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Identifying biomarkers for epilepsy after cerebral malaria in Zambian children: rationale and design of a prospective observational study

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ABSTRACT

Introduction Malaria affecting the central nervous system (CM) is a major contributor to paediatric epilepsy in resource-poor settings, with 10%–16% of survivors developing epilepsy within 2 years of infection. Despite high risk for post-malaria epilepsy (PME), biomarkers indicating which CM survivors will develop epilepsy are absent. Such biomarkers are essential to identify those at highest risk who might benefit most from close surveillance and/or preventive treatments. Electroencephalography (EEG) contains signals (specifically gamma frequency activity), which are correlated with higher risk of PME and provide a biomarker for the development of epilepsy. We propose to study the sensitivity of quantitative and qualitative EEG metrics in predicting PME, and the potential increased sensitivity of this measure with additional clinical metrics. Our goal is to develop a predictive PME index composed of EEG and clinical history metrics that are highly feasible to obtain in low-resourced regions.

Methods and analyses This prospective observational study being conducted in Eastern Zambia will recruit 250 children aged 6 months to 11 years presenting with acute CM and follow them for two years. Children with pre-existing epilepsy diagnoses will be excluded. Outcome measures will include qualitative and quantitative analysis of routine EEG recordings, as well as clinical metrics in the acute and subacute period, including histidine-rich protein 2 levels of parasite burden, depth and length of coma, presence and severity of acute seizures, presence of hypoglycaemia, maximum temperature and 1-month post-CM neurodevelopmental assessment scores. We will test the performance of these EEG and clinical metrics in predicting development of epilepsy through multivariate logistic regression analyses.

Ethics and dissemination This study has been approved by the Boston Children's Hospital Institutional Review Board, University of Zambia Biomedical Research Ethics Committee, and National Health Research Authority of Zambia. Results will be disseminated locally in Zambia followed by publication in international, open access, peer-reviewed journals when feasible.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The prospective study design and data collection will allow for better understanding of postmalaria epilepsy development.
⇒ Primary data collected is standardly accessible in low-resource regions.
⇒ Both clinical and quantitative electroencephalography metrics will be tested for postmalaria risk prediction, in combination and individually.
⇒ Due to resource constraints, correlation to neuroimaging is not feasible.

INTRODUCTION

Over 45 million people live with epilepsy globally,[1–3] 80% of whom live in lower-resource countries.[4 5] Over 25% of these epilepsy cases are acquired as a result of central nervous system (CNS) infection or trauma.[6 7] Malaria, a parasitic infection caused by *Plasmodium falciparum*, contributes significantly to the burden of acquired epilepsy when it affects the CNS (CM), particularly in sub-Saharan Africa (SSA), where resources are limited.[8] The risk is highest for children under the age of 5 years.[9] Over 30% of paediatric CM survivors are estimated to develop neurodevelopmental sequelae detectable within 2 years of acute illness; for 10%–16% of survivors, these sequelae will include postmalaria epilepsy (PME).[10–17] Cerebral malaria (defined as coma and malaria parasitaemia, in absence other coma aetiology)[9] has been better studied than malaria that affects the CNS in general (manifested by alteration of consciousness or complicated seizures), yet the two conditions have been shown to have similar rates of neurodevelopmental sequelae.[10 16 18] Thus, any malaria infection affecting the CNS (CM) has high rates of

PME development in survivors. With over half a million CM infections annually and a 20% fatality rate in those with cerebral malaria,[19] there are over 40 000 newly acquired, and potentially preventable, paediatric epilepsy cases attributable to malaria per year in regions where the rates of epilepsy are highest and where limited resources are available to tackle this burden.

In general, there remains a need for reliable biomarkers of epileptogenesis for people with risk of an epilepsy syndrome (ie, after traumatic brain injury or brain infection).[20] As in most acquired epilepsies, PME emerges in select children after a months-long seizure-free period following acute CM infection.[10 12 14 21 22] Ascertaining predictive factors for those at highest risk of developing PME has significant potential to impact clinical care in this condition, as well as potentially advance knowledge of epileptogenesis in acquired brain injuries overall.

