Glycerol adjuvant therapy in adult bacterial meningitis in Malawi

Thesis submitted in accordance with the requirements of University of Liverpool for the degree of Doctor of Medicine

by

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Randomised trial of glycerol adjuvant therapy in adult bacterial meningitis in Malawi carried out between March 2006 and August 2008. The thesis is based on this study which was carried out at the Queen Elizabeth Central Hospital (QECH), in Blantyre, Malawi. The trial was funded by The Meningitis Research Foundation, UK.

The trial was termed Glycerol adjuvant therapy in adult bacterial meningitis in Malawi (GLAM).

ABSTRACT

Background: Southern Africa has a high incidence of bacterial meningitis in adults, often associated with HIV co-infection. Mortality exceeds 50%, even with appropriate antibiotic therapy, and is not improved with corticosteroids. Glycerol adjuvant therapy reduces long-term morbidity in bacterial meningitis in children, and its use is being promoted. We aimed to assess the effectiveness of glycerol as an adjuvant therapy for adults with bacterial meningitis in Africa. In addition, we aimed to measure the effect of glycerol on reducing intracranial pressure and deafness.

Methods: The study was done in two phases. First, in an open-label dose-finding study, 45 adult patients with symptoms, signs, and cerebrospinal fluid findings consistent with bacterial meningitis received 50, 75, or 100 ml of glycerol four times a day for 4 days. We then did a randomised, double-blind, placebo-controlled trial of oral glycerol in adults with bacterial meningitis. Patients with clinical and cerebrospinal fluid findings suggestive of bacterial meningitis were randomly assigned in blocks of 12 by use of a random number list produced by an independent statistician to receive either glycerol or an equivalent volume of sugar solution. Glycerol and placebo were indistinguishable by colour or taste. The primary outcome was mortality at 40 days, with secondary outcomes including disability and mortality restricted to pneumococcal disease; reduction in cerebrospinal fluid opening pressure and optic nerve sheath diameter measurement at Day 2 and hearing loss at Day 40. All patients were analysed for the primary outcome excluding those who were lost to follow-up. This trial is registered at controlled-trials.com, number ISRCTN70121840.

Findings: 75 mL glycerol four times a day was the highest tolerated dose, and was used for the main study. 265 patients were assigned treatment: 137 glycerol and 128 placebo. The trial was stopped early on the advice of the data and safety monitoring board after a planned interim analysis. By Day 40, 61 (49%) of 125 patients in the placebo group and 86 (63%) of 136 in the glycerol group had died (adjusted odds ratio 2·4, 95% CI 1·3–4·2, p=0·003). There was no benefit from glycerol for death and disability by Day 40, and glycerol did not improve death and disability by Day 40 or death at Day 40 in patients with proven bacterial disease or pneumococcal disease. Two serious adverse events occurred that were possibly due to the study drug. Optic nerve sheath diameter and CSF opening pressure were not reduced by glycerol. There was a trend towards less hearing loss at Day 40 in the glycerol arm five (5.6%) compared with placebo 16 (21.3%) (p=0.026).

Interpretation: Oral glycerol therapy cannot be recommended as an adjuvant therapy in adults with bacterial meningitis in resource-poor settings with a high HIV prevalence.

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GLOSSARY

%	Percent
µL	Microlitre
AIDS	Acquired Immunodeficiency Syndrome
ART	Anti-retroviral treatment
BBB	Blood-brain barrier
^{0}C	degrees Celsius
CI	Confidence interval
COMREC	College of Medicine Research and Ethics Committee
CSF	Cerebrospinal fluid
CT	Computed tomography
DSMB	Data and Safety Monitoring Board
GCS	Glasgow Coma Scale
GOS	Glasgow Outcome Score
GLAM	Glycerol in adult bacterial meningitis study
HAART	Highly active anti-retroviral therapy
HIV	Human Immunodeficiency Virus
IV	Intravenous
mg	Milligram
MLW	Malawi-Liverpool-Wellcome Trust
NAC	National AIDS Commission (Malawi)
NG	Naso-gastric
NPV	Negative predictive value
ONSD	Optic nerve sheath diameter
PATH	Program for Appropriate Technology in Health
PCR	Polymerase chain reaction
PI	Principal Investigator
PO	Per os (by mouth)
pOSM	Plasma osmolarity
PPV	Positive predictive value
QECH	Queen Elizabeth Central Hospital
TBM	Tuberculous meningitis
TSG	Trial Steering Group
UK	United Kingdom
UN	United Nations
USA	United States of America
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 Overview of Malawi, Blantyre and QECH

1.1.1 Malawi

Malawi is one of the most densely populated countries in sub-Saharan Africa with an estimated population of 12.3 million. A landlocked country, it is bordered by Zambia, Tanzania and Mozambique with most of its eastern border made up of Lake Malawi, the third largest lake in Africa. Malawi achieved independence from the United Kingdom in 1963. The major commercial centre is Blantyre and the capital city is Lilongwe with a population of around 400,000. Malawi relies heavily on agriculture for income and the main export crops are tobacco, tea and sugar. 85% of the population is estimated to work within the agricultural sector although the majority are subsistence farmers and the economy relies on support from the World Bank, International Monetary Fund and individual donor nations. It is ranked as one of the least developed nations and roughly 80% of total income is donor based. The overall GDP per capita was $201.80 in 2005 [1]. Malawi's climate is tropical and malaria is endemic. The dry season is between April and October.

According to World Health Statistics for 2006, life expectancy for women and men was 41 years. HIV prevalence is estimated at around 14% [2] but these rates are higher in urban areas. Both infant and maternal mortality rates are some of the highest in the world: maternal mortality rates 1100/100,000 live births [3] and infant mortality of

76/1000 [4]. Overall, there are considerable health and economic concerns within Malawi.

1.1.2 Blantyre

Blantyre, named after the birthplace in Scotland of Dr David Livingstone, is the main commercial, industrial and tourist centre of Malawi. Located in Southern region, it is the oldest and largest city in Malawi. The current population is estimated at 900,000 with significant economic migration from rural areas and other areas for employment opportunities.

Surrounding Blantyre, unplanned townships provide accommodation for a large proportion of the local population. Poverty, high unemployment, alcohol misuse and overcrowding contribute to the high rates of communicable diseases including HIV around Blantyre. Compared with the rest of the country, Blantyre district has the highest numbers of HIV infected adults with a prevalence of 19% and an estimated 150,000 HIV infected adults live in Blantyre [5].

1.1.3 Queen Elizabeth Central Hospital and the College of Medicine

Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi is a Government-run teaching hospital with a bed capacity of 1200. It offers free medical care to approximately 10,000 adult inpatients per year, of whom approximately 70% have HIV infection [6]. The department of Medicine admits approximately 4500 patients per month onto five wards which have two hundred beds. Clinicians have access to

limited laboratory and radiological investigations and, at the time of this study, frequently faced limitations with prescribing due to essential drug shortages. Medical staffing in QECH is provided by academic physicians from the College of Medicine (Ministry of Education) and from the Ministry of Health.

The College of Medicine, Blantyre was established in 1991 with the aim of providing training of medical students alongside the on-going research. Academic physicians provided teaching, clinical care for patients and carried out research. Within the QECH site, working alongside and often in collaboration with College of Medicine are the Malawi-Liverpool-Wellcome (MLW) Trust and Johns Hopkins Research Project.

1.2 Bacterial meningitis

1.2.1 Historical advances

Prior to the introduction of specific therapy, mortality from acute bacterial meningitis caused by pneumococcus and *Haemophilus influenzae* was almost universal and was up to 80% for meningococcus. In 1913, Flexner administered intrathecal equine meningococcal antiserum to 1300 patients and reduced mortality to 31%. Consequently, intrathecal antisera proved beneficial in *H. influenzae,* reducing mortality to 85%, but had little effect on improving mortality from pneumococcal disease [7].

With the introduction of sulphonamides and subsequently chloramphenicol in the 1930s and 1940s, mortality due to meningococcal and *H. influenzae* meningitis fell

to <10% [8]. Improvement in mortality from pneumococcal meningitis came following high dose penicillin therapy in the mid-1940s which resulted in mortality falling to 38% [9]. Bacterial meningitis was then considered potentially curable but with variable mortality and morbidity for individual pathogens and patient groups. The mortality from adult bacterial meningitis has remained relatively unchanged since the 1980s despite advances in supportive treatment, improved understanding of pathophysiology and development of new antibiotics. Pneumococcal meningitis still carries a mortality over 25% [10]. This highlights the need to search for alternative approaches to treatment to reduce mortality.

1.2.2 Incidence and mortality

Worldwide, bacterial meningitis is amongst the top ten causes of death related to infection [11]. In developed countries the incidence is estimated to be 2–5/100000 per year and may be ten times that in developing countries [12]. Between 1974 and 1998, the incidence of bacterial meningitis increased by 800% in Southern Malawi and this is mostly attributable to the HIV epidemic. One in forty of acute medical admissions to the adult medical wards in QECH are due to meningitis, half of these are likely to be bacterial [13]. Overall mortality from bacterial meningitis in Malawi is around 53% and is 61% for pneumococcal meningitis with 23% of survivors suffering neurological disability and 34% hearing loss [12, 14]. By comparison, overall mortality due to bacterial meningitis amongst adults in the West is 15% [15] with up to 50% of survivors suffering permanent neurological disability on recovery [16]. Such adverse outcomes may result directly from the infectious process or from other inflammatory

consequences such as cerebral oedema, increased intracranial pressure (ICP), altered cerebral blood flow, and neuronal injury [17].

1.2.3 Meningitis in Malawi

Cryptococcal meningitis is the most common type of meningitis in Malawi and is almost exclusively associated with HIV infection [13, 18]. Tuberculous meningitis (TBM) also causes a considerable burden and in this setting occurs predominantly in patients co-infected with HIV. These two causes of meningitis will not be discussed further. The most common bacterial pathogens seen in meningitis in sub-Saharan Africa are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis* and Gram-negative rods, particularly non-*typhi Salmonella spp.* Numerous factors have contributed to the shifting epidemiology of bacterial meningitis in recent years and although vaccination has impacted on the incidence of invasive pneumococcal disease in adults in the developed world [10, 19], in sub-Saharan Africa, where vaccines are least accessible, HIV is the single most important factor influencing the changing epidemiology of meningitis.

1.2.4 Corticosteroids in meningitis

Evidence for the benefit of corticosteroid adjuvant therapy has come from paediatric studies. No studies have demonstrated a clear improvement in mortality. A meta-analysis of eighteen studies involving 2750 cases of which 2074 were children, demonstrated a significant reduction in severe hearing loss in those receiving steroid. In sub-group analyses, this effect was restricted to children with haemophilus

meningitis [20]. The incidence of haemophilus meningitis has fallen since the introduction of Hib vaccine and therefore these conclusions should be considered carefully in the context of current epidemiology.

Reduced incidence of hearing loss [21] or the incidence of neurological complications [22] in children recovering from bacterial meningitis have been seen with steroids in two studies where *H. influenzae* type b caused the majority of infection. These results were not reproduced in another European trial carried out prior to the introduction of Hib vaccination [23], or in the paediatric trial in Malawi where haemophilus meningitis was relatively infrequent (28% of cases) [24].

Trials of steroid adjuvant therapy in adults have been less conclusive. Of eleven published trials identified, only four have been sufficiently powered to detect a mortality benefit. The main trial which has led to the introduction of steroid adjuvant therapy being recommended for suspected pneumococcal meningitis in Europe, is the prospective European trial by de Gans *et al* [15]. Dexamethasone significantly reduced mortality from 15% to 7% and unfavourable outcome (death or neurological disability) from 25% to 15%. Patients with pneumococcal disease achieved greatest benefit from dexamethasone. A further study on a subset of this cohort found no difference in long term neurological sequelae, including motor impairment, deafness and cognitive difficulties [25].

A study in Vietnam in 435 patients over age 14 years with suspected bacterial meningitis demonstrated that (by intention-to-treat analysis) dexamethasone had no statistically significant mortality advantage. However, patients receiving dexamethasone with definite bacterial meningitis had a statistically significant mortality advantage (relative risk of death at 1 month 0.43 (p=0.033)) whereas patients with probable meningitis had a (statistically non-significant) trend towards a higher mortality. Dexamethasone reduced the incidence of hearing loss amongst survivors [26]. A randomised controlled trial of steroid therapy in adults in Malawi [14] did not show any significant difference in mortality with corticosteroids either by intention-to-treat analysis or restricted to proven pneumococcal meningitis. There were no significant differences in hearing impairment in survivors. The overall conclusion from this trial is that dexamethasone adjuvant therapy for bacterial meningitis in adults from an area with a high prevalence of HIV did not reduce mortality or morbidity. Corticosteroids are not routinely used in Malawi for bacterial meningitis.

1.2.5 Antibiotic therapy in Malawi

Prior to 2006, bacterial meningitis in Malawi was treated according to standard guidelines with parenteral penicillin and chloramphenicol for a minimum of 10 days. During 2006, the national recommendation changed and ceftriaxone became first-line therapy. This was facilitated by the expiry of the patent for ceftriaxone and the general availability of good quality generic drug.

In the Malawian steroids in adult bacterial meningitis trial [14], 8% of pneumococcal isolates were resistant to penicillin and 46% resistant to chloramphenicol. Antibiotic resistance was detected on culture by the disc diffusion method (resistance defined by zone of inhibition of >3 mm). Dual resistance to penicillin and chloramphenicol was not seen in any pneumococcal isolate but resistance to ceftriaxone was detected in one (0.5%). No meningococcus or Gram-negative isolate was resistant to ceftriaxone.

Ceftriaxone (and cefotaxime) have a proven record of clinical efficacy in the treatment of bacterial meningitis when compared to penicillin and chloramphenicol [27-32]. Ceftriaxone can also be given once daily as effective treatment for adult bacterial meningitis [28, 29] which has advantages for both patient comfort and nursing time.

1.2.6 Effect of HIV on invasive pneumococcal disease

HIV increases the incidence of invasive pneumococcal disease. In a population-based prospective review from the United States, the incidence of invasive pneumococcal disease in HIV-infected patients was 803 per 100,000 person years compared to 35 per 100,000 person years in the general population [33]. A further retrospective review from USA demonstrated that recurrent pneumococcal bacteraemia was more likely in HIV infected patients: 23 out of 432 patients had recurrent episodes of pneumococcal bacteraemia and 87% of these episodes occurred

in HIV-infected patients [34]. It is therefore unsurprising that the prevalence of HIV in sub-Saharan Africa impacts on both the incidence and recurrence rate of invasive pneumococcal disease, thereby influencing the epidemiology of pneumococcal meningitis.

Since the start of the HIV epidemic, there has been an eightfold increase in the rates of hospital admission for meningitis in Malawi [13] and over 80% of adults with bacterial meningitis in this region are HIV co-infected [14, 35].

HIV infection is recognised as a major risk factor for pneumococcal meningitis in both adults [36] and in children [37] in Malawi. Pneumococcal meningitis is the most common and severe form of community acquired bacterial meningitis with the highest mortality rate and frequency of neurological sequelae [11]; the adverse impact of HIV infection only compounds this.

Despite this finding, it is unclear whether outcome from a single episode of invasive pneumococcal disease is affected by HIV status. A small European study of purulent meningitis (predominantly pneumococcal) in adults with HIV demonstrated a favourable outcome compared with HIV negative adults. This was however a small study with twelve episodes of purulent meningitis in nine HIV-infected patients and one death (mortality 8.3%) [38]. In contrast, in a paediatric population from Africa, outcome from bacterial meningitis was significantly worse and recurrent episodes

more frequent in HIV infected children compared to HIV uninfected children (mortality 65% in HIV positive compared with 36% in HIV negative) [37].

1.3 Impact of other interventions on invasive pneumococcal disease and bacterial meningitis

1.3.1 Antiretroviral therapy

In the USA, antiretroviral therapy (ART) has almost halved the incidence of invasive pneumococcal disease in patients with advanced HIV [39]. ART is not yet available to the majority of HIV infected individuals in Africa, although access to ART continues to improve particularly as a result of the Gates partnership. National scale-up of ART in Malawi commenced in June 2004, and (following completion of the glycerol in adult bacterial meningitis trial) in December 2008, 223,437 patients were registered for treatment [40]. Progressive roll-out of ART remains a priority.

1.3.2 Prophylactic antibiotics

No analysis on the impact of prophylactic antibiotics on pneumococcal meningitis has been reported. Daily co-trimoxazole prophylaxis reduced HIV related morbidity and bacteraemia in Cote d'Ivoire [41] although this did not specifically assess the impact on invasive pneumococcal disease. No benefit was demonstrated in a similar trial of co-trimoxazole primary prophylaxis among adults in Senegal [42].

A randomised clinical trial in children in Zambia demonstrated strong evidence that daily co-trimoxazole prophylaxis is effective in reducing both mortality and morbidity [43]. The main protective effect was seen in the reduction in severe pneumonia despite high levels (60–80%) of *in vitro* resistance of common bacterial infections to co-trimoxazole.

In adults, studies using historical controls and observational cohort studies across Africa have consistently demonstrated the effectiveness of co-trimoxazole prophylaxis in reducing mortality and morbidity [41, 43-50]. This prompted the development of WHO recommendations in 2006 for prophylactic co-trimoxazole for those with symptomatic HIV (WHO clinical stages 2–4) or based on CD4 counts (where available) [51]. There have been no studies to suggest any effect of prophylactic co-trimoxazole on the prevention of bacterial meningitis.

1.3.3 Vaccination

The goal of any vaccine programme is to prevent a potentially fatal disease. The majority of vaccines are developed within the West and may not be appropriate for resource poor settings due to vaccines being expensive, ineffective or not serotype appropriate [52] or have restricted availability due to manufacturing and distribution priorities. In addition, the development of successful vaccines in the West is liable to reduce the research interest into the diagnosis and management of bacterial meningitis in general. Vaccination can certainly change the epidemiology of

bacterial meningitis but is unlikely to be a health priority in Africa for some considerable time outside the setting of epidemic meningitis in the Sahel region.

