietin concentration (OR 0.28, 95%CI 0.09-0.79), depth of coma (OR 7.74, 95%CI 2.50-23.98) and seizures during admission (OR 3.42, 95%CI 1.11-10.48) as the main factors independently associated with development of neurological sequelae (table 2). The median level of erythropoietin in plasma was lower in patients with seizures during admission (204, IQR 39.8–1655U/L) compared to patients without recurrence of seizures (258, IQR 83.6–1779 U/L).

Age was associated with erythropoietin concentration ($r = -0.18, P=0.04$) and BCS on admission ($r = -0.22, P=0.01$). We therefore performed the same analyses in a conditional logistic regression model matching by age. This analysis again identified plasma erythropoietin (OR 0.21, 95%CI 0.05-0.86), seizures (OR 6.9, 95%CI 1.37-34.92) and depth of coma (OR 18.6, 95% 2.91-118.8) as variables independently associated with neurological sequelae (table 2).

The risk of developing sequelae given a certain concentration of erythropoietin was lower in the age-matched compared with the unadjusted model suggesting an age-dependent dose effect. Indeed, the odds ratio for neurological sequelae at different concentrations
of plasma erythropoietin, after adjusting for hypoglycaemia, depth of coma, seizures during admission and age in a multiple logistic regression analysis, was much lower in children under 2 years of age at concentrations of erythropoietin >500 U/L (figure 2).

Plasma erythropoietin was negatively associated with Hb concentrations (r = -0.59, P<0.001). We introduced Hb level as an ordinal independent variable (categorised as Hb<5, 5-7, 7-9 and >9 g/dL) in the logistic regression model to predict sequelae and found that children with Hb concentrations between 5 and 7 g/dL were associated with a significant reduction in the risk of developing neurological sequelae (OR 0.17, 95%CI 0.03-0.87). The median erythropoietin concentration for this subgroup was 2,097 (IQR 287-3,693). We therefore matched each case of cerebral with neurological sequelae and two cerebral malaria controls within the same Hb range.

Table 2. Factors associated with the development of neurological sequelae in Kenyan children with CM.

<table>
<thead>
<tr>
<th></th>
<th>Unmatched*</th>
<th>Matched</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR  P   95%  CI</td>
<td>OR  P   95%  CI</td>
</tr>
<tr>
<td>EPO (&gt;200 U/L)</td>
<td>0.28 0.01 0.09 0.79</td>
<td>0.21 0.03 0.05 0.86</td>
</tr>
<tr>
<td>Hypoglycaemia</td>
<td>3.11 0.11 0.76 12.71</td>
<td>0.57 35.65 9.27 0.057</td>
</tr>
<tr>
<td>Seizures</td>
<td>3.42 0.03 1.11 10.48</td>
<td>1.37 34.92 16.35 0.001</td>
</tr>
<tr>
<td>Depth of coma</td>
<td>7.74 0.00 2.50 23.98</td>
<td>18.6 0.002 2.91 118.80</td>
</tr>
</tbody>
</table>

Logistic regression analyses displays the odds ratio of high levels of plasma erythropoietin on the development of neurological sequelae (dependent variable) controlling for the effects of potentially confounding variables: depth of coma (BCS <2), seizures during admission and hypoglycaemia. To adjust for age and anaemia, a conditional (fixed-effects) logistic regression model was used to match each case of cerebral malaria with neurological sequelae and two controls (children with cerebral malaria and no neurological sequelae at discharge) by age and anaemia. Hosmer-Lemeshow = 5.54, P=0.476 a Cases of cerebral malaria with neurological sequelae (N=32) are matched by age (within 18 months) to 2 cases of CM discharged without neurological sequelae (N=64). b Cases of cerebral malaria with neurological sequelae (N=32) are matched by the Hb level (within groups Hb < 5, 5.1 to 7, 7.1 to 9 and >9.1 g/dL) to 2 cases of CM discharged without neurological sequelae (N=64). EPO=Erythropoietin.

Figure 2 Effect of age on the odds ration for neurological sequelae at different concentrations of plasma erythropoietin.

Odds ratio represents values after adjusting for hypoglycaemia, depth of coma, seizures during admission and age in a multiple logistic regression analysis using different cut-off values for age. * P <0.05 ≠ P 0.05-0.08 (P values are the significance of the OR in the logistic regression analyses for erythropoietin>500 and erythropoietin>1,000 U/L).
Erythropoietin concentrations were also measured in CSF from the same patients. Levels of plasma erythropoietin correlated with erythropoietin levels in CSF ($r = 0.40$, $P < 0.001$) in children discharged without neurological sequelae ($n = 76$) but not in those who developed neurological sequelae ($n = 32$) ($r = 0.21$, $P = 0.23$). CSF erythropoietin levels were not associated with neurological sequelae.

