Predicting Acute and Post-Recovery Outcomes in Cerebral Malaria and Other Comas by Optical Coherence Tomography

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Short title: Optical coherence tomography in cerebral malaria (OCT in CM)

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Collaborators

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Neuroradiologist with expertise in MRI imaging in children who will provide expertise in analysis of MRI brain scans including brain volume and cerebral atrophy.

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Biostatistician with expertise in clinical studies, including in ophthalmology, and multivariate statistical modelling, who will lead on statistical aspects of data collection and analysis.

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Neurodevelopmental paediatrician with expertise in assessing children's neurological function in LMICs who will lead the acquisition and analysis of neuro-developmental data collected on children in follow up.

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Ophthalmologist subspecialised in paediatric and neuro-ophthalmology who has pioneered the use of OCT in childhood disease. Prof Gottlob will provide expertise in interpretation of OCT images.

Prof Simon Harding	Professor of Clinical Ophthalmology, Dept Eye and Vision Science, University of Liverpool, UK

Ophthalmologist subspecialised in retinal diseases providing expertise on malarial retinopathy and its image analysis.

Dr Christopher Moxon	Senior Lecturer, University of Glasgow and Malawi-Liverpool-Wellcome Trust Clinical Research Programme, UK

Infectious diseases physician researching haematological and immune dysfunction in severe malaria, who will provide insights into malaria pathogenesis including brain swelling, coagulopathy and immune dysfunction

Prof Yaochun Shen	Prof Electrical Engineering, Dept Electrical Engineering and Electronics, University of Liverpool. UK

Electrical Engineer with expertise in novel medical imaging devices who will be providing oversight of the development of a low-cost, robust hand-held OCT.

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Research sites: Queen Elizabeth Central Hospital, Malawi; Blantyre Malaria Project, Malawi.

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Abbreviations

ABC	Assessment battery for children
AUC	Area under the curve
CM	Cerebral malaria
COM	College of Medicine, now Kamuzu University of Health Sciences
COMREC	College of Medicine Research Ethics Committee
CTIMP	Clinical Trial of Investigational Medicinal Product
CRSU	Clinical Research Support Unit
ICP	Intracranial pressure
GCP	Good Clinical Practice
KUHeS	Kamuzu University of Health Sciences
LMIC	Low and middle income countries
MDAT	Malawi developmental assessment tool
MLW	Malawi-Liverpool-Wellcome Trust Clinical Research Programme
MRI	Magnetic resonance imaging
NDD	Neurodevelopmental deficit
OCT	Optical coherence tomography
ONH	Optic nerve head
REC	Research ethics committee
ROC	Receiver operating characteristic
SAE	Serious Adverse Event
QECH	Queen Elizabeth Central Hospital, Blantyre
UoL	University of Liverpool
WP	Work Package

Executive Summary

Type of study
This is an observational cohort study of children with cerebral malaria (CM) and other comas conducted at a single-site. We will assess ocular imaging techniques as methods to identify severe brain swelling during the acute illness, and predict neurodevelopmental deficits after CM.

Problem
Children in Africa continue to die in large numbers from CM, mostly with severe brain swelling. New treatments for severe brain swelling and to reduce intracranial pressure (ICP) in CM are under clinical trial, but rely on MRI scans to identify severe brain swelling. Hardly any children with CM have access to MRI scanning, so those likely to benefit from new treatments are not identifiable. Children in coma for other causes would also benefit from the identification of raised intracranial pressure.

Children who survive CM are at high risk of neurological and developmental complications. It is thought that this may be due to cerebral ischaemia but at present there is no method to identify these children other than waiting for their deficit to become manifest. Identifying CM patients at risk of neurological deficit or developmental delay would enable early intervention. Identifying a link between cerebral ischaemia and neurodevelopmental deficit (NDD) would also support the development of new treatments for CM which mitigate ischaemic injury.

Optical Coherence Tomography (OCT) is a non-invasive ocular imaging modality which uses low-coherence light to obtain detailed cross-sectional images of the retina and optic nerve head (ONH). The ONH swells with raised intracranial pressure (papilloedema).

Objectives
Broad objective
To develop an OCT based bedside test to identify and quantify brain swelling in CM and other comas, and predict NDD in CM.
Specific objectives
1. To determine the accuracy of OCT measures of ONH swelling to detect severe brain swelling in children with CM compared to brain MRI.
2. To ascertain the accuracy of OCT measures of macular ischaemia to predict NDD in children with CM at 1 and 2 years post-recovery.
3. To establish if large accumulation of retinal haemorrhages predicts progression to severe brain swelling in children with CM.
4. To determine if OCT measures of ONH swelling can detect and quantify raised ICP in other comas in children.
5. To establish if a low-cost handheld OCT with AI analytics and tested to ISO standard can replace the commercially available OCT and human image analysis.

Methodology
Participants will be recruited on admission with CM and other comas. During the acute illness participants will have OCT of the ONH and macula, and fundus imaging, in addition to standard care which includes funduscopy and an MRI brain scan. Children with CM will be followed up over 2 years with OCT, MRI and neurodevelopmental assessments.

We will test the value of OCT-measured ONH parameters to identify severe brain swelling determined by MRI (gold standard). We will use Receiver Operating Characteristic (ROC) curves to determine the utility of OCT of the ONH to identify severe brain swelling, and which parameter(s) have the best specificity and sensitivity.

OCT of the retina can identify retinal ischaemia manifest as hyper-reflective signal. Similar statistical methods will be employed to determine the value of OCT of the central retinal (macula) to identify patients developing NDD (either at discharge or during follow up). Children will have Malawi Developmental Assessment Tool (MDAT)(≤5yrs) or Kaufman Assessment Battery for Children (ABC)(>5yrs) developmental assessment as well as Liverpool Outcome Score to identify NDD over two years.

