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The Effect Of Impaired Blood-Brain Barrier In Seizure Control And Response To Anti-Seizure Medication During Paediatric Acute Symptomatic Seizures Due To Severe Falciparum Malaria

Authors: Symon M Kariuki[1,2,3,4], Charles RJC Newton[1,2,3,4]

Affiliations:

1. Neuroscience Unit, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya.
2. Research Division, African Population and Health Research Centre, Nairobi, Kenya.
3. Department of Psychiatry, Oxford University, Oxford, United Kingdom.
4. Department of Public Health, Pwani University, Kilifi, Kenya.

Corresponding author: Symon M Kariuki. Email: skariuki@aphrc.org
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ABSTRACT

Background: Blood–brain barrier disruption is documented in severe falciparum malaria, but the role of the cerebrospinal fluid (CSF) proteins in pathogenesis and control of acute symptomatic seizures is not studied. We hypothesised that elevated CSF proteins (markers of blood-brain breakdown) in children admitted to the hospital with severe malaria may affect the risk for seizures in malaria and seizure control or response to anti-seizure medications (ASM**). Objective:** We measured CSF and plasma total protein, albumin, ferritin, and S100B concentrations in children with severe malaria to determine their associations with the risk of acute symptomatic seizures, seizure control, and response to ASM. **Methods**: We measured CSF levels of proteins such as albumin, ferritin, and S100B (a glial biomarker), in 45 children with carefully phenotyped seizures in the context of severe falciparum malaria admitted to hospital and documented the response of these seizures to ASM. We compared the distribution of these proteins in those with and without seizures, and in those requiring second-line ASM versus those controlled on first-line ASM. We further documented the occurrence of acute symptomatic seizures in those with evidence of blood–brain barrier disruption. **Results:** In the 45 children with severe falciparum malaria who had CSF protein levels measured, 36 of 45 (80.0%) had acute symptomatic seizures, of which 32 of 36 (88.9%) were complex seizures (focal, repetitive and/or prolonged). One unit increase in CSF Protein S100B levels (ng/mL) was associated with acute symptomatic seizures ((adjusted risk ratio (aRR)=1.06 (95%CI:1.01-1.11); P=0.012)). Acute symptomatic seizures, stopping spontaneously or without requiring second-line ASM, was associated with a one-unit increase in CSF ferritin levels (ng/mL) (aRR=1.12 (95%CI:1.03-1.23); P=0.008), CSF S100B levels (ng/mL) (aRR=1.33 (95%CI:1.00-1.78); P=0.051) and CSF total proteins levels (mg/L) (aRR=1.01 (95%CI:1.00-1.01); P=0.023). Seizures, responding to first-line ASM or stopping spontaneously, were more frequent in those with blood–brain barrier disruption compared to those without disruption (aRR=2.72 (95%CI:1.08-6.83); P=0.032). Evidence of blood–brain barrier disruption was associated with simple seizures. **Conclusion:** Elevated CSF proteins may be associated with anti-seizure activity in Kenyan children with acute symptomatic seizures admitted to hospital with severe falciparum malaria. Protein S100BB appears to be context-dependent in being involved in both the pathogenesis of acute symptomatic seizures in malaria, and in promoting a compensatory response for seizure control. Future well-powered longitudinal studies are required to confirm these findings.

Keywords: Cerebrospinal fluid proteins; acute symptomatic seizures; anti-seizure medications; Africa

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INTRODUCTION

Acute symptomatic seizures are common in children in the community and those admitted to hospitals in sub-Saharan Africa (1, 2), and falciparum malaria is the commonest cause of these seizures (3). Acute symptomatic seizures are associated with mental health, epilepsy, and neurocognitive comorbidity (1, 4, 5), complications which could be prevented by early treatment with anti-seizure medication (ASM) (6). Pharmacokinetic studies have identified safe doses of ASM that reach therapeutic ranges in children with falciparum malaria (7-9). Despite these drugs reaching the required therapeutic ranges in plasma, first-line ASMs such as diazepam failed to stop acute symptomatic seizures in up to 60% of children with falciparum malaria (10), but the underlying mechanisms are not fully understood.