There are around 90 known serotypes of pneumococcus, although a relatively limited number of these cause the most serious disease due to this organism. Antibody to the capsular polysaccharide provides only serotype-specific protection from disease. Pneumococcal vaccines, both conjugate and polysaccharide vaccines, show variable benefit in preventing invasive pneumococcal disease. In the developed world, the 23-valent polysaccharide pneumococcal vaccine has been shown to protect against bacteraemia and meningitis [53, 54]; however, in Uganda, significantly more pneumonia and a trend towards more invasive pneumococcal disease was seen in vaccinated HIV infected adults (not on ART) [55]. Mortality was not affected by vaccination in the Ugandan study. A subsequent study in Taiwan demonstrated a reduction in pneumococcal disease in HIV infected patients receiving 23-valent pneumococcal polysaccharide vaccine and a non-significant decrease in mortality [56].

Conjugate pneumococcal vaccines have been used in the USA since 2000 and have been included in UK childhood vaccine schedule since 2006. They have been highly effective in USA reducing invasive pneumococcal disease in children and demonstrating herd immunity effects in unvaccinated adults. The serotypes in this vaccine cover the principal circulating strains in North America which includes serotypes most often associated with reduced penicillin sensitivity [57]. Although apparently highly effective in America, this vaccine may not be appropriate for use

in Africa. At the time of this study, the 7-valent vaccine was available: this covered only 25% of invasive pneumococcal isolates in adults in sub-Saharan Africa at the time. 9-valent vaccine has been shown to cover 55% of invasive isolates from adults [52] and significant benefit was shown in children in South Africa vaccinated with a 9-valent conjugate vaccine designed for use in the USA [58]. A trial of the 7-valent conjugate vaccine in adults with a history of invasive pneumococcal disease has recently been completed in Malawi and shows significant benefit in terms of preventing recurrence of invasive pneumococcal disease amongst HIV infected patients not on ART [59]. Following on from this, the 13-valent pneumococcal conjugate vaccine was introduced as part of the national vaccination programme in Malawi from November 2011.

With the widespread use of conjugate vaccine, epidemiological changes are occurring as serotypes causing invasive pneumococcal disease are increasingly serotypes not covered in the vaccine [60]. This has prompted further vaccines to be developed as rapidly as possible even though there is no suggestion to date that this current vaccine will become redundant [61]. In 2007, WHO recommended pneumococcal (conjugate) vaccination in all, based on the high levels of disease burden in developing countries and proven efficacy and safety of the pneumococcal conjugate vaccines.

Meningococcal meningitis periodically reaches epidemic proportions, mainly in the classical meningitis belt, an area that stretches from Senegal in the West to Ethiopia

in the East [62, 63]. Effective vaccination is likely to reduce the occurrence or extent of epidemics but this is not straightforward. With the polysaccharide vaccine currently used during epidemics, repeated dosing is required in young children due to weak immunogenicity [64]. The more recently developed conjugate meningococcal vaccines result in a more durable antibody response in addition to blocking colonisation and producing herd immunity [65, 66], but the logistics for implementing mass vaccination is a challenge. Conjugate meningococcal A vaccine ("MenAfriVac"), was developed through a product development partnership between WHO and the Program for Appropriate Technology in Health (PATH). Multiple agencies including the Global Alliance for Vaccine and Immunisation are working with the WHO, the PATH and the Serum Institute of India alongside local governments and health services to achieve the goal of vaccinating at risk populations [67].

As with meningococcus, the incidence of meningitis due to *H. influenzae* does not appear to be affected by HIV co-infection and although *H. influenzae* causes a significant burden of disease in the paediatric population [37] incidence has fallen significantly in areas with the implementation of an effective vaccination programme [10, 68].

Meningitis caused by non-typhoid salmonella is strongly associated with HIV infection. Although uncommon in adults, it carries a case fatality rate of almost 80% [13].

1.4 Establishing the microbiological diagnosis

The ability to make a microbiological diagnosis in bacterial meningitis is variable in resource-poor settings. In the West, much effort is expended in identifying the causative organism so that appropriate therapy can be instituted early. Many institutions in sub-Saharan Africa have no culture facilities and clinicians must therefore rely heavily upon the history, the naked eye appearance of the cerebrospinal fluid (CSF) and, if available, microscopy. In some centres, including Blantyre, the use of urine dipsticks provided a semi-quantitative measure of CSF glucose and protein[69, 70], a valuable, rapid and relatively cheap contribution to aid in the diagnostic process. During the period of this study, the method of glucose and protein measurement in CSF changed to quantitative measurement. However, despite the availability of diagnostic tools such as these, significant difficulty in distinguishing different types of meningitis remains.

In Malawi, where cryptococcal meningitis is the most common CNS infection in adults [13], distinguishing TBM from cryptococcal meningitis remains a major challenge. Both are usually, but not always, associated with a lymphocytic pleocytosis and both tend to have a clinical history of more than 5 days [71, 72].

The sensitivity of the India Ink stain limits its usefulness [73] and cryptococcal antigen testing or culture is not possible in the majority of centres. Reliance is commonly placed on high-protein and low glucose (detected by urine dipsticks) to predict TBM rather than bacterial or cryptococcal meningitis. The sensitivity of urine dipsticks in

distinguishing cryptococcal meningitis from either TBM or from bacterial meningitis has not been formally addressed. However, a diagnostic scoring system has been developed in Vietnam which has shown reasonable sensitivity and specificity in distinguishing TBM from bacterial meningitis [74]. The scoring system relies upon five key features which are available at or soon after presentation of a patient with suspected meningitis: age, time to presentation, peripheral and CSF white cell count and percentage neutrophilia in the CSF. This scoring system has been validated in a population with a similarly low incidence of HIV infection to that seen in Vietnam [75]; however, when evaluated in the context of high HIV prevalence setting [14], the Vietnam diagnostic index did not perform well with low specificities for diagnosing either bacterial meningitis (52%) or TBM (43%).

Diagnostic strategies for TBM include direct Ziehl-Neelsen staining, CSF culture and molecular methods such as polymerase chain reaction (PCR). The former has a low sensitivity, culture is too slow to be useful in making therapeutic decisions and as with PCR, is often not available in resource-poor settings.

1.5 Osmotherapy for raised ICP

1.5.1 Raised ICP in meningitis

Raised ICP impairs cerebral bloodflow and may be a significant contributory factor to mortality and morbidity from all forms of meningitis including bacterial meningitis [76, 77]. The pathophysiology of raised ICP is complex and involves cytotoxins and interstitial oedema due to increased permeability of the blood–brain-

barrier with subsequent elevations in ICP; in addition to increased intracranial blood volume and disturbances in CSF flow [78].

Early reduction of the ICP should theoretically improve outcome. In their study, Lindvall *et al* [79], demonstrated a worse outcome with higher ICP. Fifteen patients with bacterial meningitis and reduced conscious level (Glasgow Coma Scale [GCS] <8/15) were selected for Intensive Care management, invasive monitoring of ICP and interventions (including thiopental) to reduce ICP to <20 mmHg. Overall mortality was 33% (five patients died). ICP was significantly higher in the five patients who died compared with survivors both initially and after 19 hours treatment; initial ICP was 46±8.6 mmHg in non-survivors versus 20.3±4.6 mmHg in survivors ($p<0.05$), and after treatment was 61.2±12.8 mmHg in non-survivors versus 19.4±1.35 mmHg in survivors ($p<0.001$). ICP reduced linearly with ICP-reducing therapy in survivors whereas no reduction in ICP was seen in those who died. The authors state an improvement in outcome with ICP-reducing therapy in bacterial meningitis however this was not a randomised study, there was no control arm and no explanation of expected ICP or mortality rate without intervention [79].

Osmotic therapy has been used extensively for raised intracranial pressure in acute brain injury due to trauma [80-82], investigated comprehensively for use in stroke [83-86] and tried (unsuccessfully) to improve outcome in cerebral malaria [87]. Osmotic agents used to control raised intracranial pressure includes mannitol [81], glycerol [88],

sorbitol [89], sodium lactate [90] and hypertonic saline [91]. All are administered parenterally but glycerol has the advantage of being able to be taken orally.

Glycerol, a hyperosmolar liquid widely used as a food additive, has an excellent safety profile, is low cost, easily administered and widely available. If effective, it would be an ideal adjuvant therapy, particularly in resource poor settings. Glycerol has been suggested as a potentially promising adjuvant treatment in bacterial meningitis in children. In three paediatric studies, glycerol reduced neurological sequelae but not death when given both alone and in combination with dexamethasone [92-94].

No trials of glycerol adjuvant therapy in bacterial meningitis have previously been reported in adults.

1.5.2 Glycerol

Glycerol (glycerine) is a clear, colourless, odourless, viscous liquid when pure, and has a warm, sweet taste. A naturally occurring liquid, it is primarily synthesized from propylene (obtained from petroleum) but can also be obtained as a by-product in the manufacture of soap from animal or vegetable oils and fats and is produced during the fermentation of sugars. Glycerol is cheap and widely available in sub-Saharan Africa.

Used in an extensive range of industries (including the food and beverage industry, medicine, cosmetics, cellophane and paper, explosives, lubricants, electrical equipment and metals), glycerol has been used as a food additive and for cosmetic purposes for many years and is widely used amongst sportsmen and sportswomen to improve performance and thermoregulation by hyper-hydration prior to endurance events [95]. Medicinally, it has been used as a laxative, in the treatment of acute glaucoma and in the diagnosis of Menière's disease. It is well absorbed orally and poorly absorbed across rectal mucosa.

Adverse effects of glycerol

Toxicity data for oral glycerol is limited but indicates that it is safe in the dosages used in this trial [96]. Side effects are infrequent, most commonly gastrointestinal in nature and generally mild. They include nausea, vomiting, diarrhoea, bloating and headache; with additional rare reports of confusion, dizziness, hyperglycaemia, polydipsia, hyperosmolar non-ketotic coma and dry mouth [88, 97]. There is one case report of reversible hemiparesis and seizures in a male following inadvertent ingestion of 500 ml pure glycerol [98] and a case of acute encephalopathy attributed to accidental glycerol overdose [99, 100]. A 72 year old male presented with confusion following a glycerol test for Menière's disease and glycerol overdose (of approximately 3.75 g/kg glycerol rather than 0.5–1.5 g/kg which is usually used in this context) was inferred from elevated triglyceride levels. The patient spontaneously recovered although he was amnesic of the event [99, 100].

With intravenous administration of glycerol, intravascular haemolysis, haemoglobinuria and renal failure can occur. This is not seen with oral administration of glycerol [101]. The effect of glycerol on blood glucose is controversial. Glycerol affects glucose metabolism which can theoretically lead to an increase in serum glucose levels [102-104]. However, a study in both healthy and well-controlled Type 2 diabetic patients did not show any clinically significant increases in blood glucose following oral glycerol in either group [105].

There have been a few reports of hyperosmolar non-ketotic coma reported in the context of glycerol therapy. One occurred in a diabetic with chronic renal failure when glycerol was given repeatedly for glaucoma [106] and another in two elderly patients treated with glycerol for cerebral oedema [107]. Following administration of parenteral glycerol, the sudden expansion of extracellular fluid may precipitate cardiac failure due to hypervolaemia in those with pre-existing cardiac disease; in renal insufficiency accumulation may lead to overexpansion of extracellular fluid and circulatory overload [108].

Pharmacology of glycerol

Few studies have been published on glycerol's pharmacokinetics and most pharmacodynamic information is from animal studies on lowering intracranial or ophthalmic pressure. The pharmacokinetics of glycerol in humans is still incompletely described; its mechanism of action is thought to be through an osmotic reduction of intracranial pressure.

Glycerol has a half-life of 0.2–1 hour; it is predominantly metabolised by the liver and undergoes renal filtration although the risk of accumulation in renal insufficiency is low. There is little evidence that glycerol causes an osmotic diuresis. There are no known significant interactions and limited data in pregnancy in either humans or animals [101, 109, 110].

After oral administration, glycerol absorption in the intestine is rapid; maximum serum concentrations of glycerol are reached after 15–20 min [100]. Following entry into cells, glycerol may enter cellular glycolysis or gluconeogenesis. Glycerol metabolism is mainly by three enzymes:

- glycerol kinase which converts glycerol to glycerol-3-phosphate
- glycerol-3-phosphate dehydrogenase which converts glycerol-3-phosphate into dihydroxyacetone phosphate
- triose phosphate isomerase which converts dihydroxyacetone phosphate into glyceraldehyde-3-phosphate.

Either glyceraldehyde-3-phosphate or dihydroxyacetone phosphate may be used as substrates for glycolysis or gluconeogenesis. The majority of glycerol is released from its storage within triglycerides during lipolysis. Glycerol metabolites are utilized for the synthesis of glucose, glycogen, and body fats [111].

The exact mechanism of action is unclear but glycerol is an osmotic dehydrating agent which increases osmotic pressure. Its laxative effect occurs by drawing fluid into the colon and thereby stimulating evacuation. Within the blood, glycerol is thought to exert much of its effect by increasing serum osmolarity. Glycerol has previously been used in stroke, head injury and glaucoma to reduce elevated tissue pressure [81, 82, 84, 86, 112-116].

In a small prospective study of oral glycerol therapy in both healthy individuals and those with Type 2 diabetes, plasma osmolarity (pOsm) increased significantly with maximal change seen 105 min following ingestion [105]. Peak change in pOSM was 8.6% for healthy individuals (receiving 3 ml/kg glycerol), and 8.5% and 4.2% for diabetic patients (receiving 3 ml/kg and 1.5 ml/kg respectively) supporting a dose-response relationship.

In a randomised double blind placebo controlled paediatric study of 36 children with bacterial meningitis, Singhi *et al* [117] investigated whether increase in serum osmolality is glycerol's mode of action in bacterial meningitis. While receiving study drug (glycerol, dexamethasone, both agents or neither agent), urine output, blood pressure and pOsm were monitored. Plasma osmolality increased in the glycerol group – the maximum level was seen at 6 hours and this was maintained up until at least 18 hours but not beyond 24 hours. Osmolality increased by a maximum of 3% (from 294±22 mOsm/kg to 302±31 mOsm/kg) in the glycerol only group, and by 1% (from 295±19 mOsm/kg to 298±21 mOsm/kg) in the glycerol-dexamethasone

group. The osmolality change was significant at 18 hours (p=0.01) but did not reach statistical significance at other time points. There was no evidence of osmotic diuresis and no change in blood pressure. The authors conclude that increased pOsm causing reduced CSF excretion and improved cerebral blood-flow with reduction in cerebral oedema is the possible mechanism for the beneficial effects of glycerol in this setting. Clinical success of therapy was a secondary endpoint not reported.

In addition to its osmotic action, there are several additional potential mechanisms of action of glycerol in the brain which have been suggested in the literature. Glycerol readily crosses the blood brain barrier (more readily than other osmotherapy agents) [118] where it could provide an alternative source of energy for brain tissue if glucose is lacking – glycerol can be converted to glucose and pyruvate and as a component of fat, glycerol is normally produced and metabolised in tissues. Parenteral glycerol has been shown to reduce the cerebral symptoms of hypoglycaemia in rabbits [119, 120].

Glycerol may increase regional cerebral blood flow and regional cerebral blood volume in ischaemic brain by reducing focal cerebral oedema facilitating redistribution of cerebral blood flow (however, no reduction of healthy brain volume was observed in magnetic resonance imaging studies) [121]. Such improvement in cerebral blood flow could occur by modulation of the leukocyte-endothelium interaction. This interaction plays a critical role in the pathogenesis of brain injury by ischaemia and reperfusion leading to secondary brain damage. Compared with saline, intravenous glycerol has been shown to significantly reduce adherent

leukocytes within microvasculature in rats. This modulation of adherence prevents leukocytes from interfering with the blood cell and plasma flow and is thought to be a factor in the neuroprotective effect of glycerol [122].

ICP rising above original pressure, or 'rebound phenomenon' is often cited as a potential adverse effect of glycerol (and other osmotic agents) but there is conflicting published data, mostly following head injury or in animals. Some have not found any evidence of rebound phenomenon [113, 123-125] and others dispute the existence of rebound phenomenon.

Kaufmann and Cardoso [126] demonstrated that repeated doses of mannitol lead to accumulation of mannitol within the extravascular compartment and the development of cerebral oedema as a consequence. This has subsequently been refuted [81, 127]. It would appear that glycerol and mannitol pass through a damaged blood-brain barrier (BBB) [125]; elimination from CSF is slower than from serum but because glycerol can be metabolised by neuronal cells (leading to a reduction in extravascular concentration) this may make it less likely to produce rebound phenomenon than mannitol. Despite this, it has been suggested that during the elimination phase of glycerol, CSF:serum osmotic gradient can be temporarily reversed causing a paradoxical rise of ICP. In stroke patients given 50 g glycerol by intravenous (IV) infusion, intracranial pressure fell by almost 50% and lasted just over an hour; but a small rebound in ICP occurred after 40 min. This was explained

by a temporary reversal of the serum to CSF fluid concentration gradient during glycerol elimination [128].

Berger *et al* [129] concluded that IV glycerol in stroke patients has a brief effect on serum osmolarity but a more sustained effect on raised ICP. However, they did not recommend its use in stroke because of lack of evidence of long-term benefit and concerns around accumulation of glycerol in brain tissue causing potential rebound effects following initial reduction in ICP.

In a retrospective study of 16 patients with brain injury, Troupp *et al* [124] concluded that glycerol could be used short-term (for a few days) but not long term to reduce intracranial pressure. Mannitol, glycerol and urea were given either alone or in combination; three received glycerol. One patient with frontal haematoma (which was surgically removed) had elevation of ICP to >50 mmHg following the first dose of glycerol but not the second dose. This was explained as possible rebound phenomenon but also possibly secondary to neurosurgery; however outcome was not reported in the other two patients.

Garcia-Sola *et al* [125] performed a comparative study of mannitol and glycerol in 18 goats and found no evidence of any rebound in ICP. ICP was measured with an epidural latex balloon immediately post-infusion and long-term (following repeated infusions). Glycerol reduced ICP less than mannitol but with no rebound phenomenon and less elevation of blood pressure.

In a clinical investigation reported by Wald *et al* [82], 15 patients with head injury were administered glycerol at a dose of 0.5–1.0 g every 3–4 hours depending on intracranial pressure and duration of drug effect (dose range 4–70 g, mean 54 g). ICP was reduced by over 50% in more than 70% of patients; no rebound elevation in ICP was reported. The study concluded that glycerol is both safe and effective as an adjunct to reduce ICP following trauma.