Identification of those who would benefit from close observation is an essential consideration in lower-resource regions, where routine follow-up for all patients is not feasible. Identification of biomarkers that predict epilepsy risk could also be used to test potential antiepileptogenic neuroprotective therapies or select appropriate children for clinical trials evaluating such interventions. As revealed from the EPISTOP trial in tuberous sclerosis, disease-modifying therapy has potential not only to impact epilepsy development but to reduce severity of neurodevelopmental impairments.[23 24]

We propose a practical approach for utilising metrics to create a predictive model that would be feasible across settings, including low-resource regions. Specifically, we propose to use data acquired from standardly acquired electroencephalograms (EEGs), through both quantitative and qualitative analyses, in conjunction with clinical metrics of acute infection and early recovery phases during malaria for development of an individual risk prediction model. Quantitative EEG predictors of neurodisability in adults and children after cardiac arrest have been described,[25–27] and similar EEG techniques have recently been used to demonstrate frequency band metrics associated with mortality and neurological morbidity during hospitalisation for acute CM.[28]

We propose that quantitative EEG metrics hold even more promise as a biomarker for PME. Activity of fast-spiking, parvalbumin-positive (FS-PV+) GABAergic inhibitory interneurons, a cell population that progressively declines over the course of epileptogenesis,[29 30] has been shown to be reflected by low frequency EEG gamma (30–60 Hz) activity.[31–33] Animal models of acquired epilepsy demonstrate an initial peak of gamma frequency activity, suggestive of initial hyperactivation mediated by acute glutamate toxicity of the FS-PV +interneuron population, followed by a progressive *decrease* of gamma EEG power, reflecting ultimate excitotoxic injury and cell death.[34] Our group looked at 70 standardly acquired EEGs from Malawi, acquired within the first 24 hours of acute hospitalisation for paediatric cerebral malaria, to test this measure. We found that not only is extracting low

frequency gamma power through spectral analyses from EEGs obtained in SSA with minimal computing requirements feasible, but notably that an initial acute increase in gamma frequency is predictive of PME.[35] Our finding of acute increase correlation of gamma EEG power with PME development matches the preclinical data from animal models,[34] suggesting promise of this technique as a biomarker for PME.

We now propose to assess this measure in a larger-scale, prospective observational study, hypothesising that we will find the same two-phased curve as demonstrated by animal studies, with an initial peak and then slow decline of gamma frequency activity (figure 1). Through serial EEG monitoring, we anticipate narrowing the window of time during which we identify the occurrence of epileptogenesis, providing a mechanism for monitoring and potential intervention.

Furthermore, while we predict that EEG gamma frequency activity will be associated with risk of PME, we hypothesise that other quantitative and qualitative EEG metrics as well as clinical features of acute CM presentation, including maximum temperature, length and severity of coma, presence of acute seizures, presence of hypoglycaemia and prior medical history risk factors (ie, HIV, prior neurodevelopmental disability), would all increase risk of PME in this population. Therefore, we will test these various metrics both individually and in combination via logistic regression to select the combination of EEG and clinical features most predictive for PME development and develop a multivariable model for individual PME risk. Such a measure of individual PME risk would allow monitoring and management of this high-risk population.

METHODS AND ANALYSES
Study design
This observational prospective cohort study will recruit a goal sample size of 300 children admitted for CM at Chipata Central Hospital (CCH), Chipata, Zambia, over a 3 year period. CCH is a 600-bed third-level provincial referral hospital in Eastern Zambia, serving approximately 1.5 million people and encountering a high burden of severe malaria cases annually. The research will be conducted through the paediatrics department, which has a full EEG lab for research and clinical purposes, routine laboratory diagnostic services, and a CT scanner.

