Treating Brain Swelling in Pediatric Cerebral Malaria (TBS)

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Principal Investigator: Terrie E. Taylor, DO

DMID Program Officer: Greg Deye, MD

DMID Medical Monitor: Mo Elsafy, MD, MSc

DMID Clinical Project Manager: Walter Jones, PhD

DMID Regulatory Affairs Specialist: Blossom Smith, MS

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- NIH Clinical Terms of Award
- Malawi Pharmacy and Medicines Regulatory Authority

All key personnel have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:

Signed: _____ Date: _____

Name
Title

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

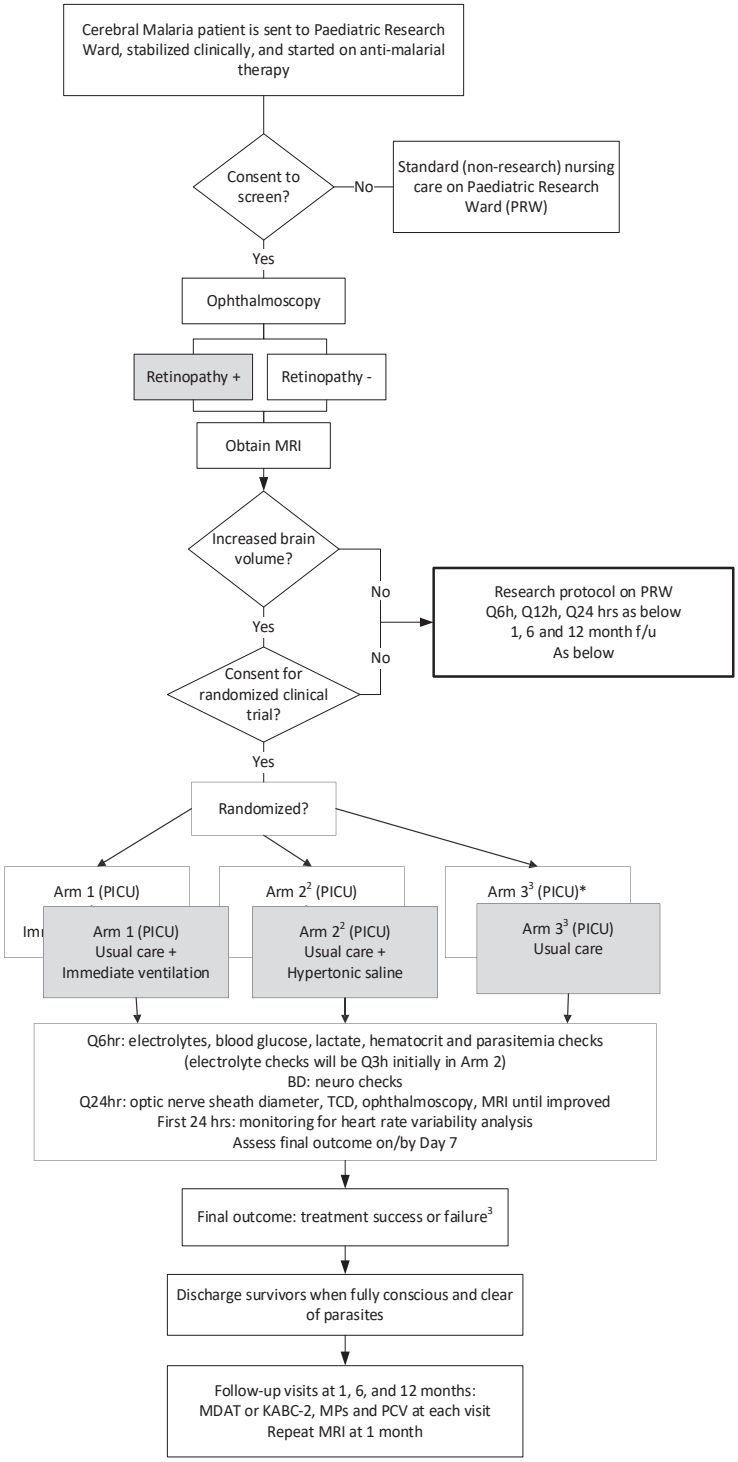
AE	Adverse Event/Adverse Experience
AQP4	Aquaporin 4
BBB	Blood-Brain Barrier
BCS	Blantyre Coma Score
BIO	Binocular Indirect Ophthalmoscopic (eye examination)
BMP	Blantyre Malaria Project (a research affiliate within the University of Malawi College of Medicine)
BRIC	Biomedical Research and Informatics Core (at Michigan State University)
CM	Cerebral Malaria
COMREC	College of Medicine Research and Ethics Committee (in the University of Malawi)
CQMP	Clinical Quality Management Plan
CRF	Case Report Form
CRI™	Compensatory Reserve Index
CROMS	Clinical Research Operations and Management Support
CSF	Cerebral Spinal Fluid
CT	Computed Tomography
DMID	Division of Microbiology and Infectious Diseases
DMU	Data Management Unit
DSMB	Data and Safety Monitoring Board
DWI	Diffusion-Weighted Imaging
eCRF	Electronic Case Report Form
EKG	Electrocardiogram
GOS	Glasgow Outcome Scale
HIV	Human Immunodeficiency Virus
HOB	Head of the Bed
HPS	Hypertonic saline arm of the clinical trial
HTTPS	Secure hypertext transfer protocol
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
ICP	Intra-Cranial Pressure
IM	Intramuscular (injection)
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IV	Intravenous (lines, infusions)
JAMA	Journal of the American Medical Association
KABC-2	Kaufman Assessment Battery for Children 2
LUS	Protocolized lung ultrasound
MBP	Myelin basic protein

MDAT	Malawi Developmental Assessment Tool
MOP	Manual of Procedures
MP	Malaria Parasitemia
MR	Malarial Retinopathy
MRI	Magnetic Resonance Imaging
MSU	Michigan State University
N	Number (typically refers to subjects)
NDA	New Drug Application
NSE	Neuron-specific enolase
NIAID	National Institute of Allergy and Infectious Diseases,
NIH	National Institutes of Health
NIRS	Near infrared spectroscopy
OCRA	Office of Clinical Research Affairs,
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ONSD	Optic Nerve Sheath Diameter
OR	Odds Ratio
ORA	Office of Regulatory Affairs,
PHI	Protected Health Information
PCV	Packed Cell Volume
PNGT	Per Naso-Gastric Tube
PO	Per Os (by mouth)
PR	Per Rectum
PRW	Paediatric Research Ward (Queen Elizabeth Central Hospital)
PI	Principal Investigator
PICU	Paediatric Intensive Care Unit
PK	Pharmacokinetics
QA	Quality Assurance
QECH	Queen Elizabeth Central Hospital
QC	Quality Control
RBC	Red Blood Cell
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SSL	Secure sockets layer
TBI	Traumatic Brain Injury
TNF	Tumor Necrosis Factor
UC	Usual care arm of the clinical trial
US	United States
VENT	Mechanical ventilation arm of the clinical trial
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	Treating Brain Swelling in Pediatric Cerebral Malaria
Phase:	3
Population:	Malawian children aged 6 months to 12 years, with cerebral malaria and MRI evidence of increased brain volume (brain volume score ≥ 6). Total of 128 children.
Number of Sites:	Single site: Paediatric Research Ward, Queen Elizabeth Central Hospital, Blantyre Malawi
Study Duration:	Six years participant accrual; one-year data analysis
Subject Participation Duration:	55 weeks: duration of hospital stay, and follow-up visits at 1, 6, and 12-months post-randomization.
Description of Agent or Intervention:	Arm 1: Usual care + immediate ventilatory support for a maximum of 7 days. Brain death or death will be considered a failure of the primary intervention. Arm 2: Usual care + infusion of 3% hypertonic saline to achieve a blood sodium concentration of 150-160 mmol/L. Ventilatory support will be provided to patients who experience a respiratory arrest, and this will be considered a failure of the primary intervention. Arm 3: Usual care (elevation of the head of the bed 30 degrees and intravenous antimalarial drugs). Ventilatory support will be provided to patients who experience a respiratory arrest, and this will be considered a failure of the primary intervention. Final outcomes will be determined at 7 days post-randomization: The primary study outcome will be recovery *vs* treatment failure, where treatment failure is a composite of death, ventilatory rescue (Arms 2 and 3), and brain death

Objectives:	Primary Objective Compare final outcomes in pediatric CM patients receiving usual care (Arm 3) with both of two interventions (Arm 1 and Arm 2). Secondary Objectives: 1. Among survivors of this interventional study, compare rates of adverse neurological outcomes in those assigned to the two interventions and those assigned to usual care. 2. Among all children admitted to the Paediatric Research Ward and screened for eligibility for randomization, evaluate and validate biomarkers of increased brain volume. 3. Assess the safety of early mechanical ventilation as well as intravenous hypertonic saline in children with CM. 4. Establish association between specific pathogenic mechanisms and TCD-derived phenotypes.
Description of Study Design:	Prospective, randomized, controlled non-blinded clinical trial
Estimated Time to Complete Enrollment:	Six years



PICU[1]: will be on continuous monitoring 24hrs, then transferred to PRW
[2]Pts in Arms 2 and 3 who experience respiratory arrest will be placed on ventilators.
[3]Treatment failure is a composite of death, ventilatory rescue or brain death

1 KEY ROLES

Individuals:

Principal Investigator:

Terrie E. Taylor, DO
University Distinguished Professor
MSU Foundation Professor
Department of Osteopathic Medical Specialties
College of Osteopathic Medicine
909 Wilson Road
B309-B W. Fee Hall
E. Lansing MI 48824-1315 USA
Tel : 517 353 3211
In Malawi : +265 999 969 220
FAX: 517 432 1062
Email ttmalawi@msu.edu

Co-Investigators:

Pediatrician

Yamikani Chimalizeni FCPaed (SA)
Senior Lecturer
Department of Pediatrics
Kamuzu University of Health Sciences
Private Bag 360
Chichiri, Blantyre 3
Malawi
Tel: +265 992 233 221
Fax: +265 1 875 774
Email: gaveta3@yahoo.com

Pediatric Neurologists

Douglas G. Postels, MD, MS
Associate Professor
George Washington University
Children's National Medical Center
111 Michigan Avenue NW
Department of Neurology
Washington DC
20011
Tel: 202 476 6231
FAX 202 476 2864
Email : dpostels@childrensnational.org

Macpherson Mallewa, MD,MRCPCH, PhD

Consultant Pediatric Neurologist
Associate Professor
Department of Paediatrics
Kamuzu University of Health Sciences
P/Bag 360
Blantyre 3
Malawi
Tel: +265 995 83 50 83
FAX: +265 1 87 5774
Email: macphers@liverpool.ac.uk or
mmallewa@mlw.medcol.mw

Pediatric Intensivists
Adnan Bhutta, MBBS
Clinical Professor of Pediatrics
Division Chief, Pediatric Critical Care Medicine
Department of Pediatrics
Affiliate Faculty Member, Center for Global Health Equity
Indiana University School of Medicine/ Riley Children's Health
705 Riley Hospital Drive, Suite 4900 phase II
Indianapolis, IN 46202
Email-Adbhutta@iu.edu

Allan Doctor, MD
Professor of Pediatrics, Biochemistry (UMB) and
Bioengineering (UMCP)
University of Maryland Schools of Medicine and Engineering
Director, Center for Blood Oxygen Transport and Hemostasis
HSF III, 8th Floor
670 West Baltimore Street
Baltimore, MD 21201
Phone: 410-706-7084
Fax: 410-706-6417
adoctor@som.umaryland.edu

Nicole O'Brien, MD
Associate Professor of Clinical Medicine
Division of Critical Care Medicine
Chief, Neurocritical Care
Nationwide Children's Hospital
700 Children's Drive, FOB 2C2
Columbus, OH 43205
Tel: 614-722-2000

FAX: 614-722-3443
Email: Nicole.obrien@nationwidechildrens.org

Radiologists

Manu S. Goyal, MD, MSc
Assistant Professor of Radiology, Neurology and Neuroscience
Neuroimaging Laboratories
Mallinckrodt Institute of Radiology
Washington University School of Medicine
510 S Kings Highway Blvd
St. Louis, MO 63110-1076
Phone: (314) 362-5950
Fax: (314) 362-4886
Email: goyalm@wustl.edu

Lorenna L. M. Vidal, MD, DABR
Attending Physician
Pediatric Neuroradiology
Children's Hospital of Philadelphia
Department of Radiology, Neuroradiology Division
Clinical Instructor, Radiology
Perelman School of Medicine
University of Pennsylvania
3401 Civic Center Blvd., Suite W225 (Neuroradiology Suite)
Philadelphia PA 19104
Phone: (267) 426-3883
Email: vidall@email.chop.edu

Ophthalmologists :

Simon Harding
Professor of Clinical Ophthalmology
Department of Eye and Vision Science
William Henry Duncan Building
Royal Liverpool University Hospital
Liverpool L7 8TX
UK

Petros Kayange, MBBS, MMed(Ophth), FCOphth.(EDSA), FICO(Hons)
Head of Ophthalmology, Senior Lecturer
Kamuzu University of Health Sciences
Ophthalmology Unit, Dept of Surgery
P/B 360
Blantyre 3

Malawi

Nicholas Beare
Senior Lecturer and Consultant Ophthalmologist
Department of Eye and Vision Science
William Henry Duncan Building
University of Liverpool
Liverpool L7 8TX
UK
Tel +44 (0)7855 019379
Email: n.beare@liv.ac.uk

Senior Study Clinician
Karl Seydel, MD, PhD
Assistant Professor
Department of Osteopathic Medical Specialties
College of Osteopathic Medicine
909 Wilson Road
B309 W. Fee Hall
East Lansing MI 48824
USA
Tel : +1 517 353 3211
Email: seydel@msu.edu

Heart rate variability expert :
Peter Hu, Ph.D
Associate Professor of Anesthesiology, Surgery and Epidemiology
University of Maryland School of Medicine
11 S. Paca Street, Suite LL01
Baltimore MD 20219
USA
Tel : 410 328 7264
Fax : 410 328 7230
Email : phu@som.umaryland.edu

Vascular physiology collaborator:
Hans C. Ackerman, MD DPhil MSc
NIH-Lasker Clinical Research Scholar
Investigator and Chief, Physiology Unit
Laboratory of Malaria and Vector Research
Division of Intramural Research
National Institute of Allergy and Infectious Diseases
12735 Twinbrook Parkway, Lab 3E-20
Rockville, MD 20853

USA
Tel: (301) 761-5646
Email: hans.ackerman@nih.gov

Statistician:
Joseph C. Gardiner, PhD
University Distinguished Professor
Michigan State University
909 Wilson Road,
Room B629 West Fee
East Lansing, MI 48824-6410 USA
Tel: 517-884-3967
Fax: 517-432-1130
Email gardine3@ msu.edu

Administrative Support:
University of Malawi College of Medicine:
Mrs. Esther Gondwe
Blantyre Malaria Project (BMP)
PO Box 32256
Blantyre 3
MALAWI
Tele: +265-(0)999-969222
Email: egondwe@bmp.medcol.mw

Michigan State University:
Ms. Karen Hassankhani
Department of Osteopathic Medical Specialties
College of Osteopathic Medicine
909 Wilson Road
B309-B W. Fee Hall
E. Lansing MI 48824-1315 USA
Tel : +1 517 353 3211
Fax: +1-517-432-1062
Email: Karen.Hassankhani@hc.msu.edu

University of Maryland:
Tori Boyer, DNP, BSN-RN
Research Associate | Departments of Pediatrics
Director, Biospecimen Repository and Clinical Research Core
Center for Blood Oxygen Transport and Hemostasis
University of Maryland School of Medicine
HSF III, 8th Floor
670 West Baltimore Street

Baltimore, MD 21201
Tori.boyer@som.umaryland.edu
Office: (410) 706-7093
Cell (preferred): (618) 977-7952
Fax: (410) 706-4148

Medical Monitor:

Mo Elsafy, MD, MSc.
Medical Monitor
Office of Clinical Research Affairs
Division of Microbiology and Infectious Diseases
5601 Fishers Lane, 7E56 MSC 9826
Bethesda, Maryland 20892-9826
For courier service use Rockville, MD 20852
Phone: 240-292-4241
Cell: 301-204-3735
Email: elsafymm@nih.gov

Institutions:

Michigan State University (USA)
Nationwide Children's Hospital (Columbus OH, USA)
Children's National Hospital (Washington DC, USA)
Children's Hospital of Philadelphia (Philadelphia PA, USA)
Kamuzu University of Health Sciences (Malawi)
Washington University (St. Louis MO, USA)
University of Maryland (Baltimore MD, USA)
University of Liverpool (UK)
National Institute of Allergy and Infectious Diseases (USA)

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Recent advances in treatment, prevention and control notwithstanding, malaria remains a major scourge, claiming more than 429,000 lives each year[1]. Cerebral malaria (CM) is a severe complication characterized by coma and seizures. The case fatality rate is 15-50% and the annual incidence ranges from 1-12 cases per 1,000 children in malaria-endemic regions [2, 3]. Most (85%) of the morbidity and mortality occurs in young African children [4]. Of the five species of malaria parasite which can infect humans, *Plasmodium falciparum* is the primary cause of severe disease, and is responsible for the vast majority of malaria associated mortality [4].

2.1.1 Life cycle

Transmitted by the bite of a female *Anopheles* mosquito, the malaria parasite undergoes asexual replication in hepatocytes before invading red blood cells (RBC). The erythrocytic cycle occurs entirely within the circulatory system. The parasite consumes hemoglobin as it grows and replicates within the RBC; one merozoite develops into a schizont over the course of approximately 48 hours in *P. falciparum* infections. The infected RBC eventually ruptures, releasing 16-32 daughter merozoites. The diagnosis of malaria infection is usually made by detecting the intra-erythrocytic parasites in peripheral blood samples, examined microscopically. The life cycle of *P. falciparum* is distinguished histopathologically from the four other species of *Plasmodia* which infect humans by the capacity of RBCs containing mature forms of the parasite (generally 32 hours or more into the life cycle) to adhere to endothelial cells in the microvasculature of various organs. This phenomenon is known as sequestration, and *P. falciparum* does this so effectively that the mature trophozoites and schizonts are only very rarely seen in the peripheral blood. The life cycle stages most commonly seen in diagnostic blood films are the younger, ring-form stages.

2.1.2 Epidemiology

CM is a disease primarily of young African children as older individuals living in malaria-endemic areas have acquired immunity to severe disease [5]. The semi-immune population remains susceptible to infection, but rarely do those infections progress to severe and complicated malaria. Asymptomatic parasitemic individuals are commonly identified during population-based surveys in malaria-endemic areas. The biological basis for antimalarial immunity remains largely unknown, but the epidemiology is consistent: in areas where malaria transmission is intense and regular, the burden of severe disease falls on younger children.

For reasons yet to be fully elucidated, even within the vulnerable population of non-immune young children, only a relatively small proportion of those infected with *P. falciparum* develop a life-threatening illness. Why some non-immunes are more susceptible than others to severe disease is not known; the relative contributions of host resistance and parasite virulence are

unclear. Only one percent of symptomatic infections progresses to severe disease [6]. Most falciparum infections result in a mild flu-like febrile illness and asymptomatic infections are common, even in young children [7].

Because the level of antimalarial immunity in individuals cannot be measured, and because non-immunes are differently susceptible to severe disease, the diagnosis of a malaria *illness* is not as straightforward as merely identifying malaria *infection* by detecting parasites in a blood film. Peripheral parasitemia is necessary in order to identify an infected individual, but, in a symptomatic individual, the presence of parasitemia alone does not establish a causal connection to the symptoms.

2.1.3 Pathogenesis

There are many hypotheses regarding the pathogenesis of CM, but the unifying feature is the sequestration of parasitized RBCs in the microvasculature of various organs, including the brain [8]. During the latter half of the 48-hour life cycle, parasite proteins stud the surface of the host RBC, and mediate the adherence of infected RBCs to host endothelial cells, primarily in capillaries and post-capillary venules (Fig.1) [9]. This interaction is hypothesized to cause localized impaired perfusion, leading to hypoxia [9] and acidosis [10]. It is also thought to activate endothelial cells [11, 12], leading to subsequent blood-brain barrier (BBB) breakdown [13] and increased brain volume as a result of the sequestered mass and increased cerebral blood flow related to anemia, seizures and fever [14]. Endothelial cell activation may also lead to a systemic inflammatory response [15, 16] and a pro-thrombotic state [17-20].

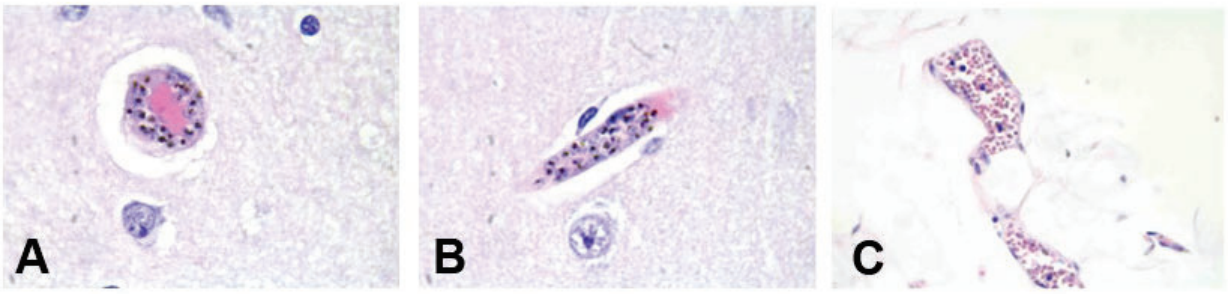


Figure 1. (A, B, C) Histologic evidence of parasite sequestration in small blood vessels of the brain.

2.1.4 Antimalarial treatment

Intravenous quinine has been the mainstay of treatment for CM for two hundred years, but recently completed clinical trials established the superiority of artesunate over quinine as the primary antimalarial treatment in both adults [21] and children [22] with severe malaria. The benefits of artemisinin treatment are thought to be related to the more rapid parasite clearance times, and to the fact that the artemisinin drugs exert their activity over a broader period of the parasite life cycle [23], thus diminishing ongoing sequestration. Even under the ”best case scenario” of prompt treatment with artesunate, the case fatality rate in the subgroup of African children with CM in the AQUAMAT study was 18% [22]. Additional impact on the case fatality rate will likely require adjunctive therapies targeting key pathogenic mechanisms.

2.1.5 Adjunctive therapy

A number of clinical trials targeting various putative pathogenic mechanisms have been carried out [24]:

- Modulating the immune response
  - Dexamethasone to decrease cerebral edema [25, 26]
  - Intravenous immune globulin to reverse sequestration [27]
  - Monoclonal antibodies to tumor necrosis factor (TNF) to decrease immune activation [28]
  - Pentoxifylline to decrease TNF production and improve RBC deformability [29, 30]
- Countering a pro-thrombotic state, using heparin or aspirin [31]
- Decreasing cerebral edema and reducing raised intracranial pressure, using osmotic agents [14, 32, 33]
- Ameliorating acidosis by expanding intravascular volume [34, 35]

None of these trials improved clinical outcomes and in fact, recently published data suggest that intravascular volume expansion in pediatric malaria participants with impaired perfusion may be counterproductive [36].

There are several possible explanations for these failed attempts:

1. <u>The trials were underpowered.</u> In a recent review of the quality of randomized clinical trials of adjunctive therapy for the treatment of CM, sample sizes were recalculated to determine the study power to detect 25% and 50% reductions in mortality. Of nine trials, only two had sufficient power to detect a 50% reduction in mortality and none could detect a 25% reduction [24]. None of the trials carried out to date have included an ocular funduscopic examination for the detection of malarial retinopathy (see 2.1.1.7); the loss of the added specificity such an examination provides would further decrease the power of these studies.

2. <u>The putative mechanism is not critical to the pathogenesis of fatal malaria.</u> Hypotheses often emerge from clinical studies in which an association is noted between a factor (e.g., TNF concentration) and disease severity or outcome [28, 37]. The hypothesis may be bolstered by data from an animal model in which an intervention has an impact on the exposure (in this case TNF) in question (e.g., intravenous immune globulin in *Saimiri* monkeys)[38]. The clinical trial may be successful in terms of modifying the exposure, but the impact on the outcome of the malaria participant is not clinically significant because the mechanism itself was not on the causal pathway between malaria infection and death or neurological morbidity[28].

3. <u>The mechanism is relevant, but the intervention was timed ineffectively.</u> In the murine and non-human primate malaria worlds, interventions are often initiated simultaneously with the induced malaria infection and, in effect, *prevent* the evolution of severe malaria [39]. In the world of human malaria, CM can develop very rapidly, and although the child is often rushed to the hospital, they may have been comatose for several hours before

treatment can be started. Interventions in this setting need to be able to reverse the key process in order to rescue the participant.

2.1.6 Fatal pediatric malaria

Case fatality rates for children meeting the standard clinical case definition of CM, and with evidence of at least one feature of malarial retinopathy (see 2.1.1.7) are approximately 15% [40]. Risk factors for a poor outcome include a Blantyre Coma Score (BCS) $\leq$ 2, an elevated white blood cell count, pre-treatment hypoglycemia, hyperlactatemia, and papilledema [41-43]. The most commonly observed mode of death is respiratory arrest with subsequent bradycardia and eventually asystole [44].

A case/control study of fatal CM has been ongoing in Blantyre, Malawi since 1996. Cases are children who meet the standard clinical case definition of CM, *P. falciparum* parasitemia, BCS $\leq$ 2 with no other obvious cause of coma; controls are aparasitemic children with non-malarial causes of coma. An early finding of this study was that 24% of children meeting the standard clinical case definition of malaria had no evidence of the post-mortem histological *sine qua non* of CM, the sequestration of parasitized RBCs in the cerebral microvasculature; all of these children had a non-malaria cause of death identified at autopsy. Two different histological patterns were observed in the majority of children with cerebral sequestration: sequestration alone (known as “CM1”); or sequestration with intra- and peri-vascular pathology (“CM2”) (Fig 2) [8].