One case report describes rebound phenomenon following IV and oral glycerol and IV mannitol in a patient with glioblastoma multiforme [130]. Osmotic therapy was administered to reduce ICP. During IV glycerol infusion the ICP initially fell to almost normal levels but subsequently increased above the initial level despite elevated pOsm. Four hours following oral glycerol, CSF pressure increased to levels higher than originally. Administration of mannitol and recent neurosurgery may have influenced this result.

There remains conflicting data on the cause of any possible 'rebound phenomenon'. In animal and human studies where it has been observed, the postulated pathophysiology is an inversion of the osmotic gradient between plasma and brain at some point during osmotherapy [128, 131, 132].

1.5.3 Glycerol in stroke

There have been multiple clinical trials using glycerol for both haemorrhagic and ischaemic acute stroke (see Table 1). None have demonstrated significant benefit either by reducing mortality long-term or improving outcome in terms of neurological sequelae. A Cochrane review identified 10 randomised studies in acute ischaemic stroke in which a total of 482 glycerol-treated patients were compared with 463 control patients [88]. There was no evidence of an effect of glycerol on mortality at the end of planned follow-up. Functional outcome was only directly comparable in two trials; there was a non-significant reduction in odds of death or dependence (odds ratio (OR), 0.73; CI, 0.37–1.42). As many of these trials were performed prior to routine use of computed tomography (CT), most patients did not have radiological confirmation of the diagnosis of stroke. Overall, there was no reliable evidence of efficacy of glycerol in patients with cerebral oedema in acute stroke [88].

Table 1. Glycerol in acute cerebrovascular disease

Group	Type	No. of patients	Dose/pathway of glycerol	Results
Meyer et al (1971) [116]	Descriptive	36	IV/PO given within 72 h, for 4 days	Better mental status and motor strength. No clear long-term benefit.
Mathew et al (1972) [133]	Double-blind randomised	54	IV glycerol vs placebo for 4–6 days	Significant improvement in neurological examination. No long-term data. No improvement in mortality.

Larsson et al (1976) [134]	Randomised	27	IV glycerol vs IV dextrose, given within 6 h, for 6 days	No immediate (1–10 days) or late (3 months) benefit. No difference in mortality.
Bayer et al (1987) [86]	Double-blind randomised	173	IV glycerol vs placebo started within 48 h	Significant improvement in cognition, motor strength, and speech during early treatment. Significant improvement in early mortality. No differences in morbidity or mortality at 12 months.
Yu et al (1993) [84]	Double-blind randomised	113	IV glycerol vs. placebo	No significant differences (Barthel Index and Scandinavian Stroke Scale).

1.5.4 Glycerol in meningitis

Evidence for the benefit of glycerol adjuvant therapy in human bacterial meningitis is limited. There are three published studies to date on outcome in children where adjuvant glycerol therapy has been used [92-94]. In each, bacterial meningitis was treated with ceftriaxone and patients were randomised to receive oral glycerol, dexamethasone IV, both agents or placebo. Apart from the earliest study by Kilpi *et al* [92], a daily dose of glycerol 6 mg/kg daily divided into four doses was used. None of the studies demonstrated a clear mortality advantage. All three studies found a benefit in terms of reduction in hearing loss or neurological sequelae.

The first published trial using glycerol adjuvant therapy in bacterial meningitis was carried out by The Finnish Study Group in 122 children [92]. Glycerol therapy – at a lower dose than used in subsequent trials (4.5 g/kg in three divided doses) – prevented neurological abnormalities (0/61 vs 5/57; p=0.024). Oral glycerol reduced hearing loss when analysis was restricted to severe and profound bilateral hearing impairment (0% vs 7%; p=0.049). Overall, there was little difference between the treatment arms for hearing loss and neurological sequelae at follow-up; 7% (4/61) who received glycerol, and 19% (11/57) who did not receive glycerol, had neurological or hearing impairment (p=0.052). The relative risk of sequelae without glycerol was calculated as 2.94 (95% CIs, 0.99–8.72).

Subsequently a multi-centred, randomised, double blinded study in Latin America by Peltola *et al* [93] recruited 654 children with bacterial meningitis to four treatment arms - oral glycerol, IV dexamethasone, oral glycerol plus IV dexamethasone or oral placebo (carboxymethylcellulose). There were no significant mortality benefits. Fewer neurological sequelae were seen in those children receiving glycerol or glycerol plus dexamethasone (OR, 0.31 [95% CI, 0.13–0.76]; p<0.01 and 0.39 [95% CI, 0.17–0.93] p<0.03) respectively, compared with those patients who received placebo. Profound hearing loss occurred with similar regularity in all four groups. The authors concluded that glycerol prevents severe neurologic sequelae in childhood meningitis and advocated its use particularly in resource-poor settings. Of note, the majority of cases were caused by *H. influenzae* type b (221/654, 34%) with 20% (132) pneumococcal and 17% meningococcal. Not only is this is in contrast to

many areas where Hib vaccination programmes have significantly reduced the prevalence of *H. influenzae* type b but also led to study limitations which could have affected interpretation of results.

There is good evidence that dexamethasone adjuvant therapy reduces audiologic and/or neurologic sequelae in Hib meningitis [20, 135, 136]. The omission of corticosteroid adjuvant therapy raises ethical concerns in this setting with high Hib prevalence; it is therefore reasonable that one of the 10 participating centres opted out of the placebo-placebo arm. However, the published trial results amalgamated all data and did not provide individual centre data. Thus any difference that may have been present in the centre that followed a different treatment protocol was not able to be reviewed. In addition, use of pre-admission parenteral antibiotics was an exclusion criterion (although oral antibiotics were permitted); this may have affected culture results and may have added bias in that potentially more severe cases were excluded.

A small prospective randomised double blind study in India by Sankar *et al* [94] did not find any significant difference between the dexamethasone and glycerol groups. No significant difference was seen in neurological (p=0.29) or hearing (p=0.68) outcome with glycerol in children with acute bacterial meningitis treated with ceftriaxone [94]. Fifty-eight children were randomised to receive oral glycerol 1.5 g/kg 6 hourly, dexamethasone, both or placebo. An organism was isolated in half of all patients; 42% (10/24) pneumococcal, 29% (7/24) *H. influenzae* type b, 29% (7/24)

other organisms. Seven (12%) patients had neurological sequelae and 10 patients (17%) had hearing impairment.

A further study from India was designed to demonstrate glycerol's mechanism of action in bacterial meningitis in children [117]. Mortality was an unreported secondary outcome.

A meta-analysis of currently available data is in preparation [137]. Overall, glycerol may reduce deafness in meningitis but due to the small size of most trials, the evidence is low quality. No mortality benefit has been seen.

1.6 Measurement of intracranial pressure

1.6.1 Background

Raised ICP may be one of the factors contributing to mortality in bacterial meningitis. There are invasive and non-invasive methods to measure ICP both directly and indirectly and these include intracranial methods, lumbar puncture, transcranial Doppler sonography (mainly in children) and optic nerve sheath diameter measurement. Not all of these methods are readily available, particularly in resource poor settings.

ICP is most accurately measured directly with an intracranial device. Intraventricular pressure monitoring using direct ventricular manometry was developed in the 1960s and although ventricular catheterisation is still used, this

invasive technique carries risk of both haemorrhage and infection. Extra-ventricular devices were developed in the 1990s and include minimally invasive electronic or fiberoptic microsensor devices which are placed in brain parenchyma. Although widely used throughout the world in neurosurgical units, such methods are not readily available out-with specialised and well resourced centres [138].

Although not strictly a direct measure of ICP, lumbar puncture with CSF opening pressure correlates well with lumbar epidural pressure and ICP. However, lumbar puncture cannot be repeated frequently and can be contraindicated, for example with an intracranial mass lesion. Readings can vary with position and orthostatic changes have complex interactions with intracranial pressure. When seated, the difference in intracranial pressure and lumbar CSF pressure is equivalent to the height of the hydrostatic column. When a person is tilted vertically head down, ICP rises three times higher compared with the magnitude of reduction that occurs when they are tilted head-up [95]. Hydrostatic pressure, elasticity of the lumbar thecal sac and venous compression along the neuraxis all contribute to these changes in ICP. In addition to postural effects, regional cerebral blood-flow, fall in PaO2, rise in PaCO2 and body temperature are all stimuli which can increase ICP [139]. Some of these parameters have been manipulated therapeutically with variable success to try to reduce elevated ICP [140]. Depending on the underlying aetiology, correcting the patient's position, lowering core temperature, hyperventilation to induce hypocapnoea, use of hyper-osmolar agents (mannitol) and drainage of CSF have all been used in intensive therapy settings to treat intracranial hypertension.

Normal CSF opening pressure is generally accepted as 5–20 cm CSF with an upper limit of normal 18–20 cm [141]. Body mass index (BMI) does not appear to affect this significantly. A study by Whitely *et al* [142] in 242 adults undergoing their first lumbar puncture found increased BMI was only weakly associated with an increase in ICP but that this was not sufficiently different to be relevant to clinical practice. Their population was overweight with a median BMI 26 kg/m^2 (normal BMI = 20–25 kg/m^2) with CSF opening pressures of 12–25cm CSF were normal.

Computed tomography can allow raised ICP to be inferred from radiological signs but this has limitations and such imaging is not routinely available in resource-poor settings.

1.6.2 Optic nerve sheath diameter measurement

More recently, optic nerve sheath diameter (ONSD) measurement by ultrasound has been used to evaluate ICP. This simple, non-invasive technique can be repeated as often as required to evaluate ICP. Several studies suggest that ultrasound measurement of ONSD provides accurate, reproducible information in the presence of elevated ICP (Table 2). Only basic ultrasonography skills are required and it can be done with standard paediatric ultrasound equipment. Other advantages to this non-invasive technique include no radiation exposure; being accessible at the bedside and therefore able to be taken to critically ill patients (rather than moving them); and ability to be easily repeated to re-evaluate a patient.

Table 2. Ultrasound measurement of optic nerve sheath diameter

Authors	Patient group	Optic nerve sheath measurements	Sensitivity and specificity	Study weaknesses
Paediatric studies				
Beare et al 2008, Malawi [143]	Case control study: African children with acute neurological disease; control group of children with no neurological disease.	Raised ICP: ≥4.5 mm indicative of raised ICP Mean 5.4 mm (4.3–6.2) Controls : 3.5 mm (2.5–4.1)	Sensitivity 100% and specificity 86% (if 4.2 mm ULN)	Small study (n=51; 21 cases)
Newman et al 2002, UK [144]	Case control study: children with hydrocephalus; control group with no neurological disease	Raised ICP: ULN 4 mm in <1 year ULN 4.5 mm in ≥1 year Mean 5.6±0.6 mm Controls: Mean 2.9 mm (2.2–3.4) in <1 year Mean 3.1 mm (2.3–4) ≥1 year		Small study (n=125; 23 cases)

Authors	Patient group	Optic nerve sheath measurements	Sensitivity and specificity	Study weaknesses
Malayeri et al 2005, Iran [145]	Case control study	Raised ICP: 5.6±0.6 mm (4.55–7.6) Controls 2–4.3 mm		
Ballantyne et al 1999, UK [146]	Prospective study establish normal values for ONSD up to 15 years of age	Raised ICP: >4 mm in < 1 year > 4.5 mm in > 1 year Normal: 2.1–4.3 mm, mean 3.08 (SD 0.36).		
Adult studies				
Kimberley et al 2008,USA [147]	Prospective observational study to validate ONSD >5 mm indicating raised ICP: Adults with invasive ICP monitoring (as part of care). ONSD by ultrasound compared with direct ICP measurement	Raised ICP: >5 mm	ONSD >5 mm had sensitivity of 88% and specificity of 93% for diagnosis of ICP >20 cm H20	Small sample size (n=15). Convenience sample so possible selection bias.
Tayal et al 2007,USA [148]	Observational study: Adult patients with suspected raised ICP after head injury. ONSD cf CT findings suggestive of raised ICP	Raised ICP: >5 mm	Sensitivity of 100% and Specificity of 63% for detecting signs of raised ICP on CT	CT poor predictor of raised ICP

Authors	Patient group	Optic nerve sheath measurements	Sensitivity and specificity	Study weaknesses
Moretti et al 2009, Italy [149]	Adults with intracranial haemorrhage and invasive ICP monitoring as part of care	Raised ICP: >5.2 mm	93.1% sensitivity and 73.85% specificity for ONSD 5.2 mm to predict ICP >20 mmHg	
Blaivas et al, 2003,USA [150]	Observational Study: Adults thought to have elevated ICP possible focal intracranial pathology. ONSD cf CT findings suggestive of raised ICP	Raised ICP: >5 mm predicted all Mean 6.27 mm (5.6–6.89 95% CI)	Sensitivity of 100% and specificity of 95% for detecting signs of raised ICP on CT PPV 93%, NPV 100%	Small sample size (n=35). Convenience sample so possible selection bias. CT poor predictor of raised ICP
Geeraerts et al 2008, France [151]	Observational study: Adults with traumatic brain injury requiring ICP monitoring. ONSD by ultrasound cf initial invasive ICP reading	Raised ICP: >5 mm	100% sensitivity and negative predictive value (of ONSD >5 mm detecting ICP >20 cm H20)	Small sample size (n=34). Convenience sample so possible selection bias.
Goel et al 2008,India [152]	Adults with head injury who could undergo CT brain	Raised ICP (on CT): 5.8±0.57 mm No CT evidence of raised ICP: 3.5±0.75 mm	Sensitivity (of detecting radiologically proven) elevated ICP 98.6%, specificity 92.8%, PPV 97.26%, NPV 96.3%.	CT poor indicator of raised ICP. Selection bias

Authors	Patient group	Optic nerve sheath measurements	Sensitivity and specificity	Study weaknesses
Girisgin et al 2007,Turkey [153]	Case control study: Adults with elevated ICP from any cause diagnosed by emergency CT; control group of healthy volunteers. ONSD by ultrasound compared with CT findings in both groups	Raised ICP (on CT): 6.4±0.7 mm 4.6±0.3 mm in control group ($p<0.001$)		Small study. CT diagnosis prior to ONSD measurement (unblinded). Potential bias: those with wide ONSD measurements but normal CT scans excluded from study
Soldatos et al 2008, Greece [154]	Case control study: Adults with brain injury; control group without intracranial pathology. ONSD by ultrasound compared with transcranial Doppler sonography and internal ICP measurement (in brain injury patients)	Raised ICP: 5.7 mm in those with raised ICP 3.6±0.6 mm in control group	74.1% sensitivity and 100% specificity with cut-off 5.7 mm	
Hansen et al 1997,Germany [155]	Observational Study: Adult patients undergoing neurological testing with intrathecal puncture. ONSD by ultrasound compared with subsequent intrathecal CSF pressure	Raised ICP: >5 cm	Mean linear regression correlation of 0.78 between ONSD and CSF pressures across all subjects	Small study. Raised ICP due to intrathecal infusion ?relevance to traumatic brain injury

The optic nerve sheath is continuous with the brain's dura mater. CSF pressure variations are transmitted to the sub-arachnoid space surrounding the optic nerve and influence the ONSD. Increased ICP causes expansion of the optic nerve sheath 3 mm posterior to the papilla which can be measured using a standard paediatric ultrasound probe in the transverse plane (Figure 1).

Sonographic optic nerve sheath diameter measurement has been used successfully in emergency rooms [148, 150], children with hydrocephalus [144], with other causes of raised intracranial pressure [145] and in one paediatric study in a resource-poor setting [143]. There is well documented intra-observer and inter-observer reproducibility of optic nerve sonography in the literature [154, 156-158].

Figure 1. The technique of ocular ultrasound and the image

The cursors are set at 3 mm behind the globe to measure the ONSD

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Newman WD et al. Br J Opthalmol 2002;86:1109-1113[144]

Newman *et al* [144] found that the mean ONS enlarged with age, but most of this increase occurred in the first year of life with less increase thereafter to age 16 years. Table 2 summarises the findings of the main published studies using ultrasound to measure ONSD. Overall, the literature supports an optic nerve sheath diameter >5 mm in adults as indicating elevated ICP.

Other conditions which can also cause ONS enlargement include tumours (ONS meningioma, optic nerve glioma, neurofibroma, metastases, leukaemia and lymphoma); neuritis; sarcoid; trauma including haemorrhage in and around the optic nerve; thyroid compressive neuropathy (in Grave's disease) and idiopathic inflammation [159].

1.6.3 Summary

In bacterial meningitis, raised intracranial pressure is usually detected by CT brain, an expensive, time-consuming investigation not readily available in resource-poor settings; opthalmoscopy for papilloedema, which is difficult to detect in early stages even by experienced examiners and has a low sensitivity; and lumbar puncture with opening pressure which cannot be repeatedly performed. Ultrasound measurement of the ONSD is a simple, non-invasive technique which provides reproducible measurements to indicate if there is elevation of ICP. Expansion of the ONS occurs before the appearance of papilloedema and therefore ONSD measurement is able to diagnose raised ICP earlier than ophthalmoscopy [145, 160]. Within our trial, we aimed to determine whether ONSD was affected by glycerol therapy and if it correlated

with CSF opening pressure at lumbar puncture. In addition, we aimed to demonstrate whether it can be used as a prognostic tool in this context.

1.7 Hearing and disability in bacterial meningitis

Hearing loss is the most common sequela of bacterial meningitis in children. It occurs in up to 35% of survivors and is more common with pneumococcal than meningococcal infection [161-163].

In the trial of adjuvant steroid therapy in adults with bacterial meningitis in Malawi, 36% (36/99) of surviving adults were found to have deafness and this increased to 43% (28/65) of those with proven pneumococcal meningitis [14]. The exact pathophysiology is likely due to a combination of direct labyrinth involvement, cochlear neuroepithelial damage, and vascular insult [164]. Hearing loss typically occurs early within the course of the illness, usually within the first 2 days [165, 166]. This is supported by animal studies where rabbits show signs of hearing loss within 12 hours of intrathecal injection with *Streptococcus* [167]. Hearing is regained if appropriate antibiotics are administered within 12 hours whereas untreated rabbits remain deaf.

Predictors of hearing loss in children include length of hospitalisation, development of seizures, cranial nerve neuropathies, elevated CSF protein, and decreased CSF glucose [168]. In the Latin American study, reduction in GCS at

presentation and young age were also found to be predictive [169]. There is no similar literature for adults.