Response to ASM in malaria-associated seizures may be determined by several mechanisms. First, the complex seizures represent severe neurological dysfunction and may be refractory to treatment compared to simple seizures (11). Animal models of severe malaria have shown a down-regulation of GABA receptors, which are often bound by these drugs (12), but the mechanisms in humans are not fully understood. There may be perturbations of extracellular ions in acute malaria infections (13), which may inhibit or enhance the action potential that generates seizure activity (14). The breakdown of the blood–brain barrier in severe falciparum malaria (15) may result in extravasation of macromolecules, particularly proteins, which may have anti-seizure activity when they change extracellular osmolality or be proconvulsant when they bind and interfere with the function of ASM in the brain tissues (16). Additionally, breakdown of the blood–brain barrier may increase access of ASM into the brain, thereby improving seizure control.

Levels of proteins such as albumin and ferritin in the cerebrospinal fluid (CSF) are biomarkers of impaired integrity of the blood–brain barrier and are elevated in about half of children with severe malaria (17). The accumulation of albumin or ferritin in the brain tissues may be proconvulsant, through reducing the bioavailability of ASM, or causing transcriptional changes that affect the generation of seizure activity (18). On the contrary, albumin is a colloid and may increase the extracellular osmotic concentration, which has been associated with decreased propensity for seizures (19). Since low ferritin levels are associated with seizures (20), elevated levels may improve neurotransmission and seizure control. Experimental studies show that elevated protein levels are associated with malaria risk (21), which can be avoided by co-administration of iron supplementation interventions to improve brain function, with antimalarial prophylaxis in endemic areas. Protein S100 beta (S100B) is a glial biomarker for neurological dysfunction (as a damage-associated molecular pattern) or inflammation (through interaction with intracellular signalling pathways for production of proinflammatory cytokines) (22).

We hypothesised that the blood–brain barrier dysfunction in severe falciparum malaria may result in extravasation of CSF proteins such as albumin and ferritin into the brain tissue, and cause (i) a pharmacokinetic binding effects that can reduce the bioavailability and efficacy of first line ASM (18); or (ii) an osmotic effect that can be associated with improved seizure control through repolarisation (19) and (iii) a neuro-inflammatory modulation that can provoke seizures (22). Clinical studies are needed to investigate these hypotheses to improve the understanding of the pathogenesis of acute seizures in severe malaria and inform the choice of ASM for seizure control.

A previous study in Kilifi, Kenya, examined the extent of damage to the blood–brain barrier in children admitted with clinical presentation suggestive of neurological involvement, including severe malaria (17). This study did not determine the risk for the occurrence of seizures and response to treatment with ASM in children with severe malaria with neurological involvement. We measured CSF and plasma total proteins, albumin, ferritin, and S100B concentrations in children with severe malaria to determine their association with risk for acute symptomatic seizures and the extent of disruption of the blood–brain barrier. The ratio of CSF/plasma levels was computed to approximate the extent of protein penetration into the brain tissues. Occurrence of complex seizures between those with impaired blood–brain barrier and those without was examined as well. We then

determined the association of these proteins with risk for seizures, seizure control and response to ASM.

METHODOLOGY

Study design:

This was an observational cohort study. Clinical information, including seizures, treatment, and collection and storage of blood samples, was prospectively collected. However, a retrospective analysis was conducted.

Study setting:

The 45 children in this retrospective analysis were admitted to the paediatric dependency ward of the Kilifi County Hospital between 1998 and 1999 with features of severe falciparum malaria. The hospital has a paediatric dependency unit for the management of severe childhood illnesses, including severe malaria, pneumonia and malnutrition. The hospital draws its admissions from Kilifi County, including a demographic surveillance area with about 300,000 people. Admissions with severe malaria peak during rainy seasons (from May to July every year), when transmission intensity for malaria is high.

Participants

Inclusion Criteria: Participants were included if they had falciparum parasitaemia and neurological signs that necessitated performing a lumbar puncture to exclude other causes of coma (17). The 45 children with malaria and signs of neurological involvement were drawn from children with (i) impaired awareness, (ii) malarial seizures in those under 2 years, (iii) prostration in those under 6 months, and (iv) clinical suspicion of meningitis.

Exclusion Criteria: Participants were excluded if they had no falciparum parasitaemia or neurological signs.