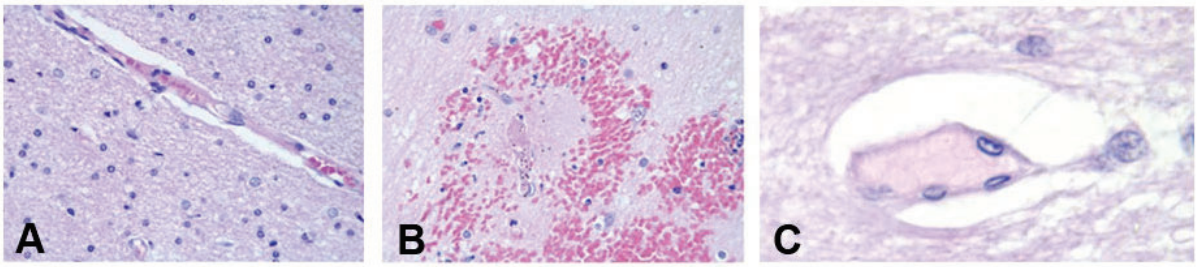


Figure 2. Different histological patterns in children meeting the standard clinical case definition of cerebral malaria. A: CM1, sequestration alone. B: CM2, sequestration with intra- and peri-vascular pathology. C: CM3, no sequestration.

2.1.7 Linking ocular funduscopic findings to autopsy results

In conjunction with the autopsy study described above, ophthalmologists observed a constellation of ocular funduscopic features in many Malawian children with classically defined CM. Four components were described: white-centered hemorrhages (Fig 3A), vessel color changes (Fig 3D), retinal whitening (peri- and extra-macular)(Fig 3B,3C), and papilledema. These were observed individually and in various combinations [42, 45-48].

Post mortem examinations of participants with CM1 and CM2 revealed histologic parallels between the eye findings and the cerebrum. Retinal hemorrhages (Fig 3B) resembled the classically described cerebral ring hemorrhages, where endothelial disruption leads to the extravasation of non-parasitized RBCs [49]. Retinal vessel color changes similar to those

previously seen in the cerebral microvasculature were evident and are caused by the sequestration of parasitized RBCs. As the parasite consumes hemoglobin, the cytoplasm of the parasitized RBC becomes less red, thus during funduscopy the color of the column of blood in the eye appears yellow or orange (Fig 3C).

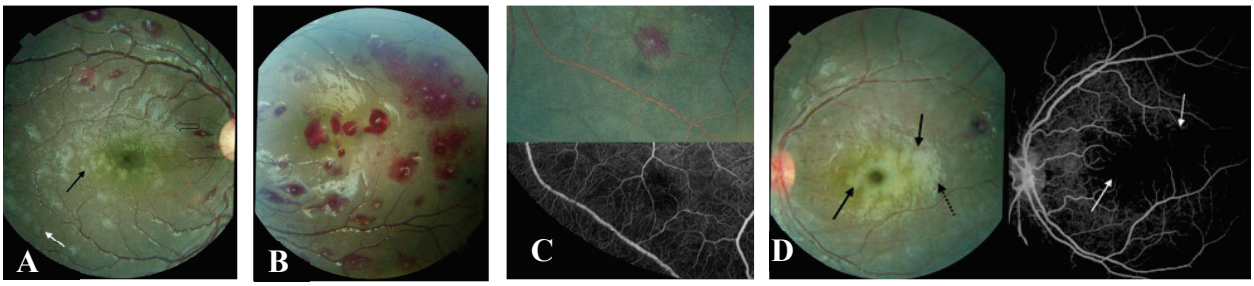


Figure 3. Fundus photographs illustrating features of malarial retinopathy. A: Extensive macular whitening (black arrow) and extra-macular whitening (white arrow). B: Multiple retinal hemorrhages; white areas are reflection artifacts. C: A single hemorrhage and vessel changes; lower image is the corresponding fluorescein angiogram showing a mottled appearance, likely due to sequestered parasitized red cells. D: Peri-macular whitening (black arrows) and fluorescein angiography of the same area, showing impaired perfusion which corresponds to the areas of macular whitening (white arrows)

The etiology of the macular and extra-macular whitening was revealed by fluorescein angiography to reflect areas of non-perfusion beyond vascular obstruction, caused by the sequestration of parasitized RBCs (Fig 3D) [50].

Papilledema is not a feature of malarial retinopathy *per se*, but the finding is associated with worse clinical outcomes when it is found in conjunction with any of the other three retinal features [48].

Among participants in the aforementioned ongoing autopsy study, there was a very strong association between the presence of any of the three features of malarial retinopathy during life (hemorrhages, vessel changes, whitening) and the presence of cerebral sequestration (CM1 or CM2) identified post-mortem (Table 1, and [8])

Table 1. Malarial retinopathy and histological evidence of sequestration in postmortem samples

	Malarial retinopathy present	**Malarial retinopathy absent**
Sequestration present	40	1
No cerebral sequestration	2	14

Sensitivity: 98%	Positive predictive value: 95%
Specificity 88%	Negative predictive value: 93%

The ongoing autopsy-based study of the clinicopathological correlates of fatal CM in Blantyre, using the gold standard of sequestered parasites in the cerebral microvasculature of fatal cases to diagnose CM, has established that the standard clinical case definition misclassifies 25-30% of participants and that the added presence of malarial retinopathy increases the specificity of the case definition from 61% to 95%. Studies conducted prior to the recognition of malarial retinopathy are likely compromised by this misclassification bias [46].

A biomarker for malarial retinopathy: Histidine-rich protein 2 (HRP2) is a *P. falciparum* protein released primarily (but not exclusively) at the time of schizont rupture[51]. Plasma concentrations are significantly higher in patients with CM1/CM2 on histology, compared to autopsy subjects with the CM3 (no sequestration) pattern; plasma HRP2 concentrations are also significantly higher in patients with evidence of malarial retinopathy, compared to those with retinopathy-negative CM[52].

2.1.8 Post*mortem* features of fatal pediatric CM

There are only a few systematic, standardized autopsy-based studies of fatal pediatric malaria [53-63] and only one [61] has classified participants on the basis of the presence or absence of malarial retinopathy. There, 37 participants with retinopathy-positive CM were compared with 13 comatose, retinopathy-negative participants (individuals who were parasitemic, but in whom the autopsies identified non-malarial etiologies for coma and death), and with 18 participants who were aparasitemic and comatose.

- Brain weights (adjusted for age) were increased in all three groups, and differences between groups were not statistically significant.
- 8 of 37 retinopathy-positive CM participants had evidence of mild tonsillar and/or uncal herniation, compared to one of 13 retinopathy-negative participants and one of 18 participants with non-malarial coma
- Sequestration of parasitized red cells was present in all cases of retinopathy-positive CM, and in none of the retinopathy-negative parasitemic participants or in the aparasitemic controls. In the retinopathy-positive CM cases, 25% were CM1, 75% were CM2 (evidence of intravascular thrombin, ring hemorrhages and the intravascular accumulation of hemozoin-laden macrophages).
- Diffuse myelin and axonal damage were seen in association with ring hemorrhages and thrombi, and in areas of intense sequestration.
- The permeability of the blood-brain barrier (BBB) was assessed by immunostaining for fibrinogen, and BBB breaches were seen in association with ring hemorrhages, fibrin thrombi, and areas of intense sequestration.
- There was no evidence of an extravascular localized immune response to the vascular, myelin, and axonal pathology. Microvessels were often distended by the accumulation of CD45/CD68-positive monocytes, which appeared to occupy most of

the lumen of capillaries and venules but did not migrate out of the vessels into the brain tissue.
- Endothelial cells of parasitized vessels often displayed large vesicular nuclei, suggestive of activation.

These findings do not definitively establish causation, or even a sequence of events, but are consistent with pathogenic mechanisms involving thrombotic events, BBB disruption, endothelial cell activation and edema formation. Additional studies in pre-fatal and non-fatal cases of CM were suggested to identify mechanisms by which malaria infections cause coma, brain damage and death [61].

2.1.9 Imaging studies

Owing to the resource-limited settings in which pediatric CM occurs, research involving imaging studies has been limited to computed tomography (CT) case series and occasional magnetic resonance imaging (MRI) case reports [64-67]. Until recently the largest published imaging case series on pediatric CM was a computed tomography (CT) study from Malawi that included 14 participants, 3 of whom were studied in the acute illness and 11 of whom were studied up to 18 months after discharge [68].

In June 2008, MRI technology became available in Blantyre, Malawi. We were able to describe the acute brain MRI findings in children with CM admitted to the Paediatric Research Ward (PRW) of Blantyre's Queen Elizabeth Central Hospital (QECH) between 2009 and 2011. Findings from 120 children with retinopathy-positive CM were compared to the more heterogeneous group of comatose, parasitemic children who met the standard clinical case definition of CM but lacked evidence of retinopathy and thus were presumed to have a non-malarial cause of coma (n=32) [69] .

None of the children with retinopathy-positive CM had a normal MRI. Findings which were more commonly seen in the retinopathy-positive participants include the following:

- Increased brain volume (odds ratio (OR) 9.9; 95% confidence interval (CI) 4.0-24.4): Moderate to severe increases in brain volume were seen in 47% of participants. Ten percent had severely increased volume, *i.e*. their scans exhibited loss of all sulcal markings and had cisternal effacement. Diffuse volume increases were the predominant pattern and were observed in 79% of participants with increased volume. Given the limitations of the 0.35T MRI in Malawi (low field strength, abandoned platforms), it is not possible to delineate the pathogenic mechanism underlying the volume changes (e.g., vasogenic or cytotoxic edema) at this time.

- Basal ganglia involvement (OR 8.9; CI 3.7-21.1) including diffusion-weighted imaging (DWI) changes (OR 4.7; CI 1.5-14.2): The most common finding was abnormal T2 signal in the basal ganglia, present in 84% of the children with retinopathy-positive CM.

- Cortical abnormalities on T2 (OR 4.1; CI 1.7-9.7): Supratentorial cortical thickening and T2 signal abnormalities were present in 62% of children. This generally did not follow a typical vascular distribution. Cortical DWI abnormalities were seen in 21% of children. These also often failed to correspond to a typical vascular distribution. Specifically, there were no cases of watershed ischemia

- Periventricular white matter changes (OR 3.7; CI 1.6-8.3): Increased T2 signal in the white matter was present in 72% of children and was often associated with DWI abnormalities, which were present in 45%. White matter DWI abnormalities tended to occur in the absence of cortical DWI abnormalities.

- Corpus callosum changes on T2 and DWI (OR 4.2; CI 1.6-10.9 and OR 3.3; CI 1.3-8.6, respectively): T2 signal changes in the corpus callosum were present in 49%, and often occurred in the absence of other white matter changes. Involvement of the corpus callosum was closely associated with corresponding positive DWI changes, seen in 43% of total cases. Although some cases of corpus callosum abnormality had diffuse involvement, the splenium was predominantly affected in a majority (38/52, 73.1%) of cases.

- Cerebellum (posterior fossa) changes (OR 3.5; CI 1.4-8.6)**:** Increased T2 signal was identified in the cerebellum in nearly half of children. This ranged from diffuse involvement with associated edema to multifocal areas of deep involvement with localized mass effect.

2.1.10 Imaging features associated with fatal outcomes in pediatric CM

Of the MRI features associated with retinopathy-positive CM described above, only one was statistically significantly associated with outcome (survival *vs* death): severely increased brain volume[52] (Table 2). This sample included 168 participants, enrolled from 2009-2011. Twenty-one of the 25 fatalities had severely increased brain volume on the initial MRI (OR 7.5). All the retinopathy-positive children with lesser degrees of increased volume survived. These findings are consistent with the brain swelling observed at autopsy [61] and with earlier clinical observations suggesting raised intracranial pressure as a step on the pathway to death in CM [14, 70]

Table 2. MRI features associated with fatal outcomes in Malawian children with retinopathy-positive CM (multiple regression analyses)

	Ret-pos CM survivors (n=143)	**Ret-pos CM fatalities (n=25)**	**OR (CI)**
Pre-pontine CSF (mm)	10.0	8.5	0.5 (0.3-0.8)
Posterior predominance	16	8	1.6 (0.7-3.8)
Severely increased brain volume	39	21	3.4 (0.9-12.7)

A neuroimaging study performed in children with retinopathy-negative CM revealed that severe neurological morbidity in survivors is associated with increased brain volume on admission brain MRI [71]. In this study there were only three deaths and one of these three children had greatly increased brain volume on admission scan. Brain MRI factors associated with death in children with retinopathy negative CM are still unclear due to the low number of scans interpreted in children with this condition. Therefore, in the clinical trial proposed here, sample size and power calculations were performed on children with retinopathy-positive CM. We postulate that the same association between increased brain volume and death will be valid in children with retinopathy-negative CM.

2.1.11 The natural history of malaria-associated increased brain volume

The evolution of increased brain volume in pediatric CM has been captured in sequential MRIs from 35 of the 168 retinopathy-positive CM participants scanned in Blantyre between 2009 and 2011. These participants received antimalarial drugs and (as needed), antipyretics, anticonvulsants and antibiotics [8], but were not treated specifically for the volume changes observed on MRI scans. The post-admission changes in the mean pre-pontine CSF measurement of the 30 patients who survived (dashed black line) was significantly different from that in the 5 patients who died (solid yellow line) (P=0.02) (Fig 4).

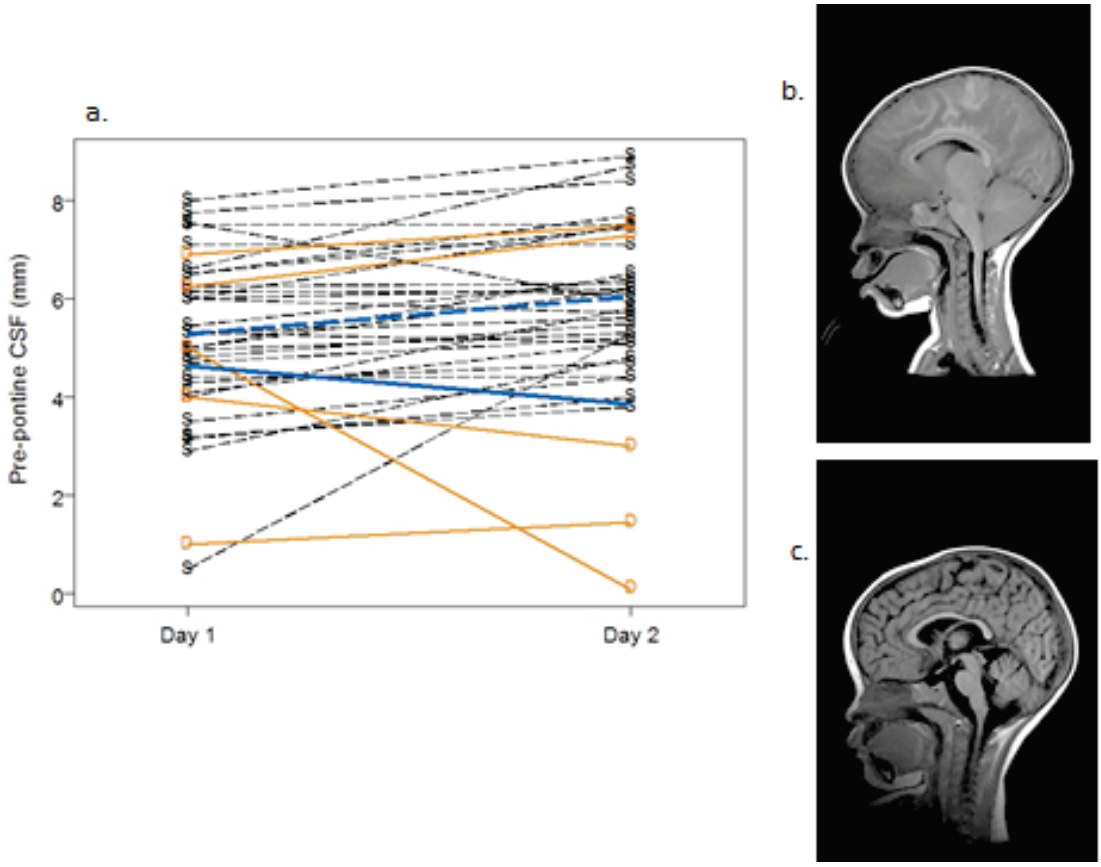


Figure 4. Evolution of increased brain volume in pediatric CM.
The line graph (a) shows the distribution of pre-pontine CSF measures on first and second MRIs. Second MRIs were obtained 16 to 30 hours after the first. Dashed black lines reflect subjects who survived; solid orange lines are subjects who died. The post-admission changes in mean pre-pontine CSF measures in retinopathy-positive CM patients who recovered and were discharged (N = 30, dashed blue line) was statistically significantly different from those patients who died (N = 5, solid blue line) (P = 0.02). The corresponding MR images (b, c) show rapid resolution of the increased volume over 48 hours in one patient.

2.1.12 Quantitative vascular physiology measurements

Transcranial Doppler Ultrasound (TCD)

Transcranial Doppler Ultrasound (TCD) is a portable, non-invasive, easy to use tool that quantifies cerebral blood flow velocities (CBFV)[72]. TCD has been widely used to assess cerebrovascular physiology in number of neurologic illnesses in children[73-75]. Specific alterations to TCD derived flow velocities and waveform morphologies enable characterization of specific pathologic changes to the neurovasculature during illness. In 160 children with retinopathy positive cerebral malaria, five distinct changes to expected flow velocity patterns were noted (Figure 5).[76]

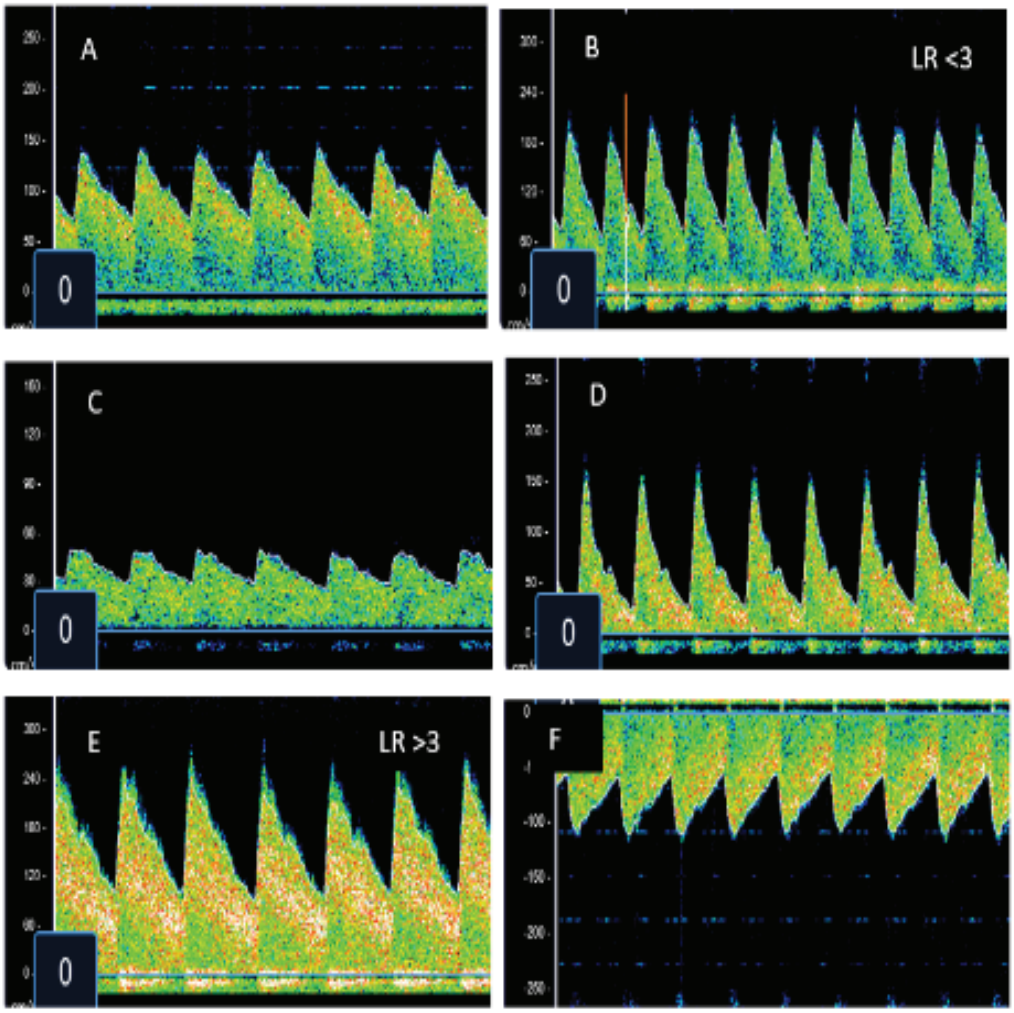


Figure 5. A. Normal middle cerebral artery (MCA) transcranial Doppler ultrasound (TCD) flow velocities and waveform for a 3-year-old child. Peak= systolic, EDV= end diastolic velocity, PI= pulsatility index . TCD with normal systolic flow velocity, reduced diastolic flow velocity, increased pulsatility index. These findings represent a child categorized as having microvascular obstruction. C. TCD with increased systolic flow velocity, increased diastolic flow velocity, loss of dichrotic notch. Lindegaard ratio (LR) <3. These findings represent a child categorized as having hyperemia. D. TCD with increased systolic flow velocity, increased diastolic flow velocity, presence of dichrotic notch. LR >3. These findings represent a child categorized as having cerebral vasospasm. E. TCD with decreased systolic flow velocity, decreased diastolic flow velocity, decreased mean flow velocity. These findings represent a child categorized as having low flow. F. TCD with increased systolic flow velocity, increased diastolic flow velocity, increased mean flow velocity in the basilar artery. At the same time, all measurements in the middle cerebral arteries were normal. These findings represent a child categorized as having isolated posterior hyperemia.

Each flow velocity phenotype was identified with different frequency (microvascular obstruction 22%, hyperemia 26%, vasospasm 13%, low flow 26%, isolated posterior hyperemia 4%) and was associated with different neurologic outcomes. Children found to have low flow were most

likely to die (Table 3). Children diagnosed with vasospasm were most likely to survive, but with neurologic sequelae.

Table 3: Predicted probabilities (with 95% confidence intervals) of neurologic sequelae or death in children with cerebral malaria in each TCD diagnostic group.

	Probability of Neurosequelae, %		Probability of Death, %	
Microvascular obstruction	16	(5, 26.)	22	(10, 34.)
Hyperemia	17	(6, 29)	27	(14, 41)
Vasospasm	45	(25, 66)	18	(2, 34)
Low Flow	24.62	(14, 35)	321	(21, 44)
IPH	20	(0, 45)	20	(0, 45)

IPH; isolated posterior hyperemia

These findings suggest that multiple different neurovascular changes may contribute to neurologic injury across a cohort of pediatric patients with CM. However, 96% were able to be placed into a single phenotypic group and remained in that group until normalization of flow or progression to death. This suggests that a predominant neurovascular change may occur on an individual level in most cases.

Near-infrared spectroscopy

Near-infrared spectroscopy (NIRS) is an established technology for measuring the oxygen saturation of hemoglobin in tissues. Near-infrared light can penetrate through biological tissues, including skin, bone, and muscle. To interrogate a tissue, light is applied to the region of interest and undergoes scattering and absorption before being detected by a photosensor[77]. Using different wavelengths, NIRS can differentiate between oxygenated and deoxygenated hemoglobin in blood and can measure changes in total hemoglobin concentration using the sum of oxygenated and deoxygenated hemoglobin[78]. NIRS can assess oxygen availability and consumption in living tissues.

NIRS can provide continuous assessment of the oxygen saturation of hemoglobin in cerebral tissue via a sensor placed against the scalp. One study used NIRS to monitor cerebral oxygenation in twenty adults with malaria[79]. They found that the oxygen saturation of hemoglobin was generally low (56-60%) across all clinical groups of malaria patients: cerebral malaria (Glasgow coma score < 11), non-cerebral severe malaria, and uncomplicated malaria. However, when they applied spectral analysis to calculate the amplitude of cerebral blood flow oscillations, they discovered that patients with cerebral malaria had severely diminished amplitude of oscillations in the 0.02 – 0.04 Hz band. The amplitude of this oscillation increased after recovery from coma. This may reflect an impairment of the ability cerebral vessels to vasodilate and vasoconstrict.

In a pilot study carried out on the Pediatric Research Ward in 2013-2014, we used NIRS to measure total hemoglobin concentration (the sum of oxy- and deoxy-hemoglobin) and hemoglobin oxygen saturation using a NIRS probe placed on the forehead where it interrogated the frontal cortex. We found that patients with retinopathy-positive cerebral malaria had higher total hemoglobin concentrations in the brain than healthy Malawian children ($p < 0.0001$), but the oxygen saturations did not differ (Figure 6). Higher tissue densities of total hemoglobin could reflect increased vessel diameter (e.g, hyperemia) or increased venous congestion.