The presence of fits may not be a direct marker of disease severity because the cause for seizures in bacterial meningitis may be multifactorial and includes cerebral irritation, raised intracranial pressure, metabolic disturbances and high fever (particularly in children). Cranial nerve neuropathy is however a sign of a severe infection and is highly correlated with the development of hearing loss [168].

Since the 1970s, glycerol has been suggested as a potential adjuvant therapy for the prevention of sequelae in meningitis. Herson and Todd [170] suggested it might be of benefit in Hib meningitis and the other paediatric studies were subsequently completed [92, 169].

1.8 Summary

Meningitis is one of the most common causes of adult inpatient deaths in high HIV-prevalent areas [13]. Even with effective antibiotics, in-hospital mortality from bacterial meningitis is over 50% in regions where HIV is common. Steroids, which have been found to be effective adjuvant treatment in low-HIV settings, have been found to be ineffective where most meningitis is HIV-related [14, 36]. Bacterial meningitis in adults in Malawi is most commonly due to *S. pneumoniae* and the majority of patients are co-infected with HIV. Since the start of the HIV epidemic, the incidence of bacterial meningitis in Malawi has risen markedly and mortality

from pneumococcal meningitis in this region exceeds 50%, with up to half of survivors being left with neurological sequelae such as deafness, intellectual impairment or physical disability [15, 36].

In summary, bacterial meningitis continues to be a significant health problem in developing countries. There are many reasons why mortality is high in this setting and several strategies have been considered in order to address this issue. Increasing awareness of the symptoms and epidemiology of bacterial meningitis amongst both health workers and the public, and the promotion of early hospital referral, early diagnosis and appropriate antibiotic therapy are likely to improve prognosis. Improved access to ART, vaccine development and deployment, and the use of primary antibiotic prophylaxis may reduce the incidence of bacterial meningitis.

In order for a new intervention to be employed in the developing world, it must be cheap, sustainable, easily administered and safe. Glycerol is all of these and is widely available making it a potentially highly appropriate therapy for resource poor settings. Data from paediatric studies suggest an advantage from adjuvant glycerol therapy, particularly with outcome in terms of neurological sequelae and hearing loss. Theoretically, the osmotic effect of glycerol on reducing raised ICP might confer some benefit in terms of mortality. There are no studies of adjuvant glycerol therapy in adults with bacterial meningitis. This led to us designing a study to initially assess tolerability of oral glycerol and then whether oral glycerol would improve outcome in adults with bacterial meningitis in Malawi.

CHAPTER 2: METHODS

2.1 Background

Resource poor settings such as those in sub-Saharan Africa have the highest incidence of bacterial meningitis and mortality rate – currently, mortality exceeds 50% in Malawi. More effective locally appropriate treatments are an urgent priority. The most common organism isolated in bacterial meningitis is *S. pneumoniae* [171]. Despite advances in supportive care, the availability of novel antibiotics, and our understanding of the pathophysiology of the disease, mortality from pneumococcal meningitis in adults has not improved significantly over the last half of the previous century [172]. Glycerol has been shown to reduce neurological sequelae in children with bacterial meningitis in Latin America. If similarly effective in adults, glycerol would represent a cheap, locally available adjuvant therapy appropriate for use in this setting. It has been used as a food additive and for medicinal and cosmetic purposes for many years. We set out to investigate the use of glycerol to improve outcome.

This study provides the first information on the efficacy of glycerol in meningitis in adults. The data informs investigators of the potential benefits, constraints and side effects of glycerol adjuvant therapy. It was anticipated that the results from this study may precede a larger multicentre trial in four tropical research centres aimed at

investigating the use of glycerol in bacterial, tuberculous and cryptococcal meningitis.

This chapter provides a comprehensive overview of methods used to carry out the study. It describes the aims of the study and the methodology required to obtain these aims. The original study proposal, data proforma, consent forms and patient information sheets are in Appendix 1–7.

2.2 Study design and objectives

This was a prospective double-blind placebo controlled randomised trial recruiting patients admitted to the QECH, Blantyre, Malawi **(See Chapter 1 for the description of QECH).** It was conducted in two phases.

Phase 1: open label dose-finding and tolerability study from March to July 2006.

Phase 2: Double-blind placebo controlled clinical trial commenced September 2006 and recruitment finished August 2008.

The main trial objectives were to identify if glycerol is effective at reducing mortality and improving outcome in adult Malawian patients with bacterial meningitis.

There were three specific objectives:

1. To investigate the feasibility and tolerability of glycerol as an adjuvant therapy in adult bacterial meningitis.
2. To assess the efficacy of glycerol adjuvant therapy in reducing mortality from adult bacterial meningitis in Malawi at Day 10 and Day 40.
3. To assess the degree of neurological deficit in survivors at Day 10 and Day 40.

The study had ethical approval prior to starting recruitment from the College of Medicine Research and Ethics Committee, Malawi in December 2005 and from Liverpool School of Tropical Medicine, Research Ethics Committee, UK in February 2006. The trial design was approved by the data and safety monitoring board (DSMB) and the Trial Steering Group (TSG) prior to the start of recruitment.

2.3 Participants

All medical patients with suspected bacterial meningitis routinely had a lumbar puncture with opening pressure measurement as part of their admission investigations. The decision to perform a lumbar puncture was not part of the trial algorithm. All such patients were referred to the glycerol trial staff and assessed for eligibility to enter the trial. Those eligible for enrolment were identified from the macroscopic appearance of their CSF or from CSF microscopy results. Due to delays in receiving initial laboratory results, all patients suspected of having meningitis with

hazy/cloudy CSF were recruited before CSF microscopy results were known. Individuals under follow-up within other research projects were eligible for inclusion. Most patients were recruited from the medical admissions ward; others were located elsewhere in the hospital once their cerebrospinal fluid results had been obtained by the study team from the laboratory.

Records were kept of all patients who were potential recruits to GLAM. This included those who refused consent and those who were not recruited for other reasons – died before recruitment, missed by the trial team, discharged home before CSF results known, pregnant women.

2.4 Inclusion criteria and consent for Phase 1 and 2

Inclusion criteria were clinical suspicion of meningitis plus CSF evidence of bacterial meningitis or cloudy CSF if microscopy was not immediately available i.e. within 30 min (See Table 3). Exclusion criteria were age, cryptococcal meningitis; pregnancy, heart failure, known Type 2 diabetes or capillary whole blood glucose (BM) >12 mmol/L. Patients were excluded if their CSF contained <100 white cells/μl or lymphocytic meningitis; they were <16 years old, pregnant, had a diagnosis of Type 2 diabetes (due to the possibility of precipitating hyperosmolar non-ketotic coma) or heart failure (due to the possibility of exacerbating congestive cardiac failure); or had CSF results indicating infection by cryptococcus (India ink or cryptococcal antigen positive on CSF) or mycobacteria (lymphocytic CSF). Treatment with antibiotics prior to recruitment was not a criterion for exclusion.

Table 3. Inclusion and exclusion criteria

Inclusion criteria	
Adults	Age ≥16 years old
Clinical suspicion of meningitis (any 1 of)	Headache Neck pain or stiffness Reduced conscious level Photophobia Confusion Fits Rash Fever
CSF evidence of bacterial meningitis	Cloudy CSF* >100 white cells/μl with predominant neutrophils Gram-stain showing bacteria
Informed consent given	By patients or their guardians
Patient/guardian willing to follow study protocol	
Exclusion criteria	
Pregnancy Type 2 diabetics BM >12 mmol/L Heart failure CSF results indicating infection with cryptococcus or mycobacteria†	

*if microscopy was delayed >30 min
†lymphocytic meningitis

Individuals were given or read an information sheet (Appendix 6 and 7) in Chichewa (the local language) or English and given the opportunity to ask questions. If the individual wished to enrol in the study, the study clinician interviewed them in order to ensure they understood the nature of the study and the information provided. In particular, potential recruits needed to understand the nature of a placebo-controlled trial. Verbal and written informed consent was obtained from the patient or their legal guardian a member of the trial staff or, overnight, from a specialised blood culture nurse (working on an invasive pneumococcal disease study) who were trained in obtaining informed consent. Wherever possible, consent from the patient

was obtained in the presence of their guardian. Where patients were deemed to be incapable of giving valid informed consent next-of-kin or guardians were asked to give consent on their behalf. Those unable to read or write gave witnessed verbal consent and a thumbprint as proof of consent. Once satisfied, the study clinician witnessed the completion of the consent to participate form and countersigned it (Appendix 4 and 5).

All trial staff were trained to obtain informed consent and were fully aware that properly acquired informed consent is central to the performance of ethically sound and scientifically credible clinical trials.

Refusal did not adversely affect patients' care, and these patients received antibiotic therapy according to Malawi guidelines. The purpose of the study was fully explained to patients; it was made clear that participation was entirely voluntary and that if the patient did not wish to participate, this would not influence management of their condition and they would be offered the best possible diagnostic and treatment options. Patients could withdraw from the study at any point in time. Patients were informed that they were free to ask for the results of the study when available.

2.5 Phase 1: Dose finding and tolerability study

Forty five adult patients with symptoms, signs and CSF findings compatible with bacterial meningitis were recruited for assessment of glycerol tolerability. Following

consent, fifteen patients each received glycerol at a dose of 50, 75 or 100 ml four times a day for four days. Previous paediatric studies had used 6 ml/kg/day [94] and in the average Malawian this would be achieved with a total daily dose of 300–360 ml glycerol. Glycerol was diluted with water at a ratio of 5:4 so that the consistency was indistinguishable from that of 50% sugar solution (the proposed placebo for Phase 2). Patients therefore received diluted glycerol 90, 135 or 180 ml QDS.

Clinical details of all cases, including possible or probable adverse events due to glycerol administration, were recorded. Patients were followed up to Day 10 or until discharged home.

2.6 Glycerol and placebo: presentation

Glycerol BP 99% (manufactured by Organic Chemical Corporation (Pty) Ltd, Durban, South Africa) and Malawian sugar was purchased locally. Glycerol (1mg/ml) was diluted with water in a ratio 5:4 to a consistency indistinguishable from the placebo. Placebo was sugar solution made of sugar and water (1 kg in 1000 ml water).

It was anticipated that both fluids would appear as a slightly viscous, colourless liquid but the sugar solution was a pale yellow colour. Addition of 125 ml of Orange SOBO® (a locally produced orange squash) into each bottle of glycerol and 100 ml into each bottle of 50% sugar solution ensured both liquids were the same in appearance. Both liquids were a pale orange colour and were more palatable and

indistinguishable as assessed by blinded medical and clerical staff within the Department of Medicine, QECH.

The glycerol solution and placebo were made in batches in order to keep up with demand. 2430 ml diluted glycerol and equal volumes of placebo were placed in bottles which were kept separate until labelled with the GLAM study number. This provided two extra doses per bottle which could be used if the patient vomited shortly after ingestion when the dose was repeated. 125 ml SOBO® was added to each bottle of glycerol and 100 ml to each bottle of placebo. This final solution given to patients contained 528.3 mg/ml glycerol (125 ml SOBO® + 1350 mg glycerol + 1080 ml water); each 135 ml solution therefore contained 71.3 mg glycerol.

2.7 Randomisation and blinding

Randomisation was performed by an independent statistician using Stata version 9.0 to randomise in blocks of size 12. Three independent scientists with no involvement with GLAM were nominated to label bottles as they were made. Treatment allocation was in opaque sealed envelopes which were opened sequentially by an independent person not directly involved in the trial who then labelled pre-prepared containers of glycerol and placebo with the unique Study number. Bottles were either labelled or disposed of. No other person had access to the randomisation code and all envelopes were accounted for. Study drug was then placed in consecutive order within a secure room. Clinicians and patients were blind to study allocation.

Study drug was stored in a cool dark office prior to being transferred to a small locked fridge on the ward for dispensing to patients. Once a patient fulfilled the study criteria and informed consent was obtained, the patient's name was entered into the GLAM Trial Register and the next available Trial number was allocated to them. Allocation of the next available study number constituted entering the trial and the intention-to-treat analysis was based on study allocation irrespective of subsequent treatment. The GLAM trial number was written onto each page of the proforma. A master register of all patients recruited to the trial and their allocated GLAM study number was maintained within the GLAM Trial office.

The appropriate bottle with that GLAM number on (with a dispensing cup marked at the correct level) was taken to the patient. Study drug was then given to the patient to drink – in Phase 1 patients were randomised to 90, 135 or 180 ml; in Phase 2 all patients received 135 ml four times a day. Patients who were unable to swallow had a naso-gastric (NG) tube inserted to give the study drug. Treatment with glycerol or placebo was administered by trial staff.

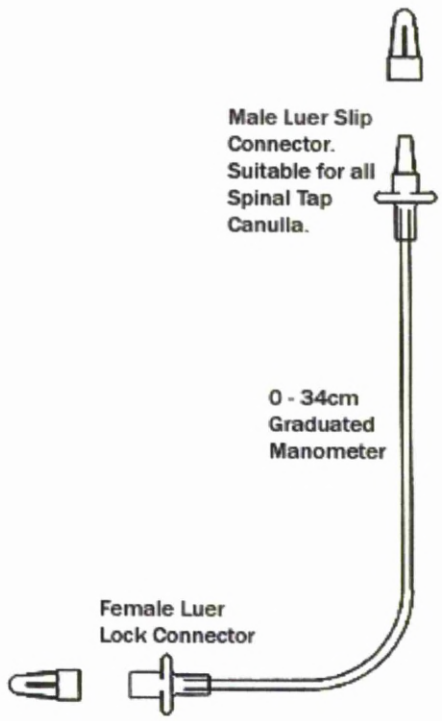
2.8 Identification of participants

Trial staff were informed of any patient suspected of having bacterial meningitis who was admitted to the medical admission ward. Each patient underwent a lumbar puncture with opening pressure prior to recruitment to GLAM. Opening pressure was measured using disposable gamma sterilised flexible calibrated spinal

manometers (0–34cm calibrations) purchased from Baldwin Medical (www.baldwinmedical.com.au) (Figure 2).

2 g IV ceftriaxone was administered to any patient suspected of having bacterial meningitis following lumbar puncture. Any adult patient with cloudy CSF was considered eligible for enrolment to the study. All CSF was taken the laboratory for analysis and once results of microscopy were known, additional patients were then recruited to the study if they fulfilled the inclusion criteria.

Figure 2. Spinal manometer



The laboratory was open during the day and therefore patients admitted overnight had their samples processed the following day. Patients recruited to the study with cloudy CSF who were subsequently found to have CSF microscopy suggesting non-bacterial meningitis had their study drug withdrawn and their treatment modified as necessary. All such patients continued to be followed up as if they were part of the trial (intention-to-treat).

2.9 Enrolment

Following consent, each patient or, in the event of the patient being unconscious, their legal guardian underwent an interview by a clinical officer or trial nurse to document baseline demographic characteristics, current medical problems, duration of symptoms, past medical history including AIDS-defining conditions and neurological sequelae, social history and a full drug history. Each patient was examined by a trial clinical officer or the principal investigator (PI). All data was documented in a study proforma (Appendix 3).

Malawi has no residential address system and therefore keeping track of study participants has always been challenging. Individuals may return to their home villages for long intervals during planting or harvesting seasons. At recruitment, a map was drawn within the proforma to record where participants lived in order to locate them for follow-up if the need arose.

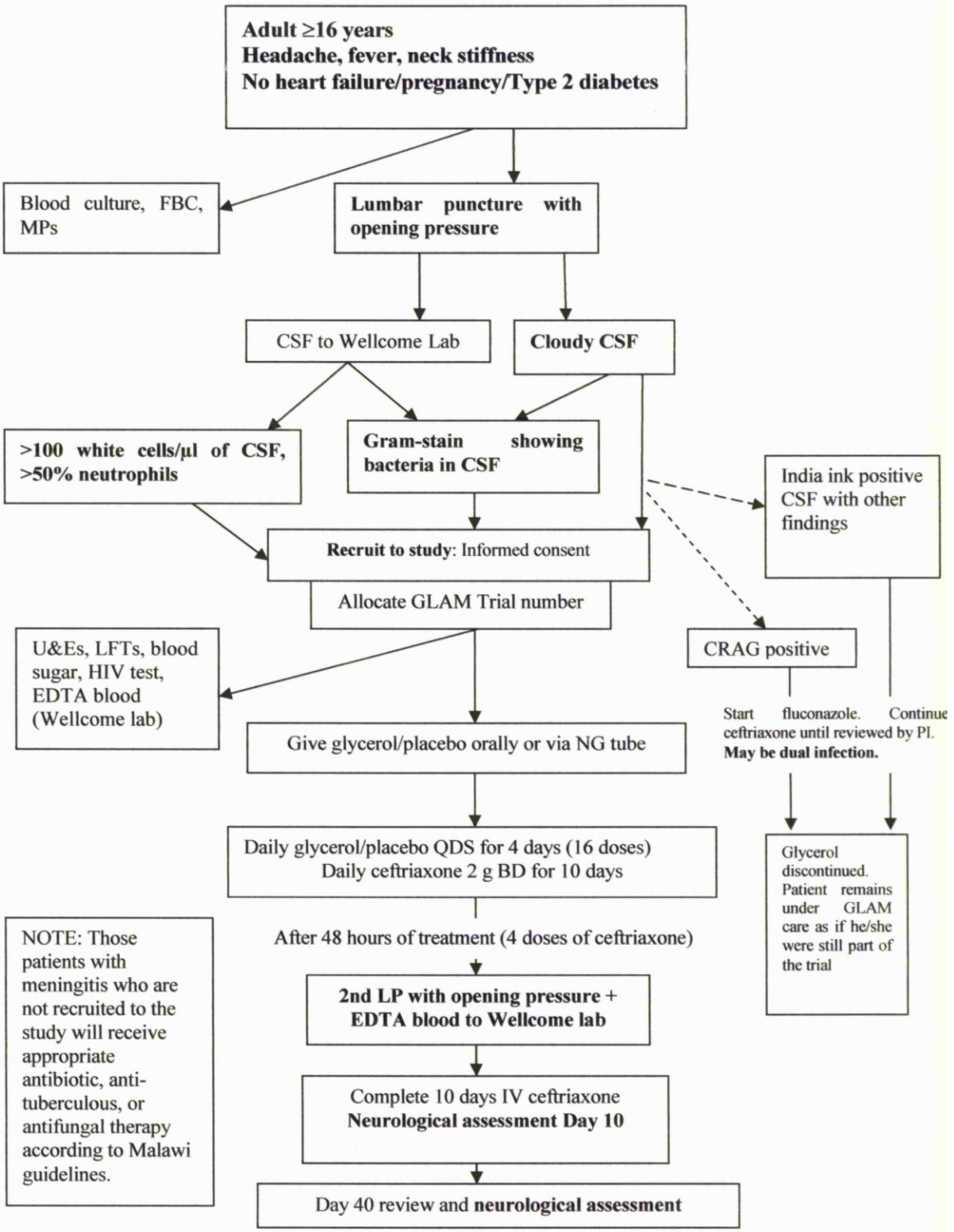
2.10 Intervention trial management

Following consent, glycerol/placebo was given to the patient to swallow. If the patient was unable to swallow or had a GCS of <8/15, a NG tube was inserted for administration. If they had not already received a 2 g dose of ceftriaxone, this was administered at the same time or immediately after.