Recruitment
Subjects will be enrolled on a rolling basis, as malaria is endemic year-round in this region of Zambia. The local study team will be notified of any child presenting with malaria (by rapid diagnostic test and confirmatory blood smear) and neurological symptoms (depressed level of consciousness or seizures); those aged 6 months to 11 years are further screened for inclusion in the study. This screening includes confirmation of malaria diagnosis, eligible age and meeting criteria for either (1)
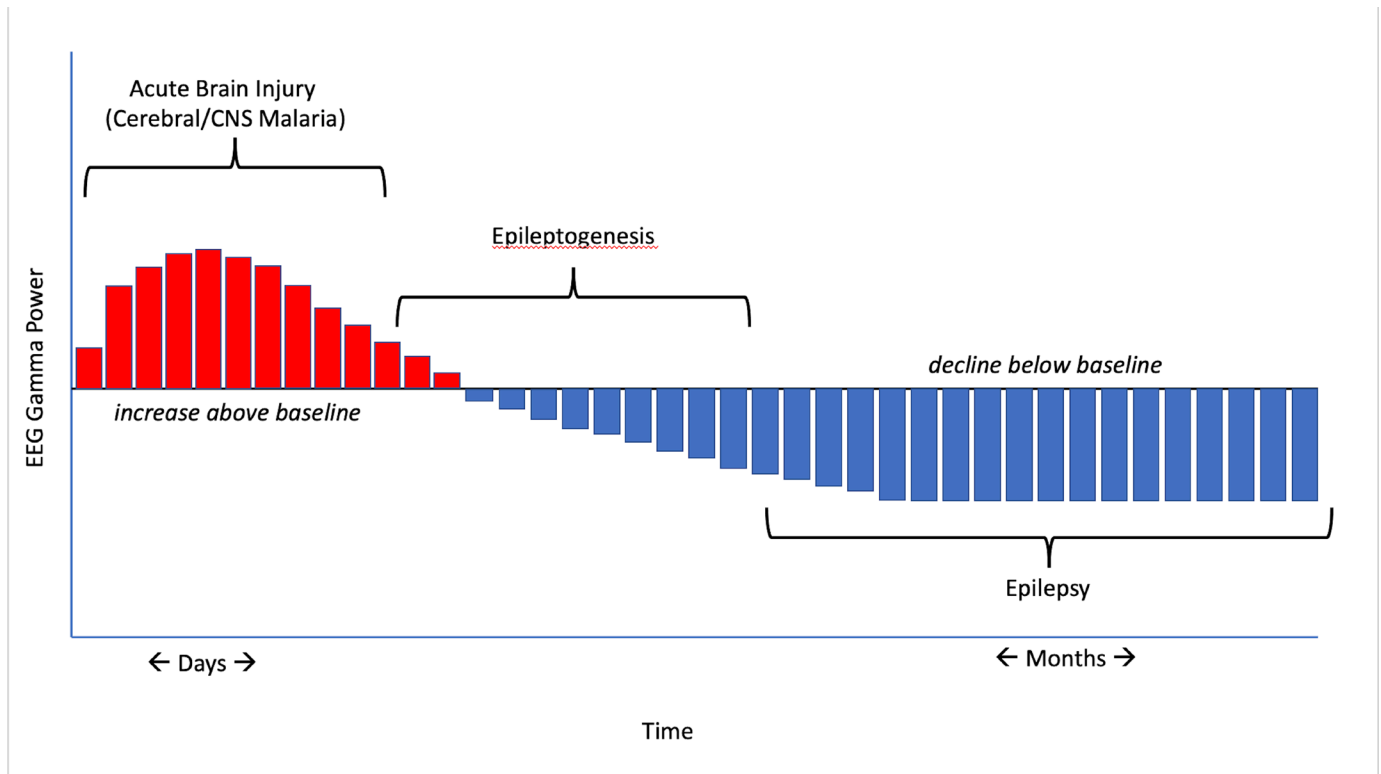
Figure 1 An initial peak of EEG gamma frequency activity is predicted to be seen during malarial infection affecting the central nervous system, followed by subsequent decline during epileptogenesis, in children who will ultimately develop postmalaria epilepsy. CNS, central nervous system; EEG, electroencephalography.