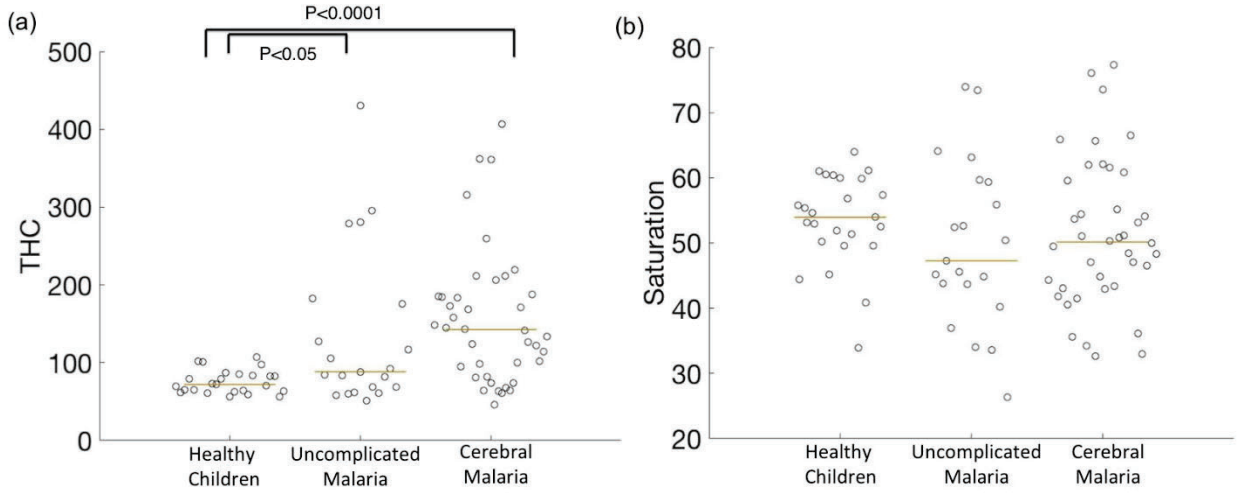


Figure 6. Cerebral total hemoglobin concentration (THC) (a) and cerebral hemoglobin oxygen saturation (b). THC and oxygen saturation were determined from a 30-minute recording obtained from a NIRS probe applied to the forehead of each patient. Horizontal lines represent median values for each group.

To investigate the frequencies and patterns of periodic oscillations in cerebral hemoglobin oxygen saturations, we applied detrended fluctuation analysis (DFA) to the continuous recordings. DFA is a mathematical method that analyzes the long-range correlation and long-range memory of a signal. It has been used to analyze diverse signals from heart-rate dynamics to neural spiking to intercranial pressure. DFA analysis determines a single exponent, called ***α***, which reflects the level of self-correlation and randomness in the signal[80]. When α is greater than 0.5, the signal is correlated, i.e., has repeating patterns; an α of 0.5 represents random fluctuations. Increased randomness in cerebral blood-flow indicates a failure of cerebral autoregulation. We applied DFA to both the THC (which reflect fluctuations in hemoglobin tissue density, e.g., pulsatility of vessels) and the hemoglobin oxygen saturation (which reflects fluctuations changes in oxygen supply and demand).

Patients with retinopathy-positive cerebral malaria had lower α_{THC} than healthy Malawian children ($p < 0.001$) or children with uncomplicated malaria ($p < 0.0001$) (Figure 7). Moreover, the pattern of fluctuations clustered around $\alpha = 0.5$, indicating that the fluctuations in THC were random. The α_{sat} revealed a similar pattern whereby patients with cerebral malaria again had nearly random fluctuations in cerebral hemoglobin oxygen saturation, much lower than healthy Malawian children ($p < 0.001$) or children with uncomplicated malaria ($p < 0.01$). Together, the results of detrended fluctuation analysis of the total hemoglobin concentration and the cerebral hemoglobin oxygen saturation suggest a loss of cerebrovascular autoregulation.

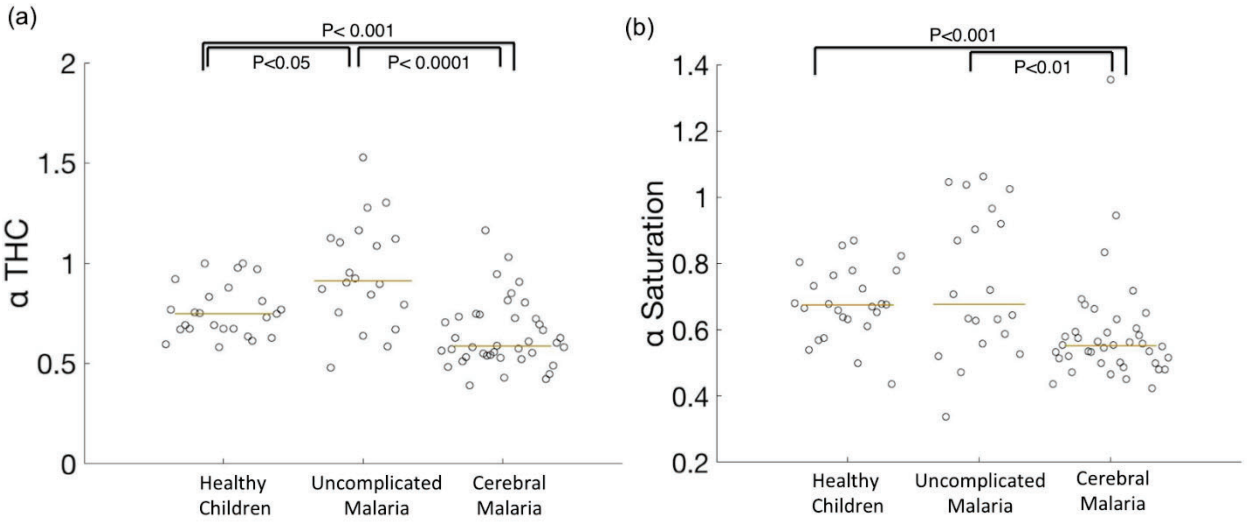


Figure 7. Detrended fluctuation analysis of the instantaneous changes in the total hemoglobin concentration (THC) (a) and cerebral hemoglobin oxygen saturation (b). Horizontal lines represent median values for each group.

NIRS can also be applied to measure arterial vasoreactivity in the peripheral circulation. This is accomplished by inflating a pneumatic cuff around the upper arm or thigh while monitoring muscle oxygenation distal to the occlusive cuff. During occlusion, blood flow stops and oxygenation decreases in the tissue distal to the occlusion. After release of the occlusion (3 minutes duration), both blood flow and tissue oxygenation increase rapidly and exceed their baseline resting value. The rate of increase is a quantitative measure of endothelium-dependent vasodilation.

In a pilot study carried out on the Pediatric Research Ward during 2013-2104, we measured skeletal muscle reperfusion dynamics in children with retinopathy-positive cerebral malaria and compared them against reperfusion dynamics in healthy Malawian children and children with uncomplicated malaria. Measurements were performed within six hours of admission to the Pediatric Research Ward. Peak muscle reoxygenation rate was lower in patients with cerebral malaria compared to healthy Malawian children ($p < 0.0001$) or compared to children with uncomplicated malaria ($p < 0.01$) (Figure 8). The muscle reoxygenation rate improved on hospital day 2 ($p < 0.01$), and at follow up on day 28 ($p < 0.05$) among children with cerebral malaria (Figure 9).

Together, these studies of skeletal muscle reperfusion in children with retinopathy-positive cerebral malaria identify a defect in muscle reperfusion. This may be attributable to impaired endothelium dependent vasodilation, a physiological hallmark of endothelial injury or dysfunction, changes in blood rheology, or changes in autonomic control of blood flow. Like the cerebral NIRS oxygenation fluctuations described above, decreased skeletal muscle reoxygenation is a feature that distinguishes uncomplicated malaria from cerebral malaria. In the current study, we plan to measure both cerebral oxygenation fluctuations and skeletal muscle reperfusion rates as a vascular physiological biomarker of cerebral malaria; in addition, we will examine correlations between NIRS and TCD measurements to further characterize the 4 major neurovascular abnormalities identified by TCD.

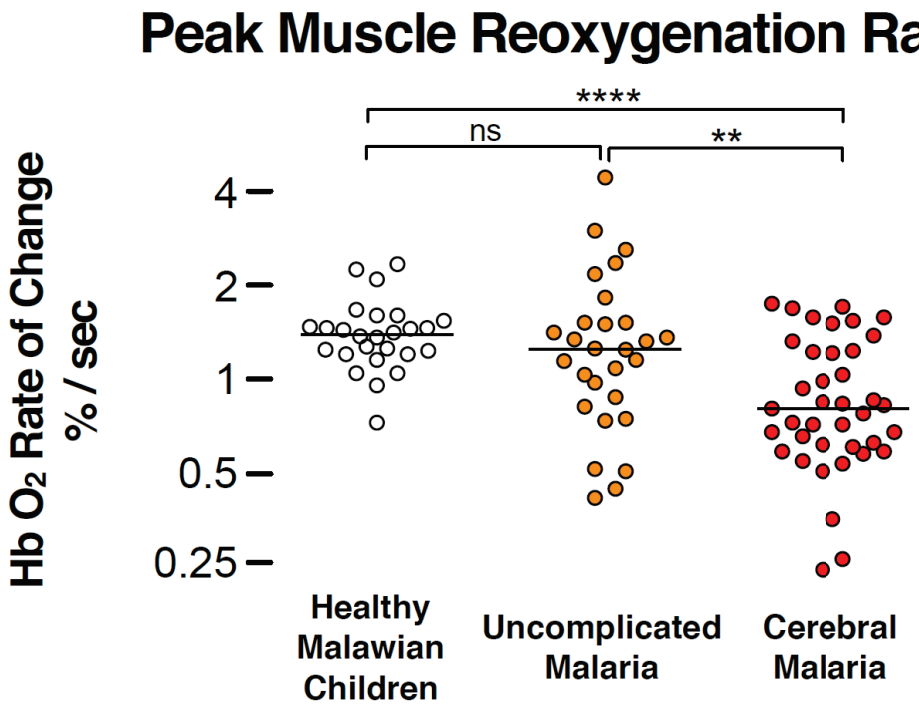


Figure 8. Peak muscle reoxygenation rates on the day of admission to hospital. Peak muscle reoxygenation rate was identified as the maximal rate of change in muscle hemoglobin oxygen saturation (%) during reperfusion following a 3-minute occlusion with a pneumatic cuff.

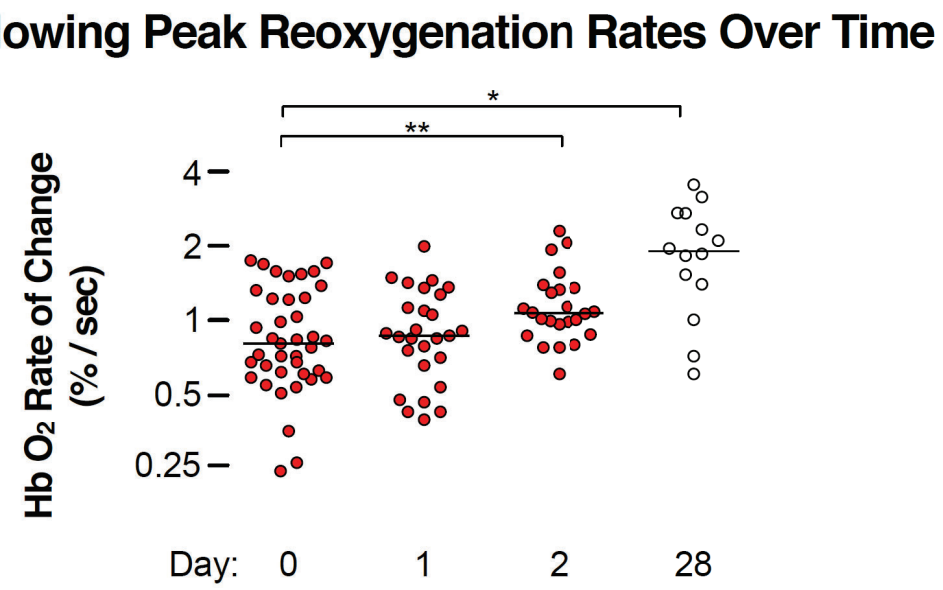


Figure 9. Peak muscle reoxygenation rates among children with retinopathy-positive cerebral malaria on hospital days 0-2 and at follow on day 28. Peak reoxygenation rate increased by hospital day 2 and at day 28 follow-up analyzing paired data using Wilcoxon's test.

2.1.13 Hypothesis

Description of the Study Refining the standard clinical case definition of CM to include malarial retinopathy may improve specificity and focusing on retinopathy-positive CM participants with moderately severe increased brain volume identifies the group at highest risk of dying.

Our data suggest that these children experience a disruption of the respiratory control center manifesting as a respiratory arrest. The final cause of death appears to be respiratory arrest secondary to intracranial hypertension.

Hypothesis: Death in children with CM occurs when raised intracranial pressure from severely increased brain volume impairs the function of the medullary respiratory center; interventions which reduce brain volume or support ventilation will improve survival rates in CM patients.

Using numbers derived from the ongoing studies in Malawi, of 100 children who meet the standard clinical case definition of CM, approximately 70-75 will be retinopathy-positive. Of these, ~ 47% (34-35 children) will have severely increased brain volume on MRI, and, with the observed mortality rate of 37% in this clinical subset, about 12 of these children will die. The majority recover quickly and completely, suggesting that the adverse effects of increased brain volume on respiratory status are reversible and short-lived[52].

All CM patients enrolled in the study will contribute to the evaluation of surrogate markers of brain volume.

CM patients who are retinopathy-negative and who have evidence of increased brain volume on MRI will be eligible to be enrolled in an identical interventional study (of assisted ventilation or hypertonic saline). This group, when combined with the retinopathy-positive CM patients described above, represents children who satisfy the less stringent but standard WHO definition of cerebral malaria[81]. In order to increase generalizability of our study results, we will analyze data from this combined group, but perform a sensitivity analysis to determine if treatment effects vary in children of different retinopathy status.

2.2 Agent(s)/Intervention(s)

The goal of this clinical trial is to compare outcomes in children treated with the current standard of care (antimalarial drugs and elevation of the head of the bed 30 degrees) to outcomes in children randomized to receive one of two different approaches to ameliorating the effects of increased brain volume:

- Arm 1: Usual care + immediate mechanical ventilatory support during the initial high risk period, with ventilator withdrawal once brain volume has decreased (brain volume score ≤ 5), and the patient has improved clinically (Blantyre Coma Score $\underline{\geq}$ 3).

- Arm 2: Usual care + intravenous hypertonic saline, withdrawn once brain volume has decreased (brain volume score ≤ 5) and the patient has improved clinically (Blantyre Coma Score ≥ 3).

- Arm 3: Usual care (antimalarial drugs plus elevation of the head of the bed by 30 degrees)

Background

The study design was informed by (a) the strong association between increased brain volume and a fatal outcome in pediatric CM, (b) the rapid reversibility of increased brain volume in CM survivors, and (c) the importance of identifying the most feasible intervention for use in malaria-endemic settings.

One challenge is that direct monitoring of intracranial pressure (via intraventricular catheters, or subarachnoid or intraparenchymal bolts) in this setting is relatively contra-indicated --- the risk of infection is too high [82]. Indirect measurements of brain volume are required; the data to date have been derived via MRI. Though the MRI findings are useful and reliable, they are not scale-able across sub-Saharan Africa. Consequently, efforts to identify more user-friendly approaches to identifying patients with severely increased brain volume will be evaluated at the same time as this clinical trial is conducted.

In developed countries, current neurocritical care of children with raised ICP from increased brain volume incorporates multiple strategies. Some of the measures commonly used to decrease ICP are already standard of care on the QECH Pediatric Research Ward, and as such, are not appropriate for inclusion in a randomized controlled trial. These include: elevation of the head, adequate oxygenation (including use of supplemental oxygen via nasal prongs and mask), prompt blood transfusions to optimize oxygen delivery, antipyretics for fever, anticonvulsants for clinically evident seizures, EEG to identify sub-clinical seizures, and glucose monitoring to avoid hypo- and hyperglycemia. Sedation is often recommended for agitated patients with raised ICP, but CM patients are, by definition, deeply comatose and very rarely require additional sedation [83].

Potential etiologies of increased brain volume in pediatric CM

Increased brain volume can result from a number of molecular, cellular, structural or functional changes in the blood brain barrier (BBB), the microcirculation, and various autoregulatory systems [84]. Because intracranial volume is fixed, any additional increase in brain parenchyma, blood or cerebrospinal fluid can cause a non-linear increase in ICP, aggravating and accelerating the original insults and injuries.

The etiology of increased brain volume in CM patients has not been established, but autopsy and clinical information provide evidence to support four possibilities:

1. Vasogenic (extracellular) edema: Vasogenic edema results from breakdown of the BBB, and primarily involves the white matter [85]. Plasma leaks into the brain parenchyma as a result of the increased permeability. There is abundant evidence of BBB breakdown in

CM, from the ring hemorrhages observed in the cortex and cerebellum [8, 61] to fibrinogen leakage from apparently intact vessels [13, 61].

2. Cytotoxic (cellular) edema: This type of edema is often the result of cellular injury following cerebral ischemia, and may play a role in situations where the microcirculation is impeded [85]. It is characterized by sustained intracellular water accumulation involving both astrocytes and neurons [86]. Fluorescein angiography has shown impaired perfusion in the eyes of children with CM [50], and there is immunohistologic evidence of axonal damage and myelin loss associated with sequestered parasitized RBCs in fatal cases of pediatric CM [61].

3. Increased blood flow volume: The anemia, fever and seizures[87] that are commonly seen in CM patients may lead to increased cerebral blood flow volume (hyperemia) and dysfunction of autoregulatory systems[88].

4. Vascular congestion: Intense sequestration of parasitized red cells in postcapillary venules may lead to venous obstruction and vascular congestion [89, 90].

The current standard of care on the Paediatric Research Ward in Blantyre addresses the likely determinants of increased blood flow volume (seizures, fever and anemia). Potential interventions were reviewed to identify those most likely to address the other three putative etiologies: vasogenic edema, cytotoxic (cellular) edema and vascular congestion.

2.2.1 Summary of Previous Pre-clinical Studies

None

2.2.2 Summary of Relevant Clinical Studies

2.2.2.1 Mechanical ventilation

There is only one published study addressing endotracheal intubation in children with life-threatening malaria, and it is a retrospective cohort study [91]. Intubations were performed by intensivists in the ICU and by pediatricians in the emergency rooms. Intensivists tuned the ventilators, and the nurse to patient ratio in the ICU was 1:5 during the day and 1:10 during the night. Children were managed according to WHO guidelines, which included prompt blood transfusions, management of hypoglycemia, and an anticonvulsant protocol for seizures. Raised intracranial pressure (protracted episodes of decorticate or decerebrate rigidity in addition to dilated sluggish pupils) was treated with 10-20 ml/kg of 20% mannitol and short-time induced hypocapnia (pCO2 adjusted to 25-35 Torr). Parenteral antibiotics were used only for concomitant community-acquired and nosocomial infections (which were defined as occurring after 48 hours of stay).

During the study period of 5 years, a total of 502 children were hospitalized with severe malaria. Of these, 83 patients required intubation. Indications for intubation included deep coma (BCS $\leq$ 2; n=16), overt cortical or diencephalic injury (i.e., *status epilepticus* or decorticate posturing;

n=20), severe brainstem involvement (decerebrate posturing or opisthotonos; n=15), shock (systolic BP < 50 in children under five, systolic BP <80 in children over five; n=15), cardiac arrest (n=13) and severe respiratory distress (acute lung injury with pulmonary infiltrates and a PiO2/Fio2<300 Torr; n=4).

The median age was 8.6 years, the median duration of ventilation was 36 hours, and 50 (60%) patients died. The lowest mortality rate (12.5%) was in the group intubated for deep coma only. All of those with cardiac arrest subsequently died, and mortality rates for the other subsets are shown below (Fig. 10).

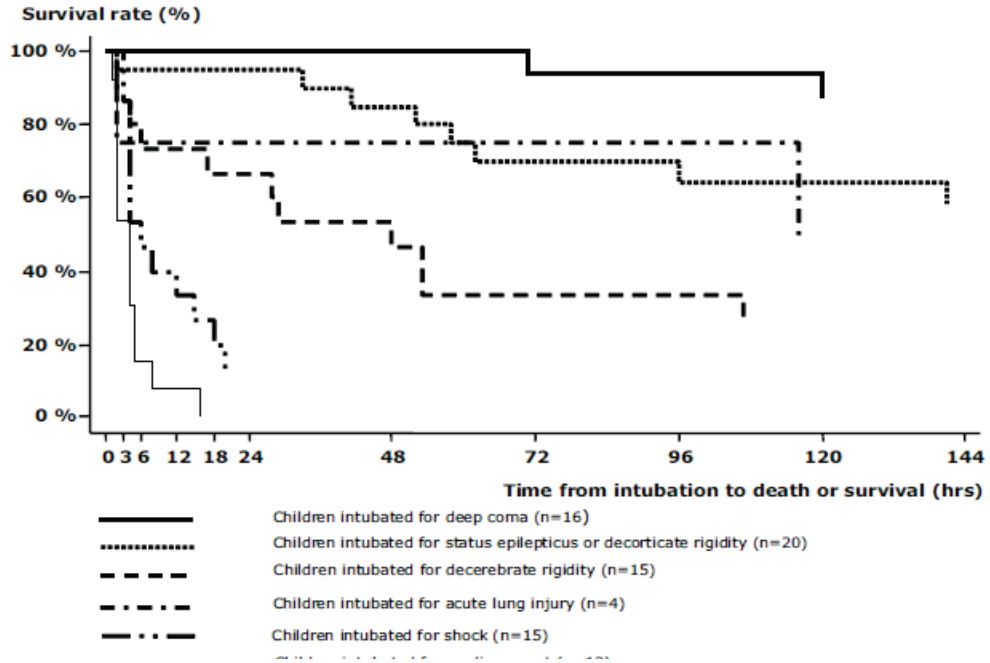


Figure 10. Kaplan-Meier curves related to reasons for intubation among 83 children with severe malaria who required intubation.

Sixty-one children survived more than two days and of these, 20 (33%) had evidence of hospital acquired infections (twelve had pneumonia, 8 had septicemia). The cumulative incidence rates were 2.3% after 48 hours, 15.3% after 72 hours, 26.6% after 92 hours and 63.4% after 12 hours.

The authors concluded that mechanical ventilation in the setting of life-threatening childhood malaria was required for a short time, and that overall outcome depended more on clinical presentation than on critical care complications. Without retinopathy data, or any other clinical indicators of raised intracranial pressure (e.g., opening CSF pressure, evidence of papilledema, neuro-imaging), it is difficult to extrapolate from this patient population to those in the proposed clinical trial – but the relatively good outcomes in the group with “coma only”, and the low rates of nosocomial infection are encouraging.

2.2.2.2 Head elevation

For decades, routine treatment of raised ICP has included maintaining the patient's head in a neutral position with elevation of the head of the bed to 30 degrees [92]. This approach has been evaluated and supported in adults [93-99] and recently in children [93]. The benefits of elevating the head, relative to the heart, are attributed to gravity-induced changes in cerebral blood and cerebrospinal fluid volume. In most clinical situations, head elevation contributes positively towards the usual therapeutic goal of maintaining ICP below 20 mm Hg while maintaining cerebral perfusion pressures between 40-65 mm Hg [100]. This approach is standard practice in neuro-intensive care units for patients with increased intracranial hypertension [101].

2.2.2.3 Corticosteroids

Glucocorticoids were first used for the control of cerebral edema associated with cerebral neoplasms [102] and their introduction coincided with a significant decline in peri-operative mortality rates [103]. Their effectiveness was eventually traced to actions on the BBB, a highly selective interface composed of brain endothelial cells. These endothelial cells, unlike those in extracerebral capillaries, are connected by tight junctions; opening of these tight junctions plays a key role in the formation of vasogenic cerebral edema [104] and corticosteroids ameliorate this disruption. Dexamethasone is the most commonly used corticosteroid in this setting. It is approximately six times as potent as prednisolone and reaches full effect within 24-72 hours [104]. Doses vary between 4 to 100 mg/day, but in patients with brain tumors, side effects begin to outweigh therapeutic effects at doses above 16 mg/day [105]. If the tumor is causing impaired consciousness or signs of increased ICP, a standard approach includes a loading dose of 10 mg (intravenously) followed by doses of 16 mg/day, in four divided doses.

Prolonged use of high dose steroids can produce serious side effects including adrenal suppression, steroid myopathy, Cushingoid facies, hyperglycemia, and peptic ulcers, and requires a tapering regimen following treatment of the underlying etiology of increased ICP (*e.g.,* tumor resection). Side-effects are similar with short-term use, but tapering is unnecessary for treatments less than three weeks [106].

Corticosteroids have also been used as adjunctive therapy for bacterial meningitis, primarily to reduce inflammation in the subarachnoid space. Several large randomized trials have had conflicting results in terms of overall outcome, but authors of a recently published meta-analysis of individual participant data from five major trials concluded that in bacterial meningitis, adjunctive dexamethasone does not significantly reduce rates of death or neurological disability [107]. Dexamethasone doses of up to 0.4 mg/kg twice daily were used for 2-4 days in the pediatric participants; in adults, the dosages ranged from 16 mg twice daily to 10 mg four times daily for 4 days. No participants were "weaned" from the corticosteroids; tapered doses were not required for regimens ranging from 2-4 days in length. Gastrointestinal bleeding was reported in all studies (1.3% of 1,021 participants on dexamethasone, 1.9% of 1,014 participants on placebo), but the difference between the two groups was not statistically significant (p=0.14). Hyperglycemia was more common in the adults receiving dexamethasone than among the

placebo recipients (20.3% *vs*. 16.0%, p = 0.02); no data on glucose concentrations after treatment were collected in the pediatric study populations.

2.2.2.3.1 Corticosteroids in malaria

There have been two randomized controlled trials of dexamethasone as adjunctive therapy for CM [25, 26]. Warrell *et al*.[25], used a total of 2 mg/kg dexamethasone for the first 48 hours (in children, 0.6 mg/kg loading, followed by 0.2 mg/kg q 6h; in adults, 0.5 mg/kg initially, followed by 10 mg q 6h), in conjunction with intravenous quinine. One hundred patents with CM were included, and the mortality rates did not differ between dexamethasone and placebo recipients. Coma recovery times were prolonged in the dexamethasone cohort, and they experienced more complications (pneumonia, gastrointestinal bleeding) than did those who received placebo.

The second study [26] used a much higher dose of dexamethasone (11.4 mg/kg in total over 48 hours, administered as 3 mg/kg initially, and then 1.4 mg/kg q 8h for 48 hours). Thirty-eight stuporous or comatose participants infected with *P. falciparum* were randomized to receive either dexamethasone or placebo. All participants were treated with intravenous quinine. Overall mortality rates were the same in the two study arms; four participants in each group died. Although there was not a statistically significant difference in the rate of complications, 3 of 19 dexamethasone recipients developed gastrointestinal bleeding, compared to 0 of 19 placebo recipients. In contrast to the results observed in the Warrell study [25], 3 of 19 placebo recipients developed pneumonia, compared to 0 of 19 dexamethasone recipients.