Study Procedures:

i) Treatment of seizures

During the period in which the study was conducted, children with severe falciparum malaria were treated with first-line intramuscular quinine or oral sulfadoxine pyrimethamine (23), plus a cover of recommended broad-spectrum antibiotics. Children admitted with seizures lasting 5 minutes were first treated with intravenous diazepam, and for those whose seizures did not stop within 10 minutes, a second dose of intravenous diazepam was given. Seizures lasting 15-20 minutes were managed with intravenous phenobarbital, and those continuing further with intravenous phenytoin. Seizures refractory to phenytoin were managed in consultation with senior clinicians, who would often recommend another dose of intravenous phenobarbital or midazolam to prevent further seizures, since artificial ventilation was not available.

ii) Laboratory measures

Plasma and CSF samples were collected during hospital admission (from 1998-1999) and stored in liquid nitrogen at −80 C until 2002, when protein assays were performed. Extension of the lifespan of critical cold storage is ensured through alarm-system-supported temperature monitoring, manual defrosting of refrigerators to prevent manual insulation, and regular servicing and cleaning of refrigerators. Those performing the assays were blinded to the clinical status of the children.

Malaria parasitaemia was determined by staining thick and thin blood slides and examined per 100WBC or 500 RBC (Beckman/Coulter, UK), and parasite density was calculated from the resulting counts expressed per total blood cell counts. The total protein in CSF was measured by turbidimetry using benzethonium chloride as described elsewhere (24). Albumin was measured using an electroimmunoassay technique (25), while ferritin and S100B were measured by sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA)(17). Reporting of the protein levels followed the reference intervals (RI) from the original study (17).

Definition of terms

Seizure status was available for all 45 children, even though 9 children may have missed one or more seizure characteristics e.g., duration and onset of seizures. Seizures were classified according to ILAE criteria in use at the time of analysis (26). Seizures were prolonged if they lasted >15 minutes, focal if they involved one body part, and repetitive if >1 seizure occurred in the same illness. Complex seizures comprised prolonged, focal and/or repetitive seizures. Simple seizures were those with generalised

manifestations (involving parts from both sides of the body), occurred once and lasted less than 15 minutes. Reduced response to ASM was defined as an inability of a single dose of a drug to stop seizures. The blood–brain barrier was considered impaired if the CSF/serum albumin ratio or quotient being above the reference interval, as previously explained (17). Impaired consciousness was defined as a Blantyre coma score of <3. Hypoglycaemia was defined as a glucose concentration <3.3mmol/L, and anaemia as haemoglobin concentration <5g/dL.

Outcomes: The primary outcomes were seizure control and response to ASM, while the secondary outcomes were risk for acute symptomatic seizures and the extent of disruption of the blood-brain barrier.

Statistical analysis:

All analyses were done with STATA (Version 13). Man-Whitney U test was used to compare continuous variables between (i) children with seizures and those without (ii) children requiring second line ASM and those whose seizures were controlled with or without first line ASM. Pearson's Chi-Square test and Fisher's exact test (when counts in a cell were infrequent) was used to compare categorical variables between these groups. Factors associated with seizures, or seizure control without need for 2nd line ASM, were determined using a generalised binomial model (as the outcomes were binary), with a log link, and specifications for robust standard errors. The risk ratios were computed from exponentiated coefficients of a generalised binomial model, which was adjusted for age, sex and weight of the child. A complete case-analysis approach was used. Based on the exploratory nature of the analysis and owing to the modest numbers, two-sided p-values for frequency distributions are provided throughout the analysis. Despite the modest sample size, a p-value of 0.05 was used for reporting or highlighting important comparisons.

Ethics:

Permission to conduct this study was obtained from the KEMRI National Ethical Review Committee (Number 966). Written informed consent was obtained from the study participants, through the parents or main givers of the children. Participants were assured of data confidentiality, protection and anonymity during and after the study.

RESULTS

Of the 45 children with malaria parasitaemia, 36 (80%) had acute symptomatic seizures, while 9 (20%) had no seizures. Complex seizures were documented in 32/36 (88.9%) children with acute symptomatic seizures, of which 29/32 (90.6%) were repetitive, 9/32 (28.1%) were focal, and 13/32 (40.6%) were prolonged. The clinical and laboratory features of severe malaria, such as hypoglycaemia, coma, base excess, and haemoglobin concentration, were similar between those with and without acute symptomatic seizures (Table 1).