Expected findings and dissemination

We expect to find that OCT of the ONH can identify patients with severe brain swelling, and to determine which ONH parameter(s) has the best sensitivity and specificity. The links between retinal, cerebral ischaemia and neurological outcomes in CM are less well established, but the study will determine whether OCT of the macula has predictive value for NDD.

The study findings and results will be shared in a timely manner with COMREC, UoL sponsor, QECH Medical Director and Wellcome Trust. Results will be presented at Malawi, UK and international scientific meetings, and by open-access publication (as required by the funder). This will include the KUHeS Research Dissemination Day and MLW Annual Scientific Meeting.

1. Background

Malaria remains a serious health problem with global mortality of 400,000 every year, mainly in African children. Reductions in global malaria cases have slowed or stalled since 2014, and reductions in mortality since 2016.(1) Children in Malawi suffer severe disease from overlapping syndromes of cerebral malaria (CM), severe anaemia and respiratory distress, but most mortality and disability are related to CM. CM remains the commonest cause of acute paediatric coma in Malawi.

The pathogenesis of CM involves the sequestration of parasitised red blood cells (pRBC) in small vessels in the brain and other organs. High parasite loads are associated with endothelial dysfunction, local inflammatory cascades, coagulation activation and microvascular occlusion. MRI studies have demonstrated that severe brain swelling is an important mechanism of death. 84% of children dying from CM have severe brain swelling evident on admission MRI.(2) The pathogenesis of brain swelling is thought to involve breakdown of the blood-brain barrier (vasogenic oedema) and/or oncotic cellular swelling (cytotoxic oedema). Our recent research suggests an additional mechanism involving fluid leak during microhaemorrhage formation.(3)

As brain swelling appears to be central to fatal outcome in CM, a randomised clinical trial is underway in Blantyre, Malawi to investigate interventions to reduce intracranial pressure (ICP) (NCT03300648). This trial is of usual care versus hypertonic saline versus mechanical ventilation, and relies on brain MRI to identify severe brain swelling (grades 7 or 8) as an inclusion criteria. However, access to MRI in Africa is very poor. MRI coverage is estimated at 1 scanner/4 million population in West Africa, with at least half in private facilities.(4, 5)

Brain swelling with raised intracranial pressure (ICP) causes swelling of the ONH in the eye, also known as papilloedema. Clinical identification of papilloedema is subjective, difficult in mild or early cases, and not quantifiable. However the presence of papilloedema is known to have a strong association with fatal outcome in CM.(6)

Neurological and developmental deficits (NDD) occur in a third to half of survivors of CM,(7, 8) evident at discharge or months after the acute illness. Acute ischaemia is thought to be the critical event in the development of NDD, which include motor & sensory impairments (25%), epilepsy (9%), cognitive/behavioural impairments (11%). Evidence shows that parental

education, support and skills training can improve outcomes for children with acquired disability.(9)

Optical Coherence Tomography (OCT) uses low coherence light to obtain cross-sectional images of the retina and optic nerve head (ONH) with resolution of micrometres. The light source is a superluminescent diode and images are obtained through the pupil with no contact. OCT imaging is used worldwide in ophthalmic practice, with handheld OCT adding versatility.

OCT can identify ONH swelling from raised ICP,(10-12) and OCT of the macula can identify ischaemic macular whitening in CM.(13) OCT generates a large amount of imaging data, but AI shows great potential for screening and diagnosis of eye diseases, and has been trialled in clinics with cost savings.(14)

2. Rationale

Across Africa very few children with CM have access to MRI scanning which could identify them as at high risk of fatal outcome from severe brain swelling. In the development of new treatments for brain swelling in CM, MRI images are used to identify potential candidates who will benefit from treatment. A low-cost bed-side test is needed instead of MRI scanning for use in secondary care in malaria endemic regions.

As well as acute mortality from CM, long-term morbidity is from neurological injury. A test which could identify patients at risk of NDD would facilitate early interventions to educate parents and maximise the child's developmental outcome. If this was coupled to tissue ischaemia it would support the scientific case for studies of neuroprotective agents in acute CM, and provide a surrogate outcome measure for these studies.

Our research vision is to develop a bedside test to identify severe brain swelling, and separately predict NDD in CM, and to make this sustainable by developing a low-cost OCT with AI-enabled algorithms. Automating this imaging technique at relatively low cost will make it scalable for LMICs. The University of Liverpool (UoL) Department of Electrical Engineering will develop a low-cost, robust OCT in parallel to the clinical study and plan to compare it to the Leica OCT in year 4.

The clinical study will further the understanding of the pathogenesis of CM, building on previous critical insights from the study of malarial retinopathy. Furthermore, a non-invasive, rapid bedside test for identifying and quantifying raised ICP in children would also be applicable to many neurological diseases. Success in this programme will lead to multicentre trials utilising OCT across the International Centers of Excellence in Malaria Research.

3. Research question and objectives

3.1 Research questions

1.Can OCT of the optic nerve head (ONH) detect and quantify brain swelling reliably, and in particular differentiate severe brain swelling in children with CM?
2. Can retinal ischaemia (hyper-reflectivity) on OCT of the macula be used as a biomarker of patients at risk of NDD? Does retinal ischaemia and later atrophy, detectable by OCT, relate to cerebral ischaemia and atrophy in CM?
3. Can a large accumulation of retinal haemorrhages, alone or in combination with ONH OCT, identify patients progressing from mild-moderate to severe brain swelling in CM?
4. Can OCT of the ONH identify and quantify raised ICP in other comas in children?
5. Can the development of OCT technology and artificial intelligence (AI) based image analysis make OCT more accessible and generalisable across settings?

3.2 Broad objective
To develop an OCT based bedside test to identify and quantify brain swelling in CM and other comas, and predict NDD in CM.