**Effect of plasma erythropoietin on mortality in cerebral malaria**

We performed an ordinal logistic regression to measure the impact of erythropoietin on poor outcome (death or neurological sequelae). In this model, mortality and discharge with and without sequelae were used as the dependent (ordinal) variable. In the subgroup of children with cerebral malaria who died ($N=16$) the median plasma concentration of erythropoietin was 124 (IQR 30-1,726) U/L. Blood transfusion, deep (acidotic) breathing and hyperparasitaemia were also more frequent in this group (Table 1). In addition to the independent variables used to identify risk factors for neurological sequelae, in this model we also adjusted for deep breathing and hyperparasitaemia as these factors may be associated with an increased fatality rate in severe malaria. Here, the logistic regression model identified the following variables to be independently associated with poor outcome: seizures during admission (OR 6.77, 95%CI 2.18-20.98), depth of coma (OR 5.65, 95%CI 2.24-14.21), deep breathing (OR 6.09, 95%CI 2.08-17.79) and hyperparasitaemia (OR 5.19, 95%CI 1.26-21.34). In this model, plasma erythropoietin was also independently associated with a better outcome (OR 0.21, 95%CI 0.08-0.54).

**Effect of VEGF and TNF-α on neurological sequelae**

VEGF is both neuroprotective and pro-inflammatory in the brain. Plasma VEGF (>100 ng/mL) was not associated with development of neurological sequelae but was strongly associated with seizures during admission (OR 4.1, 95%CI 1.75-9.60). Similarly, plasma VEGF (>100 ng/mL) was associated with a 4.5 fold increase in the risk signs of raised intracranial pressure by fundoscopy (95%CI 1.41-14.74) and a 12.1 fold increase in the risk of finding papilloedema (95%CI 1.84-79.37). VEGF concentrations in plasma were correlated with plasma TNF ($r = 0.23$, $P<0.001$) and inversely correlated with plasma erythropoietin ($r = -0.17$, $P = 0.051$). However, plasma VEGF was associated with higher concentrations of erythropoietin in CSF ($r = 0.24$, $P = 0.007$) and TNF in CSF ($r = 0.38$, $P <0.001$).
We measured the TNF in plasma and CSF to assess the role of inflammation in relation to the outcome of children with cerebral malaria. Plasma levels of TNF were higher in children with neurological sequelae compared with those healthy at discharge and were correlated with TNF concentration in CSF ($r = 0.35$, $P < 0.001$). High concentrations of plasma TNF (>100 pg/mL) and detectable TNF in CSF were associated with a 2.7-fold (95% CI 0.9-9.6) and a 3.4-fold (95% CI 0.72-16.0) increase in the risk of neurological sequelae, respectively.

**DISCUSSION**

This study was designed to seek evidence for the neuroprotective role of erythropoietin and VEGF in children with cerebral malaria. Here, we report a strong association between high concentrations of plasma erythropoietin and a reduced risk of neurological sequelae in Kenyan children with cerebral malaria, and the association of VEGF with seizures and signs of raised intracranial pressure but not neuroprotection.

The potential neuroprotective mechanism(s) of erythropoietin and VEGF in response to hypoxia are related to their neurotrophic and pro-angiogenic activities. In the last decade a number of experimental and pre-clinical studies have investigated the tissue protective activities of erythropoietin (reviewed in) and VEGF (reviewed in). In this study we have found that high erythropoietin levels in plasma are associated with a 70% reduction of the risk of being discharged with neurological sequelae, and 79% and 82% reduction when the analyses are matched by age or level of Hb, respectively. Erythropoietin is thought to prevent neuronal apoptosis and to down-regulate the inflammatory response in the brain which may explain differences in the clinical presentation and outcome in cerebral malaria. Neuronal apoptosis has been recently reported in a mouse model with experimental cerebral malaria. However, a recent trial in a similar model suggested that protection is due to the anti-inflammatory (rather than the anti-apoptotic) effect of erythropoietin.