CSF proteins and the risk of seizures in malaria

The concentration of CSF total protein, albumin, and ferritin was similar between those with and those without acute symptomatic seizures (Table 1). The ratio of CSF/plasma albumin levels did not differ between those with and without seizures. The concentration of CSF protein S100B was significantly increased in those with acute symptomatic seizures compared with those without (p=0.051). Similarly, in generalised linear models adjusted for age, sex and weight of the child, a unit increase in CSF protein S100B was associated with acute symptomatic seizures (adjusted risk ratio=1.06 (95%CI:1.01-1.11); P=0.012), but other proteins (total protein, albumin, and ferritin) were not (Table 2).

Protein concentrations were similar between complex acute seizures and controls (Fisher's exact P=0.47 for serum albumin; P=0.96 for CSF albumin; P=0.46 for total proteins and P=0.68 for ferritin), and with no significant associations in the adjusted models (Table 2). Among those with seizures, a unit increase in protein concentrations appeared to protect against complex seizures compared to simple seizures (adjusted risk ratio=0.64 (95%CI:0.48-0.85); P=0.002) for protein S100B, but not with other proteins (Table 2).

CSF proteins and control of seizures in malaria

The concentration of CSF albumin (z statistic P=0.037) and ferritin (z statistic P=0.003) was significantly elevated in those whose acute symptomatic seizures stopped spontaneously or stopped by first-line administration of diazepam compared to those requiring second-line ASM (Figure 1). Most other CSF proteins, except S100B, appeared increased in those with seizures that were easily stopped compared to those who required a second-line ASM (Table 3). In adjusted GLM models, seizures stopping spontaneously were associated with a unit increase in CSF ferritin (adjusted risk ratio=1.12 (95%CI:1.03-1.23); P=0.008), and CSF total proteins (adjusted risk ratio=1.01 (95%CI:1.00-1.01); P=0.023), with that for CSF S100B (adjusted risk ratio=1.33 (95%CI:1.00-1.78); P=0.051) showing borderline statistical significance.

Seizures and blood–brain barrier integrity

Significantly more seizures were easily stopped in those with evidence of blood–brain barrier disruption (as measured by elevated CSF total proteins and albumin and ratio of CSF/plasma albumin levels) compared to those without (Fisher's exact P=0.047) (Table 4). In adjusted GLM models, there was a significant association of blood–brain barrier disruption and seizures stopping easily (adjusted risk ratio=2.72 (95%CI:1.08-6.83); P=0.032).

Simple seizures were significantly more frequent in those with blood–brain barrier disruption compared to those without (Fisher's exact p=0.043) (Table 4). Other seizure phenotypes were not elevated with blood–brain barrier integrity. Among those with seizures, complex seizures appeared to have less blood–brain barrier impairment compared to simple seizures (adjusted risk ratio=0.57 (95%CI:0.33-0.98); P=0.042), but no other seizure phenotype was associated with blood–brain barrier in adjusted GLM models (Table 4).

Table 1: Comparison of participants' characteristics and CSF protein levels between those with and without acute symptomatic seizures in severe malaria

Features	Seizures (N=36)	No Seizures (N=9)	P-value
Age (months)	30.5 (18.5-43.7)	18.1 (6.6-28.9)	0.118
Male sex	16 (44.4%)	3 (33.3%)	0.710
Weight (Kg): median (IQR)	10.2 (8.3-13.0)	7.9 (5.5-10.2)	0.073
Neonatal jaundice present	2 (5.6)	1 (11.1)	0.497
Hypoglycaemia (<3.3mm/L)	6 (16.6%)	0	0.306
Coma	16 (44.4%)	2 (22.2)	0.279
BCS: median (IQR)	3 (2-4)	3 (1.5-3)	0.164
Base excess: median (IQR)	-7.8 (-12.8, -4.9)	-9.4 (-20.1, -5.5)	0.154
Haemoglobin (g/dL): median (IQR)	6.6 (5.4-12.2)	7.9 (5.4-12.2)	0.070
Treated with ASM	19 (52.7%)	-	-
CSF total proteins (mg/L): median (IQR)	364 (275.5-498.5)	387.0 (322-634)	0.590
CSF albumin (mg/L): median (IQR)	156.5 (135.5-206.0)	152.0 (135.0-280.0)	0.943
CSF ferritin (ng/mL): median (IQR)	2.5 (2.0-4.7)	2.4 (1.7-3.4)	0.629
CSF S100B (ng/mL): median (IQR)	0.19 (0.12-0.35)	0.13 (0.11-0.16)	0.051
CSF/serum albumin ratio: median (IQR)	4.7 (3.7-6.6)	3.8 (3.5-7.3)	0.681