3.3 Specific objectives
1. To determine the accuracy of OCT measures of ONH swelling to detect severe brain swelling in children with CM compared to brain MRI.
2. To ascertain the accuracy of OCT measures of macular ischaemia to predict NDD in children with CM at 1 and 2 years post-recovery.
3. To establish if large accumulation of retinal haemorrhages predicts progression to severe brain swelling in children with CM.
4. To determine if OCT measures of ONH swelling can detect and quantify raised ICP in other comas in children.
5. To establish if a low-cost handheld OCT with AI analytics and tested to ISO standard can replace the commercially available OCT and human image analysis.

4. Methodology

4.1 Study Type and Design

We will conduct an observational, single-site, cohort study, with an acute-phase and 1-2 year follow up, to evaluate the value of OCT of the ocular fundus to detect severe brain swelling and predict neurological outcomes in CM. We will recruit 120 paediatric patients with WHO defined CM, and 90 with other acute comas over 3 years admitted to Queen Elizabeth Central Hospital (QECH). We will recruit 60 healthy age-matched controls. Recruits will have 2 years follow up (1 year for final year recruits).

Standard care for CM patients after hospital discharge is one follow up appointment at 1 month post discharge, when patients are weighed, have a malaria slide and neurological examination by a clinician. In the OCT in CM study, patients will also have an OCT scan, Liverpool outcome score and neurodevelopmental assessment at that follow up visit. They will have additional follow up visits at 6 months (OCT only), 12 months (OCT, MRI, LOS, developmental assessment) and 24 months (OCT, MRI, LOS, developmental assessment).

Work package 1: Identifying brain swelling
For this work package the population is children with CM and acute coma of other causes under 16 years of age.
The anticipated sample is 120 children with CM, and 90 with coma.
Inclusion criteria
1. Parent or guardian gives informed consent for their child's participation.
2. CM: under 16 years, BCS≤2, Plasmodium falciparum parasitaemia on blood film, no other cause of coma evident.
or
3. Other comas: under 16 years, BCS≤3 with acute presentation.
4. Healthy controls age-matched under 16 years.
Exclusion criteria
1. Hypoglycaemia, post-ictal, or other transient state accounting for coma.
2. Contraindication to MRI.
3. History or evidence of pre-existing hydrocephalus, significant neurological disease, gross neurological disability or learning difficulties.
4. History or evidence of pre-existing significant ocular disease.
5. History or evidence of severe life-limiting or chronic disease including but not limited to advanced HIV/AIDS (stage 4), disseminated TB and malignancy.

Consent for participation in the OCT in CM study will be from parents/guardians prior to any study procedures are undertaken (appendix 1). Assent will be sought for children 7 years and over once they are fully alert which may not be until day of discharge from hospital (appendix 2). Children under 7 years of age or not fully awake on day of discharge will not be asked for written assent.

Patients admitted to the Paediatric Research Ward with CM or coma of other causes will admitted, assessed, and treated according to standard care. Once the patient is assessed to be stable, and treatment underway, parents or guardians will be approached for consent for the patient to participate in this observational study. Consented patients will then have an examination of pupil reactions and pupil dilation with tropicamide 1% and phenylephrine 2.5% eye drops followed by
1. indirect funduscopy and standardised grading of malarial retinopathy
2. OCT scans of the ONH and central retina or macula will be performed using Leica Envisu 2300 taking approximately 10mins.
3. Video imaging of their fundi using an Epicam fundus camera.
As part of standard care patients will also have brain MRI scans which will be assessed and graded for severe brain swelling.(2) It is envisaged that this study will run alongside the Treating Brain Swelling study (NCT03300648), a separate interventional study assessing different treatments for severe brain swelling. In accordance with local protocols, MRI scans may be conducted after sedation with chloral hydrate to reduce image degradation due to movement artefact.

For objective 1, the population is patients with CM, the condition is severe brain swelling versus no/mild/moderate brain swelling, the diagnostic test under evaluation is OCT of the ONH, and the gold standard is graded brain MRI images. We will prospectively evaluate a number of ONH parameters detectable by OCT to determine which has the best AUC to detect severe brain swelling. A similar analysis will be undertaken for patients with other comas.

OCT scans and fundus video imaging will be repeated twice a day during coma in CM patients to address objective 3: to see if changes in number of retinal haemorrhages, with or without OCT changes can predict progression to severe brain swelling.

WP1 addresses objectives 1, 3 and 4.

<u>Work package 2: Predicting neurodevelopmental deficit</u>
The population is children with CM enrolled in WP1 and healthy controls under 16 years old. The sample size of CM patients is the same 120 from WP1; and controls is 60.
Inclusion criteria
1. Parent or guardian gives informed consent for their child's participation.
2. CM: under 16 years, BCS≤2, Plasmodium falciparum parasitaemia on blood film, no other cause of coma evident.
3. Healthy controls age-matched under 16 years.
Exclusion criteria
1. Hypoglycaemia, post-ictal, or other transient state accounting for coma.
2. Contraindication to MRI.
3. History or evidence of pre-existing hydrocephalus, significant neurological disease, gross neurological disability or learning difficulties.
4. History or evidence of pre-existing significant ocular disease.
5. History or evidence of severe life-limiting or chronic disease including but not limited to advanced HIV/AIDS (stage 4), disseminated TB and malignancy.

CM participants will already be consented (and assented if 7 years or older) during enrolment to OCT in CM study WP1. For healthy controls, parents or guardians will be asked for consent prior to any study procedures, and children aged 7 years or over will be asked to assent at the same time.