'Cerebral malaria'—defined as impaired consciousness with Blantyre Coma Score (BCS) of ≤2 in children under 2 years of age, or a Glasgow Coma Score (GCS)≤10 in children ≥2 years, without any other explanation for coma, or (2) 'CNS Malaria', defined as complicated seizures (either prolonged ≥15 min, focal or multiple) or impaired consciousness without frank coma (ie, BCS 3–4, GCS 11–14). To identify children with a pre-existing epilepsy for exclusion, caregivers will be explicitly asked if the child is/has been on antiseizure medications, has had two or more seizures without fever or trauma, or has been given a diagnosis of epilepsy by a clinician previously in effort to capture all pre-existing epilepsy patients. Additional exclusion criteria include another acute CNS infection, clinically identifiable toxin ingestion and head trauma within twenty-four hours. If qualified, caregivers are invited to participate once the child is clinically stable and will be consented on agreement.

Patients and public involvement
The study design has been developed with significant input from medical providers in the region, and Information about this study has been dispersed to the public locally for awareness with feedback from the community and local providers used to ensure methodology, and particularly enrolment procedures, is culturally appropriate. While outcome measures were chosen based on prior evidence, finalisation of relevant measures and mechanisms was done with the local study team who are

part of the community to ensure that these would have benefit. Dissemination of results will be performed locally through the hospital to the community.

Power and sample size calculation
Power and sample size calculation was conducted based on the hypothesis test for detecting the difference of gamma-delta power ratio in two study groups: CM survivors who develop epilepsy and CM survivors who do not develop epilepsy. Our preliminary studies assessing spectral EEG analyses between these groups revealed a significantly higher gamma-delta power ratio in CM survivors who developed epilepsy (mean: 0.23, SD: 0.10) than in those who did not (mean: 0.16, SD: 0.06).[35] Based on this data and reported rates of PME in CM survivors within 2 years (10%–16%),[10 12–15 18] our current calculation shows a sample size of 250 (25 for CM +Epi and 225 for CM-Epi; ratio is 1:9) achieves 90% power to reject the null hypothesis of equal means in gamma-delta power ratio for the two groups, with a significance level (type I error) of 0.05 using a two-sided two-sample unequal-variance t-test. Power and sample size calculations were performed using PASS V.15 Power Analysis and Sample Size Software (NCSS).

Outcomes measured
The primary end point of interest in this study is the development of PME in CM survivors. Secondary outcomes include neurodevelopmental impairment, including

Table 1 Schedule of outcome measurements

	Acute Hospitalisation	1-month postadmission	6-month postadmission	12-month postadmission	18-month postadmission	24-month postadmission
Patient characteristics						
Sex	X	X	X	X	X	X
Weight	X	X	X	X	X	X
Pre-CM illness history (HIV status, prior neurological disease including epilepsy or ASD, family history of seizures/epilepsy)	X					
Caregiver perception of wellness/recovery		X	X	X	X	X
Sleep quality		X	X	X	X	X
School attendance		X	X	X	X	X
Clinical metrics						
Age	X	X	X	X	X	X
Weight	X	X	X	X	X	X
Coma score	X					
Coma duration	X					
Maximum temperature	X					
Presence/absence of acute symptomatic seizures	X					
Use of antiseizure medications	X	X	X	X	X	X
Diagnostic metrics						
Glucose level	X					
Parasite burden (HRP2 level)	X					
EEG	X	X	X	X	X	X
Developmental Impairment and ASD Screening						
Ten Questions Screen	X					
23Q Screen		X	X	X	X	X
Epilepsy assessment						
WHO Epilepsy Screen		X	X	X	X	X
Neurological assessment						
Malawi Developmental Assessment Tool if ≤6 years old, neurological exam for subtle signs if >6 years old		X	X	X	X	X

CM: malaria affecting the central nervous system (including cerebral malaria as defined by WHO as well as any malaria associated with prolonged seizure activity)
ASD, autism spectrum disorder; EEG, electroencephalography; HRP2', histidine-rich protein 2.

autism and motor impairment. Data will be collected during acute CM infection and over the subsequent 2 years (table 1).