A Cochrane review combined results from these two trials to assess the effects of corticosteroids in participants with CM on death, life-threatening complications and residual disability in survivors [108]. The meta-analysis showed that deaths were distributed evenly between the corticosteroid and control groups in both trials (RR 0.89, 95% CI 0.48-1.68, 143 participants). Gastrointestinal bleeding and seizures were more common in the corticosteroid group (RR 8.17, 95% CI 1.05-63.6 for bleeding; RR 3.32, 95% CI 1.05-10.47 for seizures). For pneumonia, the confidence intervals were very wide, and no statistically significant difference was observed between the two groups. For pulmonary edema, urinary tract infections and bacteremia/septicemia, there were no differences between groups.

The authors of the Cochrane review concluded that there is insufficient evidence to support the use of corticosteroids in the routine management of CM but recognized that the two trials did not have sufficient power to exclude a beneficial effect. Given the unclear efficacy and consistent evidence of adverse effects of adjunctive steroid therapy, we have chosen two non-steroid interventions for testing in this clinical trial.

At present, corticosteroids are recommended to treat intracranial hypertension in only two settings: acute mountain sickness[109] and brain tumors[110]; in all other circumstances studied (e.g. stroke[111], traumatic brain injury[112], brain swelling following cardiac arrest[113], sub-arachnoid hemorrhage[114]), and in the majority of CNS infections (with niche exceptions, and in those exceptions steroids are not believed to act by reducing intracranial hypertension)[115, 116], the risk benefit ratio is unambiguously unfavorable and not considered a component of standard care, nor worthy of further investigation.

2.2.2.4 **Osmotic agents** (mannitol and hypertonic saline)

Osmotic agents play an important role in reducing raised ICP and treating cerebral edema [85, 117-119]. The salutary effects of osmotherapy on ICP are thought to result from brain shrinkage after the shift of water out of the brain parenchyma. The ideal osmotic agent remains in the intravascular compartment, thus promoting movement of water from the intracellular to the extracellular compartment. As fluid moves into the vascular space, the tissue shrinks. The ideal agent is inert, nontoxic, and has minimal systemic side effects. The two main agents in clinical use today are mannitol and hypertonic saline [85, 117-119].

2.2.2.4.1 Mannitol

Mannitol is a freely filtered, non-metabolized solute that decreases the reabsorption of water across the renal tubules, creating diuresis. It has a biphasic effect on ICP. Initially it affects RBCs, and reduces blood viscosity [120]. At the same time, intravascular volume increases because mannitol increases plasma osmolality. The combined effect is an increase in cardiac output which, if autoregulatory pathways are intact, leads to compensatory cerebral vasoconstriction, and a drop in ICP [121]. This effect typically wanes 4 hours after administration [120].

The second phase of ICP reduction occurs as mannitol extracts water from the cerebral extracellular space into the intravascular compartment. This occurs due to an induced osmotic gradient. An intact BBB is required for this to occur [85].

In traumatic brain injury (TBI), bolus dosing is preferred. Mannitol becomes less effective with repeat dosing and works best when cerebral vascular autoregulation is intact.

Mannitol administration decreases cerebrospinal fluid production by up to 50%, which, *via* the Monro-Kellie Doctrine, can lead to prolonged ICP decreases [122, 123].

In combination with hyperventilation, mannitol was shown to reverse transtentorial herniation in a heterogeneous cohort of 28 participants with cerebral edema related to neoplasms, intracerebral hemorrhage, TBI and ischemic stroke [124].

Side-effects: Mannitol can cause significant diuresis, necessitating close observation of fluid balance to avoid hypovolemia. Target osmolality in mannitol recipients is generally between 300 and 320 mOsm [125]. Hyperosmolality can cause acute renal failure via dose-dependent vasoconstriction of the renal arteries [126] or by massive diuresis [127]. Accumulation of mannitol in cerebral tissue may lead to a rebound phenomenon of increased ICP, but this is controversial [117, 119].

2.2.2.4.2 Mannitol in malaria

The earliest use of osmotherapy in malaria involved urea in invert sugar (i.e., a mixture of glucose and fructose derived from sucrose) [128, 129], but these studies lacked controls. More recently, mannitol has been used in a study of intracranial hypertension in Kenyan children with

CM[14] and in two randomized clinical trials in CM, one involving adults [33] and the other involving children[14].

Newton, *et al* [14] found that mannitol controlled the ICP in children with moderate intracranial hypertension (ICP above 20 mm Hg and cerebral perfusion pressure less than 50 mm Hg, lasting longer than 15 minutes continuously), but it did not prevent the development of intractable intracranial hypertension in children with severe elevations of intracranial pressure (ICP about 40 mm Hg and cerebral perfusion pressure less than 40 mm Hg lasting longer than 15 minutes continuously).

A large, randomized, double-blind controlled trial comparing mannitol to placebo was conducted in Uganda, involving 156 pediatric participants with CM [32]. The mannitol recipients were given a single dose (1g/kg) of mannitol, and those in the placebo group received an equal volume (5 ml/kg) of normal saline. All children were treated with intravenous quinine. The trial was powered to detect a difference in coma resolution time in mannitol recipients (66% were predicted to regain full consciousness in 24 hours) compared to those receiving placebo (where 41% were predicted to regain full consciousness in 24 hours). Mortality rates in the two groups were similar (13.2% in those receiving mannitol, 16.3% in the placebo group). The median time to regain consciousness was shorter in the mannitol group (18.9 hours) than in the placebo group (20.5 hours) but the difference was not statistically significant. Intracranial pressure was not measured in the participants, as CSF opening pressures were not reported in participants who had lumbar punctures.

Mohanty, *et al* [33] used CT scan evidence of cerebral edema to identify adult CM participants for an open, randomized trial comparing intravenous mannitol (1.5 g/kg, followed by 0.5 g/kg every 8 hours) to controls receiving standard of care.. Thirty-one participants received mannitol and 30 received standard care; all received intravenous quinine. There was no difference between the two groups in terms of survival, and the median coma resolution time was longer in the mannitol recipients than in those receiving quinine only (90 hours *vs* 32 hours, p = 0.02). Analysis of the CT scan findings from the participants enrolled in this study suggested that mild to moderate cerebral swelling was common in adult participants with CM but did not correlate with coma depth or disease outcome.

A Cochrane review of mannitol and other osmotic diuretics as adjuncts for treating CM was published in 2011[130], before Mohanty *et al* [33]. Only one trial, the Uganda study described above, met the inclusion criteria, and the conclusion of the review was that there are insufficient data on the effects of osmotic diuretics in children with CM.

We did not select mannitol for our osmotherapy agent given the aforementioned weak support for efficacy, the challenging management of intravascular volume required when using mannitol (which may result in hypotension – and thereby exacerbate brain injury associated with increased intracranial pressure)[131, 132] and the mounting body of evidence demonstrating superiority of hypertonic saline as first line osmotherapy for cerebral edema in numerous settings[133].

2.2.2.4.3 Hypertonic saline

Hypertonic saline gained popularity initially as a volume expander in acute resuscitation [134] and investigators noted improved survival rates in participants with traumatic brain injuries who had received hypertonic saline [135]. Like mannitol, hypertonic saline has a two-phase effect on ICP. The first is *via* rheology, and the second by osmotic activity across the BBB [136, 137]. The BBB is less permeable to hypertonic saline than to mannitol, and as a result, the potential for water to "follow" the solute into the brain, thus worsening cerebral edema, is less [138].

Hypertonic saline has been effective in reversing transtentorial herniation. In a retrospective analysis involving 75 herniation events in 68 participants, boluses (30-60 mL) of 23.4% hypertonic saline reversed 75% of the clinical herniation events [139]. These participants had a variety of neurologic illnesses including intracerebral hemorrhage, subarachnoid hemorrhage, stroke, tumor, subdural and epidural hematomas, and meningitis.

Hypertonic saline has been demonstrated to act via salutary effects on aquaporin water channels, which are newly recognized as therapeutic targets in cerebral edema [140-142]. Abundant in brain, the aquaporin 4 (AQP4) water channels play multiple roles regulating brain water homeostasis, astrocyte migration, neuronal excitability, and neuroinflammation [143]. AQP4 is highly expressed in ependymal cells and astrocytes with polarized distribution in end-feet membranes facing cerebral capillaries and pia mater [144]. The strategic location at the blood–brain interface and the cerebrospinal fluid–brain interface makes AQP4 a major factor in regulation of water movement into and out of the brain. AQP4 has been investigated in brain edema associated with multiple forms of brain injury such as stroke, meningitis, neuromyelitis optica, and brain tumor[145].

A role for AQP4 in CM has recently been suggested and AQP4 has been demonstrated to be protective in murine models of CM. In addition to its effect on AQP4, hypertonic saline has been demonstrated to have significant salutary effects upon neuro-inflammation[146-150] and endothelial injury (a putative mechanistic basis for vasogenic edema in cerebral malaria)[151-154].

Side-effects: A theoretical danger of hypertonic saline administration is that rapid increases in sodium concentration may induce central pontine myelinolysis. This devastating neurological abnormality is a rare consequence of overly rapid correction of chronic hyponatremia. To date, there are no reports of central pontine myelinolysis occurring in participants receiving hypertonic saline for the treatment of increased ICP [139, 155, 156]. Additionally, there are no data clearly linking the use of hypertonic saline to the development of acute renal failure or to rebound increases in ICP in humans [85, 117, 119].

The hypernatremia induced by hypertonic saline has been associated with coagulopathies and excessive intravascular volume [85, 119]. Electrolyte abnormalities (hyperchloremic metabolic acidosis and hypokalemia) can develop, necessitating careful monitoring [85, 118, 119].

2.2.2.4.4. Mannitol *vs.* hypertonic saline

There are numerous biochemical and physiologic considerations when choosing between hypertonic saline and mannitol for the treatment of elevated ICP. Hypertonic saline infusions result in significant volume expansion, along with improved cardiac output, regional cerebral blood flow, and increased cerebrospinal fluid absorption [123]. Mannitol's advantages include improved microvascular cerebral blood flow [121, 157] and blood rheology [117, 158], reduced blood viscosity [120] and cerebrospinal fluid production [158], free-radical scavenging [159], and inhibition of apoptosis [160].

Hyperosmolar therapy has been studied most extensively in the setting of TBI [161]. The first head to head comparison of mannitol and hypertonic saline in TBI was a randomized trial comparing 20% mannitol to 7.5% hypertonic saline (both as boluses) [162]. The volumes delivered were identical, but the osmolar loads were different; the mannitol group received 4.8 mOsm/kg and the hypertonic saline group received 2.3 mOsm/kg. The mean number and duration of episodes of intracranial hypertension were significantly lower in the hypertonic saline group, but overall outcomes of the two treatment groups were similar.

The effects of sustained equimolar doses of mannitol and hypertonic saline on cerebral blood flow and metabolism following TBI were compared in 47 adult participants [163]. Both interventions effectively and equally reduced increased ICP while improving cerebral perfusion pressure and cerebral blood flow, but the effect was significantly stronger and of longer duration after hypertonic saline. The effect of hypertonic saline on raised ICP appeared to be more robust in participants with diffuse brain injury. This study was published after the meta-analysis described immediately below (2.2.2.4.6)

2.2.2.4.5 Meta-analysis of mannitol *vs.* hypertonic saline in adults

Five trials enrolling a total of 112 adults participants, comparing equimolar doses of mannitol and hypertonic saline for the treatment of elevated ICP in settings where quantitative ICP measurements were feasible were identified [157, 164-167]. None of the studies was blinded, and all were small; the largest enrolled 40 participants. Many studies amplified the sample size by including multiple episodes of raised ICP per participant. Most studies included a heterogeneous group of participants (TBI, stroke, intracerebral hemorrhage, subarachnoid hemorrhage).

Mannitol was effective in controlling elevated ICP in 69 of 89 episodes (78%, 95% CI 67-86%), whereas hypertonic saline was effective in 88 of 95 episodes (93%, 95% CI 85%-97%). The pooled relative risk of ICP control using hypertonic saline compared to mannitol was 1.16 (95% CI 1.00-1.33, p=0.046), and the weighted mean difference in ICP reduction using hypertonic saline compared to mannitol was 2.0 mm Hg (95% CI -1.6 – 5.7 mm Hg, p=0.276). When a fixed-effects model was applied, the point estimate for the relative risk did not change, but the confidence intervals narrowed (95% CI 1.05-1.35, p = 0.007). With the fixed effects model, the weighted mean difference in ICP reduction using hypertonic saline compared to mannitol remained 2.0 mm Hg, but again, the confidence interval narrowed (95% CI 0.1 – 3.8 mm Hg, p=0.036).

Conclusions: This meta-analysis found that hypertonic saline was more effective than mannitol in controlling episodes of elevated ICP and identified a trend toward greater quantitative ICP reduction in the hypertonic saline group. These findings are corroborated by the results of seven observational studies demonstrating the effectiveness of hypertonic saline in lowering ICP after failure of standard mannitol therapy [139, 168-173].

2.2.2.4.6. Systematic review of mannitol vs hypertonic saline in children

Gwer *et al* [174] recently completed a very thorough review of the use of osmotic agents in children with acute encephalopathies. The goal of this review was to examine the effectiveness of osmotic agents in reducing ICP in children with acute encephalopathies, and to determine the effect of osmotic agents on clinical outcome in the same population.

They identified four randomized trials [32, 175-177], three prospective observational studies [14, 155, 178], two retrospective studies [156, 179] and one case report [180]. Of these ten studies, four involved non-traumatic encephalopathies [14, 32, 176, 179] and ICP was monitored in seven. Both the observational study of mannitol in pediatric CM [14], and the Ugandan clinical trial of mannitol in pediatric CM [32] described above were included in this systematic review

Effects on ICP: One study [175] was a crossover trial involving 18 children with TBI; there was a significant drop from the initial ICP following treatment with hypertonic saline ($p = 0.003$) compared to normal saline ($p = 0.32$). Another randomized, controlled trial [177] compared hypertonic saline with Ringer's lactate; more interventions for raised ICP were used in the Ringer's lactate group compared to the hypertonic saline group ($p < 0.001$). Hypertonic saline was administered as a continuous infusion to ten children with TBI with raised ICP that was refractory to mannitol [155]; it produced a sustained reduction of ICP that was maintained over 72 hours. As noted above, bolus therapy with mannitol reduced moderately raised ICP in children with CM, but did not impact those with severely increased ICP [14]. Oral glycerol exerted a short-term effect on ICP in 3 children with TBI; ICP decreased by at least 50% within the first half hour of administration, but the reduction was not maintained beyond 90 minutes [178]. Another case report describing two children with TBI showed a dose response relationship between both hypertonic saline and mannitol and ICP [180], but mannitol appeared to cause a reduction in cerebral perfusion pressure.

Effects on outcome: Clinical outcome was evaluated only in the four randomized, controlled trials as these were the only studies with comparison groups. In one trial of children with bacterial meningitis that compared glycerol alone versus glycerol plus dexamethasone versus placebo, the mortality was lower in those given glycerol compared to placebo (RR 0.64, 95% CI 0.54 – 0.76), and in those given glycerol and dexamethasone, compared to placebo (RR 0.79, 95% CI 0.68 - 0.92) [176]. There were only two deaths in the trial comparing hypertonic saline and Ringer's lactate, both in the Ringer's lactate group [177]. As noted earlier, the clinical trial of mannitol in pediatric CM was not powered to detect an impact on outcome, and it did not demonstrate any differences between the mannitol recipients and those receiving the normal saline placebo.

Conclusions: Hypertonic saline appeared to achieve greater reduction in ICP than other osmotic agents, and all examined agents demonstrated a dose response in the reduction of ICP. When compared to mannitol, hypertonic saline maintained or improved cerebral perfusion pressure, an important determinant of neurological outcome [180].The duration of the effect on ICP was transient in a number of cases, and continuous infusions of hypertonic saline appeared to achieve sustained reductions in ICP more commonly than other osmotic agents. The review supports the use of oral glycerol in acute bacterial meningitis, and the use of hypertonic saline in acute traumatic and non-traumatic encephalopathies. To date, the data are not felt to be sufficient to generate specific guidelines, and clinical trials were recommended to establish the safest and most efficacious concentrations, and to determine optimum routes and rates of administration.

As described in detail above, as a pharmacologic rescue for severe CM, there is a (1) plausible mechanistic rationale, (2) empiric efficacy and safety data as well as (3) a pragmatic basis for selecting osmotherapy over corticosteroid therapy and more specifically, for selecting hypertonic saline over mannitol.

As stated in the package insert for hypertonic saline: Safety and effectiveness of sodium chloride injections in pediatric patients have not been established by adequate and well controlled trials, however, the use of electrolyte solutions in the pediatric population is referenced in the medical literature. The warnings, precautions and adverse reactions identified in the label copy should be observed in the pediatric population.

2.2.3 Summary of Epidemiological Data

NA

2.3 Rationale

This clinical trial of adjuvant therapy (immediate ventilatory support, or hypertonic saline) for Malawian children with CM is directed toward the supportive treatment of increased brain volume, and it avoids the pitfalls that have bedeviled previous trials because:

- The interventions target a mechanism recently recognized as being strongly associated with a fatal outcome, severely increased brain volume; this mechanism is self-limited in CM survivors, suggesting that supportive care or rescue therapy may be effective.

The clinical trial setting in Malawi has been transformed because, beginning in July 2017, mechanical ventilation became available in the new Paediatric Intensive Care Unit (PICU) at the Queen Elizabeth Central Hospital.

This introduces new ethical issues: mechanical ventilation cannot be withheld in patients who suffer a respiratory arrest, and ventilatory support may result in a cadre of survivors with severe neurological sequelae. Therefore, "treatment failure" (failure of the primary intervention) is defined differently in the different therapeutic arms. In Arms 2 and 3, in which participants are not randomized to immediate ventilatory support, any participants who suffer respiratory arrest and are placed on ventilators *or* who die *or* are brain dead will be classified as treatment failures. In Arm 1, the patients who are randomized to receive immediate ventilatory support, "treatment failure" is defined as brain death at any time, *or* death at 7 days, with ventilatory support withdrawn.

The hypothesis underlying this prospective, randomized clinical trial is that death in children with CM occurs when raised intracranial pressure from severely increased brain volume impairs the function of the medullary respiratory center and that interventions which reduce ICP or support ventilation will improve survival rates in CM patients.

Support for this mechanism of death is based on MRI evidence of severely increased volume in all fatal cases and on clinical observations consistent with pre-morbid brainstem compression and cerebral herniation [14, 44, 52, 181]. Neuroimaging was required to identify this mechanism: full autopsies with detailed neuropathological studies did not reveal the cause of death in participants with CM. Cerebral edema was a constant feature of all fatal CM cases at autopsy, but gross findings consistent with transtentorial or uncal herniation were less common. This suggests that medullary impingement is a transient event, or that the autopsy itself, performed within 12 hours of death, became effectively a decompressive craniotomy, and may have obscured physical evidence of herniation.

The proposed trial will simultaneously test the hypothesis that increased ICP is the cause of death in children with CM and evaluate two therapeutic approaches: one treatment regimen

targets raised ICP directly and the other targets the effect of ICP (respiratory arrest). If the trial is successful, at least one effective treatment for children with CM at high risk of death will be identified.

The study population

The *mortality rate* in children with CM who have highly increased brain volume (37%) is high enough to support a single-site three-armed clinical trial over six years.

The *survival rate* in this subset (63% survive, and nearly all are discharged within 72 hours of admission) suggests that in survivors, severe edema rapidly subsides spontaneously, and that interventions which (a) reduce edema enough to prevent cerebral herniation or (b) support ventilation during the vulnerable period, might be sufficient to save lives.

Using malarial retinopathy to identify the high risk group: Recognizing the importance of being able to scale up the findings of this study, and appreciating the operational difficulties of identifying patients with malarial retinopathy, children with retinopathy-negative CM will also be enrolled and randomized to one of the three study arms. Randomization for the retinopathy-positive patients will be carried out independently of randomization for the retinopathy-negative patients. Should the clinical trial results reveal a positive therapeutic effect of either intervention (Arm 1 or Arm 2), uptake of study results across Africa will be enhanced if treatment decisions do not depend on the results of an ocular funduscopic examination, which requires expensive equipment and extensive training. This trial represents an opportunity to assess efficacy in the entire CM population (combined retinopathy-positive and retinopathy-negative). If efficacy can be demonstrated in the entire CM population, scalability will be enhanced, as indirect ophthalmoscopy will not be necessary before instituting an intervention which will have been shown to be beneficial.

Using MRI to identify the high-risk group: The data driving this study were collected using the 0.35T MRI in Malawi[52]. Evidence of severely increased brain volume is readily discernible via MRI, but clinically reliable surrogate markers of increased brain volume have yet to be identified. A secondary outcome of this study will be an evaluation of these potential surrogate markers so that, if a successful intervention is identified, its implementation will not require MRI technology to identify subjects at high risk of death.

During the first enrollment season (January-June 2018) 57 participants had MRI images interpreted in real time by a single neuroradiologist. When the season was completed, all images were independently re-interpreted by two different neuroradiologists. If there was a discrepancy in the assigned brain volume scores determined by the radiologists interpreting the images post-season, a consensus score was obtained.

Comparisons between the brain volume scores assigned in real time and the consensus score revealed an inability for neuroradiologists to differentiate a score of 6 or 7 in real time, leading to non-differential misclassification. Four subjects who had real-time assignment of brain volume

scores of 7 (and therefore were eligible for randomization in real time) had consensus scores of 6. Three subjects with real-time assignment of brain volume scores of 6 had a consensus score of 7.

Comparisons between brain volume scores assigned in real time and the consensus score showed that for scores of 6 or less, neuroradiologists interpreting images in real time generally assigned brain volume scores higher than the consensus score. During the first enrollment season, a score of 5 or 6 was assigned 17 times in real-time. Adjudicated scores were the same (4), lower (12), or higher (1). One subject assigned a brain volume score of 5 had an adjudicated score of 7.

To include all participants who should be randomized (e.g. that have an adjudicated brain volume score of 7 or 8) requires that all participants with brain volume scores of ≥ 6 are eligible for randomization. To try to avoid including participants who will eventually have an adjudicated score of 5 or less, beginning in the 2019 enrollment season, all participants will have 2 independent MRI interpretations for brain volume score performed in real-time. If both real-time interpretations have a brain volume score of ≥ 6 the participant will be eligible for randomization. If either of the independent real-time neuroradiologists assigns a brain volume score of < 6 the participant will not be eligible for randomization.

Beginning in 2021, the 0.35T fixed magnet General Electric MRI will be replaced with a portable MRI scanner manufactured by Hyperfine. Clinical trial participants may be scanned with the 0.35 T General Electric MRI machine, the Hyperfine scanner, or both.

Interventions

Our site, by virtue of its ready access to MRI and pediatric intensive care, is uniquely qualified to identify the study population and carry out the clinical trial. For the findings to be useful in other malaria-endemic settings, it will be important to identify and validate more user-friendly approaches to identifying children with life-threatening increases in brain volume, and to determine the simplest approach to treat them. These dual objectives are the basis of the primary and secondary aims of the study.

Criteria for selecting experimental interventions

The pathophysiology of intracranial hypertension is complex and depends on the mechanism of cerebral edema, the volume of intracranial contents, the integrity of the BBB, and cerebral perfusion pressure. The balance of Starling forces (the transcapillary hydrostatic pressure gradient that is counterbalanced by an osmotic pressure gradient) determines the magnitude of extracellular fluid flow into the brain substance.

The following issues were considered:

- The etiology of increased brain volume in this patient population has not been established, and the relative contribution of any of the four putative mechanisms, at any single point in the disease process, is unknown. Furthermore, the relative contributions may change over the course of the illness. Since no animal model of CM exists, it is

unlikely that insights into the mechanism of increased brain volume in children with CM will be rapidly forthcoming. Although ongoing research may eventually illuminate the specific mechanisms contributing to the elevated ICP of these patients, our previous work has identified a group of children at high-risk for mortality and broadly effective interventions exist.

- The treatment for increased brain volume that is most likely to be effective in this setting is hypertonic saline.
- Given the rapid reversibility of the increased brain volume, that its etiology is unclear, and that respiratory arrest is the likely cause of death, the other trial arm is ventilatory support.

- Given the high mortality rate in children with CM with highly increased brain volume and the now ready availability of ventilatory support in the ICU in Malawi, those randomized to "usual care" or "hypertonic saline" will receive 'rescue ventilation' if they experience a respiratory arrest.

Experimental regimens

Duration of therapy: Serial MRIs have shown that, in survivors, the increased brain volume generally resolves within 48 hours. A summary of data from CM participants on the research ward shows that 85% have either died or been discharged within 72 hours (data not shown). To maximize the chance of observing a positive result, the experimental regimens will continue for 7 days, or until the child experiences death, brain death, or clinical improvement (brain volume score $\leq$ 5 and Blantyre Coma Score $\geq$ 3).

Elevation of the head of the bed: In the PICU and PRW, the head of the bed will be elevated to 30^{o}, and the head maintained in the midline with towels.

Hyperosmolar therapy: The choice of hypertonic saline (over mannitol) as the hyperosmolar agent was made on the basis of data demonstrating the efficacy of hypertonic saline in a wide variety of clinical settings [138, 155, 156, 162, 163, 174, 182-187], especially in reversing herniation[133], and its volume expansion effects (which may increase its efficacy in CM, where sequestration of parasitized erythrocytes impairs cerebral microcirculation). A recent review, focusing on pediatric encephalopathies, recommended hypertonic saline over mannitol [174]. Participants receiving mannitol are often subject to diuresis and maintaining the appropriate intravascular volume in a resource-challenged setting such as ours is complicated.