IQR: interquartile range; BCS: Blantyre Coma Score

Table 2: Association between acute symptomatic seizures and protein levels

Proteins	All acute symptomatic seizures vs no seizures: RR (95%CI)	Complex acute symptomatic seizures vs no seizures: RR (95%CI)	Complex acute symptomatic seizures vs simple seizures: RR (95%CI)
CSF total proteins (mg/L)	0.9999 (0.9994-1.0004)	0.9999 (0.9994-1.0004)	0.9999 (0.9995-1.0002)
CSF albumin (mg/L)	0.9999 (0.9979-1.0016)	0.9995 (0.9974-1.0017)	0.9992 (0.9978-1.0007)
Serum albumin (mg/L)	1.0010 (0.9880-1.0142)	1.0017 (0.9867-1.0168)	1.0019 (0.9831-1.0210)
CSF ferritin(ng/mL)	0.9950 (0.9705-1.0201)	0.9904 (0.9607-1.0210)	0.9884 (0.9537-1.0244)
CSF S100B (ng/mL)	**1.0581 (1.0124-1.1059)**	1.8538 (0.8619-3.9873)	**0.6413 (0.4812-0.8547)**
CSF/serum albumin ratio	0.9974 (0.9341-1.0651)	0.9923 (0.9226-1.0671)	0.9799 (0.9386-1.0230)

RR: adjusted risk ratio; protein levels were entered as continuous variables into the model; risk ratio estimates are provided up to 4 decimal places for ease of interpretation; there were separate regression models for each protein

Table 3: Distribution and associations of CSF protein levels with seizure control status

Features	Seizures stopped with or without first-line ASM (n=23)	Required second-line ASM (n=13)	P-value[a]	50th Percentile protein level difference (95%CI)[c]	Adjusted associations with seizures stopping with or without first-line ASM (RR (95%CI))	P-value[b]
Median CSF total proteins (mg/L, IQR)	389 (295-592)	332 (247-380)	0.106	-160 (-421, -17)	**1.0048 (1.0006-1.0090)**	**0.023**
Median CSF albumin mg/L, IQR	**178 (141-214)**	**144 (118-152)**	**0.0377**	-36.5 (-72, -8)	1.0088 (0.9940-1.0238)	0.244
Median serum albumin (mg/L, IQR)	32.5 (31-34.5)	36.5 (34-39)	0.4720	3.5 (-2, 7)	0.9340 (0.7941-1.0944)	0.399
Median CSF ferritin (ng/mL, IQR)	**3.4 (2.4-5.3)**	**1.8 (1.6-2.2)**	**0.003**	-1.75 (-6.8, -0.2)	**1.1275 (1.0312-1.2327)**	**0.008**
Median CSF S100B (ng/mL, IQR)	0.19 (0.12-0.37)	0.18 (0.12-0.34)	0.717	-0.03 (-4.67, 0.09)	**1.3335 (0.9984-1.7811)**	**0.051**
CSF/serum albumin ratio	5.3 (3.9-6.8)	4.0 (3.2-5.8)	0.130	-1.05 (-2.36, 0.59)	1.2570 (0.7539-2.0958)	0.380

[a]Mann-Whitney U test comparisons; [b]p-value from log binomial models; RR: risk ratio; CI: confidence interval; risk ratio estimates are provided up to 4 decimal places for ease of interpretation; [c]Hodges–Lehmann median group differences for the 50th percentile; there were separate regression models for each protein; proteins were entered into the models as continuous variables

Table 4: Distribution and associations of acute symptomatic seizures with blood–brain barrier integrity

Features	Blood–brain barrier impaired (n=24)	Blood–brain barrier intact (n=21)	P-value[a]	Associations with blood–brain barrier disruption (RR (95%CI))	P-value[b]
All seizures	19 (79.2%)	17 (80.9%)	1.00	1.04 (0.54-2.00)	0.885
Status epilepticus	8/23 (34.8%)	5 (23.8%)	0.518	1.17 (0.67-2.06)	0.567
Repetitive seizures	14/23 (60.9%)	15 (71.4%)	0.535	0.81 (0.43-1.52)	0.521
Focal seizures	2/23 (8.7%)	7 (33.3%)	0.043	0.41 (0.12-1.43)	0.164
All complex seizures	16/19 (84.2%)	16/17 (94.1%)	0.043	0.95 (0.48-1.87)	0.902
Seizures didn't require second-line ASM	**15/19 (78.9%)**	**8/17 (47.1%)**	**0.047**	**2.72 (1.08-6.83)**	**0.032**