This work package is to evaluate macula ischaemia, detectable as hyper-reflectivity on OCT of the macula, as a predictor of neurological deficit in CM. It will only be undertaken on CM patients and controls. CM survivors will return one month after discharge for OCT scans and neuro-developmental assessment. This will consist of Malawi Developmental Assessment Tool (MDAT) for those under 5 years, Kaufmann Assessment Battery for Children (ABC) on those 5 years of age and older, and Liverpool Outcome Score. From the results we will create an age-adjusted measure of global development and cognition.(8) Patients will return at 6 months for OCT and 12 and 24 months for repeat OCT scans, brain MRI scans and neurodevelopmental assessment.

Controls will be recruited from patient siblings and attendees with unrelated illnesses at QECH and be age-matched. They will have a medical and developmental history taken, pupil dilation, funduscopy, OCT of ONH and macula, and neurodevelopmental assessment. These will be undertaken at baseline and 12 and 24 months. The neurocognitive status of healthy controls will help to define neurocognitive deficit in CM cases. Neurocognitive data on our 60 controls will be added to existing data on 100 healthy subjects from the same population(8) and case z-scores determined [z=(case score –population mean)/standard deviation]. Two z-scores below the mean defines abnormal.(15)

WP2 addresses objective 2

<u>Work package 3: Development and evaluation of low-cost handheld OCT</u>
Population is CM and other coma patients recruited to WP1 and control children recruited to WP2, during year 3 or 4.

The anticipated sample is CM patients 40, other coma patients 30 and controls 20.
Inclusion is only if already enrolled in the OCT in CM study.
The participants will already have given consent on enrolment to OCT in CM study (and assent in children aged 7 years or over once fully conscious and alert).

A novel OCT which is robust and low-cost will be developed in Liverpool in collaboration with the University of Liverpool Dept of Electrical Engineering. The key novelties of the device will be 1) fibreoptics based spectrometer and new microelectromechanical systems (MEMS) with integrated drivers for robustness and compactness targeted to applications in low resource settings; 2) Novel optics design to provide larger field-of-view image, and allow greater engineering tolerance making it easier maintenance and less expensive to manufacture; 3) optional integration with a scanning laser true-colour fundus camera developed by us for retinopathy screening.

Development of the handheld OCT will be based on our current table-mounted prototype developed for diabetic retinopathy screening in China. We will follow a medical device design approach and build the prototype complying with regulatory requirements. The design and development processes will be accompanied by risk management activities according to ISO 14971.The software being classified as class B will be constructed to IEC 62304:2006 requirements,

Laboratory evaluation will be carried out to ensure the measured light power output is within recognised safety standards (see below). OCT scans will be taken on eye models and porcine eyes to validate the design and performance of the device. In addition, laboratory evaluation will also include light safety assessment to ensure the ISO 15004-2 (International Standards Organisation) standard is met. ISO 15004-2 is the light safety standard for ophthalmic instruments. We will ensure this OCT device also meets class 1 standard when assessed against IEC 60825-1 (International Electrotechnical Commission), in the same way as we have done for the device above. The laser safety assessment will be certified by both an internal (UoL) laser safety expert and a certified external laser safety specialist company. The optical output of the developed device will be assured to be within class 1 limits. In Malawi the

regulation of medical devices is delegated to the Pharmacy and Medicines Regulatory Authority, and we will ensure the OCT meets their relevant regulations.

In year 3 and/or 4 of the project, the University of Liverpool OCT will be evaluated in Malawi in comparison to the Leica handheld OCT. Participants will be in those already enrolled in the OCT in CM study, including participants with CM, other comas and healthy controls. OCT scans of the ONH and macula will be taken with both devices in alternating sequence. Operators will ensure that the corneas remain lubricated during the obtaining of OCT scans with both devices by manually closing the upper eyelid or use of lubricating eye drops. The operator will record ease of use on a 5 point scale, time taken to acquire OCT scans and any factors affecting scan quality. At image analysis a quality assessment of the images will be made (good, acceptable, unacceptable/ungradable). Measured parameters from the optic nerve head and extracted data on hyper-reflectivity and other parameters in the macula will be compared between the two devices using intraclass correlation coefficients. Kappa statistics (and other comparative statistics) will be used to compare the human image analysis with the computerised AI analysis. This will be done according to the Standards for Reporting Diagnostic Accuracy Studies–Artificial Intelligence if available (currently under development). This part of WP3 is limited in scope to demonstrate the equivalence of the UoL OCT to the commercial Leica OCT which is the same imaging modality. It is not conducted with a view to commercialisation of the UoL OCT. If the OCT in CM study as a whole has positive results then a new clinical study would be planned (with new protocol and approvals) to test the UoL OCT with a view to widespread or commercial use.

4.2 Study Place

The study will be conducted in the Paediatric Research Ward and Paediatric Intensive Care (Mercy James Unit) at Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi. Developmental assessments will be conducted in Blantyre Malaria Project, QECH.

4.3 Study Population

Objective 1,2, 3 and 5: children admitted to the Paediatric Research Ward with CM.
Objective 4: children admitted to the Paediatric Research Ward with coma other than CM.
Healthy controls: age-matched children who are siblings of patients in hospital or children attending QECH with unrelated conditions or children of hospital staff. Healthy controls have to satisfy exclusion criteria 3,4 and 5. That is have no history or evidence of pre-existing hydrocephalus, significant neurological disease, gross neurological disability or learning difficulties; no history or evidence of pre-existing significant ocular disease; no history or evidence of severe life-limiting or chronic disease including but not limited to advanced HIV/AIDS (stage 4), disseminated TB and malignancy.

4.4 Withdrawal Criteria

Subjects (patients and guardians) can choose to withdraw from the study at any time. Reasons for withdrawal will be recorded.