Patients received IV ceftriaxone 2 g twice a day for at least 10 days plus glycerol/placebo orally four times a day for 4 days (16 doses). Treatment was modified if an enrolled patient was subsequently found to have an alternative diagnosis to bacterial meningitis (Figure 3). Cryptococcal meningitis was treated with fluconazole according to national protocol. All patients continued to be monitored and followed up and managed by the study team.

After 48 hours of glycerol therapy (and at least four doses of ceftriaxone), a repeat LP was carried out for CSF examination (microscopy, culture and storage for glycerol levels) and to measure CSF opening pressure. This was done in the morning in order that CSF samples reached MLW laboratory in a timely manner for processing; some patients therefore had their second LP after more than 48 hours of therapy because of the time they were admitted. Blood for serum biochemistry and glycerol levels were taken simultaneously. In view of the short half-life of glycerol (elimination half-life 30–45 min) [173], CSF and blood samples were to reach the laboratory for processing within 10 min of being taken and freezing (at –80°C) was requested within 1 hour of the sample being taken.

Figure 3. Intervention trial: Patient journey



2.11 Clinical procedures

The patient interview was performed by a clinical officer or Trial nurse. Patient examination and lumbar punctures were carried out by clinical officers. There were no absolute contraindications for lumbar puncture. CT scan was not available as an emergency investigation. A history of fits, altered conscious level or focal neurological deficit were not considered a contraindication for LP in this setting because it was felt that the need for a CSF examination to diagnose meningitis outweighed the risks of potential complications associated with lumbar puncture. The PI performed fundoscopy and sonographic ONSD measurements and also regularly carried out neurological examinations to ensure standards were maintained. Trial clinical officers were regularly appraised particularly in clinical examination skills as part of continuing medical education.

Blood sampling and NG tube insertion was predominantly carried out by the Trial nurses but also by Trial clinical officers. All nursing staff had already received training in venepuncture prior to working for this study. Clinical officers were already experienced at taking patient histories and carrying out a physical examination, they were also experienced at performing lumbar punctures.

NG tubes were inserted and secured by a GLAM nurse or clinical officer. Once inserted, the tube was marked (with a pen) at the nose to demarcate the level to which it had been inserted. NG tube position was checked by:

i) Aspiration of the tube and seeing that the aspirate turned blue litmus paper pink.

ii) Injecting 30 ml of air into the NG tube and by hearing the air bubbling while auscultating with a stethoscope over the epigastric area.

Litmus paper and stethoscopes were kept within the locked GLAM filing cabinets on the admissions, male and female wards.

The procedure and level to which the tube had been inserted was documented on the treatment chart. In this way, the next nurse to look after the patient would know if the tube had fallen out at all. Position of the tube was checked daily before administration of study drug.

Chest radiographs are not done routinely on admission and were only requested if indicated. The most common reasons for requesting a chest radiograph were suspected pulmonary tuberculosis or aspiration pneumonia. If tuberculosis entered the differential diagnosis then three sputa were requested to be examined for acid fast bacilli; mycobacterial culture was not available.

2.11.1 Optic nerve sheath diameter measurement

ONSD measurements were made by the PI using a handheld Sonosite-180 ultrasound machine (Sonosite Inc., WA, USA) with a 7 MHz curved array transducer (4–7 MHz 11 mm array transducer set to highest resolution frequency of 7 MHz). This design is for general paediatric use and was used by Beare *et al* [143] to validate ONSD measurements in African children with raised ICP. The scan type was set to

'Neonatal' throughout, with maximum scan depth set to 4 cm with the gain control on the default setting. Conductive ultrasound gel was placed over a closed eye lid and ONSD measurements were obtained from the temporal side with the ultrasound probe held against the closed eye lid. This achieved an axial view through the eye with a longitudinal section of optic nerve. The left eye was usually imaged.

The optic disc and optic nerve (the longest section achievable) was included in the freeze-frame. The best image was obtained by scrolling through the preceding 10 seconds of images. ONSD measurements were made using electronic callipers 3 mm posterior to the globe. Measurements were taken perpendicularly across the optic nerve and the mean of three measurements from different scans was calculated. On-screen measurements were concealed from the operator until each was completed.

ONSD measurements were made within 24 hours of recruitment to GLAM (Day 0), after 48 hours of therapy, at Day 10 and at follow-up on Day 40. Simultaneous direct ophthalmoscopy was also carried out on dilated pupils. Lumbar puncture with opening pressure was performed prior to recruitment to GLAM and after 48 hours of therapy.

2.11.2 Hearing assessment

Hearing was assessed by live voice speech presentation audiometry [174, 175] and, where possible, by single frequency (1000 Hz) pure tone audiometry. Audiometry

was performed by the PI or appropriately trained GLAM trial nurse at Day 10 and at follow-up on or after Day 40. Only those followed up at Day 40 in the clinic and able to cooperate with the test were included. We were not able to conduct pure tone audiometry in patients followed up in the community as the instrument was dependent upon an electrical supply which was not available in most patients' homes; nor did we perform pure tone audiometry in patients who remained on the ward at the time of follow-up because of the requirement to perform this test in a quiet setting.

Patients were placed in a quiet environment. With the patient wearing headphones and facing away from the person testing, the audiometer was set to 'Pure Tone', with 100 Hz frequency and initially 50 dB intensity. Each ear was tested in turn and the patient asked to indicate verbally or with a hand movement when a tone was heard. The tone switch was held for 2 seconds; if heard, the 'Intensity' was reduced by 10 dB and the process repeated in a stepwise manner until the tone was not heard. The 'Intensity' was then increased by 5 dB and retested – if heard, the intensity was reduced by 10 dB and retested; this was repeated in order that the level of intensity (dB) at which the patient hears was confirmed on a total of three occasions. This was recorded as the threshold level for the given frequency on the audiograph for the corresponding ear.

Each step was repeated at 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz, 8000 Hz and finally repeated at 500 Hz.

Mild hearing loss was defined as 'unable to localise finger rub at approximately 5 cm from the ear, unable to hear whispered voice at 30cm or a hearing threshold above 40 dB'. Severe hearing loss was defined as 'unable to hear spoken voice at 30 cm from the ear or a hearing threshold of >60 dB'. For speech presentation, patients were asked to repeat three words presented to each ear from behind the patient, with occlusion of the contra lateral ear. Words were in the appropriate vernacular, consisted of two equally emphasised syllables and avoided high frequency sounds (e.g. 'f', 's', 'v', 'ch', 'th'). Failure to correctly repeat two or more words was taken to represent hearing impairment for that test. Where possible, tests were conducted in a setting where there was minimum ambient noise.

2.12 Laboratory investigations

Trial samples were analysed in the QECH laboratory or the MLW laboratory or were bedside (point-of-care) tests (Table 4).

CSF and blood for culture was sent to the MLW laboratory. Blood was cultured at 37°C for a minimum of 48 hours (BacT/alert) and sub-cultured onto 5% sheep blood agar and lysed sheep blood agar for further incubation at 37°C. Antibiotic sensitivity was determined by disc diffusion (modified Stokes method) in line with British Society of Antimicrobial and Chemotherapy guidelines. Isolates with a zone of inhibition of >3 mm less than the control organism were classified as resistant.

CSF microscopy was routinely performed using standard laboratory techniques for cell count, differential white cell count (when >20 cells/mm^3 present), India ink staining and Gram stain. Gram staining was only done if there were more than 10 white cells per μl or organisms were seen on direct microscopy. Cryptococcal Antigen Agglutination Tests (CRAG) were not routinely carried out however CSF CRAG (Pastorex Crypto Plus Biorad) were performed on a subset of patients as part of another study running concurrently [176]. CSF was cultured on sheep blood and chocolate agar for 48 hours and Sabouraud-Dextrose-Agar (for *Cryptococcus neoformans*) and subcultured onto liquid media as appropriate.

Quantitative measures of CSF glucose and protein were not available. Estimates of protein and glucose levels were measured with a urine dipstick (Multistix 8SG Bayer) which provided colour-coded results for protein and glucose respectively as: negative (0 mg/dl: 0 mmol/L); trace (30 mg/dl: 5.5 mmol/L); 1+ (31–100 mg/dl: 5.5–14 mmol/L); 2+ (101–300 mg/dl: 28 mmol/L); 3+ (301–2000 mg/dl: 55 mmol/L); and 4+ (> 2000 mg/dl: 111 mmol/L).

Blood for malaria film, full blood count and biochemistry was analysed by the QECH laboratory. HIV testing was carried out on all patients using Uni-Gold Recombigen® HIV Rapid test [Trinity Biotech plc, Ireland] and Determine® HIV-1/2 [Abbott]. Capillary blood glucose was measured at least twice a day while taking study drug.

Table 4. Trial samples

Specimen	At recruitment (if not already taken)	After 48 hours therapy
Urine	Pregnancy test*	
Blood 20 ml	8–10 ml culture, MLW lab	
	3 ml Full blood count (EDTA), QECH lab	
	Malaria blood film, QECH lab	
	4 ml (clotted) U&Es, LFTs, QECH lab	4 ml (clotted) U&Es, LFTs, QECH lab
	Capillary blood glucose: Glucostix® test	
	1 ml whole blood HIV test (Phase 2 only)	
	4 ml EDTA blood MLW lab (stored at -80°C, GLAM "A" Whole blood planned for qPCR)	1 ml EDTA blood, MLW lab (stored at –80°C; GLAM "B" plasma planned for glycerol levels)
CSF	2 ml whole CSF, MLW lab (retrieved from initial LP, stored at -80°C, planned for qPCR)	6 ml CSF, MLW lab: 4 ml microscopy, culture and India ink stain and 2 ml stored at -80°C (planned for glycerol levels)

*Bedside urinary βHCG in females of child-bearing age

2.13 HIV testing

HIV status was not required at the time of enrolment to Phase 1; all participants were encouraged to access voluntary counselling and testing for HIV. No patient who declined HIV testing was denied entry to the trial. Experience from the previous trial [14], suggested that take-up of HIV testing would be high and that over 80% would be HIV infected. During Phase 1 we found that there were a number of patients in whom knowing their HIV status would have been helpful with their management. This was particularly when CSF results suggested possible cryptococcal meningitis. Many patients in Phase 1 were not capable of informed consent due to their clinical

condition and therefore diagnostic HIV tests were carried out in some cases. HIV testing of participants' anonymised stored samples was planned at the end of the trial to give an overall unlinked percentage of HIV seropositivity in Phase 1 participants.

HIV tests were planned for all patients in Phase 2. Blood was sent to Tiyanjane clinic (voluntary counselling and testing clinic) within QECH for testing with two kits: Uni-Gold Recombigen® HIV Rapid test [Trinity Biotech plc, Ireland] and Determine® HIV-1/2 [Abbott]. Results were obtained by the GLAM trial clerk.

HIV test results were given to patients once they were well enough to attend for post-test counselling. Relatives and patients' guardians were all encouraged to attend for voluntary counselling and testing for HIV regardless of the patient's HIV status. All patients found to be HIV positive were informed of their results before discharge from hospital. This was done by a member of the GLAM team or via Tiyanjane clinic. On discharge, patients were commenced on co-trimoxazole prophylaxis and referred to the antiretroviral (ART) clinic.

At the start of the study eligibility for ARTs in Malawi was determined by WHO clinical staging system [177]; during the study, from 2006 onwards, CD4 counts became more readily available and these were increasingly used prior to commencing ARTs. Bacterial meningitis is a WHO stage III disease and patients

were therefore eligible for free and indefinite access to ARTs under the current programme. Recruitment to the trial did not result in any delay in access to ART.

2.14 In-patient care

Documentation

All documentation including the proforma, any forms and laboratory specimens were labelled by hand with the GLAM Study number.

The signed informed consent, the proforma (with enrolment data form, inclusion and exclusion check list, and results) and any additional sheets of continuation paper were placed in a plastic file on the GLAM desk close to the patient's bedside. These formed the patient's notes. Files were not stored at the end of patients' beds because of staff and patients' concerns regarding confidentiality with visitors reading the notes.

On discharge, the notes were removed from the file, given to the PI for checking and stored until the follow-up visit. On discharge, the GLAM study number and the date of the 40-day follow-up appointment was recorded in the study register and into the patient's health passport (handheld notes).

Once the follow-up visit had taken place, the notes were returned to the PI to ensure the proforma was complete before it was transferred to MLW statistics department for data to be entered into the study database.

Confidentiality

Details of individuals participating in the study remained confidential. The study was conducted in accordance with standard clinical practice in relation to patient information. In addition study participants' files which constituted patient notes were kept accessible to staff members but away from participants' bedsides and HIV test results were kept in the filing cabinet on the ward and at discharge, results were written into the proforma and the patient asked to attend Tiyanjane clinic for counselling.

Following discharge, all study participants' files were kept securely in the PI's office until transfer to MLW statistics department for data entry. Files were only accessible to the study team. Database entries did not include names or addresses, only study numbers and initials. Databases were password protected.

Patient care on the wards

Patients were treated in hospital for a minimum of 10 days and looked after on the general medical wards by the GLAM trial team during their inpatient stay.

Each day, the trial nurses carried out assessment and measurement of a participant's pulse, temperature, blood pressure, respiratory rate, urine output and blood glucose. Observations were performed twice daily while the patient was taking study drug and once a day thereafter. Results were recorded in the proforma.

Participants were examined daily by a Trial Clinical officer or the PI. This included assessment of clinical progress, GCS and potential side effects of glycerol – confusion, fits since admission, diarrhoea, nausea, vomiting, dizziness, anuria, jaundice or other event. Patients were examined for signs of aspiration pneumonia – crackles in the chest or respiratory distress – and a chest radiograph was requested if new signs in the chest developed. All details were entered into the proforma.

The Clinical Officer also dealt with any other medical problems as appropriate and in consultation with the PI if necessary. Patients could be transferred to a high dependency unit for oxygen therapy if required; antibiotic therapy could be modified if indicated by sensitivity patterns or clinical response.

Those unable to swallow safely or those with a GCS <8 had a NG tube inserted for administration of study drug; the NG tube was also be used for administration of fluids, other medication and nutrition (if the guardians consented).

Patients were closely monitored for hyperglycaemia. While taking study drug, capillary whole blood glucose (BM) was measured twice daily using disposable Glucostix® (Bayer Diagnostics). These are visually interpreted reagent strips – a large drop of blood is placed on the Glucostix® test pads, then blotted after 30 seconds, after a further 90 seconds the result is read visually from the colour blocks found on the bottle. A larger green pad measures blood glucose levels from 1–6

mmol/L the other smaller orange pad measures blood glucose levels from 8–44 mmol/L.

Discharging patients home

On the day of discharge, the health passport (handheld patient notes) were completed to record the admission, management and GLAM Trial number. Patients were given an appointment to return for a Day 40 review. Money for transport was given on Day 40 once reviewed.

The completed proforma was given to the PI.

2.15 Management of side effects

Hyperglycaemia

If the blood glucose rose above 20 mmol/L and ketonuria was present, study drug was discontinued, adequate hydration ensured and management for diabetic ketoacidosis commenced (according to protocol, Department of Medicine, College of Medicine). If ketonuria was absent, study drug was discontinued. Blood glucose was continued to be observed and adequate hydration maintained.

Ideally, an IV infusion of insulin would have been used to maintain normoglycaemia. This was not possible on the general medical wards at QECH. Following discussion with the TSG and DSMB, it was felt that attempting to give insulin for hyperglycaemia was more likely to be hazardous – the risks of

hypoglycaemia and hypokalaemia combined with the loss of practical application of results (insulin given in this context would be even more unsafe out-with a trial setting) led us to exclude insulin as part of standard care for hyperglycaemia in GLAM.

Persistent hyperglycaemia was treated as new onset diabetes and managed with oral hypoglycaemic agents (or as diabetic ketoacidosis if the patient was unable to swallow).

Aspiration pneumonia

A chest radiograph was requested on any patient who developed signs or symptoms suggestive of aspiration – cough, shortness of breath, fast respiratory rate, or crackles in the chest. The radiograph was looked at by the PI to identify radiological signs of aspiration; it was also used to check the position of the NG tube.

If a diagnosis of aspiration pneumonia was made, Gag reflex and ability to swallow was assessed and the patient was commenced on metronidazole (orally if swallow safe, via NG or intravenously otherwise).

Swallow assessment:

Ensure GCS >7/15 (If GCS <8/15, swallow unsafe, insert a NG tube).

Sit the patient up, give a few small sips of water.

If the patient coughs or has difficulty, the swallow is unsafe.

Insert a NG tube if swallow unsafe.

Other possible side effects

Patients were reviewed daily for side effects of glycerol including headache, diarrhoea, nausea, vomiting and bloating. These were treated symptomatically:

i. Promethazine and/or other anti-emetics for nausea and vomiting

ii. Loperamide for diarrhoea

iii. Paracetamol and/or ibuprofen for headache.

Adverse events were recorded and reported to the DSMB (see Appendix 8).

2.16 Measurement of endpoints and follow-up

Trial endpoints were:

For Phase 1: Tolerability of glycerol adjuvant therapy and serious adverse events

Main trial primary endpoint: death by Day 40

Main trial secondary endpoints:

1) Death by Day 10.

2) Death or disability (Glasgow Outcome Score (GOS) 1&2) at Day 40.