The hypertonic saline regimen will be "goal directed", aiming to achieve serum sodium (Na) concentrations between 150-160 mmol/L[188]. This range is safe and has been shown to be effective in reducing ICP in pediatric participants with a variety of underlying pathologies[155, 182]. Sodium balance is rarely deranged in malaria [189], but doses of hypertonic saline will be titrated to serum sodium levels, measured with a bedside instrument (I-Stat, Abbot Labs). Adjustments for hyper- and hyponatremia will be made (see Section 9.2.4.1).

3% saline was chosen as the specific hypertonic saline product for this trial because: (1) the target range of serum sodium can be effectively achieved [155, 156, 175, 190], (2) it can be administered through peripheral intravenous lines[191, 192] therefore mitigating the risk associated with placing and maintaining central venous catheters in our setting. We will employ a continuous infusion to maintain elevated sodium levels as is common practice for other therapeutic indications.

Estimating intracranial pressure

We are in the unusual position of being able to obtain magnetic resonance images of participants without being able to measure intracranial pressure directly. We have clear cut evidence of severely increased brain volume on MRI, and a clinical syndrome compatible with raised intracranial pressure. However, the risks of infection with placement of an intracranial pressure monitoring device, in both the short- and long-term, outweigh the benefits of the direct measurements [193]. Additionally, there is no evidence to suggest that monitoring of intracranial pressures in cases of cerebral malaria or other forms of meningitis/encephalitis, are of any clinical benefit [194].

Concurrent with our clinical trial, we will test and validate other surrogate measures of increased brain volume, an approach that is gaining ground in more sophisticated intensive care settings [195]. These can all be carried out at the bedside and include optic nerve sheath diameter (ONSD) by ultrasound, cerebral flow velocities by transcranial Doppler ultrasound, NIRS monitoring of both cerebral oxygenation and skeletal muscle reperfusion dynamics, heart rate variability from the EKG, and clinical neurological examination.

Ultrasound measures of the optic nerve sheath diameter (ONSD): The optic nerve sheath is continuous with the dura mater of the brain and expands in the presence of raised ICP. The distention can be measured non-invasively by ultrasound. Several studies suggest that ONSD has potential as a screening test for elevated ICP [196-200]. ONSD was a sensitive and specific predictor of CT scan signs of raised ICP and was elevated in 25 of 27 children with papilledema [201, 202]. A small study in Uganda found increased ONSD in all patients meeting a broad case definition of CM [203]. We used this approach on the Research Ward in two different studies, and serial data have been collected on all patients since 2013, providing reassurance that this approach is feasible.

Ultrasound measures of the cerebral blood flow velocities: In both adults and children TCD-derived parameters have been shown to have utility to non-invasively estimate an absolute value for ICP.[204-206] As ICP rises, distal resistance to cerebral flow increases and results in a progressive reduction in diastolic flow velocity (Vd) and an increase in pulsatility index (PI= Systolic flow velocity-Vd / Mean flow velocity).[207] In a prospective cohort of adult patients, Spearman correlation between ∆ICP measured invasively and changes to PI were averaged across time during induced ICP increase.[208] At the 0.05 level, baseline and plateau distributions of ICP and PI were significantly correlated ($r = 0.39 \pm 0.40$). In children with hydrocephalus, TCD has been shown to be a valid measure of ICP changes during cerebrospinal fluid drainage.[209-211] In 7 patients, TCD was performed with simultaneous invasive ICP measurement through a

ventricular reservoir during ventricular taps and ventriculoperitoneal shunting. Resistive index ecreased significantly from a mean of 0.9 pre-CSF removal to 0.75 post-CSF removal.[209] In another 22 infants with hydrocephalus and a ventricular reservoir, a volume of 5ml/kg body weight was removed twice daily and ICP and TCD flow velocities were determined before and after CSF tapping.[7] A significant decrease in ICP after CSF removal was accompanied by a concomitant increase in mean flow velocity ($p < 0.05$) and Vd ($p < 0.01$). A significant decrease in resistive index was also noted ($p < 0.01$). The current expert consensus is that a ≥ 10% change in Vd or PI represented a clinically meaningful change to ICP (NOB personal communication).

Heart rate variability (HRV) patterns: Heart rate is the net result of complex interactions at every level of the organism and these interactions are severely impacted by critical illness. Ultimately, heart rate, strength of cardiac contraction and vascular resistance are regulated on a beat-by-beat basis in order to optimize cardiac output to the needs of the body (resulting in healthy HRV). A primary mediator of heart rate is the autonomic nervous system which directly innervates the sino-atrial node (the heart's pacemaker). In healthy individuals, the autonomic nervous system is responsive to the needs of the body, *i.e.*, heart rate accelerates with inhalation and decelerates with respiration (respiratory sinus arrhythmia) and also changes at a slower rate in association with oscillations in the baroreflex and other physiologic rhythms, as well as in response to external stimuli. It has been clearly shown that there is a loss of normal HRV in the presence of critical illness and that it returns in association with clinical improvement[212]. Although nothing is known about the evolution of HRV in CM, we postulate that HRV will be diminished and possibly disorganized (lacking the expected intrinsic rhythms), and that potentially HRV will be significantly worse in CM with brain swelling compared to CM without it. Furthermore, it would be expected that HRV will improve in association with underlying clinical improvement. In order to test these hypotheses, we will use monitors to record the EKG in all children for at least 24 hours. These data will be downloaded for off-line analysis. HRV will be obtained from a beat-to-beat file generated by research scanning of the EKG signal and then examined in multiple ways:

1. A tachogram of instantaneous heart rates *vs*. time will be plotted. That will permit identification of the presence of any HRV and the evolution of regular respiratory sinus arrhythmia and other trends in heart rate patterns as the patient's condition evolves or interventions are undertaken.
2. Heart rate and multiple HRV parameters will be calculated for every 2 minutes of the recording period and their evolution plotted over time. They will reflect how much HRV there is, the underlying oscillatory rhythms in the HRV pattern and the relative organization *vs*. randomness of heart rate patterns. These parameters can also be averaged over longer periods of time for statistical purposes.

Biobanking: Admission samples of plasma, urine and CSF will be bio banked for later analysis, via a separate funding source. The most widely studied of biomarkers in pediatric brain injury include S100B, neuron specific enolase (NSE), and myelin basic protein (MBP) [213]. Although the neuropathological role of these biomarkers is incompletely understood [214, 215], they have

shown utility in stratifying patients with mild traumatic brain injury (TBI), identifying TBI patients on admission, and modestly predicting outcomes in patients with TBI [216-218]. Despite these advantages, S100B, NSE, and MBP all possess significant limitations that preclude their selection for further clinical testing in this proposal. The most significant limitations include a large range of age-dependent variability for S100B [219], an inadequately high presence in erythrocytes for NSE [213], and latent rise (peaking around 48-72 hours after injury) for MBP [213]. UCH-L1 is a highly abundant neuronal protein thought to play a critical role in cellular protein degradation during both normal and pathological conditions [220]. It constitutes up to 10% of cytoplasmic protein in neurons, is elevated in cerebrospinal fluid (CSF) and serum following TBI, and is significantly associated with measures of injury severity and outcome in adults [221-228] and children [225]. UCH-L1 is localized to neurons in the cerebral cortex, and has demonstrated remarkable accuracy in stratifying patients with varying degrees of TBI within an hour of injury in addition to predicting outcomes [220-222, 225]. GFAP*, a* marker of astroglial injury, is a type III intermediate filament that forms part of the cytoskeleton of mature astrocytes and other glial cells. Importantly, GFAP is only found in the central nervous system [229]. Central nervous system (CNS) injury causes gliosis and subsequently up-regulates GFAP, making GFAP an attractive candidate biomarker for evaluation of acute brain injury. Data suggest that GFAP, which is released after TBI but not after trauma without brain injury, is an indicator of cell destruction, while S100B is an indicator of glial activation but not necessarily of cell destruction [230, 231]. GFAP is a product of astrocyte cytoskeleton degradation by calpain protease activation and therefore considered specific to the CNS [232]. Recent studies the investigators and others have documented elevated GFAP levels in both CSF and serum after TBI in adult [222, 225, 233-237] and pediatric patients [238-240]. GFAP is useful in stratifying patients with mild to severe TBI, may remain elevated for several days post injury, and shows better specificity than NSE and S100B in detecting TBI and predicting outcome [241-243]. Moreover, elevated concentrations of GFAP two days post injury portend a poor prognosis and, given the short half-life of GFAP in the blood stream, are thought to reflect the presence of a secondary insult (i.e. ICH and $P_{bt}O_2$) [244, 245].

2.4 Potential Risks and Benefits

2.4.1 Potential Risks

2.4.1.1 Risk from mydriatics

Instillation of dilating eye drops is required prior to the indirect and direct ophthalmoscopy. The side effects of blurred vision and photophobia are not expected to trouble these particular participants (who will be unconscious), and the risk of acute narrow angle closure glaucoma (a rare side-effect of mydriatics) is very low in the pediatric population. Cardiovascular effects have been reported in premature infants, who are not candidates for enrollment. The dilating effect of mydriatics generally dissipates within 2 hours of instillation.

2.4.1.2 Hypertonic saline

The known side-effects of hypertonic saline include:

- Hypernatremia
- Hypokalemia
- Rebound cerebral edema upon termination of therapy

These conditions are all a direct result of the increased sodium and osmolarity that are also the goal of the intervention. Assiduous monitoring of electrolytes (as described in 9.2.4) will therefore be undertaken to balance desired effects with possible side effects.

2.4.1.3 Assisted ventilation

Mechanical ventilation via an endotracheal tube has a number of associated risks which can be broadly classified into following categories[246]

- Adverse effects of positive pressure, volume and flow:
  - Pneumothorax
  - Pneumomediastinum
  - Hypotension
  - Bronchospasm
  - Pulmonary hemorrhage

- Adverse effects of endotracheal intubation
  - Endotracheal tube obstruction
  - Inadvertent endotracheal tube dislodgment
  - Bradycardia induced by vagal response
  - Mucosal inflammation
  - Stridor
  - Subglottic stenosis
  - Respiratory nosocomial infections

These events may be persistent and severe, necessitating tracheostomy.

2.4.2 Known Potential Benefits

Participants will have the benefit of the increased level of nursing care provided on the PICU and PRW. Were they not to be enrolled in this study, they would be hospitalized in the general pediatrics ward at QECH. The general ward is staffed by government employed nurses. The patient to nurse ratio is often > 50:1. In contrast, the PRW patient to nurse ratio is 2:1 at maximum and is 1:1 in the PICU. The diligent observations and monitoring that are provided on the PRW and in the PICU are impossible in the general ward. For a disease such as CM that demonstrates rapid progression, extreme clinical vigilance and the ability and resources to respond to clinical needs are crucially important. These can only be provided on the PRW/PICU and serve as the major benefit of participants to this study – independent of any benefit provided by the investigational efforts. When the guardians of potential study participants decide against providing consent, the patients are eligible to receive the “usual care” (sans study-specific investigations) on the PRW.

3 OBJECTIVES

3.1 Study Objectives

Primary Objective

Compare final outcomes in pediatric CM patients receiving usual care (Arm 3) with both of two interventions (Arm 1 and Arm 2).

Secondary Objectives:

1. Among survivors of this interventional study, compare rates of adverse neurological outcomes in those assigned to the two interventions and those assigned to usual care.
2. Among all children admitted to the PRW with CM whose parents consent to screening, evaluate and validate biomarkers of increased brain volume.
3. Assess the safety of early intubation and mechanical ventilation as well as intravenous hypertonic saline in children with CM.
4. Establish association between specific pathogenic mechanisms and TCD-derived phenotypes.
    a. microvascular obstruction phenotype with MRI findings
    b. hyperemia phenotype with hypercapnia on blood gas analysis, acidosis, and seizures on EEG
    c. vasospasm phenotype with regional EEG findings of loss of fast frequencies and increased slow frequencies in territories of affected vessels and MRI findings with localized ischemic changes
    d. low flow phenotype with compromised hemodynamics, diminished compensatory reserve and increased brain volume per MRI

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

Final outcomes will be determined within 7 days: treatment success or failure, where failure is a composite outcome of death, ventilatory rescue, and brain death. Final outcomes within 7 days in children who meet the standard WHO definition of cerebral malaria treatment success or failure, where failure is a composite outcome of death, ventilatory rescue, and brain death.

3.2.2 Secondary Outcome Measures

1. Neurological sequelae in survivors, assessed at 1, 6, and 12 months after randomization via the Malawi Developmental Assessment Tool (MDAT) for participants up to the age of 59 months old, and the Kaufman Assessment Battery for Children, Version II (KABC II) for participants over the age of 59 months. All survivors will also be assessed using the Glasgow Outcome Scale (GOS)[247]. The GOS is a global scale for functional outcome that rates patient status into one of five

categories: dead, vegetative state, severe disability, moderate disability or good recovery.

2. Surrogate measures of increased brain volume: At the time of admission (only): opening CSF pressure, archived samples of blood, urine and CSF. Daily while comatose: ophthalmoscopy for papilledema (present/absent), optic nerve sheath diameter in mm, NIRS measurements of cerebral oxygenation and muscle reperfusion, standardized neurologic observations.

3. Standard TCD phenotypes: microvascular obstruction, hyperemia, vasospasm, low flow, isolated posterior hyperemia). Potential mechanisms: increased brain volume per MRI/ischemic changes, hypercapnia/acidosis/seizures, localized ischemia on MRI, compromised hemodynamics/diminished compensatory reserve/increased brain volume per MRI

4 STUDY DESIGN

This clinical trial is a single site, prospective, open, randomized controlled study in which the impact on final outcome of two different, adjunctive therapeutic approaches aimed at reducing increased brain volume or ameliorating its effect on ventilatory status in Malawian children with CM and severely increased brain volume per MRI will be evaluated.

Children who meet the World Health Organization (WHO) clinical case definition of CM (peripheral *P. falciparum* parasitemia of any density, Blantyre Coma Score (BCS) ≤2, and no other discernible cause of coma) will be admitted to the Pediatric Research Ward (PRW) at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, and will be eligible to be screened for participation in the trial. All children whose parents/guardians' consent for screening will undergo ophthalmoscopy and MRI.

- Those without MRI evidence of increased brain volume will be cared for on the PRW, and will contribute to Secondary Objective #2, identifying surrogate markers of increased brain volume and to Secondary Objective #4, establishing association between specific pathogenic mechanisms and each TCD-derived phenotype.

- Children with clinically defined CM and increased brain volume are eligible to be randomized; those whose caregivers consent to screening but decline to participate in randomization will undergo continuous monitoring on the PRW as required for HRV analysis, and will contribute to Secondary Objective #2, identifying surrogate markers of increased brain volume. They would be eligible for rescue ventilation in the event of a respiratory arrest.

- Children with or without evidence of malarial retinopathy (see Section 2.1.1.7) and with evidence of increased brain volume per MRI (see Section 8.2.2.2 and 2.3.3) will be eligible to be randomized and participate in the clinical trial. If the parents/guardians grant informed consent, each participant will be randomized to one of three arms:

    Arm 1: Usual care plus immediate ventilatory support (VENT)

    Arm 2: Usual care plus hypertonic saline (HPS)

    Arm 3: Usual care (UC)

Participants will be monitored as follows:

- PICU-specific monitoring

    - Continuous monitoring of heart rate and rhythm from EKG, respiratory rate, and pulse oximetry via monitor, and blood pressure via arterial line or cuff. Signals will be archived for offline analyses aimed at identifying heart rate variability patterns over time associated with CM and with clinical improvement or decline in association with treatment.

- Electrolytes and acid-base balance 3-hourly during the first 24 hours and 6-hourly thereafter (in those randomized to hypertonic saline)

- PRW and PICU

  - 2–hourly assessments of vital signs (including temperature) and Blantyre Coma Score until BCS = 5 and temperature is ≤37.5ºC for 24 hours, then 6-hourly.

  - 6-hourly assessments of malaria parasitemia and hematocrit, until two consecutive parasitemia counts are negative.

  - 12-hourly neurological examinations,

  - Daily ophthalmoscopy while unconscious

  - Daily ONSD measurements (while unconscious)

  - Daily TCD examinations

  - Daily MRI examinations (while unconscious if participant is clinically stable for transportation to undergo neuroimaging)

The primary objective (treatment success or failure) will be determined within the first 7 days of hospitalization (Figure 8)

Secondary objectives (Figure 8)

1. The secondary objective involving neurological sequelae at 1, 6, and 12 months after randomization will be determined in survivors randomized in the clinical trial.
2. The secondary objective of identifying surrogate measures of increased brain volume will include participants in the clinical trial as well as study subjects who are screened but do not meet inclusion criteria for clinical trial enrollment *e.g.* their brain volume is not increased enough to quality for enrollment in the trial and are therefore not randomized.
3. The secondary objective of assessing the safety of early intubation and mechanical ventilation will include participants in the clinical trial as well as study subjects with CM who are screened but do not meet inclusion criteria for clinical trial enrollment *e.g.* their brain volume is not increased enough to quality for enrollment in the trial and are therefore not randomized.as well as intravenous hypertonic saline in children with CM.
4. TCD endpoints will be determined on all participants (see section 7.2.2.4)]

Choice of participant population and sample size considerations

By focusing on children with CM who have severely increased brain volume per MRI, the study population represents those at highest risk of dying of CM. The mortality rate of retinopathy-positive children with severely increased brain volume is 37%.

Prior to study commencement and based on recruitment patterns over the previous five years (the years that the MRI has been available for characterizing brain volume) we expected to enroll approximately 30-35 retinopathy-positive and 10-12 retinopathy-negative participants with severely increased brain volume per year.

The rate of participant enrollments has decreased, and challenges have been faced in almost all study years:

• 2017: the pediatric intensive care unit remained under construction. Given a steady decline in enrollment rates for pediatric cerebral malaria on the Research Ward, a referral network of 6 hospitals within an hours' drive of the Queen Elizabeth Central Hospital was established. No participants were recruited or randomized.

• 2018: The Malawi Pharmacy, Medicines and Poisons Board unexpectedly delayed the clearance needed to import 3% hypertonic saline (HPS). Protocol amendments were made quickly. Fifty-seven participants were recruited, and 8 were randomized: 4 to usual care and 4 to ventilatory support.

• 2019: The inability to import 3% HPS to Malawi continued. A total of 42 participants were screened, and 24 were randomized (12 to ventilatory support and 12 to usual care).

• 2020: The MRI scanner became non-functional and repair efforts were stymied by the adverse impact of the COVID-19 pandemic on international travel. (The repair technician could not travel from South Africa to Malawi after all commercial flights to Malawi ceased.) A total of 48 patients was screened, but none were randomized because brain volume could not be assessed. We will have a new, low field MRI machine (manufactured by Hyperfine) capable of assessing brain volume in place for the 2021 season.

- 2021: A low field MRI machine (manufactured by Hyperfine) capable of assessing brain volume was placed in service in late March 2021.The possibility of randomization was delayed until after the portable MRI became functional.

    a. Given these successes and challenges and the potential beneficial effects on mortality described in Section 11.2, a study comparing two interventions to Usual Care that will answer our original research questions prior to study closure will be challenging but remains feasible. Answering the question whether mechanical ventilation or intravenous hypertonic saline decreases mortality risk in children with CM requires sing participants enrolled in the BMP observational study prior to clinical trial commencement as Historical Controls (obviating the need for randomizing high numbers of participants into the Usual Care arm)

Historical Controls:
We identified potential Historical Controls from the electronic database of the completed observational study of cerebral malaria pathogenesis. In order to minimize temporal differences between participants randomized in the clinical trial, we began our search with children enrolled in the observational study in 2017 and moved backwards in time.

Neuroradiologists affiliated with the Treating Brain Swelling clinical trial assessed the brain MRI scans on over 200 children enrolled in the observational study. These images dated from 2014-2017.

Once consensus brain volume scores on these MRI scans were assigned by the neuroradiologists, we retrieved demographic and clinical data on children who had assigned brain volume scores of 6-8 from the master Blantyre Malaria Project database. Demographic, clinical, and outcome data were compared between children enrolled in the observational study who had brain volume scores of 6-8, and children randomized in the Treating Brain Swelling clinical trial and were randomized to Usual Care with the same range of brain volume scores. Comparisons were made between the following variables: age (in months), sex, Blantyre coma score, admission glucose, admission hematocrit, admission platelet count, and outcome. Statistical analysis: Comparisons between groups were made using t-tests or Wilcoxon test (continuous variables), ANOVA (categorical variables), or chi squared tests (dichotomous variables), as appropriate.

We found no difference in the variables in question between children enrolled in the observational study who had MRI brain volume scores of 6-8, and participants randomized in the clinical trial who had MRI brain volume scores of 6-8.

Table 4. Continuous variables compared between historical controls and randomized study participants

Variable	OS mean	OS SD	TBS mean	TBS SD	P value
Age (months)	48.25	27.54	59.63	27.88	0.168
Glucose	5.87	2.94	5.63	3.13	0.914[d]
Hematocrit (%)	23.36[a]	6.29	25.54	6.97	0.555[d]
Platelets (*1000/MicroL)	148.9[b]	373.7	94.3[c]	176.2	0.993[d]

OS= observational study (n=48), SD= standard deviation, TBS= treating brain swelling clinical trial (n=16)
[a] n=45, [b] n=47, [c] n=15. [d] nonparametric test.

There was no significant difference in admission Blantyre Coma score (p=0.948), sex (p=0.885) or outcome (death/survival, p=0.551) between the 48 children in the observational study and the 16 randomized participants in the Treating Brain Swelling clinical trial.

We therefore conclude that participants enrolled in the observational study (n=48) are comparable to participants randomized to Usual Care (n=16) in the Treating Brain Swelling clinical trial.

We anticipate enrollment in the Usual Care arm in the 2023 seasos (see below) at a reduced ratio compared to the intervention arms.

Possible future declaration of futility of the ventilation arm*:*

If we combine the mortality risk in Historical Controls and participants already randomized to Usual Care, the mortality risk of the combined historical control group and children randomized to Usual Care is 36%. At the end of the 2021 season, the mortality risk in the VENT arm was 26.3%. (Five of 19 participants randomized to VENT died or were brain dead.) With only these data, the test of the null hypothesis of a null difference in mortality is not significant (p-value 0.818, 2-sided test).

We believe it likely that VENT will, in the future, be declared futile. If so, randomization of participants enrolled in the Treating Brain Swelling clinical trial after the declaration of futility of VENT would be to HPS or UC. A formal approach to futility assessment for VENT is provided below.

Table 4. Projected enrollment

	2018 – 2021	2022	2023	**Total**
*Usual care (UC)	64	0	4	**68**
Ventilatory support (VENT)	19	7	6	**32**
Hypertonic saline (HPS)	3	14	15	**32**

*historical controls + study participants enrolled in 2018 and 2019.

Assuming that enrollment in 2023 follows the same trend seen since the beginning of the clinical trial, we will be able to detect a 50% or more risk reduction in either the VENT or HPS arms, compared to UC.

Choice of interventions

The three study arms of the study will be:

1. Arm 1: Usual care + immediate mechanical ventilatory support (VENT) for a maximum of 7 days, with ventilator withdrawal once brain volume has decreased (brain volume score $\leq$ 5) or the patient has improved clinically (modified Blantyre Coma Score $\geq$ 3) *or* the patient dies *or* meets criteria for severe irreversible neurologic failure.
2. Arm 2: Usual care + intravenous hypertonic saline (HPS) for a maximum of 7 days, withdrawn once brain volume has decreased (brain volume score $\leq$5) and the patient

has improved clinically (Blantyre Coma Score ≥ 3) *or* the patient dies *or* meets criteria for severe irreversible neurologic failure.

3. Arm 3: Usual care (UC; intravenous fluids, intravenous artesunate; antipyretics, anticonvulsants and blood transfusions as needed; nutritional support) for a maximum of 7 days, *or* until brain volume has decreased (brain volume score ≤5) and the patient has improved clinically (Blantyre Coma Score ≥ 3) *or* the patient dies *or* meets criteria for severe irreversible neurologic failures

Participants will remain in the study throughout their hospitalization and, in survivors, through the 12-month post-randomization follow-up visit. Children are discharged when two sequential 6-hourly blood films are free of malaria parasites, when they have been afebrile for 24 consecutive hours, and when they are able to take medications and food by mouth. Final data collection will be at 12 months post-randomization.

Stratification:
No stratification is required. The ages of retinopathy-positive CM with severely increased brain volume per MRI who survived were compared to those of participants from the same population that died. There was no difference in the age structure (data not shown, p=0.95).

Primary and secondary outcomes.

The primary outcome is treatment success or failure, determined within the first 7 days. Treatment success is recovery. Treatment failure is death, ventilatory rescue (for participants in Arms 2 and 3) or meeting criteria for severe irreversible neurologic failure.

The secondary outcomes include rates of neurological sequelae in all 3 groups; potential surrogate measures of increased intracranial pressure (opening CSF pressure, ONSD measurements from serial ultrasound examinations, NIRS measurements of cerebral oxygenation and muscle reperfusion, serial examinations for papilledema, decreased diastolic flow velocity or increased pulsatility index on TCD, heart rate variability patterns from continuous cardiac monitoring measured from enrollment through initial MRI scan, and standardized clinical observations) and development of hypotension following induction/intubation, defined by proportional deviation from the initial systolic blood pressure at enrollment.

Data collection methods.

The primary outcome will be determined by study clinicians.

For secondary objectives, the following methods will be used:

- ONSD measurements will be collected at the bedside. Data will be transferred to the electronic case report form (CRF).

- TCD measurements will be collected at the bedside. Data will be transferred to the electronic case report form (CRF).

- NIRS will be used to record cerebral oxygenation fluctuations and to measure muscle reperfusion rates after transient occlusion.

- The evolution of heart rate variability and its relationship to clinical status and outcomes will be determined by extracting the archived and time-stamped continuous ECG signal.

- The neurological findings will be captured by clinicians on an electronic case report form (CRF) at the bedside.