4.5 Study Period and Timeline

The study is funded for data collection is over 4 years. Data collection on acute admissions will be in years 1-3. Checking and analysis of data from acute admissions will be completed in year 4 (2025) followed by first manuscript preparation. Last patient last follow up visit will be in year 4 (2025) with final follow up data analysis thereafter. The development and testing of a low-cost OCT will be conducted in Liverpool in years 1-3. Pending approvals it will be evaluated against the commercial OCT in year 4. Further manuscript preparation and dissemination will be in year 4 and into 2026.

Activity	2021 Q4	2022 Yr 1	2023 Yr 2	2024 Yr 3	2025 Yr 4	2026
Approvals, staff recruitment and training						
Data collection of acute admissions	WP1					
Data collection of patients follow up	WP2					
Data checking and analysis						
Development of low-cost OCT and AI	WP3					
Comparison of OCT machines						
Manuscript preparation and dissemination						

4.6 Sample Size

The study sample size calculation is based on specific objective 1: to determine the accuracy of OCT measures of ONH swelling to detect severe brain swelling in children with CM. A diagnostic test's optimal specificity/sensitivity is based on its Receiver Operating Curve with an optimal Area-under-the-Curve (AUC) being close to 1.(16) For our primary analysis (to differentiate severe from non-severe brain swelling in CM), the minimum sample size was calculated with an estimate of the Area-Under-the-Curve of 0.8 with a precision of +/-0.07 and 95% confidence (Table 13.3 in (17)). The anticipated AUC is based on preliminary data from Blantyre, and the ratio of severe: non-severe brain swelling cases from published data.(2). For an acceptable sensitivity of 90% and specificity of 80%, 120 cases are required (42 with severe brain swelling, 78 children with non-severe brain swelling). As all recruited patients will be acute admissions to hospitals, and the outcome recorded by the end of their hospital stay, we have not included any loss-to-follow up in this calculation.

Our second hypothesis tests macula OCT abnormalities in predicting neurological deficit in CM cases at 12 and 24 months. We will measure the neurocognitive status of healthy controls which will help to define neurocognitive deficit in CM cases. Neurocognitive data on our 60 controls will be added to existing data on 100 healthy subjects from the same population(8) and case z-scores determined [z=(case score –population mean)/standard deviation]. Two z-scores below the mean defines abnormal.(15)

Inclusion Criteria

1. Parent or guardian gives informed consent for their child's participation.
2. CM: under 16 years, BCS≤2, Plasmodium falciparum parasitaemia on blood film, no other cause of coma evident.

or

3. Other comas: under 16 years, BCS≤3 with acute presentation.
4. Healthy controls age-matched under 16 years.

Exclusion Criteria

CM and other coma cases:

1. Hypoglycaemia, post-ictal, or other transient state accounting for coma.
2. Contraindication to MRI.

All:

3.History or evidence of pre-existing hydrocephalus, significant neurological disease, gross neurological disability or learning difficulties.
4.History or evidence of pre-existing significant ocular disease.

5. History or evidence of severe life-limiting or chronic disease including but not limited to advanced HIV/AIDS (stage 4), disseminated TB and malignancy.

4.7 Participation Duration

Parents and guardians of children admitted to the Paediatric Research Ward with CM or other comas will be offered the opportunity to participate in this study. Eye examination and image acquisition will be conducted on admission after stabilisation of the child's clinical condition. These will be repeated daily. Discharged patients return for OCT at 1, 6, 12, and 24 months; developmental assessment at 1, 12 and 24, and brain MRI at 12 and 24 months (only 12 months for patients recruited in year 3).

Healthy controls will have baseline funduscopy, ocular imaging and then neurodevelopmental assessment at baseline, 12 and 24 months.

5. Data management

5.1 Data Collection Procedures

Procedures for data collection will adhere to Good Clinical Practice (GCP) principles. Each study subject will be allocated a unique identifier on enrolment. Demographic and clinical data will be collected according to Paediatric Research Ward standard procedures. Funduscopic findings will be entered onto validated paper charts for malarial retinopathy and later entered onto a Redcap database (appendix 3).(18) OCT scan and Epicam images will be captured onto the device and then transferred to secure files for review and back up. Measured parameters will be manually extracted and entered onto the database. Liverpool outcome score will be completed on paper and entered onto a Redcap database (appendix 4). Developmental assessments will be completed on tablet into a Redcap database (appendix 5).(19, 20)

5.2 Data Management

Data will be managed by strict procedures according to GCP to maintain confidentiality and data security. All data collected on paper will be stored separately from consent forms in locked cabinets in research (non-public) areas. Study data will only be stored on password protected computers, UoL and MLW servers. To ensure the quality of our data we will follow protocols on best practices for data management.

We plan a data lock of data from acute admissions at the end of 2024, and of data from follow up appointments in September 2025, designated as the end of the study. Study data linked to participants (pseudo-anonymised) will be kept for a maximum of 5 years and anonymised data will be kept for a minimum of 10 years. Data will be archived electronically on MLW and UoL servers.

5.3 Data Analysis Plan

For objective 1, ROC curves of sensitivity against 1-specificity for a number of ONH parameters in detecting severe brain swelling on MRI will be plotted, and the Area Under the Curve (AUC) calculated for each. It is likely that the parameter with the AUC closest to 1 will have the most advantageous sensitivity and specificity, although high sensitivity is favoured over specificity. Similar analysis will be undertaken for objective 4, and in addition the correlation between ONH parameters and degree of brain swelling will be calculated.

For objective 2, the size and extent of macular hyper-reflectivity on OCT will be measured and compared to neurodevelopmental outcomes, particularly new epilepsy, neurological impairment, developmental delay and behavioural problems. The ability of macular hyper-reflectivity to predict neurodevelopmental abnormalities will be tested with an adjusted regression model.