[a]Fishers exact or Pearson Chi-Squared test; [b]P-value from log binomial models; RR: risk ratio; CI: confidence interval; risk ratio estimates are reported up to 2 decimal places; 9 children had missing details on seizure characteristics or protein levels

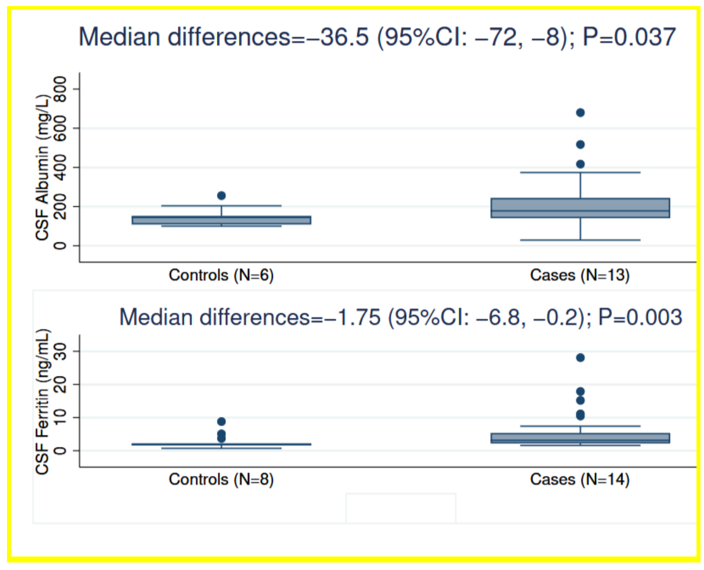

Figure 1 Distribution of albumin (top panel) and ferritin (bottom panel) by response to anti-seizure Medication. Seizures that stopped easily are grouped as the "Cases", while those that stopped after 2 or more anti-seizure medications are grouped as "Controls". Hodges–Lehmann median group differences are computed while P-values are based on Mann-Whitney U test comparisons.

DISCUSSION

The findings suggest that easier control of seizures by ASM is associated with the presence of elevated total proteins, S100B, and ferritin, but the mechanisms underlying this could not be elucidated in the study. Additionally, seizures would stop without initiation of second-line ASM if the blood–brain barrier is disrupted compared to when it is not. Only protein S100B had a borderline association with an increased risk of simple seizures, which, unlike complex seizures, are likely to stop spontaneously with or without treatment. A proinflammatory process likely contributes to damage of the blood–brain barrier, because of the association of simple seizures with proteins such as S100B. S100B may be a context-dependent marker, whereby it is associated with seizures and is also part of a compensatory response that may enhance the ASM response.

The findings suggest that elevated total proteins, S100B, and ferritin in CSF may be associated with anti-seizure activity. This is further supported by the ability of seizures to stop spontaneously or by responding to first-line ASM among those with a blood–brain barrier disruption, which is defined by elevated total proteins. These results are surprising since blood- brain barrier disruption has been associated with difficult-to-treat seizures in epilepsy in other populations and settings that are not endemic areas for malaria (27). Our findings, which are based on children with malaria-associated seizures living in a malaria-endemic area, can be interpreted in several ways. They may suggest that the presence of these proteins in the CSF does not bind the ASM to levels that can affect the bioavailability of the drugs in the brain tissues, which requires investigation in follow-up studies. Most ASMs used in this study underwent pharmacokinetic and pharmacodynamic evaluation in the context of malaria seizures (8, 9), but the impact of elevated CSF proteins on therapeutic dosages in severe malaria was not examined. The possible explanation for this finding is that a damaged blood–brain barrier allows ASM to enter the brain faster and terminate the seizures. Alternatively, as these proteins are colloid in nature, they may increase extracellular osmolality, which affects epileptiform activity in hippocampal slices (28) via repolarisation (29).