- Neurologic status at the 1, 6, and 12-month post-randomization follow-up will be assessed by one of two pediatric neurologists or clinicians trained by them. The Glasgow Outcome Scale (GOS), a global scale for functional outcome that rates patient status into one of five categories (dead, vegetative state, severe disability, moderate disability or good recovery)[247] will be used.. Study nurses will administer standardized assessments: The Malawi Developmental Assessment Tool (MDAT) for participants up to the age of 59 months; the Kaufman Assessment Battery for Children, Version II (KABC-II), for participants over the age of 59 months. Both have been validated for use in African children [248, 249].

The details of the techniques involved for the specialized assays (funduscopy, MRI, ONSD, TCD, NIRS, heart rate variability) are included in Section 8 of this Protocol.

Centralization of evaluations: MRI, ophthalmoscopy, ONSD, TCD, EEG, and neuro-checks will be carried out either at the bedside in the PRW/PICU or in the MRI suite.

Safety monitoring: A local independent safety monitoring team (ISM) will be appointed for the study. The team will consist of two local physicians with knowledge of the clinical setting and practices, but without direct involvement with this study. They will be notified of all SAEs and asked to comment on the relatedness of these events to the interventions. They will also be asked to review AEs at the discretion of the local investigators, IRBs, or the Data Safety and Monitoring Board (DSMB).

A DSMB is justified for this study for two reasons: (1) the anticipated mortality in this group of gravely ill children, without adjunctive treatment, is over 30% (2), and serious adverse events have been reported for HPS (Section 2.3.1.2) and assisted ventilation (Section 2.3.1.3). Interventions may result in survival of children who would have otherwise died, and some of these children may be severely neurologically impaired. In a country like Malawi where there is no social support for such children and their families, their survival could create a burden for the family which might worsen stress and social problems.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

For screening:

- Peripheral *P. falciparum* parasitemia of any density
- Blantyre Coma Score ≤2
- No evidence of meningitis on lumbar puncture
- Consciousness not regained after correction of hypoglycemia (if hypoglycemia is present)
- Male or female whose age on the day of screening is between 6 months and 12 years old

If the parents/guardians agree to screening, antimalarial treatment will begin (see Section 6.6), and participants will undergo an ocular funduscopic examination and a brain MRI scan.

Inclusion criteria for randomization:

- Severely increased brain volume on MRI: brain volume score ≥ 6 (see Section 8.2.2.2 for details)
- Willingness to return for follow up visits 1, 6- and 12-months post-randomization

If these criteria are met, guardians will be approached for possible enrollment by a dedicated study nurse or clinician, speaking in the local language (Chichewa).

Antimalarial therapy (standard-of-care) will have already been initiated prior to either of these consent processes

5.2 Subject Exclusion Criteria

Subjects showing evidence of the following at baseline will be excluded from the study:

- Gross malnutrition as evidenced by peripheral edema, hair color changes, or severe wasting
- Advanced Human Immunodeficiency Virus (HIV) disease – defined as known HIV positive status and evidence of severe wasting
- Evidence of recent head trauma by history or physical examination

- Evidence of severe co-morbidity at time of initial evaluation

  - Pneumonia as evidenced by oxygen saturation on room air of <85%

  - Gastroenteritis and shock as evidenced by capillary refill >3 seconds or skin tenting

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

The study will be randomized, but not blinded. Participants will be randomized after completing the eligibility screening procedures. A block randomization scheme will be used to ensure groups of equal size. Blocks sizes of six and nine will be rotated to decrease the predictability of group allocation within a block for data collectors. Children with retinopathy-positive CM will be randomized separately from those with retinopathy-negative CM.

Randomization is based on obtaining a consensus interpretation of brain volume per MRI. If this is not possible for any reason, study participants will be screened but not randomized, and will remain on the PRW. All other study procedures will be followed as described in this protocol.

Randomization is predicated on the risks and benefits as described in this protocol. Endotracheal intubation is required for participants randomized to the “intubation + mechanical ventilation” arm. The current coronavirus pandemic affects the risk/benefit ratio of this arm of the study because endotracheal intubation increases the risk of aerosol spread of the coronavirus (US CDC COVID19 guidance, 4.4.20, www.cdc.gov/coronavirus/2019-ncov/hcp/guidance-risk-assesment-hcp.html). Randomization to the mechanical ventilation arm will be adjusted in accordance with local IRB recommendations during the coronavirus pandemic.

All possible blocks with balanced combinations of group assignment will be documented. Next computer-generated random numbers will be used to select from the balanced blocks. Assignment of study subjects to randomization arm will be performed electronically, through the online data capture mechanism. If Internet service is unavailable at the time of randomization, back up sequentially numbered opaque sealed envelopes prepared before the trial begins will be available. The envelopes will be stored in a secure location with access limited to key study personnel. After a caregiver provides consent for a patient to participate in the study, study arm assignment will first be attempted electronically. If Internet service is unavailable, study personnel will open the next unopened sequentially numbered envelope to reveal the group assignment and dosing calculation sheet.

A master log of all screened and enrolled subjects will be maintained. A subject is considered randomized upon online assignment or opening the envelope to indicate treatment assignment.

Additional subjects may be enrolled into the study at the discretion of the sponsor in the case of any subject who:

- Is randomized but does not receive treatment for any reason
- Has consent for participation in the study withdrawn for reasons other than an adverse event

5.3.2 Masking Procedures

Treatment arms will not be masked.

5.3.3 Reasons for Withdrawal

A study subject will be withdrawn from the study if:

- A screening error is discovered that resulted in incorrect enrollment (subject did not meet inclusion or exclusion criteria)
- CSF from admission lumbar puncture results in growth of pathogenic bacteria
- Parental consent is withdrawn at any stage for any of the procedures or treatment
- It is discovered that the participant has an intercurrent illness that, when combined with the study interventions, might compromise the recovery of the patient
- Safety reasons as judged by the investigator, ISM, or DSMB

5.3.4 Handling of Withdrawals

Participants withdrawn from the study will remain on the PICU/PRW and will continue to benefit from the increased level of clinical care provided. If the participant is withdrawn due to an adverse event (AE; see section 9 below for more details), laboratory monitoring will continue until the event resolves. Participants will continue to have brain volumes evaluated by multiple modalities as long as this is deemed non-injurious. Participants will still be scheduled for a 28-day post randomization follow up visit and measures of neurologic sequelae and brain volume will be collected. If the participant is voluntarily withdrawn from the study by the parent/guardian, all efforts will be made to encourage the participant to return for 28-day post-randomization follow up.

Attempts will be made to recruit additional participants to replace any participants that withdraw prior to reaching the 12-month post-randomization visit. At the conclusion of the study separate analyses will be run on per-protocol and intention-to-treat populations.

5.3.5 Termination of Study

One interim analysis will be carried out as detailed in Section 11.3. Stopping rules related to toxicities attributed to the study agents are delineated in this section. Early study termination is at the discretion of DMID, either of the participating institutional review boards (IRBs), or the DSMB. The investigator will notify the IRB(s) when the study has been completed.

6 STUDY INTERVENTIONS/INVESTIGATIONAL PRODUCTS

6.1 Study Product Description – Hypertonic Saline

6.1.1 Acquisition

Hypertonic saline (HPS) (3%) will be obtained in the United States and shipped to Blantyre.

6.1.2 Formulation, Packaging, and Labeling

Product is packaged in 500ml bags. Storage is recommended at room temperature (25°C).

6.1.3 Product Storage and Stability

Bags of HS will be stored in a cabinet in the PRW pharmacy adjacent to the ward. It will be protected from direct light. The pharmacy is equipped with an air conditioning unit and is provided with back up electricity by a diesel operated generator in case of power outages. Minimum and maximum ambient temperatures will be recorded daily.

6.2 Dosage, Preparation and Administration of Study Intervention (HPS) [250, 251]

HPS will be administered through a cannula placed in a large peripheral vein. All participants will already have a peripheral cannula in place for maintenance fluid replacement. Dosage and adjustments in infusion rates will be administered per Standard Operating Procedures.

The HPS saline bag will be connected to a volumetric infusion set. Based on the weight of the subject, and the current sodium concentration, the appropriate amount of HPS will be placed in the chamber and the tubing attached to the secondary port of the intravenous line already in use for delivery of maintenance fluids. Only the amount of fluid to be installed in the current intervention will be delivered to the chamber, to avoid the possibility of overdose.

The HPS loading dose will take place over a 30-minute period, with duration regulated by adjustment of the drop rate. Maintenance fluids will continue during the infusion of the HPS loading dose to minimize the possibility of phlebitis.

After the loading dose is complete, the infusion rate will be reduced to the maintenance infusion rate (see Standard Operating Procedure).

6.3 Modification of Study Intervention for a Participant (HPS)

Although sodium abnormalities are rare in CM, in the event that a participant has a (Na^+) level of > 150 mmol/L on admission, the initial bolus will be deferred and the sliding scale dosing detailed in Table 3 will instead be followed.

The subsequent dosing of HPS is planned on a sliding scale, with the goal of maintaining serum sodium levels between 150 and 160 mmol/L. Serum sodium levels will be checked every 3 hours for the first 12 hours of the study, and then every six hours, unless designated otherwise (Table 3).

Table 5. Dosing schedule for hypertonic saline

Serum Sodium	Dose of 3% HPS	Sodium recheck
< 145 mmol/L	5 mL/kg (bolus)*	3 hours
145 – 150 mmol/L	2 mL/kg (bolus)*	3 hours
150 - 160 mmol/L	None	6 hours
>160 mmol/L	Hold infusion until serum sodium is ≤ 155, restart at 0.5 mL/kg/hr	1 hour

*If > 3 boluses are required to maintain serum sodium between 150-160, then increase infusion by 0.25 mL/kg until no further boluses are required.

Further modifications to the HPS dose will be made in the event of the following laboratory abnormalities (electrolyte abnormality grading detailed in Appendix B):

- Grade 4 hypokalemia (see Toxicity Table in Appendix B)
- A pH drop of more than 0.2 within 6 hours
- Chloride > 125 mmol/L
- Serum osmolality > 340 mOsm

In these instances, planned HPS doses will be held, and corrective actions (detailed in Section 9.2.4) will be taken. Electrolytes will be rechecked in 3 hours and the normal dosing schedule will be reinstated if electrolytes are no longer in the Grade 4 toxicity range.

If the pH < 7.25 or falls by 0.2 within 6 hours or if the serum chloride is > 125 mmol/L, the 3% saline infusion will be stopped and converted to a 1:1 ratio of 3% sodium chloride/sodium acetate solution.

6. 4 Accountability Procedures for the Study (Intervention/Investigational Product(s) (HPS)

Based on the anticipated recruitment for a calendar year, supplies will be ordered according to anticipated need and in accordance with expiration dates. Bags will remain stored in the storage case in the locked cabinet in the pharmacy until needed on the ward. On enrollment of a participant a bag will be brought to the ward for initiation of therapy. Removal from the pharmacy will be documented on a product inventory log. The lot number and expiration date of the bag will be recorded on the participant CRF. Bags will not be shared between participants. At the end of the calendar year or when the expiry date is reached (whichever comes first), the remaining product will be discarded.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product (HPS)

HPS will be administered by dedicated, trained study nurses, with appropriate documentation. Any irregularities in administration will be recorded and reported as a protocol deviation.

6.6 Study Product Description – Ventilatory Support

6.6.1 Acquisition

Age-appropriate mechanical ventilators will be used in at the bedside in the PICU; an MRI-compatible ventilator will be used to address transport needs of study subjects from the PICU to the MRI suite.

6.6.2 Formulation, Packaging, and Labeling

NA

6.6.3 Product Storage and Stability

NA

6.7 Dosage, Preparation and Administration of Study Intervention/Investigational Product (VENT)

Mechanical ventilation will be initiated immediately after randomization to Study Arm 1, or in the event of a respiratory arrest for subjects allocated to Arms 2 and 3.

Endotracheal intubation will be performed by a pediatric intensivist after administration of IV anesthetic agents in doses described in the sedation section (Section 6.3). The size of the endotracheal tube to be used for each patient will be determined by the pediatric intensivist on-site using standard formulas. A variety of sizes of both cuffed and uncuffed endotracheal tubes will be available (ranging from size 3.0 to 7.5) along with laryngoscopes and different sizes of blades

At the discretion of the intensivist, initial ventilator settings may be set to deliver a Tidal Volume at 6-8 ml/kg, age-appropriate respiratory rate, Peak End Expiratory Pressure of 5 and Fraction of Inspired Oxygen at 40%. The settings will be adjusted by the pediatric intensivists to target a pCO2 (partial pressure of carbon dioxide) in arterial blood of 35-40 mmHg and maintain oxygen saturation above 90% on pulse oximetry.

At the discretion of an intensivist, an arterial line may be placed in the ventilated subjects to measure arterial blood gases every 3 hours for the first 24 hours and every 6 hours subsequently until the participant is extubated.

Participants will remain intubated until brain volume has decreased (brain volume score $\leq$ 6) and the participant has improved clinically (modified Blantyre Coma Score $\geq$ 3) *or* the patient has improved clinically (modified Blantyre Coma Score $\geq$ 4, no repeat MRI needed) *or* the patient dies *or* develops severe irreversible neurologic damage *or* for a maximum of 7 days.

Intubated participants will be moved from side to side every 2 hours (±1 hour) to reduce chances of bed sores. If tolerated, they will undergo endotracheal suctioning every 6 hours (±2 hours) and more often as needed to prevent plugging of the endotracheal tube by mucoid secretions, using an appropriate sized suction catheter attached to wall suction. Participants may be pre-medicated prior to suctioning using sedation medications described in section 6.3.

Participants will receive chest physiotherapy as needed if they develop evidence of lung atelectasis. They may be premedicated with sedation medications as described in section 6.3.

6.8 Modification of Study Intervention/Investigational Product for a Participant (VENT)

In the event that a participant develops a nosocomial pneumonia, appropriate broad-spectrum antibiotics will be initiated after respiratory cultures are sent. Antibiotics will be continued until clinical improvement is noted or for a duration determined by the pediatric intensivist.

In the event of accidental tube dislodgement, participants will be reintubated by the pediatric ICU staff under the guidance of the intensivist.

In the case of a pneumothorax, at the discretion of an intensivist a chest tube may be inserted and hooked to suction to aid lung re-expansion.

In the case of hypotension resulting from positive intrathoracic pressure from mechanical ventilation or secondary to use of sedative agents, participants may receive a 10-20 ml/kg fluid bolus using normal saline or Ringer's lactate, at the discretion of the intensivist.

Participants at risk for development of post-extubation stridor (those with a traumatic or with multiple intubations), will be eligible to receive a 4-dose course of dexamethasone IV prior to planned extubation. In case of stridor noted after extubation, nebulized racemic epinephrine will be administered as needed, at the discretion of the intensivist.

6.9 Accountability Procedures for the Study Intervention (VENT)

NA

6.10 Assessment of Subject Compliance with Study Intervention (VENT)

NA

6.11 Concomitant Medications/Treatments

While on the PICU/PRW, all participants will receive standard of care anti-malarial treatment. This includes the following:

- Anti-malarial treatment: Artesunate will be administered according to national treatment guidelines upon admission, at 12- and 24-hours post-admission, and once a day thereafter until the child is able to take oral medication, or for a total of 5 days. After the child is able to take oral medications, they will be prescribed Co-Artem® (a fixed dose combination tablet containing artemether, 20 mg and lumefantrine, 120 mg). Dosing will be weight-based according to national treatment guidelines. Oral therapy is for three days.

- Antipyretics: An aggressive fever control regimen will be undertaken in both arms – as pyrexia has been shown to be a major predictor of poor outcome in TBI participants [252]. Anti-pyretic therapy will be initiated for any temperature >38°C. The two components will be:

    - Paracetamol – 25mg/kg per rectum (PR) loading dose and 15 mg/kg PR Q6 hours thereafter

    - Ibuprofen – 10mg/kg per naso-gastric tube (PNGT) Q6 hours

  Therapy will be initiated with paracetamol. If euthermia is not achieved in 3 hours, ibuprofen will be added. The two modalities may be scheduled on alternating Q6 hour regimens, at the discretion of the intensivist.

- Anti-convulsants: Locally available anti-convulsants include paraldehyde, diazepam, phenobarbital, and phenytoin. Choice of a specific anti-convulsant is determined on a participant-to-participant basis based on the duration of seizures, available routes of administration, and other clinical characteristics (e.g., respiratory status) of the participant.

- Antibiotics: Participants initially unable to undergo lumbar puncture to rule out meningitis will be placed on ceftriaxone (100mg/kg IV, daily) until a lumbar puncture can be performed or for a maximum of seven days, whichever comes first. A blood culture will be collected initially on all patients, and appropriate antibiotics will be prescribed on the basis of a positive culture. Participants with fever refractory to anti-malarials will have a second venous blood sample collected for culture and the initiation of empiric antibiotic treatment until culture results are available. Continued antibiotic administration will be dependent on blood culture results and clinical status. See Section 6.2.5 for antibiotic therapy for intubated patients who develop a nosocomial pneumonia.

- Sedation: Participants requiring ventilator support will be intubated after induction of anesthesia with ketamine IV [253] along with diazepam. Atropine will be available in case of bradycardia associated with direct laryngoscopy. While patients remain intubated, they may be pre-treated (at the discretion of the intensivist) with morphine *or* diazepam *or* lidocaine prior to noxious stimulation such as endotracheal tube suctioning or placement of gastric tubes, intravenous lines, etc.

- Fluids/Nutrition:

    - At admission, all participants will be placed on maintenance fluids

    - Infusion rates will adhere to the QECH Dept. of Paediatrics protocol: 4mls/hr for the first 10kg, 2ml/hr for next 10kg, 1ml/hr for any weight above 20kg. Adjustments may be performed at the discretion of the intensivist.

    - If a participant remains comatose and is therefore unable to take oral feeds at 36 hours post-randomization, a nasogastric tube will be placed, and a milk-based nutritional supplement will be administered at the same rate as peripheral IV fluids. IV fluids infusion rates will be decreased as NG feed volumes are increased. Nasogastric feeds will be initiated when deemed safe by the PICU physician.

    - 50% Dextrose will be used as a rescue therapy for subjects experiencing hypoglycemia of < 3.0 mmol/L.

- Blood transfusions will be administered as required on clinical grounds.

- Oxygen therapy: Oxygen saturation will be measured continuously in the PICU; it will be measured and recorded on the PRW every two hours by a dedicated study nurse using a portable oxygen monitor with a pediatric finger probe. From the TBI literature adequate

oxygenation is important for treating raised intracranial pressure as injured tissue is highly susceptible to secondary insults including hypoxia [254-256]. Supplemental oxygen will be provided by nasal cannula, endotracheal tube, or mask to maintain peripheral oxygen saturations above 90%.

- Chloral hydrate: Chloral hydrate will be used in children returning for 1-month follow-up visits if sedation is required for the follow-up MRI.

- Omeprazole: Omeprazole may be added at the intensivist's discretion should any participants experience gastrointestinal bleeding. Study Schedule

7 STUDY SCHEDULE

7.1 Screening

Participants admitted to the PRW who meet the following initial inclusion criteria will be approached for consent to further screening.

- Peripheral *P. falciparum* parasitemia of any density
- Blantyre Coma Score ≤2
- No evidence of meningitis on lumbar puncture
- Does not regain consciousness after correction of hypoglycemia (if hypoglycemia was present)
- Age between 6 months and 12 years
- Oxygen saturation ≥ 85% on room air
- Capillary refill ≤ 3 seconds and absence of abnormal skin tenting

The decision to approach the parent/guardian for screening will only be made after the participant has been stabilized clinically and anti-malarial therapy commenced. In the context of obtaining informed consent, the wide spectrum of CM disease presentation will be explained and our desire to perform two more exams (a retinal exam and an MRI) to further characterize the child's illness will be presented. The fact that neither of these exams are treatment, but that they might lead to further treatment, will be explained.

If the parent/guardian consents to the screening exams, mydriatic eye drops will be added to the participant's eyes and a direct and indirect ophthalmologic exam will be performed. The participant will be taken to MRI.

7.2 Enrollment/Baseline

If initial inclusion criteria for randomization are met (severely increased brain volume is confirmed on MRI), the parent/guardian will be approached for consent to participate in the clinical trial.

Initial evaluations to be completed at the time of enrollment are detailed in the Schedule of Events attached as Appendix A.

7.3 Follow-up

The first follow up visit will be scheduled for 1 month after randomization. The participant will undergo detailed physical, neurological, and developmental examinations performed by a pediatric neurologist (or their designee), a repeat MRI, NIRS measurements of cerebral oxygenation and muscle reperfusion, and a repeat ONSD determination.

Subsequent visits will be scheduled for 6- and 12-months post-randomization, at which time the patient will undergo detailed physical and neurological, and developmental examinations performed by a pediatric neurologist or their designee. The 12-month post-randomization visit will be the final visit.

All follow-up visits will be performed ± 14 days from the scheduled post-randomization visit date.

7.4 Final Study Visit

As described in Section 7.3, the final study visit will occur at the 12-month (±14 days) post-randomization follow-up visit. Evaluations to be completed at follow up are detailed in Appendix A – Schedule of Events.

7.4.1 Death

If a death occurs in any of the study arms, transportation will be provided for the deceased and the family.

7.5 Early Termination Visit

If a participant/parent/guardian elects to terminate the study, or study participation is terminated early due to any of the events listed in Section 9.2.3, the parent/guardian will be asked if they are willing to have another MRI performed. They will also be asked if they would be willing to return to the PRW at 1 month (±14 days) post-randomization, as this is standard-of-care for all participants on the PRW.

7.6 Unscheduled Visit

Participants will be invited to return to the PRW for any needed medical care in the interim between discharge from the ward and the final study visit at 12 months (±14 days) post-randomization. Any unscheduled visit will be recorded in the participant CRF and may be recorded as an AE or SAE if appropriate.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

History: A brief medical history pertaining to the immediate clinical condition is obtained immediately on arrival to the PRW. More detailed medical history is obtained after the participant has been stabilized. History includes a history of the immediate illness, previous similar illnesses, and a brief developmental history. History is obtained by interview and supplemented by any records in the participant's health passport.

Medication history is obtained on admission to the PRW and recorded on the same enrollment form. This history relies heavily on the written record provided in the health passport of the participant.

Physical Examination: A full physical examination is performed on arrival to the PRW and after immediate stabilization. Specific vital signs and organ systems evaluated can be found on the physical exam CRF). Twice daily after consent, a targeted physical exam is conducted by a study physician. The results of these daily examinations are recorded in free form on the continuation sheets. At minimum these exams will evaluate the neurologic status of the participant (as determined by BCS), examination of the heart, lungs, liver, and spleen.

Vital Signs: Vital signs (temperature, pulse rate, blood pressure, respiratory rate, oxygen concentration, and BCS) will be evaluated and recorded by a dedicated study nurse every two hours until the participant regains consciousness (BCS≥ 3).

Table 6. Blantyre Coma Score (maximum = 5):

Motor Response		Verbal Response		Eye Movements	
Nil/extend	0	Nil/gasp	0	Unable to track or follow	0
Withdraw	1	Abnormal cry	1	Able to track or follow	1
Localize	2	Normal cry or speech	2		

Table 7. Modified Blantyre Coma Score (for intubated patients, maximum = 5):

Motor Response		Verbal Response		Eye Movements	
Nil/extend	0	Nil/gasp	0	Unable to track or follow	0
Withdraw	1	Attempt to cry, grimace	1	Able to track or follow	1
Localize	2	Clear mouthing of words around ET tube	2		

IV anti-malarial therapy: Artesunate will be administered according to national treatment guidelines upon admission, at 12- and 24-hours post-admission, once a day thereafter until the child is able to take oral medication, or for a total of 5 days.

Oral anti-malarial therapy: After the child is able to take oral medications, they will be prescribed Co-Artem® (a fixed dose combination tablet containing artemether, 20 mg and lumefantrine, 120 mg). Dosing will be weight-based, in accordance with national treatment guidelines. Oral therapy is for three days.

Malaria parasite (MP) and packed cell volume (PCV): An evaluation of malaria parasitemia (MP) and hematocrit (packed cell volume (PCV)) will be performed at initial screening and every six hours thereafter. These exams will continue until two consecutive examinations show an absence of malaria parasites. MP evaluation is performed on a thick blood smear. PCV is determined in a microhematocrit tube. Blood for both of these exams is obtained by fingerprick.

Lactate and glucose check: Blood lactate and glucose levels will be measured at the bedside using point-of-care tests. They will be evaluated on admission and every 6 hours thereafter until the participant has maintained a BCS of 3 for greater than 4 hours.

Lumbar puncture: Participants will be placed in the decubitus position, the L3-4 spinal interspace determined by palpation, and the skin cleaned three times with alcohol. A twenty-one-gauge needle will be used in the procedure. Four ml (± 2 ml) of spinal fluid will be obtained. If at least 4 ml is obtained, 1 ml will be sent for each of culture and sensitivity, cell count plus differential, and protein and glucose determinations. 1 ml of spinal fluid will be archived for determination of biomarkers of increased brain volume.

Retinal exam: Fundoscopy will be performed at admission and every 24 hours thereafter until the patient reaches a BCS of 3 for greater than 4 hours. Pupils of participants will be dilated with mydriatics (a combination of 1.0% tropicamide hydrochloride and 2.5% phenylephrine hydrochloride) in both eyes. A trained clinician will perform direct and indirect ophthalmoscopy on fully dilated eyes.

MRI: After consent for screening is obtained and medical stabilization has occurred, brain MRI images will be obtained.

ONSD: Measurement of optic nerve sheath diameter is being investigated as a non-invasive measure of increased brain volume. The procedure takes 10-15 minutes to perform. A small amount of ultrasound gel is placed on the eyelid and the probe gently pressed superior to the globe. The procedure will be repeated daily until the child maintains a BCS of 3 for four hours.

Transcranial Doppler: TCD examination will be performed at enrollment and every 24 hours thereafter while the patient is hospitalized. Middle cerebral arteries (MCAs), extracranial internal carotid arteries (EC-ICAs), and basilar arteries (BAs) will be insonated at 1 mm intervals using the transtemporal or occipital acoustic windows. Systolic (Vs), diastolic (Vd), mean flow velocities (Vm) and pulsatility index (PI) will be recorded at each interval. Based on findings of TCD examinations, subjects will be placed into phenotypic groups.