The neuroprotective activities of erythropoietin are time and dose-dependent. In a rodent model of stroke, neurons within the ischaemic penumbra undergo apoptosis unless exposed to erythropoietin within 3 hours. In our study it was impossible to ascertain if the patients were anaemic (and therefore had high levels of erythropoietin) prior to the malaria episode. We have shown that erythropoietin levels on admission above 200 U/L are independently associated with a reduced risk of sequelae. In vitro studies suggest that concentrations ranging from 100 U/L to 1,000 U/L are associated with the neuroprotective activities of erythropoietin. Although our data suggest...
that erythropoietin concentrations up to 1,000 U/L were significantly associated with protection, a larger study would be required to establish the actual range of protection. The biological relevance of plasma erythropoietin is usually difficult to interpret in the absence of any direct measurement of erythropoietin in the brain. Erythropoietin is known to cross the blood-brain barrier by active translocation possibly via erythropoietin receptors expressed in the brain vasculature. We found CSF and plasma erythropoietin to be correlated in those cases discharged without neurological sequelae, but this association was moderately influenced by 5 cases with erythropoietin concentrations above 5,000 U/L. Moreover, erythropoietin in CSF was not associated with a reduced risk of neurological sequelae.

The association of younger age with higher erythropoietin concentrations and a greater protective effect of erythropoietin in children under 2 years of age are intriguing and there is no immediate biological explanation for these observations. However, these data do offer a possible reason for the widely observed and yet unexplained age-related presentation of severe malarial anaemia and cerebral malaria.

We have previously shown that malaria infection per se is associated with an increased level of erythropoietin secretion for any haemoglobin concentration. The specific mechanisms responsible for the increased erythropoietin concentration in patients with acute malaria are unclear but may include tissue hypoxia, hypoglycaemia and possibly oxidative damage. These three mechanisms have been shown to induce the expression of hypoxia inducible factor (HIF-1), which up-regulates the production of erythropoietin and other hypoxia-related proteins. Similarly, iron deficiency may also contribute to the up-regulation of erythropoietin and other hypoxia-related proteins by inhibiting the function of HIF-prolyl 4-hydroxylases. The beneficial effect of iron chelation therapy in cerebral malaria is unclear although one study suggested partial neuroprotection with desferoxamine. It is possible that up-regulation of HIF-1 and erythropoietin concentrations account for some of the tissue protective activities of iron chelation but these results may be partially obscured by the timing of the intervention.

The activation of VEGF has been reported in patients with cerebral malaria. VEGF is also up-regulated in response to tissue hypoxia and we initially predicted that the angiogenic and neurotrophic properties of VEGF would have a protective role in cerebral malaria. However, our data suggest that VEGF is not associated with neuroprotection but rather with features associated with a poor outcome. We have found that VEGF is associated with a 4-fold increase in the risk of seizures during admission. Pilocarpine-induced seizures in experimental animals are associated with a marked increase of VEGF levels in neuronal and glial cells and consequently vascular permeability and BBB
leakage. It is thus possible that increased VEGF concentrations may be the consequence rather than the cause of seizures. However, it is also likely that VEGF directly contributes to the pathophysiology of cerebral malaria by increasing the permeability of the BBB. Intracranial hypertension is a common finding in cerebral malaria in children. Here we report that concentrations of plasma VEGF (>100 pg/mL) are associated with a 4.5-fold increase in the risk signs of raised intracranial pressure and a 12-fold increase in the risk of papilloedema.

In this study, TNF was not identified as a risk factor for neurological sequelae. TNF (and other pro-inflammatory cytokines) have been shown to up-regulate VEGF, which may contribute to the development of neuropathological signs associated with inflammation in cerebral malaria. However, some of these effects may be partially antagonized by the TNF-induced up-regulation of erythropoietin receptor in the brain, and may explain the lack of association of TNF with poor outcome in this study.

Our data suggest that in addition to the inflammatory response to the parasite, molecules that orchestrate adaptation to hypoxia may influence the clinical presentation and outcome of severe malaria syndromes. Moreover, this study supports the preliminary data from murine models of cerebral malaria to indicate a neuroprotective role for erythropoietin in cerebral malaria. While rapidly effective antimalarial drugs would be used to clear sequestered and non-sequestered parasites, the use of erythropoietin in cerebral malaria would aim to protect potentially viable brain tissue to prevent development of neurological sequelae. The potential use of erythropoietin (and non-erythropoietic erythropoietin derivatives) as adjuvant treatment in cerebral malaria to prevent neurological sequelae should be investigated further.

ACKNOWLEDGEMENTS

This study was supported by The Wellcome Trust-UK and Kenya Medical Research Institute. This paper is published with the permission of the director of KEMRI. Prof. David Roberts is supported by the Howard Hughes Medical Institute and the National Blood Service and this works benefits from NHS R&D funding. Prof. CRJC Newton holds a Wellcome Trust Career Post in Clinical Tropical Medicine (No. 070114).

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