We will enrol children who present with acute CM infection. As part of the study procedures, the local community has been made aware of this study for sensitisation purposes. When a child presents with acute CM, the caregiver will be approached when the child is deemed stable and clinical team feels that approach is appropriate. The caregiver will be taken to a designated quiet spot away from the patient to review the study and offer enrolment in the local language (Nyanja). Of note, due to the nature of acute CM presentation, assent is not feasible.

During enrolment, baseline patient characteristics, including age, sex, HIV status, prior neurological conditions and developmental status (by the validated and regionally used Ten Questions Questionnaire[10 36] will be recorded. Testing for SARS-COV2 among children with malaria is not standard of care in this setting and recent research has demonstrated strong parental opposition to testing due to COVID-19-related stigma particularly ostracism of the parent–child dyad by other families on the inpatient service when positive tests occur. As such, SARS-COV2 testing will not be completing for research purposes. If collected for clinical care purposes, the information will be captured.

Acute CM clinical metrics of interest will be recorded throughout hospital admission, including coma score and duration, maximum temperature, blood glucose measurements and presence or absence of acute clinical seizures. Additionally, blood sample by finger prick will be collected within 24 hours of admission to obtain a histidine-rich protein 2 (HRP2) level (by ELISA) as a marker of parasite burden.[37] The results will not be available in real time as samples are delivered to a collaborating laboratory site in Malawi, due to absence of malaria microscopy expertise at CCH; thus, these levels will be used only for study analyses purposes.

Within 24 hours, a standard 30 min EEG will be recorded by trained EEG technologists, using Natus equipment, XLTEK software and a standard international 10–20 system, at a sampling rate of 512 Hz will be used.

We will conduct five follow-up visits of survivors (at 1-month, 6-month, 12-month, 18-month and 24-month postinfection time points) after initial CM presentation. Each follow-up evaluation consists of standard 30 min awake and sleep EEG and clinical neurodevelopmental screening. Melatonin will be used as needed for induction of sleep during the EEG, with administration done prior to the beginning of setting up the EEG (1 mg for children ≤3 years and 3 mg for those >3 years with option to repeat once if no signs of falling asleep within 25 min).[38 39] The neurodevelopmental screening consists of (1) general follow-up information including weight, any antiseizure medication use, overall caregiver impression of recovery, sleep quality and school attendance (when applicable), (2) a standardised Epilepsy (WHO) screening questionnaire used in prior paediatric cerebral malaria studies in the region,[10] (3) 23Q Developmental Screen, which consists of the Ten Questions Questionnaire with expansion to screen for autism spectrum disorder, validated in Uganda,[40 41] and a neurodevelopmental assessment via either the Malawi Development Assessment Tool[36 41] if ≤6 years old or the Neurological Exam for Subtle Signs[42] if >6 years old. Any positive epilepsy or neurodevelopmental screens will result in confirmatory diagnosis by a board-certified paediatric neurologist (AAP). The primary outcome of epilepsy will be made by the board-certified neurologist based on International League Against Epilepsy criteria.[43]

Of note, currently, there is no standard follow-up of these patients, nor is there any paediatric neurologist available in the region for a specialist review. Any positive findings will be managed by available resources at CCH, including physiotherapy for any motor impairments and paediatric/psychiatric referral for any behavioural diagnoses. Positive epilepsy diagnoses will have appropriate treatment initiated by the study neurologist in conjunction with a local paediatrician, who will then follow the child. These evaluations and interventions are above the current available standard of care.

Acquired EEG will be analysed using commercial and in-house software to assess power in each frequency band by wavelet transform, specifically looking at trends of power band ratios for delta, theta, alpha, beta and lower range gamma frequencies (30–60 Hz). Each study will be deidentified with only age available for interpretation, and remotely undergo visual interpretation via secure web-based access by a clinical neurophysiologist. A standardised form for interpretation is used for the study, documenting the presence or absence of the variables of interest. EEGs are read in real time for clinical purposes, and any impact on treatment recorded separately. All antiseizure medication use will be documented throughout study participation as an independent variable for analyses of impact on outcomes and predictive modelling.