Neurological exam: A focused neurological examination will be performed at admission, and at 0800 and 1600 daily (±2 hours) while the patient is comatose (BCS ≤ 2). A detailed neurological examination will be performed at each of the 1, 6, and 12 month (± 2 weeks) post-randomization follow-up visits. While hospitalized, the examination will focus on candidate neurological exam markers of increased brain volume: restriction or changes in eye movements, abnormal posturing (decerebrate, decorticate), changes in reflexes (corneal, gag, deep tendon). The follow-up neurological history and examinations will focus on determination of hard neurological abnormalities: tone, reflexes, movement, posture, interim unprovoked seizures, Neurological

development will be determined by administration of the Malawi Developmental Assessment Test or Kaufman Assessment Battery for children, depending on the age of the subject at the time of enrollment.

Brain swelling intervention: After brain MRI screening, subjects with severely increased brain volume scores (≥ 6) will be eligible for randomization. The parent/ caregiver will be approached for informed consent using the Randomization Consent document. If consent for randomization is granted, randomization assignment will be determined online or opening the next sequentially numbered sealed randomization envelope (stratified by malarial retinopathy status) and the subject's assignment to each of the three study arms determined.

Subjects may be randomized to:

- Arm 1: Usual care + immediate ventilatory support for a maximum of 7 days. These subjects will undergo rapid sequence intubation and be placed on a mechanical ventilator to maintain physiological pCO_2 levels for a maximum of 7 days. Arterial blood gas determinations will take place every 3 hours for the first 24 hours, then every 6 hours until the participant is extubated. Non-scheduled blood gas determinations may occur at the discretion of the PICU physician. Participants will remain in the Pediatric ICU during mechanical ventilation and after its discontinuance.

- Arm 2: Usual care + infusion of 3% hypertonic saline to achieve a target blood concentration of 150-160 mmol/L. Participants will remain in the Pediatric ICU during hypertonic saline infusion. Participants randomized to hypertonic saline administration will have electrolytes evaluated on admission and every six hours thereafter until they regain consciousness (BCS≥ 3)

- Arm 3: Usual care (elevation of the head of the bed 30 degrees and intravenous antimalarial drugs).

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Approximately three mls of blood will be obtained at admission, and a fingerpick blood sample will be taken every 6 hours for the next 48 hours. Fingerprick samples will be approximately 200 µl in volume, resulting in an approximate total blood volume obtained of 3.6 mls.

An evaluation of malaria parasitemia (MP) and hematocrit (packed cell volume (PCV)) will be performed at initial screening and every six hours thereafter. These exams will continue until two consecutive examinations show an absence of malaria parasites. MP evaluation is performed on a thick blood smear. PCV is determined in a microhematocrit tube.

Lumbar puncture: A lumbar puncture will be attempted at the time of admission on all children admitted to the PRW, and if successful, the opening pressure (mm CSF) will be recorded. This is performed to eliminate meningitis as an alternate etiology of coma. Cerebral spinal fluid (CSF) will be sent for cell count, differential, and culture. 1 ml of the total 4 ml volume will be archived for subsequent studies of biomarkers of increased brain volume.

Respiratory swab: All participants will have a nasopharyngeal swab collected at the time of admission. These will be batch analyzed (after hospitalization) to detect respiratory viral pathogens.

Red Blood Cell (RBC) Cryopreservation: RBCs will be cryopreserved and sent to an outside institution for bio-banking/future analysis.

Blood for culture will be obtained on all participants admitted to the PRW. Blood will be obtained from either a peripheral or femoral vein and transferred to a pediatric BacTec bottle for subsequent analysis.

Plasma for archive: One ml of blood will be collected in a lithium heparin anticoagulated tube. Plasma will be separated and archived for subsequent studies of biomarkers of increased brain volume.

Urine for archive: Urine will be collected either while inserting an indwelling urinary catheter *or* by attaching a sterile urine collection bag to the perineum, and at least one ml will be archived for subsequent studies of biomarkers of increased brain volume.

Blood glucose levels will be measured using point of care testing on admission and every 6 hours thereafter until the participant has maintained a BCS of 3 for greater than 4 hours. Machines will be checked for accuracy each morning with a manufacturer supplied test strip.

Lactate levels will be determined on admission and every six hours thereafter until the participant has maintained a BCS of 3 for greater than 4 hours. Testing is done with a point of care testing machine. This test uses one drop of blood obtained by finger prick. The monitor will be tested for accuracy on manufacturer supplied test strips every morning.

HIV testing will be performed on all participants, subject to permission being granted by the parent/ guardian present. The test will be performed after recovery of the participant is anticipated by the clinical staff unless test results are thought to have relevance for acute clinical care. Two rapid tests will be performed on a blood sample obtained by finger prick. In the event of discordant results an HIV polymerase chain reaction test will be performed.

Full Blood Count: Participants will have blood drawn for a full blood count (FBC) on admission. FBC includes hemoglobin, hematocrit, RBC indices, white blood cell (WBC) with 5-part differential, and platelet count.

Electrolytes: Participants enrolled in the study who are randomized to hypertonic saline administration will have electrolytes evaluated on admission and every six hours thereafter until they regain consciousness. As this is a crucial measurement in the appropriate administration of the investigational product, a back-up machine will be available.

Respiratory infection screening: On admission, two nasopharyngeal swabs: one for viral identification and one for bacterial detection and quantification by real time PCR. For viral identification we will use the respiratory PCR panel (FilmArray) which includes simultaneous detection of 18 viruses (adenovirus, 4 endemic coronaviruses, SARS-CoV-2, hmpv, RSV, 5 influenza viruses, rhinovirus/enterovirus and 4 parainfluenza viruses). Individual bacterial real time PCR analysis and quantitation will be performed for *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis.*

8.3 Special Assays or Procedures

8.3.1 Fundoscopy

Fundoscopy will be performed at admission and every 24 hours (±12 hours) thereafter until the patient reaches a BCS of 3 for greater than 4 hours. Pupils of participants will be dilated with mydriatics (a combination of 1.0% tropicamide hydrochloride and 2.5% phenylephrine hydrochloride) in both eyes. A trained clinician will perform direct and indirect ophthalmoscopy on fully dilated eyes. The elements of that will qualify a participant for being retinopathy positive in the study are presence one or more of the following:

- Retinal hemorrhages
- Whitening
- Vessel changes

Examples of each of these are included as Figure 3, Section 2.1g.

The presence or absence of papilledema will also be noted. Presence of papilledema alone without any of the other three elements will not qualify the participant for classification as retinopathy positive. The presence or absence of papilledema will however be evaluated as a measure of increased brain volume.

8.3.2 MRI

MRI of the brain will be the “gold standard” for assessing the presence of brain volume. The final determination of brain volume will be made initially by one of two experienced radiologists. They will use the following scale[52]:

1 = severe atrophy markedly abnormal for age with diffuse prominence of the cisternae and sulci

2 = mild atrophy – subtle prominence of the cisternae and sulci for age

3 = normal brain volume

4 = mild increased volume but with maintenance of cisternae and sulci

5 = mild swelling – some loss of cisternae and sulci but not diffuse

6 = moderate swelling – diffuse involvement with some loss of cisternae and sulci

7 = severe swelling – loss of all sulci, presence of cisternal effacement, decreased gray/white matter delineation

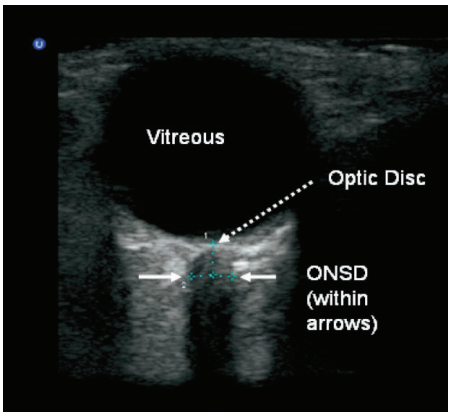
8 = severe swelling – loss of all sulci, presence of cisternal effacement, presence of uncal herniation, loss of gray/white matter delineation

The presence of severely increased volume (edema score 7 or 8) identifies the subset of children who are most susceptible to herniation and therefore death (Figure 5). Real-time interpretations during the 2018 enrollment season revealed that neuroradiologists could not reliably differentiate a brain volume score of 6 or 7. In order to include all subjects who might benefit from the interventions, subjects with brain volume scores of 6-8 will be eligible for randomization (see Section 2.3.3).

Two study radiologists will be available at all times to review MRIs and assign a brain volume score. If both independently assigned brain volume scores are ≥ 6, the participant will be eligible for randomization. If either independently assigned, real-time score is < 6 the participant will not be eligible for randomization.

Three other study radiologists will review all screening and study scans and will arrive at a consensus ‘brain volume score’ for each MRI scan on a regular basis and will complete the task within 1-month of the end of the enrollment period each year. If there is a discrepancy regarding the initial brain volume score and the consensus score in enrolled subjects any subjects incorrectly enrolled will be excluded from the ‘per protocol’ analyses but will remain in the ‘intention to treat’ analyses. If there is a discrepancy in the serial MRIs that affects the decision to withdraw the experimental treatment (VENT or HPS), the discrepancy will be noted; any subjects incorrectly withdrawn will be excluded from the ‘per protocol’ analyses, but will remain in the ‘intention to treat’ analyses.

8.3.3 Optic nerve sheath diameter (ONSD)



ONSD will be measured using a digital ultrasound machine with an appropriate transducer. Details of the ONSD determination procedure are as described in Tayal *et al* [196].

Figure 11 Illustration of the image and proper area for measurement in ONSD determination[257].

8.3.4 Transcranial Doppler Ultrasound (TCD)

TCD will be measured using a commercially available TCD unit with an appropriate transducer. Details of the TCD examination are as described in Aaslid et al[72]. The following diagnostic criteria will be used to categorize studies into phenotypes:

Microvascular Obstruction

1) Systolic flow in middle cerebral arteries was within standard deviations (SD) of normal for age and gender AND
2) Diastolic flow in middle cerebral arteries was < 2 SD below normal for age and gender AND
3) Middle cerebral artery Gosling's pulsatility index was ≥1.2
Pulsatility Index (PI)= (Systolic flow velocity-Diastolic flow velocity/Mean flow velocity)

Hyperemia

1) Mean flow in middle cerebral arteries was ≥ 2 SD above the age and gender normal value AND
2) Ratio of flow between the middle cerebral artery and extra-cranial carotid artery (Lindegaard ratio (LR)) was <3 AND
3) The dichrotic notch was absent on waveform morphology analysis of the middle cerebral artery AND

Cerebral Vasospasm

1) Mean flow in the middle cerebral arteries was ≥ 2 SD above age and gender normal value AND
2) LR was ≥3 AND
3) The dichrotic notch was present on waveform morphology analysis of the middle cerebral artery

Low Flow

1) Systolic, diastolic, and mean flows were equally reduced in the middle cerebral arteries ≤ 2SD below the age and gender normal value AND
2) The PI was <1.2

Isolated Posterior Hyperemia

1) Mean flow velocity in the basilar artery was ≥2SD above age and gender normal value AND
2) Mean flow velocity in both middle cerebral arteries was within 2SD of age and gender normal value

Terminal Intracranial Hypertension

1) Systolic flow in the middle cerebral artery was <2SD below age and gender normal values WITH associated systolic spikes on waveform analysis AND
2) Absence of or reversal of diastolic flow was noted

8.3.5 Near-infrared spectroscopy

The cerebral hemoglobin oxygenation will be assessed by near-infrared spectroscopy of cerebral tissue via one or two sensors placed on the skin overlying the skull. Cerebral oxygenation will be recorded for 30 minutes (range 15-60 minutes) on each study day per schedule in Appendix A. Average saturation as well as the amplitude and frequency of oscillations in tissue oxygenation will be analyzed.

Skeletal muscle reperfusion dynamics will be assessed by measuring the changes in muscle hemoglobin oxygenation by near-infrared spectroscopy after inflation and deflation of an occlusive thigh cuff on each study day per schedule in Appendix A. The skeletal muscle recording will last approximately 30 minutes and includes a 3-minute inflation of a pneumatic cuff on the lower limb.

8.3.6 Heart rate variability

When patients are admitted or transferred to the PICU, they are connected to bedside monitoring which includes electrocardiogram (EKG), respiration waveforms, oxygen levels, and blood pressures. The ICU monitors display instantaneous values for these clinical parameters and plots of their trends. These waveforms will be archived, compressed and uploaded for offline analysis of patterns of heart rate variability and integration of results with the clinical course. A 60 minute reading will be taken in the supine position. Readings will undertaken every six hours for the first 24 hours of admission, then daily until the patient's Blantyre Coma Score is ≥ 3, and again upon discharge. The aim is to undertake the recordings at the same time every day; currently 0600, 1200, 1800 and 0000. Readings will be discontinued upon death or improvement of Blantyre coma score to ≥ 3.

8.3.7 Heart and lung ultrasound

A protocolized heart and lung ultrasound (HLUS) examination will be performed within 4 hours of admission. All sonographic images will be acquired with a Butterfly iQ (USA) phased array transducer using transverse view. Each HLUS will be performed by the same trained investigator. Based on the international evidence-based recommendations for POCUS of the lungs, a complete eight-zone examination will be used. The anterior and lateral chest wall will be divided and imagining obtained bilaterally in a parallel plane. Each zone will be scored based on the most agreed upon scoring system for LUS which uses four ultrasound aeration patterns:

1. Normal = the presence of lung sliding with A-lines or fewer than two isolated B-lines, scored as 0
2. Moderate loss of lung aeration = multiple well-defined B-lines (B1-lines) present, scored as 1
3. Severe loss of lung aeration = the presence of multiple coalescent B-lines (B2-lines), scored as 2
4. Lung consolidation = tissue pattern characterized by dynamic air bronchograms, scored as 3.

The worst ultrasound pattern observed in each zone will be recorded and used to calculate the sum of the scores with a maximum total score of 24. Each child's lung ultrasound will be repeated and scored daily until discharge, death, normalization, or day 4 of admission, whichever comes first.

The cardiac function will be evaluated using a non-invasive cardiac output monitor (USCOM, Australia). The ultrasound probe will be placed on the suprasternal notch and the blood flow will be captured. The machine performs calculations and reports cardiac output, cardiac index, stroke volume, stroke volume index, systemic vascular resistance, and stroke volume variability. Each child's cardiac ultrasound will be repeated, and results recorded daily until discharge, death, normalization, or day 4 of admission, whichever comes first.

8.4 Specimen Preparation, Handling, and Shipping

8.4.1 Instructions for Specimen Preparation, Handling, and Storage

Admission CSF, urine and plasma samples from each participant will be archived. The one ml blood sample dedicated for plasma archive will be aliquoted into a cryovial for long term storage in a -80°C freezer. One ml of CSF will immediately be transferred to a cryovial prior to distributing the remainder of the sample for culture and cytology. Urine will be collected in the process of inserting a Foley catheter or via a sterile bag affixed to the patient and transferred to a cryovial.

Cryovials for plasma, urine and CSF will be identified with a unique bar code and the location in the freezer recorded with dedicated sample tracking software. Thick and thin blood smears to evaluate the presence of malaria parasitemia will also be stored past the conclusion of the study. They will be labeled with a unique bar code and stored in a slide file cabinet in a locked storeroom adjacent to the PRW as detailed in Section 14.7.

Detailed protocols for both procedures are included in the study's Manual of Operating Procedures (MOP).

8.4.2 Specimen Shipment

Samples will be shipped in accordance with IATA requirements and with any required Materials Transfer Agreements in place.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

The primary safety parameter for this study is a comparison of survival of the participants in the three treatment arms. In addition, we will be assessing long term neurological sequelae for all participants.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

ICH E6 defines an adverse event (AE) as any untoward medical occurrence in a participant or clinical investigation subject exposed to an intervention or a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease, temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

Because the safety profiles of the two study interventions are fairly well-defined, and the patient population is critically ill, only AE considered to be possibly attributed to the study intervention will be collected. These will include AE known to be related to the study interventions and pre-specified in 2.4.1.2 and any other unanticipated AE that in the opinion of the investigator was attributed to study interventions. All AEs occurring while on study will be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition present at the time that the participant is screened should be considered as baseline and not reported as an AE. However, if the medical condition deteriorates at any time during the study, it will be recorded as an AE.

All AEs will be graded for severity and relationship to study interventions.

Since mortality is an endpoint, death will not routinely be considered an Adverse Event for study purposes. Only events meeting leading to death, hospitalization or prolongation of hospitalization that are outlined in section 2.4.1.2, or that were unanticipated, but that are considered to be possibly related to the study intervention will be reported as SAE.

9.2.1.1 Severity

For electrolyte abnormalities, toxicity tables (Appendix B) will be used. For other, non-protocol specific AEs the following grading system will be used:

- Mild: events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Life threatening: any adverse drug experience that places the participant or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Onset and duration of each episode of intermittent adverse events will be documented.

9.2.1.2 Relatedness

The relatedness of the AE will be assessed by a study clinician based on the temporal relationship of the AE and any interventions, as well as the likelihood of other causal etiologies of the specific AE.

Related – There is a reasonable possibility that the intervention caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.1.3 Expected Adverse Events

All adverse events meeting the criteria listed above will be collected. Special concern will be paid to the following adverse events as they are known side effect of the investigational drugs:

- Potentially attributable the hypertonic saline intervention:
  - Hypernatremia
  - Hypokalemia
  - Hyperkalemia
  - Phlebitis
- Potentially attributable to the intubation intervention:
  - Pneumothorax
  - Pneumomediastinum
  - Subglottic stenosis
  - Stridor
  - Tracheostomy
  - Bronchospasm
  - Pulmonary hemorrhage

- Respiratory nosocomial infections
- Hypotension
- Endotracheal tube obstruction
- Inadvertent endotracheal tube dislodgment
- Bradycardia induced by vagal response

Adverse Event grading tables (attached as Appendix B) will be used to define these conditions. These events will be captured in the CRF and subsequently in tabular format, listing time of event onset, time of event resolution, brief description of the event, clinician's assessment of severity, and clinician's assessment of relation to the intervention. They will be reported to the local ISM on a monthly basis, and an annual report of these events will be presented to the DSMB, as well as both IRBs. The events will be captured for the duration of the participants' stay on the PICU/PRW, independent of whether the participant is still receiving interventional therapy.

9.2.2 Reactogenicity (for Vaccine Studies and Some Therapeutic Trials)

NA

9.2.3 Serious Adverse Events

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening adverse event,
- Hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Only events that are not attributable to the malarial illness, that are outlined in section 2.4.1.2, or that were unanticipated, but that are considered to be possibly related to the study intervention will be graded and reported as Adverse Events to the sponsor, IRBs, and independent safety monitors.

- Life-threatening adverse event: An adverse event is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the participant or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

9.2.3.1 Protocol defined SAEs

CM is a disease with a high mortality rate. Reported mortality rates range from 15 – 30%, with our site having a rate of 18% over the past 25 years. We anticipate the mortality rate in randomization arm 3 will be approximately 37%. All deaths not attributable to the malarial illness will be reported to independent safety monitors, IRBs, and the sponsor as required.

- Death not caused by respiratory arrest secondary to intracranial hypertension.
- Prolongation of existing hospitalization for more than 7 days due to an intervention.
- A life-threatening adverse event that is not part of the study outcomes defined in the study objectives, an attributable to an intervention.

9.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

9.2.4.1 Hypernatremia

Table 3 (Section 6.1.5) categorizes the possible serum sodium levels and actions to be taken if they are observed. A serum sodium measurement of greater than 190 mmol/L will be considered an SAE and reported as such. The 3% HPS infusion will be stopped. Maintenance fluid infusion will continue at maintenance rate. Sodium will be rechecked hourly until its serum level is < 160 mmol/L and therapy will be resumed as per Table 3.

9.2.4.2 Hypokalemia

As per the toxicity table attached as Appendix B, a serum potassium measurement of <2.0 mmol/L will be defined as a Grade 4 adverse event. Corrective action will be in the form of adding 40 mmol/L of KCl to the maintenance IV fluids (an increase from the 17mmol/L K^+ present in ½ Strength Darrow’s). IV fluid infusion rate will remain the same. HPS boluses will continue, and electrolytes will be monitored at 3 hour intervals until K^+ returns to below Grade 3 disturbances.

9.2.4.3 Phlebitis

3% hypertonic saline when administered through peripheral veins over prolonged periods of time may lead to phlebitis. If phlebitis or swelling of the IV catheter site is noted, the IV cannula will be removed and an alternate placed.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

The reporting period for SAEs will be between the time of randomization and the twelve month (±2 weeks) post-randomization follow up visit.

SAE reporting will be carried out as follows

The study clinician will complete a Serious Adverse Event Form (Appendix D) and SAEs thought to be related to study related procedures will be reported within 24 hours of their recognition, to the independent safety monitor, the IRBs at Michigan State University and the University of Malawi College of Medicine, and the sponsor, unless advised otherwise by the DSMB.

Any AE that meets a protocol-defined serious criterion will be submitted on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and will be provided as soon as possible.

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group, the independent safety monitor, and the IRBs of the University of Malawi and Michigan State University.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

NA

9.3.3 Regulatory Reporting for Studies Not Conducted Under DMID-Sponsored IND

NA

9.3.4 Other Adverse Events (if applicable)

NA

9.3.5 Reporting of Pregnancy

Participant population is pediatric and not prone to pregnancy.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic. CM is a disease with a high burden of long-term neurological deficits [258] . Participants will all be asked to return to clinic at 1, 3, 6 and 12 months (±2 weeks) post-randomization for evaluation of recovery; the 12-month (±2 weeks) visit will serve as the final study visit.

9.5 Halting Rules

The following eventuality would trigger temporary suspension of enrollment until a safety review is convened and further evaluation undertaken:

- Five subjects where current HPS regimen is unable to achieve the target Na^+ levels of 150-160 mmol/L.

- Any SAE deemed to be related to each of the experimental medical interventions (ventilator support or infusion of 3% hypertonic saline solution).

DMID retains the authority to suspend additional enrollment and study interventions during the entire study, as applicable.

9.6 Safety Oversight (ISM plus SMC or DSMB)

Safety oversight will be under the direction of a DSMB composed of a pediatrician, a pediatric intensivist, a statistician, and others at the discretion of NIAID. The DSMB will meet annually to assess safety and efficacy data on each arm of the study. The DSMB will review aggregate safety data for increased rate of occurrence of serious unexpected adverse reactions. If halting rules are initiated, more frequent meetings may be held. The DMSB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will advise DMID of its findings.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitors from the Research Support Centre at the University of Malawi College of Medicine will visit the clinical research site to review the individual subject records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, participants' hospital charts), to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

11 STATISTICAL CONSIDERATIONS

11.1 General Design and Objectives

We propose a single site randomized controlled trial (RCT) of two adjunctive therapeutic approaches compared to usual care (UC) in treating increased brain volume in Malawian children with CM and MRI-supported evidence of severely increased brain volume. Children will be randomized to one three arms: Arm 1: UC plus immediate ventilator support (VENT), Arm 2: UC plus hypertonic saline (HPS), Arm 3: UC. Arm 2 and Arm 3 would receive rescue ventilation in the event of a respiratory arrest.

11.2 Stratification and Randomization

Stratification by retinopathy status (negative/positive) is necessary as the mortality rates vary between children of varying retinopathy status. For this study, stratification by gender or age is not necessary.

11.3 Testing the adequacy of randomization

Prior to analysis, the adequacy of the randomization will be evaluated by comparing all baseline characteristics of the three study arms. These variables include characteristics such as age, gender, weight, malarial retinopathy status, height and duration of symptoms prior to admission. Where substantive differences exist in these variables, they may be controlled for in subsequent analyses by regression techniques. The degree of similarity between the study arms will be tested using, as appropriate, ANOVA, nonparametric tests and chi-square tests. However, following Altman[259],we will be guided by the degree of dissimilarity rather than strict statistical significance.

11.4 Outcomes

The primary endpoint is death within one week following randomization. In Arm 2 and Arm 3, the endpoint will include failure of the initial treatment allocation such that the patient requires rescue ventilation. Several secondary outcomes will be assessed in the trial. Table 8 gives a brief description and preferred analytic strategies. Additional details are provided elsewhere in the Protocol.

11.5 Sample Size and Power

For the primary outcome death/failure of the intervention, we conduct two parallel studies: (i) VENT versus control UC, (ii) HPS versus control UC. Approximately 128 children will be enrolled in the studies. This includes the 64 children in UC comprised of 16 randomized to UC in the 2018-2019 malaria seasons and augmented by 48 historical controls from previous seasons (2014-2017). We also randomized 16 children to VENT in the 2018-2019 malaria seasons.

Table 8: Summary of outcome measures and analytic plan		
Outcome measure	**Outcome type/scale**	**Analytic method**
PRIMARY OUTCOME		
Success of primary intervention or survival in the first 7 days after randomization	Binary	Logistic regression
SECONDARY OUTCOMES		
Brain volume determination (MRI on admission, then daily)	Ordinal	Ordinal regression
Optic Nerve Sheath Diameter (on admission, then daily)	Continuous	Linear regression. Serial assessments by repeated measures analysis
Transcranial Doppler ultrasound (on admission, then daily)	Continuous	Linear regression. Serial assessments by repeated measures analysis
Opening CSF pressure (mm CSF) (on admission)	Continuous	Linear regression
Heart rate variability patterns	Continuous	Linear regression. Serial assessments by repeated measures analysis
Neurologic exam * (on admission, then twice daily)	21 binary and 5 continuous variables	Linear or logistic regression
Papilledema (on admission, then daily)	Binary	Logistic regression
Severe neurologic sequelae at 16 and 12 months	Binary	Logistic regression. Serial assessments by repeated measures analysis
Hypotension that occurs within 30 minutes of sedation administration, initiation of mechanical ventilation or ventilator pressure adjustment.	Binary	Logistic regression.