Objective 3 is an exploratory objective to determine if an increase in retinal haemorrhages can predict development of severe brain swelling with or without data from OCT of the ONH. The number of haemorrhages on admission and change in number over time will be compared to progression in MRI detected brain swelling in patients having 2 or more MRI scans. Signs of early disc swelling on OCT will be factored in to see if additional ONH data can improve any predictive value of haemorrhages.

6. Presentation and dissemination of results

6.1 Presentation of Results

Results of work package 1 will be presented as tabulated sensitivities and specificities and AUCs of different ROCs for OCT measured parameters of the ONH in predicting severe brain swelling. Results of work package 2 will be also be presented as a table indicating the relation between degree of retinal ischaemia and risk of NDD. AUCs will also be tabulated for different cut-offs of retinal ischaemia in predicting NDD. In work package 3 the comparison between the low-cost OCT and commercial OCT with be presented in a table.

6.2 Dissemination of Results

The findings of the study will be shared and disseminated through presentation at relevant national and international meetings and publication in peer review journals. In Malawi this will include the KUHeS Research Dissemination Day and Malawi-Liverpool-Wellcome Annual Scientific Meeting. Results will be presented at relevant international meetings particularly those with a focus on malaria. Publications will be open access as required by the funder.

A final report and any publications arising will be shared with College of Medicine Research Ethics Committee (COMREC), the sponsor (UoL), funder (Wellcome Trust), QECH Medical Director and KUHeS library.

We will take the opportunity to participate in MLW's public engagement programme, including science cafés, mobile outreach exhibitions and other events. These activities will provide an opportunity for the public and journalists to meet with members of our research team and discuss the clinical study and technology we are developing. We will also utilise social media platforms to reach out to the public in Malawi, Africa and the UK, enabling dissemination and discourse about the science.

7. Ethical considerations

This study will be conducted in compliance with the ethical principles outlined in the Declaration of Helsinki, and with GCP Guidelines. Ethical approval for this study will be sought from COMREC and the University of Liverpool Research Ethics Committee.

Written informed consent will be sought from accompanying parents or guardians prior to any study procedures. Parent/guardian information sheet and consent form is attached (appendix 1). Consent will be sought sensitively and only patients of freely consenting parents or guardians will be enrolled. COM, and Blantyre Malaria Project have recently conducted qualitative studies with parents and guardians to better understand the challenge of obtaining informed consent in a paediatric critical care setting in LIMCs.(21) This will inform our consent

process. Unconscious children are vulnerable subjects, unable to give informed consent, however participants aged 7 to 16 years will be asked for their assent to participate in the study once they are fully conscious and alert. This will be confirmed by completing the Assent form (appendix 2).

This is an observational study and will not influence the patient care or enrolment into interventional studies in any way. Ocular examination and imaging will only be initiated once the clinician in charge is satisfied that the patient has been stabilised and commenced on treatment. Pupil dilation and funduscopy are well established standard care procedures on the Paediatric Research Ward in Blantyre. OCT scans are performed at the patient bedside with a handheld device, which is non-contact and utilises low coherence light to obtain cross-sectional images. The Epicam camera is a handheld video fundus camera which obtains fundus images. It is anticipated that ocular examination and imaging will take 30-45 minutes.

Healthy age-matched controls will be sought from siblings of QECH patients and patients with unrelated conditions. Conscious children tolerate the light and instrumentation of OCT scanning well including in pilot studies in Blantyre. Funduscopy involves a brighter light, but sufficient examination can usually be obtained with patience.

Participants will be renumerated for participation in this study to cover time, travel and inconvenience according to KUHeS guidelines.

Participant confidentiality will be maintained throughout the study which will adhere to Data Protection and GCP principles. No patient identifiable data will be included in presentations or publications. Identifiable photographs of patients will not be used without express written consent for publication.

Both CM cases and controls will undergo three neurodevelopmental testing sessions over 2 years in the presence of their parent/guardian. It is expected that some CM cases will be identified as having a neurodevelopmental delay or disorder, and it is possible that some control children might also. These children will be referred to appropriate clinical services provided by community and paediatric units. Blantyre Malaria Project already has these referral pipelines in place through previous projects and we will utilise these networks and relationships to ensure provision of timely and effective interventions to children identified to be in need. We will also offer a counselling session for parents with children identified with a developmental disorder. The session will cover four main points: 1) getting children engaged, 2) preventing challenging behaviours, 3) teaching new skills, and 4) helping children with motor problems.

Both coma cases and control parents/guardians will be reimbursed for time and travel for attending study visits according to KUHeS guidelines.

7.1 Ethical Approval

Ethical approval is sought from COMREC and University of Liverpool Research Ethics Committee. The Head of Paediatrics, QECH has provided a letter of support, and one is sought from the QECH Medical Director.

The study may be subject to inspection and audit by the University of Liverpool under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Framework for Health and Social Care Research.

7.2 Indemnity

Indemnity for this study is provided by UoL through their insurer Griffiths and Armour.

8. Possible constraints

8.1 Recruitment

Recruitment targets to achieve sample size requirements are realistic given the number of admissions for CM over the last five years (>40). We have included patients with coma of other causes in this study partly to mitigate the risk of reduced numbers of CM cases.

8.2 Risks and benefits to study participants

Risks of ocular examination and imaging are minimal but could rarely include corneal abrasion. Corneal abrasion would be treated with antibiotic eye ointment and usually resolves in 24-48 hours. Unrelated significant ocular disease may be detected which would result in exclusion from the study. Parents/guardians will be counselled about any newly detected ocular disease, and patients would be referred to Lions Sight First Eye Hospital if required.

Benefits for participants include detailed ocular examination and imaging in which ocular health will be assessed. Undetected ocular disease may be diagnosed sooner than would be the case for non-participants, potentially benefitting from earlier ophthalmological treatment. Participants also benefit from detailed neurodevelopmental assessments over 1 or 2 years and detection of neurological or developmental deficits which otherwise may have been undetected by a standard gross neurological examination at the usual one month follow up.