Statistical analysis plan

The clinical metrics of interest will be tested for association with the primary outcome of interest, PME, by t-test and one-way analysis of variance (ANOVA); and if needed by their non-parametric alternatives Mann-Whitney test and Kruskal-Wallis test. Analysis of receiver operating characteristic will be used to investigate the prediction performance of gamma activity on epilepsy development. Relative gamma power (30–60 Hz) normalised to the whole power band will be plotted against whether PME develops, using the data points for the EEGs at each time point of interest. These will be analysed to determine which time point of assessment reveals the largest difference between groups (CM survivors with PME and those without) in gamma frequency activity. Conventional EEG metrics and spectral analyses of each power band (delta, theta, beta, gamma) will also be assessed and will be analysed individually using chi-squared test or one-way ANOVA, and in combination by logistic regression with variable selection for association with risk of PME development.

Interim data analyses will routinely be performed to identify the most relevant metrics for risk prediction. Multivariate logistic regression analysis will be conducted with these EEG and clinical metrics ascertained at each time point and, using initial data sets, the predictive algorithm will be built on variable selection. Prediction performance will be evaluated with the interim data sets to ultimately identify the combination of clinical and EEG metrics with highest predictive capacity for determining risk of PME. Ultimately, a multivariable logistic regression model will be built with the combination of metrics of highest predictive capacity.

Data management and monitoring

Data will be stored directly into a secure, password-protected electronic database. Data quality checks of all entered forms in the electronic database will be performed on a biweekly basis to ensure accuracy and reliability. Any missing data will be reviewed by the clinical study team, and best efforts to complete accurately with hospital data files will be performed. Any missing data that cannot be directly confirmed will be categorised as missing and excluded from analyses. Interim analyses will be performed every 6–12 months, with frequency based on rate of recruitment, with any outlying data reviewed by two study members and confirmed by source data if necessary.

Ethics and dissemination

This study has been approved by the Boston Children's Hospital Institutional Review Board (IRB-P00038309), University of Zambia Biomedical Research Ethics Committee and National Health Research Authority of Zambia (2529/2022). All publications and reports that result from this work will be produced with involvement and approval of all key personnel of the study. Results will be shared with relevant personnel and NHRA throughout

DISCUSSION

Paediatric CM survivors present a unique population through which to study epileptogenesis and identify biomarkers forecasting PME due to the identifiable aetiology of injury and relatively high prevalence and risk of epilepsy in survivors within a relatively short timeframe. Identification of such biomarkers has significant implications for clinical practice because it can provide a mechanism to indicate which patients warrant closer observation due to higher risk of developing epilepsy, in addition to providing measures that can facilitate antiepileptogenic trials in PME, with potential applicability to other forms of acquired epilepsies.

As the highest burden of highly morbid and fatal CM is in SSA, it is essential that a predictive epilepsy model for PME consider the resource restrictions of the region where it is most prevalent,[9] and be feasible and applicable within this setting going forward. Therefore, we propose metrics that can be obtained with relative ease at most tertiary care centres in SSA, where children with cerebral malaria are predominantly treated due to the complexity of their care needs. This includes EEG (available in over 80% of African countries,[44] clinical metrics and basic laboratory measures). Neuroimaging is not included in our proposed study, as the goal is to use metrics of routine care, and MRI and CT capacity is variable with inconsistent use for clinical care, even in tertiary care centres across Africa.[44]

If successful, this study has the potential to provide not only a mechanism for improved stratification of risk of epilepsy after CM, but also provide a biomarker of epileptogenesis in this population. Such a biomarker would provide the means to subsequently test antiepileptogenic therapies, including a range of inexpensive compounds whose anti-epileptogenic potential is supported by preclinical and clinical data.[45] Additionally, this study will be one of the only to prospectively look at risk of autism after CM and will further assess if quantitative EEG metrics have value in predicting risk of neurodevelopmental impairment in addition to epilepsy.

The proposed study and metrics to be studied over a 2-year period will provide a better understanding of the risk factors involved for development of neurological sequelae after CM, particularly PME and will provide potential avenues for both improved monitoring and potential intervention, key needs for a disease that continues to affect a large number of children in low-resourced regions annually.

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