Since the beginning of this study, we faced several logistical challenges but had success in recruiting 32 children in 2018-2019 (16 each in VENT and UC). Based on our current data, the estimated mortality in UC and VENT are respectively, 36% and 25%. Maintaining the mortality rate in UC, a one-sample lower test at significance level α=0.05 of a null difference has 80% power to detect a reduction in mortality to 18% in VENT with 33 subjects in VENT.

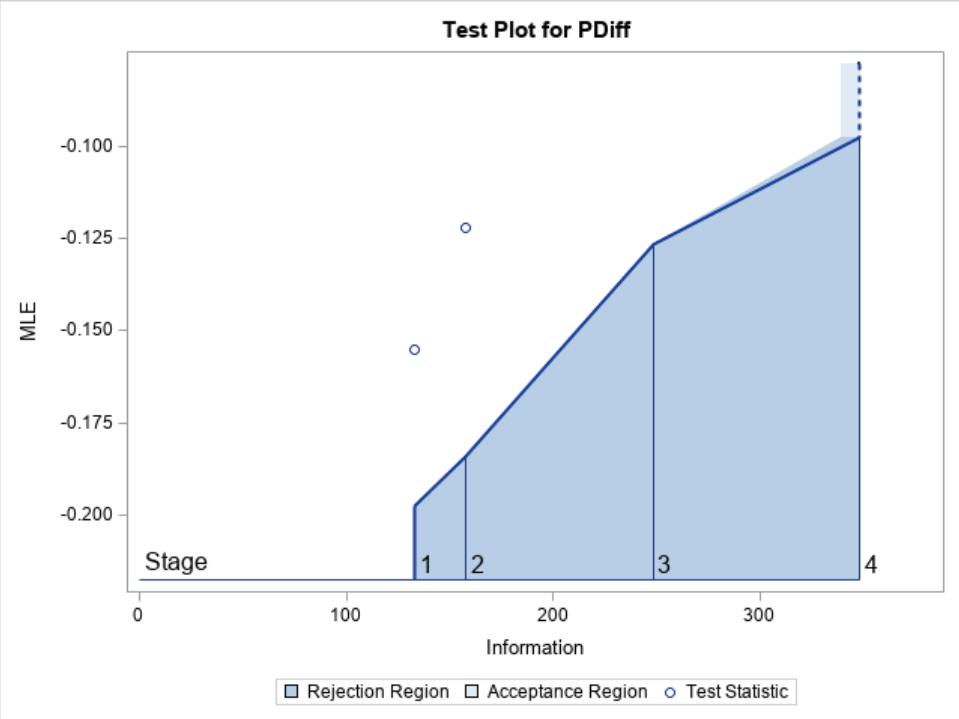
11.6 Interim Analyses

The TBS design is a 4-stage sequential plan to compare VENT to UC on the outcome of mortality. Based on historical controls and initial randomization to UC before 2020, mortality is

assumed fixed at p_0 =0.28. We test the hypothesis that mortality p in VENT is lower, i.e., we test $H_0 : p = p_0$ *versus* $H_0 : p < p_0$ at significance level α=.05. VENT will be declared superior to UC, if we can demonstrate a relative risk reduction of 50% with 80% power. This needs a sample of 38 subjects.

Boundaries for the 4-stage plan are derived from the power family. Early stopping is to reject the null hypothesis. Our first interim analysis was conducted with data from the 2019-2020 malaria seasons. Observed mortality in VENT was 12.5% (2/16). The conclusion was to continue to the next stage. Our current estimate, at the end of the 2021 season is 15.8% (3/19). In accordance with the design, we must continue to the next stage (i.e., 2022 season). The revised boundary information is depicted in the figure.

Figure: Boundary plot at conclusion of stage 2



Legend: The scale is the maximum likelihood estimate (MLE) of the mortality difference (PDIFF), $\theta = p - p_0$ against the actual information level. At stage 1, observed θ=0.125–0.28=–0.155, and at stage 2, θ= 0.158–0.28= –0.122. The rejection boundaries are not crossed.

Conditional power and predictive power

Conditional power (CP) is the power to reject the null hypothesis H_0: θ =0 at all future stages given the cumulative interim data. This calculation shows: (a) at the current estimated difference in mortality θ=–0.122, CP=76.1%, (b) at zero difference in mortality, CP=18.6%, (c) at the hypothesized difference, θ=–0.14, CP=82.9%. Predictive power (PP) is the posterior probability

of rejecting the null at the next stage given the current data, assuming a non-informative prior on the mortality difference. PP=66.1% at the current stage.
Based on these assessments it behooves that we proceed to stage 3.

Hypertonic saline (HPS) vs UC
We have our first sample of 3 subjects, one of whom died. Although hardly necessary, a formal analysis provides the exact 95% CI for mortality in HPS, (0.008, 0.906).

11.6.1 Futility Analysis

Futility Analysis: Although VENT seems an effective treatment, does it promise at this stage *substantial benefit* compared to UC? To answer this question we calculated the *conditional power* to reject the null hypothesis at all future stages give the current interim data. Conditional power (CP) is calculated on hypothesized values of the alternative θ. Our current value is –0.122. CP at the current value is 76.1%. At the hypothesized difference, 0.14–0.28=–0.14, CP=82.9%. These do suggest promise of VENT. However, if the true difference in mortality is actually zero (θ=0) then CP=18.6%. The literature advises that if CP is small (e.g., <20%) over a plausible range of θ values, then it would be *futile* to continue the trial. *The evidence that we have now does not point to futility.*

11.7 Statistical Analysis

For the primary outcome, the test of the null hypothesis $H_0 : \theta = 0$ *vs* $H_1 : \theta < 0$ for the mortality difference $\theta = p - p_0$ follows from the sequential design described in 11.6. The preferred analytic method for secondary outcomes by type/scale is included in Table 8. The unit of analysis is the individual child (indexed by *i*) with a single outcome measure Y_i and covariates $\mathbf{x}_i$ which includes an indicator for treatment arm (VENT, HPS, UC), socio-demographic and clinical variables whose prognostic influence on the outcome is of interest or needs to be controlled. The ensuing data on the *N* children are $\{(Y_i, \mathbf{x}_i) : 1 \le i \le N\}$. We expect *N*=128 across all three arms. Depending on whether the early stopping and futility rules are triggered in 11.6, sample size will vary by treatment arm. Minimum size in UC is 64 if no new recruitment into this arm is made in 2022 and 2023. Minimum size in HPS is 32, and in 21 in VENT if futility is triggered. With these caveats we describe broadly our statistical analyses.

Analysis of binary outcomes in Table 8 is by logistic regression. Continuous variables such as optic nerve sheath diameter on admission, heart rate variability patterns, and opening CSF pressure on admission are analyzed using linear models. To mitigate the effects of skewness in their distributions, if found, a logarithmic or square-root transformation *g* will be applied to Y_i and analysis carried out on the transformed variable. To assess the effect of treatment on outcome we will fit the linear model $E(g(Y_i) \mid \mathbf{x}_i)) = \mathbf{x}_i'\beta$ and test the hypothesis $H_0 : \beta_1 = 0, \beta_2 = 0$ where β_1 and β_2 are the coefficients for the VENT and HPS, respectively. Point estimates and

associated 95% confidence intervals for response mean on the original scale will be calculated and would involve smearing.[260-262]

For assessments made serially over time, the outcome is a vector $\mathbf{Y}_i = (Y_{i1}, Y_{i2}, \ldots, Y_{im})'$ of repeated continuous (e.g., sheath diameter on admission and then twice daily) or repeated binary indicators (e.g., severe neurologic sequelae at 1, 6 and 12 months). Generalized linear models $g(E(Y_{ij} \mid \mathbf{x}_i)) = \mathbf{x}'_{ij}\beta + \mathbf{z}'_{ij}\mathbf{b}_i$ are apropos with right hand side covariates specific to the *j*-th time, $\mathbf{z}_{ij}$ will include an intercept and terms involving time permitting assessment of the evolution of the mean outcome (or probability for binary outcomes) over time, both within-subject and across the study population (i.e., population average). The link *g* is the identity function for continuous responses, and the logit for binary responses. Correlations between components of $\mathbf{Y}_i$ are incorporated via the random effects. A judicious choice of covariance structure for $\mathbf{b}_i$ will be elicited from information criteria.[263-265] Alternating logistic regression will be considered the case of binary responses.[266] Repeated ordinal measures (e.g., brain volume) will be analyzed by proportional odds or partial proportional odds models.[267,268]

In all analytic procedures we will examine the overall goodness-of-fit, and presence of influential and/or outlying data points with graphical techniques and formal residual analyses.

11.7.1 Missing Data

Although quite unlikely, mechanisms for handling missingness in our response data will be appropriate to the underlying analytic strategy[269] but tempered by available sample size. In linear and nonlinear mixed models likelihood-based inference using only the non-missing responses is valid under the MAR, i.e., missingness is dependent only on the observed data. The alternative missingness mechanism--missing completely at random (MCAR) assumes missingness is does not depend the responses, observed or not. Other approaches such as inverse-probability weighting[270,271] will be investigated to accommodate missing data patterns that are neither MCAR or MAR[272,273] an approach that we used in analyzing longitudinal censored medical costs data.[274,275]

11.7.2 Additional exploratory modeling

Since Arm 2 (HPS) and Arm 3 (UC) are likely to have an intermediate endogenous binary outcome Y_{i1} of treatment success prior to final outcome of survival Y_{i2}, we will consider joint modeling for (Y_{i1}, Y_{i2}) via a two-part specification: (1) conditional model for $P[Y_{i2} = 1 \mid Y_{i1} = 1, \mathbf{x}_i]$ -- i.e., probability of survival given treatment success and (2) $P[Y_{i1} = 1 \mid \mathbf{x}_i]$ –i.e., the probability of treatment success. Both two-stage and full likelihood-based estimation of the joint model are feasible.[270,276]

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

We will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored, DMID-affiliated, or manufacturer-sponsored study, we will permit authorized representatives of the sponsor(s), DMID, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. The electronic CRFs and subsequent database will be stored on a password protected computer. Other sources of data will include:

- MRI images will be stored both at QECH and on a password protected server at Michigan State University.
- ONSD images will be stored on an ultrasound machine at QECH. It is password protected and stored in a locked room adjacent to the ward.
- TCD images will be stored on an ultrasound machine at QECH. It is password protected and stored in a locked room adjacent to the ward.
- Heart rate variability data will be stored on a Malawi-based server and securely transmitted to Michigan State University where it will be available for download and analysis by co-investigators.

13 QUALITY CONTROL AND QUALITY ASSURANCE

A plan for local monitoring of clinical quality has been documented in a Clinical Quality Management Plan (CQMP) (see Manual of Operations). This document details the Standard Operating Procedures (SOPs) for on-site quality management including data evaluation methods, staff training methods, documents to be reviewed, and corrective actions to be taken should deficits be detected. This document will be reviewed annually and submitted to DMID.

All clinical observations and laboratory evaluations will be recorded in accordance with the Manual of Procedures.

Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements.

We will provide direct access to source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The Data Manager will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

The PI is responsible for ensuring that routine quality management activities are conducted.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The principal investigator (PI) will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997), whichever affords the greater protection to the subject.

14.2 Institutional Review Board

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents and recruitment material by an appropriate independent ethics committee (IEC) or IRB registered with the OHRP. Any amendments to the protocol or consent materials must also be approved before they are placed into use. In the United States and in other countries, only institutions holding a current US Federal-Wide Assurance issued by OHRP may participate. All activities will be reviewed by a Michigan State University IRB as well as the local IRB (the University of Malawi College of Medicine Research and Ethics Committee [COMREC]).

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to caregivers of subjects. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the caregiver and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms will be IRB-approved and the caregiver will be asked to read and review the document. Upon reviewing the document, the nurse initiating informed consent will explain the research study to the subject and answer any questions that may arise. Caregivers will sign the informed consent document prior to any procedures being done specifically for the study. They will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. Caregivers may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the caregivers for their records. The rights and welfare of study subjects will be protected by emphasizing to caregivers that the quality of their child's medical care will not be adversely affected if they decline to participate in this study.

14.4 Detailed Informed Consent Process

There are two consent processes potentially involved in this study:

1. Consent for screening. This is the first step for all patients, and the ONLY step for patients who do not have increased brain volume on MRI. These study participants will spend the first 24 hours on the PICU or the PRW (for continuous monitoring for HRV) and if in the PICU will then be moved to the PRW unless they deteriorate, clinically, and warrant ICU care.

2. Consent to enroll in the interventional clinical trial. This option is for patients with MRI evidence of increased brain volume on MRI

In all cases the pertinent material will be supplied in both oral and written form. Forms will be available in English and the local language, Chichewa. The caregiver will be supplied with a copy of the consent form and given ample time to ask questions regarding specifics of the study. A study clinician or study nurse will review the study consent with the parents or guardians accompanying the child. If the parent/guardian is illiterate, the consent will be explained verbally. If the parent/guardian is unable to form a signature, a thumb print will be obtained instead.

Although all subjects will be minors under the age of twelve, the subjects will not be capable of providing assent to participate because all will be, by definition, comatose.

Exclusion of Women, Minorities, and Children (Special Populations)
The major burden of malaria is borne by children; therefore, only children under the age of thirteen years will be enrolled in this study. Subjects will all be Malawian children. All children will be cared for on specially adapted pediatric wards. Parents/guardians are allowed to remain with the children throughout their hospitalization, and all meals and medications are provided without cost. The licensed clinicians providing care are all trained in the management of children with CM, and each research unit is amply staffed with trained dedicated nursing staff. No participant will be excluded on the basis of sex/gender or ethnicity.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

14.6 Study Discontinuation

In the event of the discontinuation of the study, any subject currently enrolled in the study will continue in their current study arm, unless study discontinuation was due to one arm being determined to be superior to the others. In this case, study subjects will be switched to the arm showing highest efficacy.

14.7 Future Use of Stored Specimens

Plasma, urine, and CSF samples will be stored at the study site in liquid nitrogen or at minus-80^{o}C, filed by study number, and de- identified.

15 DATA HANDLING AND RECORD KEEPING

Clinical research data will be managed using the Research Electronic Data Capture (REDCap)[277] web-based software application developed by Vanderbilt University and hosted on servers located at the Malaria Alert Center, Blantyre, Malawi. For the purpose of data backup, a copy of the data will be hosted by the Biomedical Research Informatics Core (BRIC) on servers at MSU. The REDCap database developed for the Study will organize patient data entered directly into the database by clinicians using the REDCap application deployed on tablets at the patient bedside, by hospital staff using paper data collection forms, and by hospital staff and radiologists using computers for file uploads of MRI and laboratory results. The goals of the Data Management Plan are to optimize workflow for the capture of high quality data, provide a well-documented data audit trail, deliver robust measures of data security, and facilitate easy data access and data sharing for the Study research team.

15.1 Data Management Responsibilities

Roles for data management will engage various members of the study research team and will be supported by REDCap functionality. The PI will have access to all study data, and access to by other study personnel will be restricted as directed by the PI. REDCap user definitions and user permissions, which allow for role-based and form-level access permissions and restrictions, will be used to limit access to identifying information only to necessary individuals. Data exports will be configured to remove identifying information by shifting date fields and redacting all other fields marked as identifying, as determined by the PI. The Malawi Data Manager and BRIC Data Manager (DM) will be responsible for issues related to data collection and data quality and will train clinicians and other hospital staff on matters related to direct electronic data capture. The BRIC Project Supervisor and Development Supervisor will oversee and ensure implementation and monitoring of the data management plan. They will work closely with the PI, and Study team to ensure that the informatics configuration, testing, and production processes are adequately documented. They will meet regularly to review data quality.

15.2 Data Capture Methods

Data Collection

Clinical research data will be managed using a REDCap Study database and customized features to support the entry of patient data, using tablets at the patient bedside. A majority of the clinical trial data will be entered directly into the database by clinicians and other hospital staff as the data are generated. The appropriate patient database record will be queued for direct electronic data capture by the use of barcodes, which will encode the patient Study ID#. Biologic specimens and paper data collection forms used for data capture will be labeled with barcodes to identify the associated patient. Persons entering data via the tablet will require an active REDCap account username and password and/or pin-authentication, and users will be logged out after a period of inactivity. REDCap allows tracking of form data entry through a form status dashboard, and each form can be signed electronically after it is moved to a ‘completed’ status. In case of power failure, back up paper forms will be stored in locked file cabinets.

Corrections to data on paper forms will be initialed and dated, and the data error will be overwritten with a single strike-through line.

During transmission of data, REDCap uses Secure Hypertext Transfer Protocol (HTTPS) which employs a standard Secure Sockets Layer (TLS 1.0, 1.1, 1.2 - 128 bit block size) encryption technology. SSL creates the secure connection and HTTPS transmits the data securely. All subject data will eventually reside in a MySQL database server. The REDCap application includes functionality to enable 21 CFR part 11 compliance for electronic records and signatures, including a comprehensive audit log that records all user activity. Audit logs are created automatically within the REDCap application to capture the complete user history of database activity.

The final record for each study participant will comprise data collected over a period of two years: from initial data collection during hospitalization through and 6-month periodic follow-up assessments to 12-months post-randomization

Data Quality

The Malawi and MSU-BRIC DMs will work together to provide ongoing management and review of data collection processes and data quality. The MSU-BRIC DM will provide on-site initial data entry training for study personnel, and refresher trainings via annual trips. During active enrollment periods, the Malawi and MSU-BRIC DMs will conference weekly to address matters related to data entry and data quality. DMs will utilize standard REDCap features to ensure data integrity and quality. Variables will be designed with real-time field validation rules, and data will be reviewed using built-in checks for missing values and outliers. The Data Quality module will be used to execute quality control verification on all fields and to resolve discrepant data, using an audited trail of query and query resolution.

Data Security

REDCap will be hosted on a server housed in a locked and climate-controlled server room at the Malaria Alert Centre, Blantyre, Malawi. The server resides on its own private segment of the network, protected through a firewall, which routes traffic as well as provides active intrusion detection and protection. Data will be accessed through an HTTPS connection to the web application front end. Daily backups of the data are made to an alternate storage location at an off-site location to protect data in the event of a disaster that destroys the server room. A back-up copy of the Malawi REDCap study database will be maintained by MSU-BRIC, which is described in detail in the Project/Performance Site(s) and Resources section of the application. The backup of the Malawi REDCap study database will be purely file level backup and not generally available through a web interface. In the event that the service in unavailable in Malawi, BRIC will enable access to the backup copy and it will follow the same terms of service as the MSU- REDCap server. The MSU- REDCap server is housed in a facility which satisfies requirements for the storage of Health Insurance Portability and Accountability Act (HIPPA) data. The server is connected to a private segment of the BRIC network. The segment is protected through a firewall, which routes traffic and provides active intrusion detection and protection. Security staff patrols, key card access, and audio/video monitoring protect the

physical location of the server. Daily and monthly backups of the database are made and retained on a server for fast restore capabilities. Copies of the data are moved to tape daily and transported to a separate storage location. Tapes rotate after 28 days.

15.3 Types of Data

The final record for each study patient will comprise data collected during hospitalization for acute cerebral malaria and neurological assessment data collected at follow-up visits 1, 3, 6, and 12 months post-randomization. These data will include MRI images and patient clinical data collected during the course of care and intervention, and neurological and developmental assessments.

15.4 Timing/Reports

Data will be extracted for QC/QA on an annual basis (at a minimum of every 30 subjects). In addition to presenting key study outcome data, reports will consider internal data management of data quality, the timely use of data entry and cleaning, and the study enrollment schedule. The content and frequency of study quality and operational metric reports will be defined by the Project Manager. Study metric reports will be interpreted to identify the need for corrective action such as training or re-training, modification of the protocol, or modification of data collection material.

15.5 Study Records Retention

Study records in the format of paper and electronic versions are to be retained by the Malawi Data Management Unit (DMU) at the College of Medicine for a minimum of 2 years after the submission of the final report and close-out procedures. A longer retention period can be arranged in accordance with NIAID requirements. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the NIAID to inform the investigator when these study records no longer need to be retained. The retention of the original study records shall be the responsibility of the principal investigator.

REDCap is designed to export data easily in formats ready for analyses in a number of standard statistical software applications. For long term storage, data will be preserved in comma-separated-value file format. Data will be preserved in the REDCap database for approximately one-year following the cessation of active data collection. The PI will be provided with metadata and the final de-identified dataset. After obtaining the PI's release signature, live data will be removed the server, and backup files will be phased out systematically as tapes are retired. The PI will serve as the primary contact for access and questions regarding the data collected for the Study.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or protocol-specific Manual of Procedures (MOP) requirements. The noncompliance

may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with GCP:

4.5 Compliance with protocol, sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, section 5.1.1
5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site PI/study staff to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations will be promptly reported to DMID, via the TRI/ICON DMID-Clinical Research Operations and Management Support (CROMS) email (protocoldeviations@dmidcroms.com), web- (www.dmidctm.com) or fax-based system (1-215-699-6288). All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form (TRI/ICON DMID-CROMS or IDES form) will be maintained in the regulatory file, as well as in the subject's source document. All deviations will be reported to the local ISM quarterly, and to the IRBs at Michigan State University and the University of Malawi College of Medicine annually, unless advised otherwise by the local ISM.

16 PUBLICATION POLICY

Following completion of the study, the investigators will publish the results of this research in a scientific journal. In accordance with the International Committee of Medical Journal Editors (ICMJE) member journals, the trial will be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. It is the responsibility of DMID to register this trial in an acceptable registry. Financial support from NIAID will be acknowledged in all publications.

The primary manuscript will be developed by the PI in conjunction with other Key Personnel.

All publications emanating from this protocol will be submitted to the digital archive PubMed Central upon acceptance for publication, as required by the US National Institutes of Health Access Policy (Division G, Title II, Section 218 of PL 110-161 (Consolidated Appropriations Act, 2008)). PubMed Central reference numbers (PMCIDs) will be attached to citations of all manuscripts in all progress reports required for the study described with this protocol.

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18 PROTOCOL APPENDICES

Appendix A Schedule of Events
Appendix B Adverse Event Grading Table

Appendix A

Schedule of Events

Clinical and Laboratory Evaluations

A = performed on all children on PRW/PICU [(A) = only if still unconscious]

S = performed on children eligible to be screened for TBS study

I = performed as part of TBS trial [(I) = only if still unconscious]

Event	Admission	Screening	With TBS consent	Day 1	Day 2	Days 3 -7	Follow-up visits
History	A						A
Physical Exam	A			A,A	A,A	A,A	A
Vital signs	A			A – q2 hours	A – q2 hours	A – q2 hours	A
IV anti-malarial Rx	A			A,A	A,A		
PO anti-malarial Rx						A (3 day course)	
MP and PCV	A			A A A A	(A)(A) (A)(A)	(A)(A) (A)(A)	A
Lactate and glucose check	A			A A A A	(A)(A) (A)(A)	(A)(A) (A)(A)	
Lumbar Puncture	A						
Retinal exam		S		(I,)	(I,)	(I,)	
MRI		S		I	(I)	(I)	I (1 month)
NIRS		S		I	I	I	I (1 month)
TCD		?	I?	I	I	I	I (1 month)
ONSD			I	I,	(I,)	(I,)	I
Expanded neurologic exam			I	I, I	(I,I)	(I,I)	I
Heart rate variability			I	I	I	I	
Lung ultrasound			I	I	I	I	
Brain swelling intervention			I	I	I	I	

Appendix B - Adverse Event Table

Event	Grade 1	Grade 2	Grade 3	Grade 4
Hypernatremia	166-170 mEq/L	171-180 mEq/L	181-190 mEq/L	>191 mEq/L
Hyperkalemia	5.4-5.9mEq/L	6.0-6.4mEq/L	6.5-7.0mEq/L	> 7.0 mEq/L
Hypokalemia			2.0-2.4 mEq/L	< 2.0 mEq/L
Phlebitis	erythema ≥ 2 cm diameter at IV site without swelling, venous cord or fever	erythema and induration ≥ 2 cm diameter at IV site without venous cord or fever	erythema and induration ≥ 2 cm diameter at IV site with venous cord or fever ≥ 38°C	erythema and induration ≥ 2 cm diameter at IV site with venous cord and fever ≥ 38°C
Pneumothorax	NA	small pneumothorax with no cardiovascular effect -no intervention required	small pneumothorax requiring placement on 100% oxygen	pneumothorax with associated cardiovascular compromise requiring needle decompression or placement of chest tube
Pneumomediastinum	NA	simple pneumomediastinum-asymptomatic (No intervention required)	pneumomediastinum with extrathoracic subcutaneous emphysema (no intervention required)	pneumomediastinum with associated cardiovascular compromise requiring placement of drain or surgical intervention
Subglottic stenosis	Need for racemic epinephrine(X 1 dose)use post-extubation	Need for use of multiple doses of racemic epinephrine	Need for reintubation secondary to respiratory difficulty	Need for surgical resection of subglottic stenosis and/or tracheostomy secondary to sub glottic stenosis
Respiratory nosocomial infection	Post-admit pneumonitis on CXR and phys exam with O2 sat of ≥ 90% on room air	Post-admit pneumonitis on CXR and phys. Exam with O2 sat of < 90% on RA, O2 sat ≥ 90% on supplemental 02	Post-admit pneumonitis on CXR and phys. exam after admission with O2 sat < 90% on room air, O2 sat 70-90% with supplemental 02	Post-admit pneumonitis on CXR and phys exam with O2 sat of < 90% on room air, O2 sat < 70% with supplemental 02
Hypotension		Reduction of resting mean arterial blood pressure 25-49% of pre-intubation mean arterial pressure	Reduction of resting mean arterial blood pressure 50-74% of pre-intubation mean arterial pressure	Reduction of resting mean arterial blood pressure >75% of pre-intubation mean arterial pressure
Endotracheal tube obstruction	Binary (present or absent)			
Inadvertent endotracheal tube dislodgment				
Bradycardia induced by vagal response		Reduction of resting heart rate 25-49% of pre-intubation heart rate	Reduction of resting heart rate 50-74% of pre-intubation heart rate	Reduction of resting heart rate >75% of pre-intubation heart rate