Malawi society and children benefit from the potential for improved care for future children with CM.

8.3 Adverse events

This is an observational study of conditions which frequently causes death and disability. Following UK's Health Research Authority requirements for non-interventional or non-CTIMP studies, serious adverse events (SAE) will only be reported where they occur as a result of any research procedure or are unexpected. This is also in accordance with UoL standard operating procedure 007 Safety Reporting for University of Liverpool Sponsored Research (Version 3.00 Date 21/09/2017). Where death or disability occur as a result of cerebral malaria or other comas they will not be reported, although they will be recorded as outcome data for the study. Serious adverse events (SAE) resulting from study procedures are not expected, but will be reported to the chief investigator and CRSU immediately and no later than 3 days from knowledge of the event. The CI will report all study related SAEs to the KUHeS REC, UoL REC and UoL Sponsor within 15 days. All non-serious adverse events arising as a result of study procedures will be reported to the CI and MLW CRSU within 15 days, for example (but not limited to) corneal abrasion.

8.4 Conflict of Interest

The investigators declare they have no conflict of interests.

8.5 Steering committee

A Study Steering Committee (SCC) will be established who will meet remotely at least 6 monthly in year 1 and then annually. We will appoint a clinical academic with a UK appointment in a clinical speciality relevant to the study to be Chair of the SSC. We will invite the following as members: Clinician/Clinical academic with a clinical speciality relevant to the study (ideally complementing the Chair), Statistician with experience in clinical studies. These members will be from institutions different from the University of Liverpool or investigators' institutions. We will invite a Malawian clinician or clinical researcher who is independent of the study, but likely to be associated with the Kamuzu University of Health Sciences (as the only

academic medical institution in Malawi). We will seek to appoint a non-medical Malawian to act as a patients' representative, taking advice from the MLW public engagement and outreach programme. The Chief investigator and joint co-applicant will be members. We will invite a representative of the sponsor and Wellcome Trust as observers. Study investigators and collaborators will be asked to attend the SSC as appropriate.

Adverse event data and disease outcome data will be presented to the SSC for oversight. As this is an observational study we are not planning a Data Monitoring and Safety Board.

9. Requirements

9.1 Requirements

Personnel	For this project we will hire: Enrolled Nurse, part time Trial coordinator, part time Clinical Officer or clinician, part time
Equipment	Equipment for this project already in place: Handheld OCT (Leica Envisu) Fundus video camera (Epicam) MDAT & KABC Developmental assessment kits Direct and Indirect Ophthalmoscopes
Research Space and Clinical Facilities	For this project we will utilise: Paediatric Research Ward – contribution to running costs MRI scanner, currently Hyperfine MRI – scans funded Neurodevelopmental assessment room in Blantyre Malaria Project – room hire funded.

9.2 Training Provided

All study staff will complete GCP training in research practice. The clinician or clinical officer will be given training on grading for malaria retinopathy using funduscopy; obtaining OCT scans of the ONH and macula with the handheld OCT; and obtaining fundus images with the Epicam. This will be provided directly by experienced members of the study team.

The enrolled nurse conducting neurodevelopmental assessments will be trained by senior project personnel. The training protocol will include classroom training with some explanation of child development, general features of the tools (e.g. developmental construct it is measuring, what instructions to deliver, administration rules), demonstrations of tool administration and roleplay, followed by field practice.

Observed practice and refresher training by senior staff will be scheduled periodically during the entire data collection period.

There will be an opportunity for training of medical students, healthcare workers and clinicians in funduscopy, ophthalmic imaging and developmental assessment.

10 **Budget**

10.1 Estimated Budget (GBP)

	Yr1	**Yr2**	**Yr3**	**Yr4**	**Total (GBP)**	**Total (million MWK)***
SALARIES						
Snr/Spec Enrolled Nurse (BMP) 50%	6,989	6,989	6,989	6,989	27,958	30.754
Trial Coordinator 20%	6,645	6,645	6,645	6,645	26,580	29.238
Clinical Research Associate C RES 10%	4,400	4,400	4,400	4,400	17,600	19.360
Total Salaries	**18,034**	**18,034**	**18,034**	**18,034**	**72,137**	**79.351**
RESEARCH MATERIALS AND CONSUMABLES						
Stationery, printing, photocopying	724	796	876	964	3,360	3.696
Shipping and Clearing	3,000	0	2,000	0	5,000	5.500
MLW Transportation	52	58	63	69	242	0.266
Ward/Clinic expenses	10,989	12,088	13,297	14,626	51,000	56.100
MRI scans	11,509	25,320	27,852	15,319	80,000	88.000
BMP room for developmental assessments	1,034	1,138	1,251	1,377	4,800	5.280
Total Research Materials & Consumables	**27,309**	**39,399**	**45,339**	**32,354**	**144,402**	**158.842**
MISCELLANEOUS						
Recruitment	100	0	0	0	100	0.110
Telephone Communications	330	300	300	300	1,230	1.353
Translation	96	0	0	0	96	0.105
Science and public communication	1,000	1,000	1,000	1,000	4,000	4.400
Data Entry & Statistics	2,150	900	900	0	3,950	4.345
Health & Safety inc PPE	300	300	300	300	1,200	1.320
KUHeSREC processing fee	265	150	150	150	715	0.786
Study Monitoring	330	75	75	100	580	0.638
Study participant compensation	2,541	5,082	5,082	2,541	15,246	16.771
Conference fees	1,100	0	1,100	0	2,200	2.420
Total Miscellaneous Costs	**8,212**	**7,807**	**8,907**	**4,391**	**29,317**	**32.248**
Subtotal					**245,856**	**270.441**
KUHeS DIRECT RESEARCH COSTS						
KUHeS direct research costs	**6,146**	**6,146**	**6,146**	**6,147**	**24,585**	**27.044**
OVERALL TOTAL					**270,441**	**297.485**

*exchange rate GBP 1 = MK 1,100 @20/9/21

The budget is entirely supported by Wellcome Trust which has been awarded, grant ref 222530/Z/21/Z.