3) Hearing loss at Day 40.

4) Occurrence of all serious adverse events other than death (reported as part of the endpoint).

5) Incidence of adverse events in the 10 days of follow-up (during the antibiotic treatment period), including gastrointestinal side effects (defined as vomiting,

diarrhoea or nausea), and fits.

6) CSF opening pressure after 2 days of therapy.
7) Time to death.
8) Incidence of raised blood glucose (capillary blood glucose >11.1 mmol/L).
9) Per-protocol analysis day 40 deaths restricted to those not withdrawn from glycerol/placebo.
10) Days to GCS >13.

Patients were formally reviewed at 10 days and after 40 days. At review, patients had a standardised neurological examination including fundoscopy, hearing assessment, ONSD measurement and GOS.

If a patient failed to attend their Day 40 follow-up appointment, a probation period of 1 week after non-attendance at a 40-day follow-up appointment was given. After this, attempts were made to locate them by travelling to their home or village. If the patient was still not found, verbal report of a patient's death was accepted as was a verbal report that they were alive (but lost to follow-up).

For survivor's, the principal outcome measure were the GOS (Table 5), which provides a measure of functional ability and/or dependency irrespective of the nature of neurological deficit [14] and hearing loss. Disability was represented by scores of 1, 2 or 3.

Table 5. Definitions of the Glasgow Outcome Score measures

OS	Definition
4	No disability. Minor neurological deficits which had no impact on any activity of daily living were allowed e.g. isolated cranial nerve palsies, unilateral hearing loss
3	Disabled but functionally independent and able to return to work or school
2	Disabled and dependent upon others on a daily basis (including severe bilateral hearing loss). Unable to return to previous level of work or school
1	Disabled and completely dependent
0	Death

2.17 Management of the trial

This followed the principles laid down in the MRC guidelines for good clinical practice in clinical trials, which are concordant with the International Conference on Harmonisation's Good Clinical Practice Guidelines.

Trial Steering Group

The TSG provided overall supervision of the study, and in particular, provided independent advice through the chairman of the group to the Investigator, the MRF and the Host institutions. The group incorporated individuals with relevant complementary experience and skills to advise on the running of a randomised controlled trial of glycerol adjuvant therapy in adult bacterial meningitis in Africa.

Plans for TSG meetings were made prior to commencing recruitment and took place before the trial started, shortly after the trial started and towards the end of recruitment.

The TSG met at least twice per year. More frequent email correspondence ensured the TSG chair was kept informed regularly of trial progress. An annual progress report was produced detailing recruitment numbers, numbers of deaths, summary of adverse events and trial progress (see Appendix 10, TSG members and terms of reference).

Data and Safety Monitoring Board

Two of the DSMB members were situated locally enabling them to visit the trial site to ensure Trial protocol and GCP was maintained; the other member was based in the UK and was kept informed by email (Appendix 11 DSMB). The DSMB were actively involved in the trial from the outset and reviewed a summary of Phase 1 data prior to commencement of Phase 2. They also received annual progress reports and interim reports of mortality which included a summary of all deaths (age, diagnosis, treatment given, time to death, cause of death) to guide when the interim analysis was likely to be required. The interim analysis was planned after 100 deaths.

Due to geographical constraints, neither the TSG nor the DSMB were able to meet physically regularly but virtual meetings via email occurred regularly.

2.18 Data management and analysis plan

All clinical data, laboratory results and outcome measures were recorded on a GLAM trial proforma (Appendix 3). Once completed, the information was double entered into the Access (Microsoft) database by MLW statistics department. Following data entry, the forms were filed in MLW statistics department. Participants' names were not recorded on the computerised database.

The trial databases were used to perform the interim and final analysis. Data were analysed with STATA 8.0 (Statacorp) and SPSS version 18.

The following data is verifiable from source documents: signed consent forms; dates of visits including dates glycerol given; CSF opening pressure on admission and after 48 hours; laboratory results; eligibility and baseline values for all patients; all clinical endpoints; all serious/severe adverse events; clinic compliance.

An initial blinded analysis was performed to check the completeness of data, the analytical method and the linkage of the database files and this was followed by a planned unblinded analysis following 100 deaths. The DSMB then advised early discontinuation of the trial and this was agreed by the TSG. The final analysis then went ahead as an intention-to-treat analysis.

Statistical assumptions

The assumptions used to calculate the required sample size included placebo: glycerol equals 1; the death rate was around 50% and the prevalence of adult proven bacterial meningitis (in QECH) was 140 per annum (with >80% probable cases). In addition, for Phase 2, (power calculations based on α 0.05, β 0.9 indicate that) 216 patients per arm were required to detect a 30% reduction in mortality from 56% to 40%. Incidence at the time of calculation meant this would likely be achieved within 30 months of recruitment to Phase 2.

Estimates of patient recruitment rates and follow-up rates of over 95% were considered entirely realistic based upon data from the steroid trial performed in the same setting [12, 14]. Sample size calculations were revised to take into account the study design and provide 90% power to detect a fall in mortality from 56% to 40%. A change in mortality of this magnitude or greater is likely to be considered of clinical relevance to most clinicians in Malawi.

Effect of glycerol on change in CSF pressure: assuming that at least 56% of patients will remain alive and consent to the second lumbar puncture at 48 hours, the study will be powered at 90% to detect a fall in CSF pressure of 4 cm CSF with $\alpha = 0.01$.

Sample size calculations for residual neurological deficit were not stated since data from the steroid trial suggested that this is a rare event (10.2%). It was anticipated

that an increase in the number of patients surviving with neurological sequelae could occur if there was a significant reduction in mortality in the treatment arm. Sub-analyses by HIV status was not planned as over 90% of patients were likely to be HIV positive.

It was calculated that two hundred and sixteen patients per treatment arm (432 patients in total) would be required to provide data on efficacy of glycerol adjuvant therapy. Therefore, it was planned that recruitment for Phase 2 (Main trial) should continue until 450 patients had been recruited (or the trial stopped if the TSG decided to halt recruitment on the basis of a recommendation from the DSMB).

Original GLAM analysis plan:

Phase 1: Tolerability of glycerol adjuvant therapy and serious adverse events.

Phase 2 (main trial) primary endpoint: death by 1 month.

Secondary endpoints:

1) Physician decision to alter treatment based on the occurrence of complications.

2) Death or residual neurological deficit (GOS and hearing loss) at discharge, and 1 month after completing antibiotic therapy.

3) Time to death, time to discharge.

4) Effect of glycerol on change in CSF pressure. We will also aim to perform a sub-study on a limited set of patients to gain data on CSF penetration of glycerol. There is no published data available for this. Sample size calculations for residual

neurological deficit have not been stated since data from the steroid trial suggest that this is a rare event (10.2%).

Modified analysis plan 2009:

The primary endpoint was death by Day 40; with modified intention-to-treat analysis (counting patients not followed to Day 40 as missing) and with the primary analysis using logistic regression without stratification.

Secondary endpoints included time to death, number of deaths by Day 10, and death and disability or hearing loss at Day 40. Disability was defined as GOS 1 or 2. Hearing loss (in those alive and with a GOS of 3 or 4 at Day 40) was defined as *either* subjective hearing loss, *or* only able to hear speaking (not finger rub or whispering) in either ear *or* inability to hear 40 dB on audiometry. Additional secondary endpoints were occurrence of all serious adverse events felt possibly to be due to the study drug; adverse events in the 10 days of follow-up (during the antibiotic treatment period), including gastrointestinal side effects (vomiting, diarrhoea or nausea), fits and hyperglycaemia (capillary blood glucose >11.1 mmol/L). CSF opening pressure after two days of therapy and days to GCS >13 were also analysed as a measure of effect of glycerol on ICP.

Various subgroup analyses were projected including deaths by Day 10 and 40 according to HIV status and death by Day 40 according to diagnosis – whether

proven (bacteria seen or cultured from CSF or blood) or probable (>100 white cells with 50% polymorphs in CSF but no organism isolated) bacterial meningitis and whether pneumococcal disease.

Primary analyses were conducted unadjusted, and in a logistic model adjusted for the pre-specified potential confounding factors. Potential confounding factors were proposed as patients' age; gender; HIV serostatus and degree of immunosuppression measured by history of previous AIDS defining events (Stages 3 & 4, WHO classification) and whether patients were on ART; organism isolated from samples (proven, probable and pneumococcal meningitis); duration of symptoms (specified as the longest of headache, neck stiffness, confusion, fever, fits, photophobia, reduced conscious level) and pre-admission antibiotic use; severity of disease as measured by history of fits prior to admission and GCS on admission; and opening pressure in the second LP, as an exploratory variable to explain the outcome.

It was planned that CSF glycerol and blood glycerol levels would be measured to assist subsequent studies to improve dosing if glycerol was shown to significantly reduce mortality by Day 40.

Statistical issues.

Baseline data were reported without tests of association. Simple descriptive data were analysed using a Chi squared test or Fisher's exact test as appropriate. For both primary and secondary outcomes, OR and 95% CI were calculated both unadjusted

and adjusted for predefined prognostic factors in a logistic regression model. Factors in the model were gender, age, GCS, length of history, fits prior to randomisation, organisms in blood or CSF, pre-randomisation antibiotic therapy, HIV status, prior AIDS defining event and history of ART. For adjusted OR, provided no more than 10% of data on potential confounding factors for any given factor were missing, dummy outcome variables were used (which are around the median of reported variables), to avoid cases being dropped from the model.

Differences in time to death up to 40 days between the glycerol and placebo arms was tested using Cox's proportional hazard ratios. These were calculated with data in non-failures censored at the time of last known encounter. Kaplan–Meier curves for the primary outcome were shown with 95% CIs.

Subgroup analyses were performed on patients with 'proven or probable' bacterial meningitis, proven bacterial meningitis alone and proven pneumococcal meningitis. A logistic regression model was used to explore the association between predetermined baseline variables selected prior to the start of recruitment and outcome.

The significance level for the primary outcome was determined as $p<0.05$. For secondary outcomes $p<0.01$ was used to determine statistical significance if the primary outcome was not significant. All other secondary outcomes had statistical tests which are informative but were not used to claim a difference between study

arms. Data were handled and analysed by K. Ajdukiewicz, PI; C. Whitty and B. Faragher, statisticians. A locked database was sent to N. French, TSG Chairman prior to unblinding and analysis.

CHAPTER 3: RESULTS – DOSE FINDING AND TOLERABILITY STUDY

3.1 Baseline characteristics

The dose-finding study commenced in March 2006 and recruitment continued until July 2006. Forty five patients with symptoms, signs and CSF findings compatible with bacterial meningitis were randomised to the three doses of glycerol for assessment of glycerol tolerability. Baseline characteristics were similar in all groups (Table 6).

Phase 1 was expected to take 3 months but recruitment was slower than anticipated, wards were quieter than usual during this period and this was thought to be due to a number of factors: crops grew well this year and many people went back to their villages to assist with the harvest; following the harvest, guardians may not have been able to spare time to bring individuals to hospital thus reducing admissions and delaying presentation; this was followed by cold weather which often results in a fall in admissions to the hospital.

Table 6. Baseline characteristics in Phase 1

	Group 1: 50 ml glycerol four times a day (n=15)	**Group 2: 75 ml glycerol four times a day (n=15)**	**Group 3: 100 ml glycerol four times a day (n=15)**
Prior AIDS defining illness Stage modified WHO 3 or 4 (%)	8 (53.3)	6 (40)	8 (53.3)
Age [years] : median (IQR)	32 (26–37)	35 (26–40)	32 (20–47)
Female (%)	10 (66.6)	8 (53.3)	10 (66.6)
Initial GCS <12	5 (33.3)	3 (33.3)	6 (40)
CSF white cell count : median (IQR)	560 (125–2080)	1000 (160–2080)	160 (124–1040)
Proven bacterial (%)	6 (40)	7 (46.7)	7 (46.7)
Pneumococcal disease (%)	6 (40)	6 (40)	6 (40)
CSF opening pressure: median (IQR)	20 (3.5–32.5)	21.5 (16.5–32)	16 (10–20.5)
Fits or history of fits in last 2 weeks	6 (40)	3 (20)	3 (20)
Prior antibiotic use (%)	7 (46.7)	6 (40)	3 (20)
Duration of symptoms: median (IQR)	3 (3–5)	3 (2–8)	4 (3–7)

IQR=interquartile range

A causative pathogen was isolated in 22 patients (47 %): 18 *S. pneumoniae*, one *H. influenzae*, two *Cryptococcus neoformans* and one *Escherichia coli*.

3.2 Adverse events and tolerability

There was no clear relationship between the dose of glycerol and frequency of side effects (Table 7). Approximately 50 % experienced nausea, vomiting or diarrhoea. Vomiting did not occur in patients receiving glycerol via NG tube. Transiently elevated BM glucose >12.2 mmol/L was observed in 14 (31%). Hyperglycaemia ranged from 12.2 to 20.4 mmol/L in all but one patient with a single reading of

40 mmol/L at recruitment who died prior to receiving study drug. No-one received treatment with insulin and five individuals had hyperglycaemia (<20.4 mmol/L) after glycerol had been discontinued; suggesting Type 2 diabetes or a stress response was the cause of the hyperglycaemia.

The 75 ml dose was best tolerated; patients randomised to the largest volume found it difficult to swallow. Eight patients in the 50 ml arm, seven in the 75 ml arm and 10 in the 100 ml arm died in hospital. Based on volume tolerability the 75 ml (75 mg) dose was used for the subsequent trial.

Table 7. Results of the dose-finding study

	Group 1: 50 ml (50 mg) glycerol four times a day (90 ml diluted volume) (n=15)	**Group 2: 75 ml (75 mg) glycerol four times a day (135 ml diluted volume) (n=15)**	**Group 3: 100 ml (100 mg) glycerol four times a day (180 ml diluted volume) (n=15)**
Died	10 (67%)	8 (53%)	12(80%)
Gastrointestinal side effects*	7 (47%)	8 (53%)	8 (53%)
Number with capillary blood glucose >12.2 mmol/L†	5 (33%)	3 (20%)	6 (40%)
Number with proven bacterial meningitis	4 (27%)	6 (40%)	7 (47%)

*Nausea, vomiting, diarrhoea
†WHO definition for diabetes [178]

Nineteen patients (42.2%) had NG tubes inserted at some point during their admission. One patient developed new chest signs while an NG tube was in-situ.

This patient was treated for aspiration pneumonia but died before a chest radiograph was able to be performed.

3.3 Influence of Phase 1 on Phase 2 main trial

Glycerol was well tolerated. During Phase 1, patients were not recruited overnight. Anyone admitted overnight with signs and symptoms of meningitis was recruited to the study the following morning if they fulfilled the inclusion criteria. Some patients did not survive to recruitment. Following Phase 1, arrangements were made to facilitate recruitment to the study overnight – anyone fulfilling inclusion criteria with cloudy CSF was able to be entered into the study if they gave informed consent.

There is evidence that patients with hyperglycaemia following stroke, acute myocardial infarct, pneumonia and those acutely ill on intensive care do less well than those with normoglycaemia [179]. Aiming for a blood glucose level of <8 mmol/L is reasonable in the acutely ill, even in those who are not known diabetics. The mechanisms of benefit of euglycemia appear to be multifactorial [180-184]

There is limited evidence available on patients with severe sepsis or septic shock. A meta-analysis [185] found no mortality benefit to glucose – insulin – potassium (GIK) infusion in critically ill patients although they acknowledge that studies were restricted to acute myocardial infarction and cardiovascular surgery patients.

During Phase 1 we modified the exclusion criteria by introducing a capillary blood glucose measurement at recruitment and excluding those with capillary blood glucose >12 mmol/L. Although transient hyperglycaemia can occur as a stress response in sepsis and other acute illnesses, glycerol induced transient hyperglycaemia (and increased serum osmolality) is thought to be clinically significant in those with Type 2 diabetes and we therefore wanted to avoid giving glycerol to anyone with undiagnosed Type 2 diabetes. In addition, it was proposed that those who developed hyperglycaemia (blood glucose>20 mmol/L) subsequently should have their study drug discontinued and be managed with rehydration in the first instance and for diabetic ketoacidosis if ketonuria was present (according to hospital management guidelines). It was agreed, in discussion with the TSG and DSMB, that administration of IV insulin for transient hyperglycaemia would not be safe in view of limited monitoring options for serum potassium levels and blood glucose, particularly overnight.