Low ferritin concentration is associated with febrile seizures (20), and so high ferritin levels may result in improved neurotransmission, thereby reducing seizures. However, ferritin may be a marker of brain injury in falciparum malaria, and is also related to the inflammatory process (30) that results in seizures. Seizures may also have stopped more easily because proteins, particularly S100B, were positively associated with simple seizures, which stop spontaneously or respond more quickly to ASM than complex seizures (9). The role of protein S100B in acute symptomatic seizures was interesting, as it was associated with both risk for all seizures and reduced risk for complex seizures, which may be specific to the context and population of our study. The role in increased risk for all seizures is consistent with findings from previous studies (31). Recent evidence suggests that S100B is a marker of brain injury (as a damage-associated molecular pattern) and is proinflammatory (through interaction with intracellular signalling pathways for inflammatory cytokines) (32), and so the association with seizures is not surprising. The role of S100B in the activation of proinflammatory cytokines may be explained by the borderline increasing risk for simple seizures (33), while another mechanism (e.g., direct brain injury from malaria sequestration) is responsible for complex seizures. Nonetheless, higher S100B levels may track a higher probability of simple seizures that self-terminate, creating an apparent association with easier control. It is unclear if extravasation of S100B into brain tissues would have the same epileptogenic effects as ferritin (a marker of iron status) (34, 35). Further evidence on the impact of protein levels in brain tissues on increased risk of seizures is required to clarify the accumulating evidence from transcriptome analyses of protein expression, including neurofilament-light chain (20, 36).

Simple seizures and easier control of seizures were associated with blood–brain barrier breakdown and could suggest seizures with generalised onset, which self-terminate, characterise the diffuse brain injury observed in cerebral malaria. Febrile seizures, most of which have a generalised onset, have been associated with immunoglobulin abnormalities in CSF (37), including in Kenyan children (17). A neuroinflammatory response may be responsible for the blood barrier damage, since simple seizures had a borderline association with S100B (described above), which is a proinflammatory protein (33). The blood-brain barrier hypothesis in

seizure control and response to treatment in malaria seizures cannot be directly inferred in epilepsy populations from settings that are not endemic for malaria. The pathophysiological process for the blood–brain barrier in epilepsy is a consequence of prolonged seizures and ends in refractoriness to ASM (38), which may differ across populations and settings.

The strength of this study is the measurement of levels of several proteins, which are markers of blood–brain barrier damage, and the careful phenotyping of acute symptomatic seizures. These protein measurements were not serial and would not capture any changes in CSF concentration before and after the seizures, nor were they correlated with electroencephalogram (EEG) or neuroimaging findings. CSF samples may be contaminated by blood, raising levels of proteins such as ferritin, although this can be independent of the outcome of interest. The samples were stored in liquid nitrogen (at -80 C) to minimise possibilities of degradation at the time of the analysis of the protein assays, but batch-assay effects cannot be completely avoided. Findings from nearly three decades ago may not be generalisable to the present era of advanced treatments. Selection bias may be introduced by failure to perform a lumbar puncture on all eligible children, especially when neurologically unstable. The lack of association of seizure outcomes with measures of the ratio between CSF and plasma protein levels may imply there was limited penetration of these proteins into the brain tissues. The reference ranges are based on the European population since it was difficult to measure these in healthy Kenyan children. Seizure control outcomes may have been different if the study had been conducted in the era when artesunate was available and ASM management protocols had evolved. Collider bias may be introduced while accounting for variables related to exposures and outcomes. A history of preadmission seizures may have influenced the levels of proteins (39). The study did not investigate the background inflammation and malnutritional conditions, e.g. Kwashiorkor, which correlate with the levels of some measured proteins. Ongoing seizure activity within the brain could not be ruled out due to the lack of EEG and neuroimaging services. The small sample size limited the power to detect associations and adjustment for multiple testing, for example, and larger longitudinal studies, measuring many more brain injury protein markers, and relating these to neuroimaging findings are required to confirm these studies.

CONCLUSION

In conclusion, the presence of elevated CSF proteins particularly ferritin and total proteins may be associated with improved seizure control or response to ASM in Kenyan children with severe malaria, but the mechanisms underlying this should be investigated. Protein S100B is associated with seizures in malaria and its proinflammatory response may be responsible for simple seizures which stop spontaneously or respond promptly to ASM. Susceptibility to seizures in children with protein-energy malnutrition and inflammation should also be examined in future studies. Future well-powered studies are required to confirm these findings, and these studies should measure the levels of these CSF proteins and perform neuroimaging in relation to the timing of occurrence of seizures, initiation of treatment and clinical response to ASM.

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