10.2 Justification of Budget

The enrolled nurse (50%) will be conducting neurodevelopmental assessments. The clinician or clinical officer (10%) will provide support in ophthalmic examination and image acquisition to a clinical research fellow. The trial coordinator (20%) will schedule follow up appointments and administer participant travel refunds and compensation, among other organisational duties.

The largest portion of Research Equipment and Consumables is to pay for Research Ward expenses and MRI scans. Also included are room hire for neurodevelopmental assessments and stationary.

Miscellaneous costs includes recruitment, training, phone, internet, translation, public engagement, data entry, PPE, participant compensation.

11. References

1. WHO. World Malaria Report 2019. 2019 04/12/19.
2. Seydel KB, Kampondeni SD, Valim C, Potchen MJ, Milner DA, Muwalo FW, et al. Brain swelling and death in children with cerebral malaria. N Engl J Med. 2015;372(12):1126-37.
3. MacCormick IJC, Barrera V, Beare NAV, Czanner G, Potchen M, Kampondeni S, et al. How Does Blood-Retinal Barrier Breakdown Relate to Death and Disability in Pediatric Cerebral Malaria? J Infect Dis. 2020.
4. Ogbole GI, Adeyomoye AO, Badu-Peprah A, Mensah Y, Nzeh DA. Survey of magnetic resonance imaging availability in West Africa. Pan Afr Med J. 2018;30:240.
5. Sambo LG, Kirigia JM. Investing in health systems for universal health coverage in Africa. BMC Int Health Hum Rights. 2014;14:28.
6. Beare NA, Southern C, Chalira C, Taylor TE, Molyneux ME, Harding SP. Prognostic significance and course of retinopathy in children with severe malaria. Arch Ophthalmol. 2004;122(8):1141-7.
7. Birbeck GL, Molyneux ME, Kaplan PW, Seydel KB, Chimalizeni YF, Kawaza K, et al. Blantyre Malaria Project Epilepsy Study (BMPES) of neurological outcomes in retinopathy-positive paediatric cerebral malaria survivors: a prospective cohort study. Lancet Neurol. 2010;9(12):1173-81.
8. Langfitt JT, McDermott MP, Brim R, Mboma S, Potchen MJ, Kampondeni SD, et al. Neurodevelopmental Impairments 1 Year After Cerebral Malaria. Pediatrics. 2019;143(2):e20181026.
9. Kohli-Lynch M, Tann CJ, Ellis ME. Early Intervention for Children at High Risk of Developmental Disability in Low- and Middle-Income Countries: A Narrative Review. Int J Environ Res Public Health. 2019;16(22).
10. Carta A, Mora P, Aldigeri R, Gozzi F, Favilla S, Tedesco S, et al. Optical coherence tomography is a useful tool in the differentiation between true edema and pseudoedema of the optic disc. PLoS One. 2018;13(11):e0208145.
11. Sarac O, Tasci YY, Gurdal C, Can I. Differentiation of optic disc edema from optic nerve head drusen with spectral-domain optical coherence tomography. J Neuroophthalmol. 2012;32(3):207-11.
12. Lee KM, Woo SJ, Hwang JM. Differentiation of optic nerve head drusen and optic disc edema with spectral-domain optical coherence tomography. Ophthalmology. 2011;118(5):971-7.
13. Tu Z, Gormley J, Sheth V, Seydel KB, Taylor T, Beare N, et al. Cerebral malaria: insight into pathology from optical coherence tomography. Sci Rep. 2021;11(1):15722.
14. Ting DSW, Lin H, Ruamviboonsuk P, Wong TY, Sim DA. Artificial intelligence, the internet of things, and virtual clinics: ophthalmology at the digital translation forefront. Lancet Digit Health. 2020;2(1):e8-e9.
15. McLeod S. Z-score: definition, calculation and interpretation. Simply Psychology. 2019;1:1-7.
16. Bridge J, Meng Y, Zhao Y, Du Y, Zhao M, Sun R, et al. Introducing the GEV Activation Function for Highly Unbalanced Data to Develop COVID-19 Diagnostic Models. IEEE Journal of Biomedical and Health Informatics. 2020;24(10):2776-86.
17. Machin D, Campbell M, Tan S, Tan S. Reference intervals and receiver operating curves. Sample size tables for clinical studies 3rd edn West Sussex, UK: Wiley-Blackwell. 2009:158-78.
18. Beare NA, Southern C, Lochhead J, Molyneux ME, Lewallen S, Harding SP. Inter-observer concordance in grading retinopathy in cerebral malaria. Ann Trop Med Parasitol. 2002;96(1):105-8.
19. Gladstone M, Lancaster GA, Umar E, Nyirenda M, Kayira E, van den Broek NR, et al. The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. PLoS Med. 2010;7(5):e1000273.
20. Boivin MJ, Mohanty A, Sikorskii A, Vokhiwa M, Magen JG, Gladstone M. Early and middle childhood developmental, cognitive, and psychiatric outcomes of Malawian children affected by retinopathy positive cerebral malaria. Child Neuropsychol. 2019;25(1):81-102.
21. Manda-Taylor L, Liomba A, Taylor TE, Elwell K. Barriers and Facilitators to Obtaining Informed Consent in a Critical Care Pediatric Research Ward in Southern Malawi. J Empir Res Hum Res Ethics. 2019;14(2):152-68.