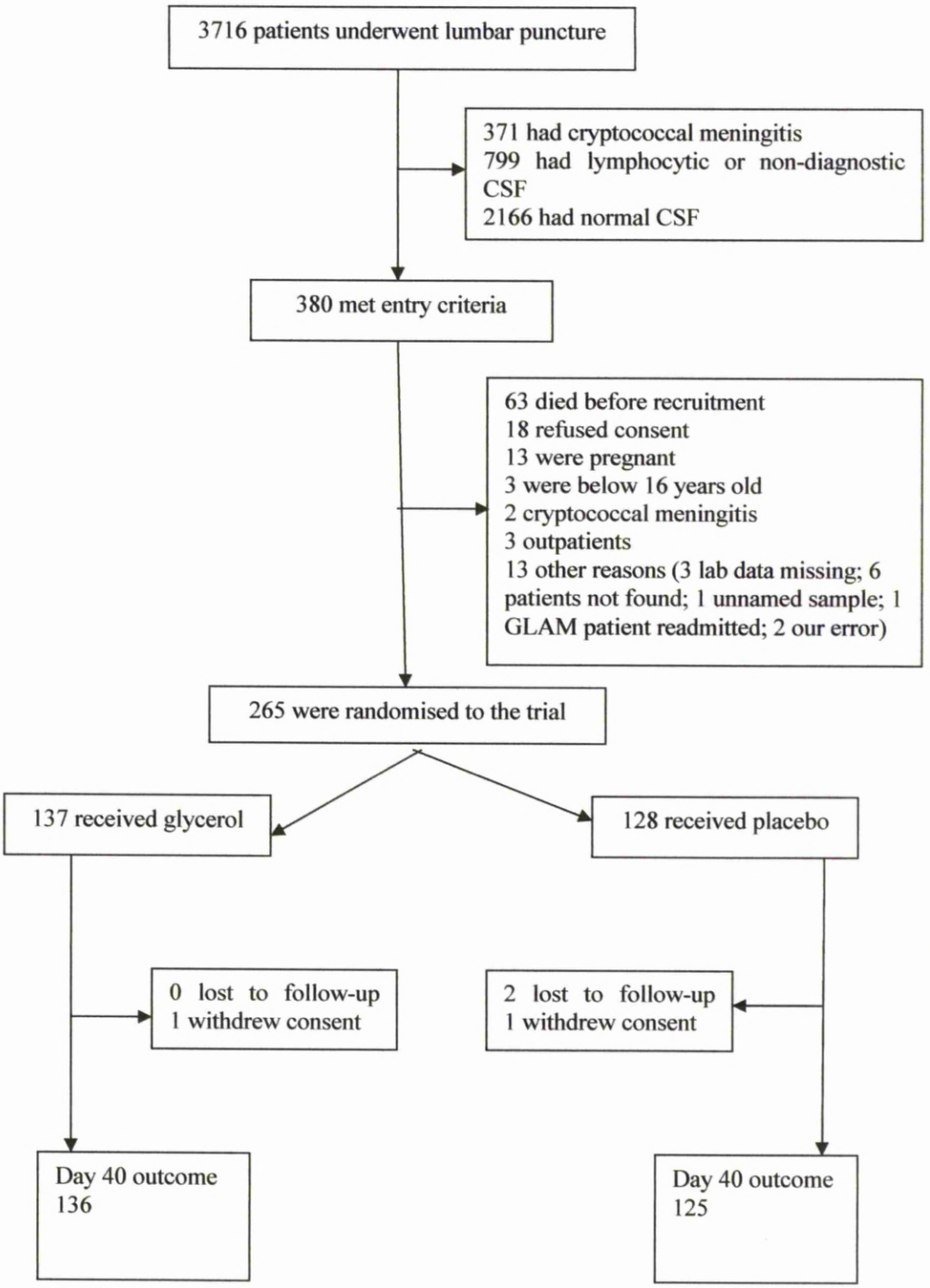
CHAPTER 4: RESULTS – INTERVENTION TRIAL

4.1 Patient recruitment and flow through the trial

The intervention trial started on 10 September 2006, and recruitment was stopped on 23 August 2008 following a planned interim analysis after 100 deaths. The data safety monitoring board advised halting recruitment on the grounds of futility; with 7 months of the trial still to run and 61% of the predetermined recruitment achieved. Patients already recruited to the trial were followed up until completion of follow-up or the primary endpoint was reached.

Three thousand seven hundred and sixteen patients were screened; 265 (70%) of 380 patients who fulfilled entry criteria, were randomised, 137 (51.7%) received glycerol and 128 (48.3%) placebo (Figure 4).

Figure 4. Flow through the trial



4.2 Baseline characteristics

Admission characteristics and laboratory results were similar in the glycerol and placebo arms (Table 8). One hundred and eighteen (44.5%) patients were diagnosed as having microbiologically proven bacterial meningitis, of whom 64 (54.2%) were randomised to the intervention arm and 54 (45.8%) to the placebo arm; 122 patients were found to have probable bacterial meningitis, of whom 63 (51.6%) were randomised to receive glycerol and 59 (48.4%) to receive placebo.

Twenty five patients (9.4% of recruited patients) were randomised but subsequently found to have diagnoses other than bacterial meningitis; 13 cryptococcal meningitis, three likely tuberculous meningitis (with lymphocytic CSF) and nine had cloudy CSF with less than 100 white cells – some of these may have had a traumatic lumbar puncture resulting in CSF turbidity. All were included in the intention-to-treat analysis.

Table 8. Baseline characteristics in intervention trial

	Placebo (n=128)	Glycerol (n=137)
HIV seropositive*	104 (83.9%)	111 (82.8%)
Prior AIDS defining illness Stage modified WHO 3 or 4	33 (25.8%)	36 (26.3%)
Age [years] : median (IQR)	32 (27–38)	32 (27–40)
Female	67 (52.3%)	72 (52.6%)
Initial GCS <12	48 (37.5%)	39 (28.5%)
CSF white cell count: median (IQR)	400 (200 - 1040)	395 (200 - 1120)
Proven bacterial	54 (42.2%)	64 (46.7%)
Pneumococcal disease	52 (40.6%)	46 (33.6%)
CSF opening pressure : median (IQR)	28 (18–34)	21.5 (12.5–34)
Fits or history of fits in last 2 weeks	51 (39.8%)	49 (35.8%)
Prior antibiotic use (%)	59 (46.1%)	53 (38.7%)
On antiretrovirals (%)	16 (12.5%)	23 (16.8%)
Duration of symptoms: median (IQR)	6 (3–8)	5 (3–7)

*HIV status not known for four patients receiving placebo and three patients receiving glycerol

4.3 Clinical presentation

4.3.1 Symptoms

The prevalence of symptoms amongst patients with proven and with probable bacterial meningitis are shown in Table 9. The most common symptoms reported were headache (257: 97.0%) and fever (244: 92.1%). Photophobia was reported in 47 (17.7%) cases and fits prior to admission in 100 (37.7%) cases.

Amongst patients with proven acute bacterial meningitis (ABM), a history of fitting was not more common in patients with pneumococcal disease (21/98: 21.4%) compared to those with non-pneumococcal bacterial meningitis (7/20: 35.0%) [Fisher exact test p=0.248].

A history of headache or fits was more common in those with probable meningitis than proven bacterial meningitis (p=0.018 and p=0.048 respectively); a history of reduced conscious level or confusion were both more likely in those with proven bacterial meningitis (p<0.001 and p=0.001 respectively).

Table 9. Demographic and historical characteristics of patients with proven and probable bacterial meningitis

	Proven ABM (n=118)	**Probable ABM (n=122)**	**Fisher exact test p-value**
History <48 hours	23 (19.5)	16 (13.1)	0.221
History headache	108 (91.5)	120 (98.4)	0.018
History fever	110 (93.2)	114 (93.4)	1.000
History neck stiffness	97 (82.2)	97 (79.5)	0.626
History photophobia	14 (11.9)	24 (19.7)	0.113
History of fits	28 (23.7)	44 (36.1)	0.048
History of reduced conscious level	87 (73.7)	56 (45.9)	<0.001
Reduced conscious level on admission (GCS <12)	53 (44.9)	30 (24.6)	0.001
History of confusion	85 (72.0)	63 (51.6)	0.001

4.3.2 Length of history and pre-hospital care

Median time to presentation was 6 days (IQR 3–9; range 0–140 days). The majority of patients (184/265; 69.4%) had sought or received some form of medical advice or therapy prior to presenting to hospital (Figure 5). Receipt of antibiotic therapy prior to recruitment was recorded in 112 (42.3%). The proportion of patients with a delayed time to presentation was significantly greater in those who had received pre-

admission antibiotics (101/112: 90.2%) than in those who had not (116/148: 78.4%) [Fisher exact test p=0.012].

Figure 5. Treatment obtained prior to admission

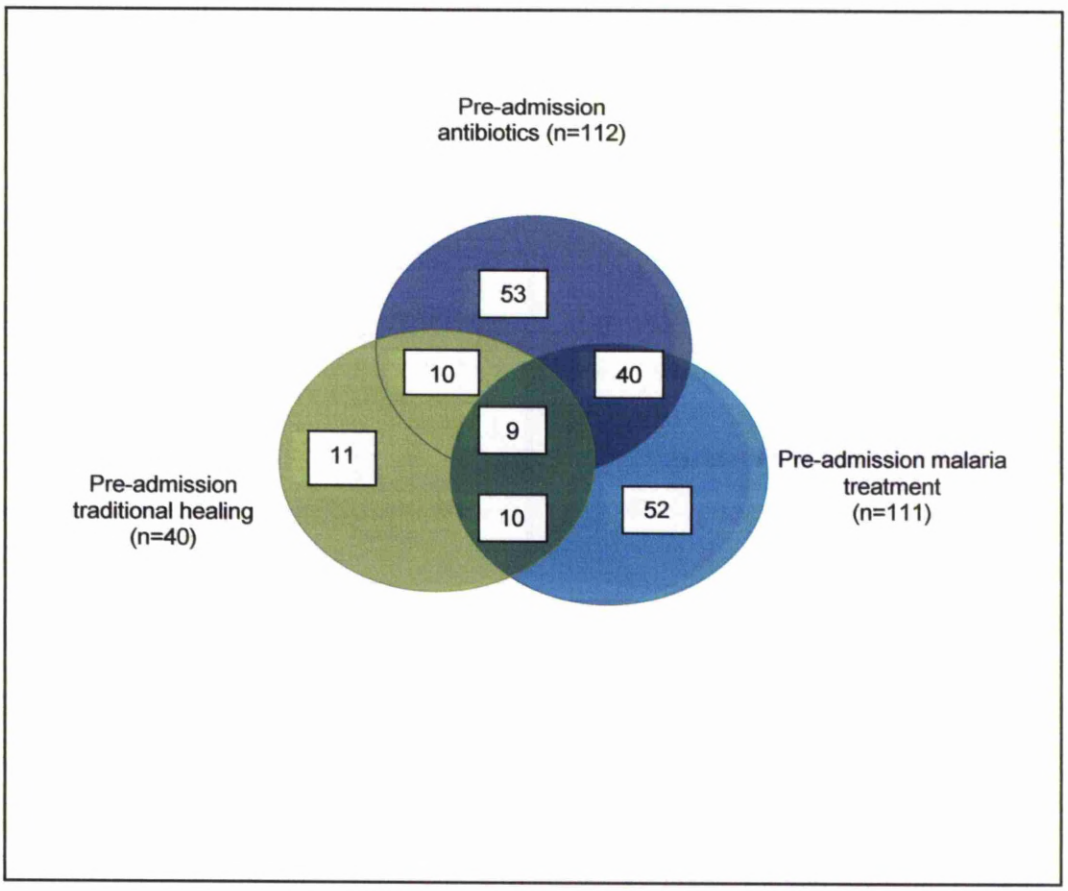


Table 10. Exposure to antimicrobials prior to recruitment

Antimicrobial	Number of patients n=112 (%)*
Penicillin V / Amoxicillin (+- clavulanic acid)	26 (23.2)
Chloramphenicol	13 (11.6)
Erythromycin	6 (5.4)
Ceftriaxone / Cefotaxime	5 (4.5)
Anti- tuberculous therapy	6 (5.4)
Doxycyline	9 (8.0)
Cotrimoxazole	49 (43.8)
Metronidazole	2 (1.8)
Ciprofloxacin	6 (5.4)
Fansidar™	70 (62.5)
Fluconazole	3 (2.7)
Benzylpenicillin	13 (11.6)
Gentamicin	5 (4.5)

*Eighty one patients had combination antibiotic therapy prior to admission; nine were taking cotrimoxazole prophylaxis

Those with probable meningitis were significantly more likely to have received antibiotics prior to admission than those with proven bacterial meningitis (65/122: 53.3% vs 36/118: 30.5%) [Fisher exact test $p<0.001$]. A wide range of antibiotics were taken; 81 patients received combination antimicrobials while nine patients were taking cotrimoxazole prophylaxis (Table 10).

Oral sulphadoxine-pyrimethamine (Fansidar™), the first line therapy for treatment of malaria in Malawi during the study, was received by 70 (26.4%) patients prior to presentation at some time during the course of the presenting illness. Thirty three (12.5%) patients had received quinine and 3 (1.1%) an artesunate-based preparation.

Attendance at a traditional healer was reported by 40 out of 254 (15.7%) patients – the majority of these (25: 62.5%) received herbal preparations to drink or eat; others had scarification or body adornments.

4.3.3 Examination and laboratory characteristics

Ninety/265 (34.0%) of patients had a recorded admission temperature of >38°C. A fever was more likely to be present in patients with proven bacterial meningitis than in patients with probable bacterial meningitis ($p<0.001$). Reduced conscious level (GCS <12) and neck stiffness were also more likely to be present in those with proven bacterial meningitis ($p=0.001$).

The triad of fever, neck stiffness and change in mental status which describes the severe meningitis phenotype [171], was present in only 39/265 (14.9%) of patients (Table 11). It was more likely to be present amongst patients with proven bacterial meningitis as compared to patients with probable bacterial meningitis (23.7% vs 7.6%; $p=0.001$).

Table 11. Prevalence of fever, reduced conscious level and neck stiffness at presentation

	Proven ABM	Probable ABM	Fisher exact test p-value
Temp >38°C	55/118 (46.6%)	27/120 (22.5%)	<0.001
GCS <12	53/115 (46.1%)	30/121 (24.8%)	0.001
Neck stiffness on examination	111/117 (94.9%)	98/121 (81.0%)	0.001
Triad of all 3	28/118 (23.7%)	9/119 (7.6%)	0.001

Neurological examination

Excluding examination of the eighth cranial nerve, 43 patients (27.9%) of 154 who were adequately assessed had a cranial nerve palsy identified on admission and 208/261 (79.7%) had focal limb paresis. Overall, in 260 patients who had a systematic examination, focal neurological deficit was identified in 210 (80.8%) patients at the time of admission. (Eighth cranial nerve palsies and abnormal deep tendon reflexes were not included in this analysis).

There were limited data available on baseline hearing assessment or visual acuity due to the inability to assess these in such a sick population. Deafness at baseline was reported by patients or their guardians in 5/134 (3.7%) in the glycerol arm and 3/127 (2.4%) in the control arm (p=0.723).

There was no significant difference between the two groups in terms of baseline neurological deficits including cranial nerve palsy, limb paresis or visual impairment. Those with proven meningitis were more likely to have a cranial nerve palsy at presentation compared with those with probable bacterial meningitis (Tables 12 and 13). There was no correlation between cranial nerve palsy and CSF OP or ONSD. In those who had CSF OP measured, 19/34 (55.9%) had CSF OP >20 cm and 15/34 (44.1%) CSF OP <20 cm (p=0.838). Nine/34 (26.5%) had ONSD >5 mm and 25/34 (73.5%) ONSD <5 mm (p=0.326).

Table 12. Baseline neurological assessment

	Placebo (%)	Glycerol (%)	Fisher exact test p-value
Cranial nerve palsy	16/69 (23.1%)	27/85 (31.8%)	0.280
Limb paresis	102/125 (81.6%)	106/136 (77.9%)	0.538
Focal deficit	102/125 (81.6%)	108/135 (80.0%)	0.756
Debility (GOS 1 or 2)	1/127 (0.8%)	2/135 (1.5%)	1.000
Blindness	3/49 (6.1%)	1/65 (1.5%)	0.313
Deafness	3/127 (2.4%)	5/134 (3.7%)	0.723

Table 13. Baseline neurological assessment according to diagnosis

	Proven ABM (%)	Probable ABM (%)	Fisher exact test p-value
Cranial nerve palsy	27/60 (45.0%)	16/78 (20.5%)	0.003
Limb paresis	96/115 (83.5%)	95/121 (78.5%)	0.408
Focal deficit	98/114 (86.0%)	95/121 (78.5%)	0.173
Debility (GOS 1 or 2)	2/117 (1.7%)	1/121 (0.8%)	0.617
Blindness	2/33 (6.1%)	1/65 (1.5%)	0.262

4.3.4 Predisposing factors

a) Local factors

Features from the history which might represent predisposition to secondary bacterial meningitis including otitis media or ear discharge were seen in 8/41 (19.5%) patients complaining of earache on admission. No patient gave a history of neurosurgery, head injury or prosthetic implants at any site.

b) Immunocompromise

No patients known to have diabetes mellitus were recruited to the study; all patients had a random capillary blood glucose measurement at admission, one patient had levels of >11.1 mmol/L. None remained persistently hyperglycaemic. No patient had a blood glucose of < 3 mmol/L at admission.

One hundred and one patients (38%) were aware they were HIV positive at recruitment but only nine were on cotrimoxazole prophylaxis and seven on antiretroviral therapy. Eight patients stated they were HIV negative; two of these tested HIV positive during the study. HIV serostatus was not known in 156 (59%) at the time of recruitment. Out of 258 patients tested, 215 (83.3%) were subsequently found to be HIV seropositive; 43 (16.7%) were HIV negative.

4.3.5 HIV serostatus

HIV test results were positive in 78/98 (79.6%) with pneumococcal meningitis and in 105/122 (86.1%) patients with probable bacterial meningitis (p=0.210). In those

with proven bacterial meningitis, those with pneumococcal meningitis were significantly more likely to be HIV infected than those with non-pneumococcal meningitis (78/98 [79.6%] vs 11/20 [55.0%]; p=0.042).

4.3.6 Blood cultures

In patients with proven bacterial meningitis, blood cultures were positive in 47/118 (39.8%) cases. The proportion with positive blood cultures was significantly lower in pneumococcal meningitis as compared to non-pneumococcal meningitis (30/98 [30.6%] vs 10/17 [58.8%] respectively; p=0.030). Positive blood culture formed part of the definition for proven bacterial meningitis.

4.3.7 CSF examination

Organisms were seen on Gram's stain in 81 out of 118 (68.6%) CSF specimens from patients with proven bacterial meningitis. A total of 25/103 (24.3%) patients had a positive CSF culture without a positive Gram stain, and seven patients had a positive blood culture with a CSF neutrophil pleocytosis but negative CSF Gram stain and culture.

Table 14. Effect of pre-admission antibiotics on blood/CSF culture and CSF gram stain positivity

	Pre-admission antibiotics	No antibiotics (%)	Fisher exact test p-value
CSF Gram stain positive	19/106 (17.9%)	60/143 (42.0%)	<0.001
CSF culture positive	33/111 (29.7%)	70/149 (47.0%)	0.007
Blood culture positive	18/103 (17.5%)	28/130 (21.5%)	0.509
CSF or blood culture positive	35/106 (33.0%)	79/142 (55.6%)	<0.001

Preadmission antibiotic therapy significantly reduced the rate of CSF gram stain and culture positivity (Table 14). Of the 112 patients who were reported to have received either antimicrobial therapy (including Fansidar but excluding quinine) within the 48 hours prior to randomisation, organisms were seen on Gram stain in 17.9% of cases as compared to 42.0% of antibiotic naive patients ($p<0.001$). Pre-admission antibiotic therapy reduced the rates of CSF or blood culture positivity from 79/142 (55.6%) to 35/106 (33.0%) ($p<0.001$).

The CSF white cell count ranged from 0 to 13600. Median CSF white well count was 400/μl (IQR 200–1070). Median CSF white cell count was 400/μl in both proven bacterial meningitis (IQR 200–1070) and pneumococcal disease (IQR 240–1320); median CSF white cell count was 440/μl in non-pneumococcal disease (IQR 220–1050).

Quantitative assessment of CSF glucose and protein was not available for the majority of the study. Multistix reagent strips [69] were used and low CSF glucose of <5.5 mmol/L was indicated if "negative" or "trace" and CSF protein >1 g/L by ≥3+ ("+++" or "++++") on Multistix.

Table 15. Low CSF glucose and high protein

	Low CSF glucose		High CSF protein		Low CSF glucose + high protein	
Pneumococcal meningitis	70/78 (89.7%)	p=0.053	80/86 (93.0%)	p=0.021	68/77 (88.3%)	p=0.123
Non-pneumococcal meningitis	12/17 (70.6%)		13/18 (72.2%)		12/17 (70.6%)	
Bacterial meningitis	82/95 (86.3%)	p<0.001	93/104 (89.4%)	p=0.002	80/94 (85.1%)	p<0.001
Probable meningitis	63/106 (59.4%)		79/110 (71.8%)		62/105 (59.0%)	

170/225 (75.6%) of patients had low CSF glucose and 185/238 (77.7%) had high CSF protein of >1 g/L (Table 15). Low CSF glucose and high protein were more likely with proven bacterial meningitis then probable meningitis (p<0.001).

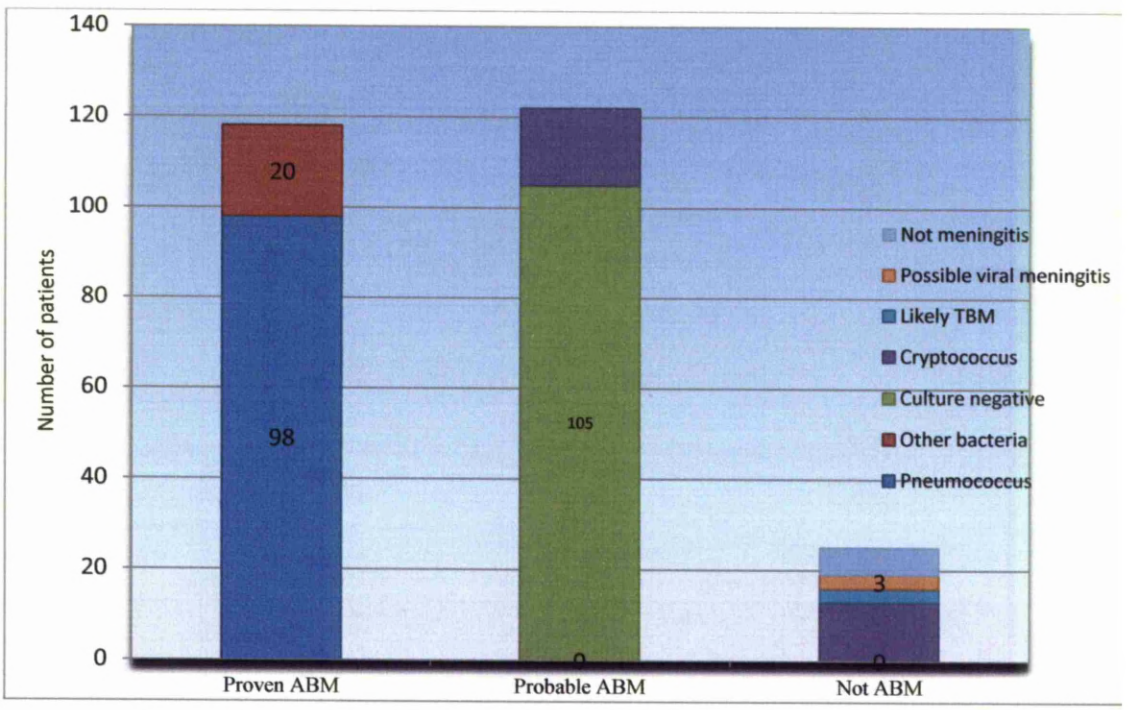
4.4 Diagnoses

Two hundred and forty (90.5%) cases fulfilled the criteria for bacterial meningitis and the diagnosis of bacterial meningitis was excluded in 42 (15.8%) subsequent to randomisation. A causative bacterial pathogen was identified in the blood or CSF of 118/265 patients (44.5%) thus defining the group of patients with proven bacterial meningitis. 122/265 (46.0%) had no bacteria isolated; these were classified as

probable bacterial meningitis (this included 17 patients with cryptococcal meningitis). Twenty five patients did not fulfil the criteria for bacterial meningitis according to CSF results – 13 of these had cryptococcus, three likely TBM, two viral meningitis, one was unknown (85% polymorphonuclear leukocytes (PMNL) without white cell count) and six did not have meningitis (Figure 6). No patient reported a previous history of meningitis.

Three patients had microbiologically proven dual infection; all had cryptococcal meningitis, two with concurrent Group A streptococcal and one with pneumococcal meningitis (see Table 16).

Figure 6. Diagnoses



Thirty three (12.5%) had a sole diagnosis of cryptococcal meningitis – all fulfilled entry criteria based on cloudy CSF but 13 were subsequently found to have CSF white cell count <100. Seventeen patients who fulfilled entry criteria based on CSF white cell count and differential were found to have cryptococcal meningitis either on CSF culture, blood culture, CSF microscopy or CRAG. For the purposes of analysis, these cases were classified as probable bacterial meningitis as per protocol.

Table 16. Organisms isolated

Organism		**Number of cases (%)**
S. pneumoniae	97 pneumococcus only 1 pneumo +crypto	98 (37)
Neisseria meningitidis		6 (2.3)
H. influenzae		0
Other Gram-negative	*E. coli* 4 Non-typhi *Salmonella* 1 *Klebsiella spp.* 1	6 (2.3)
Other Gram-positive	Group A Streptococcus 2 Group A Strep + crypto 2 *Staphylococcus aureus* 1 Other Strep 3	8 (3.0)
Cryptococcal meningitis	Cryptococcus only 30 (dual infection 3)	30 (11.3)
No organism identified	Probable meningitis 105 Likely TBM 3 Other 3 [2 likely viral; 1 had 85% PMNL no white cell count] No meningitis 6	117 (44.2)

Thirty eight CRAG tests were performed on CSF; 29 (76.3%) of these were negative. All nine positive CSF CRAG tests were in HIV infected patients and CSF

was negative for cryptococcus on microscopy (India ink stain). Only three were culture positive (blood or CSF) for cryptococcus (one on second CSF).

4.5 Principal outcomes

All randomised patients received at least one dose of their assigned treatment. Follow-up of survivors to Day 40 was 125/128 (97.7%) in the placebo group and 136/137 (99.3%) in the glycerol group. Outcome at 40 days from recruitment was thus available in 261 patients (98.5%).

Overall mortality was 147/261 (56.3%). Mortality was 64/116 (55.2%) in patients with proven bacterial meningitis, and 51/96 (53.1%) in patients with pneumococcal meningitis (Table 17). Two patients were lost to follow up (both randomised to receive placebo) and two patients withdrew consent (one randomised to receive glycerol and one to receive placebo). Withdrawal of consent was inferred from both patients – one absconded from the ward; the other patient's guardians refused to allow insertion of a NG tube for administration of study drug.

Mortality at 40 days from enrolment was 86/136 (63.2%) in the glycerol group and 61/125 (48.8%) in the placebo group (adjusted OR 2.4 [95%CI 1.3–4.2; p=0.003]).

Results of the intention-to-treat analysis and the predefined analyses for those with 'proven and probable' bacterial meningitis, and pneumococcal meningitis are shown in Table 17. Outcome in terms of mortality at 10 days or at 40 days were worse in

the glycerol arm. Further exploratory analyses describing the association between predetermined baseline factors and mortality at 40 days in the two groups of patients are shown in Table 18; mortality was significantly higher in those randomised to glycerol who were female, were HIV positive, had proven pneumococcal disease, presented late (>48 hours into their illness) or who had CSF OP >20 cm. Outcome at Day 40 for those who presented to hospital early in their illness (within 48 hours of symptom onset) was similar in the treatment and placebo arms (mortality 14/27 (51.9%) vs 9/16 (56.3%); p=0.976). There was no evidence that glycerol was effective in any subgroup.

Table 17. Outcome

	Group		OR (95%CI) [p-value]	
	Placebo	**Glycerol**	**Unadjusted**	**Adjusted***
Died before Day 40	61/125 (48.8%)	86/136 (63.2%)	1.8 (1·1-3·0) p=0·02	2·4 (1·3-4·2) p=0·003
Died before or disability Day 40†	75/124 (60.5%)	93/135 (68.9%)	1·4 (0·87–2·4) p=0·2	1·7 (0·97–3·1) p=0·07
Death by Day 10	53/126 (42.1%)	80/136 (58.8%)	2·0 (1·2–3·2) p=0·007	2·7 (1·5–4·8) p=0·001
Per-protocol analysis death to Day 40	57/106 (53.8%)	77/118 (65.3%)	1·6 (0·9–2·8) p=0·08	2·2 (1·2–4·1) p=0·01
Death by Day 40 restricted to proven bacterial disease	21/53 (39.6%)	43/63 (68.3%)	3·3 (1·5–7.0) p=0·002	5·5 (1·9–15·4) p=0·001
Death by Day 40 restricted to pneumococcal disease	20/51 (39.2%)	31/45 (68.9%)	3·4 (1·5–8·0) p=0·004	8·2 (2·4–28·5) p=0·001

*Pre-specified factors HIV, age, organism in blood or CSF, antiretrovirals, pre-treatment antibiotics, fits prior to admission, GCS, duration symptoms, sex, prior AIDS defining events.
†No Day 40 data for two patients

There was a trend towards clinically detectable hearing loss being more common in those receiving placebo (14/53 26.4% vs 4/43 9.3%; p=0.035). Hearing loss was sensori-neural in all apart from one with a dead left ear. This patient was apparently not aware of his deficit as did not self report deafness prior to testing.

At Day 40, disability was less common in those who had received glycerol (12/47 [25.5%] vs 27/53 [50.9%]; p=0.013) however, there was no evidence of benefit of glycerol in terms of death and disability at Day 40.

Table 18. Sub-group analysis for mortality at 40 days by baseline characteristics

	Placebo (%)	**Glycerol (%)**	**p-value (Kaplan–Meier)**
Overall mortality ITT	61/125 (48.8)	86/136 (63.2)	0.007
Pneumococcal disease	20/51 (39.2)	31/45 (68.9)	0.001
Other bacterial meningitis	0/2 (0)	11/18 (61.1)	0.173
Probable meningitis	32/57 (56.1)	36/63 (57.1)	0.651
Cryptococcal meningitis	9/16 (56.3)	10/17 (58.3)	0.895
Not meningitis	2/4 (50.0)	2/2 (100)	0.583
Prior antibiotics	26/58 (44.8)	30/53 (56.6)	0.141
HIV positive	52/101 (51.5)	74/110 (67.3)	0.005
HIV negative	5/20 (25)	9/23 (39.1)	0.299
Time to presentation ≤48 hour	9/16 (56.3)	14/27 (51.9)	0.976
Time to presentation >48 hour	52/107 (48.6)	70/107 (65.4)	0.006
Haemoglobin <10 g/dl	18/29 (62.1)	27/35 (77.1)	0.088
GCS <12	31/48 (64.6)	32/39 (82.1)	0.023
GCS ≥12	30/77 (39.0)	51/93 (54.8)	0.021
Male	29/60 (48.3)	37/64 (57.8)	0.248
Female	32/65 (49.2)	49/72 (68.1)	0.008
Fits prior to presentation	23/36 (63.9)	23/36 (63.9)	0.885
Temperature >38	28/46 (60.9)	31/43 (72.1)	0.196
ONSD >5 cm Day 0	7/15 (46.7)	17/30 (56.7)	0.476

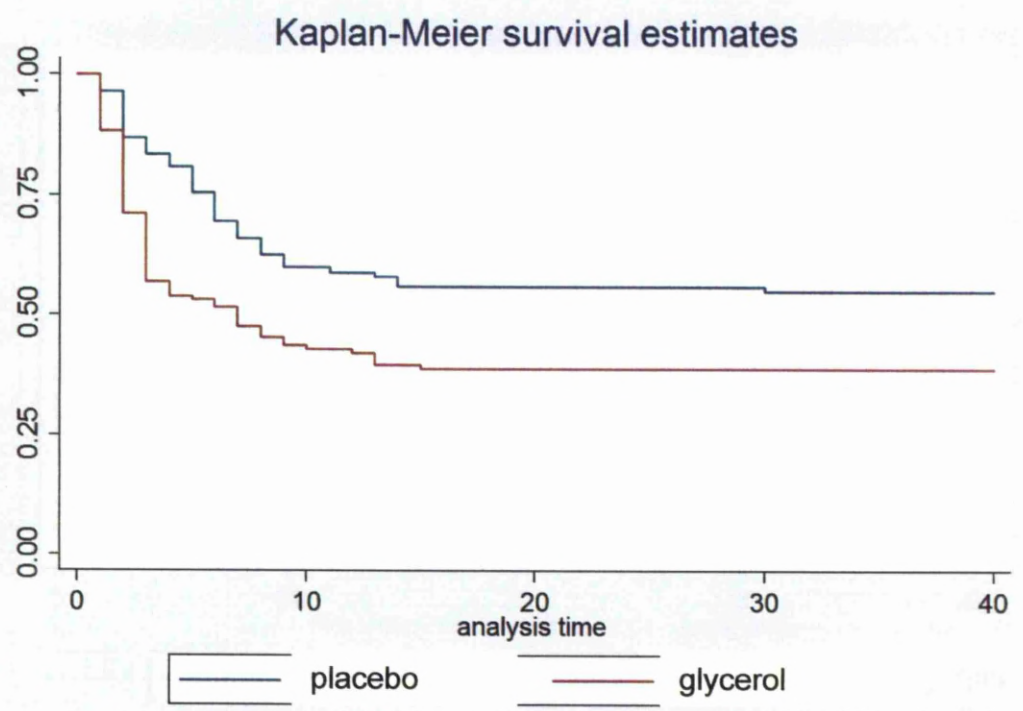
ONSD >5 cm Day 2	3/11 (27.3)	5/12 (41.7)	0.394
CSF OP >20 cm Day 0	30/62 (48.4)	35/53 (66.0)	0.025
CSF OP >20 cm Day 2	10/22 (45.5)	6/10 (60)	0.270

Most deaths occurred within the glycerol arm during the first 4 days of therapy when study drug was being administered (Table 19, Figure 7). By Day 2, survival was 67.6% in the glycerol arm falling to 54.4% by Day 3 and 51.5% by Day 4. There was clearly a greater mortality rate within the first few days – almost half of those receiving glycerol had died by Day 5.

Table 19. Survival rates (%)

Day	Placebo	Glycerol
1	91.2	83.8
2	82.4	67.6
3	79.2	54.4
4	76.8	51.5
5	71.9	50.7
6	66.3	49.3
7	63.0	45.6
8	59.8	43.4
9	58.2	42.6
10	54.9	40.4

Figure 7. Survival to Day 40 by study drug



4.6 Adverse events

Rates of adverse events potentially related to glycerol were not different between the two arms. Two adverse events thought possibly due to study drug in view of the rapid and unexpected deterioration in clinical condition resulted in withdrawal of the patients from the trial. A 70 year old HIV positive female was admitted with 1 week of headache, fever and confusion and on admission had GCS 13/15. She was randomised to receive glycerol. Her conscious level improved but on Day 2 she developed seizures refractory to anticonvulsant therapy and she became hypertensive (170/90 mmHg from a baseline of 120/80 mmHg). *Salmonella typhimurium* was

cultured from CSF; a second lumbar puncture was not carried out and study drug was discontinued on Day 3. She died on Day 12. The second case was a 35 year old HIV positive female with a 1 day history of illness and GCS 12/15 on admission who was randomised to placebo. She improved rapidly to GCS 15/15 by Day 2; *S. pneumoniae* was cultured from blood and CSF. Discharge was planned on Day 10 when she developed generalised weakness and vomiting without fever; she maintained a normal blood pressure and good urine output. On Day 11 her conscious level fell to GCS 6/15, no focal neurological deficit was identified. She died on Day 13. The likely diagnosis was felt to be a major cerebrovascular event secondary to meningitis. CT brain scan was not able to be performed in either patient due to their rapid clinical deterioration.

Gastrointestinal effects

Gastrointestinal adverse events thought possibly due to the study drug were no different between the arms (see Table 20). There were no differences in nausea, vomiting or diarrhoea between the two arms.

Glycaemic control

Random blood glucose was assessed at admission, twice daily while on study drug and once daily thereafter. Blood glucose was not found to be above 12.2 mmol/L in any patient during their admission. Therefore no patient required the addition of insulin therapy; no patient developed diabetic ketoacidosis or evidence of

hyperosmolar non-ketotic coma. No patients had an admission random blood glucose of <3 mmol/L.

Table 20. Adverse events possibly related to glycerol therapy during the first 3 days of therapy

Event	Glycerol (%)	Placebo (%)	Fisher exact test p-value
Hyperglycaemia*	0	0	1.000
Diarrhoea	18/136 (13.2%)	20/127 (15.7%)	0.602
Vomiting	58/135 (43.0%)	45/128 (35.2%)	0.208
Nausea	48/87 (55.2%)	32/76 (42.1%)	0.117
Fits	48/135 (35.6%)	25/127 (19.7%)	0.006

*Random capillary blood glucose > 12.2 mmol/L

Fits during therapy

Fits were more common in the glycerol arm particularly within the first 4 days of therapy (Figure 8, Table 21). The difference in rates was statistically significant on Days 2 and 4 ($p=0.006$ and $p<0.001$ respectively).

Fitting on Days 1–3 was a significant risk factor for death ($p<0.001$) and was associated with worst survival outcomes. This effect was of greater importance in the placebo arm where the presence of fits on Days 1–3 increased the risk of death by 4.118 (95% CI, 2.408–7.041; $p<0.001$) whereas in the glycerol arm it was only 1.631 (95% CI, 1.054–2.524; $p=0.028$).

Figure 8. Prevalence of fits Days 1–10

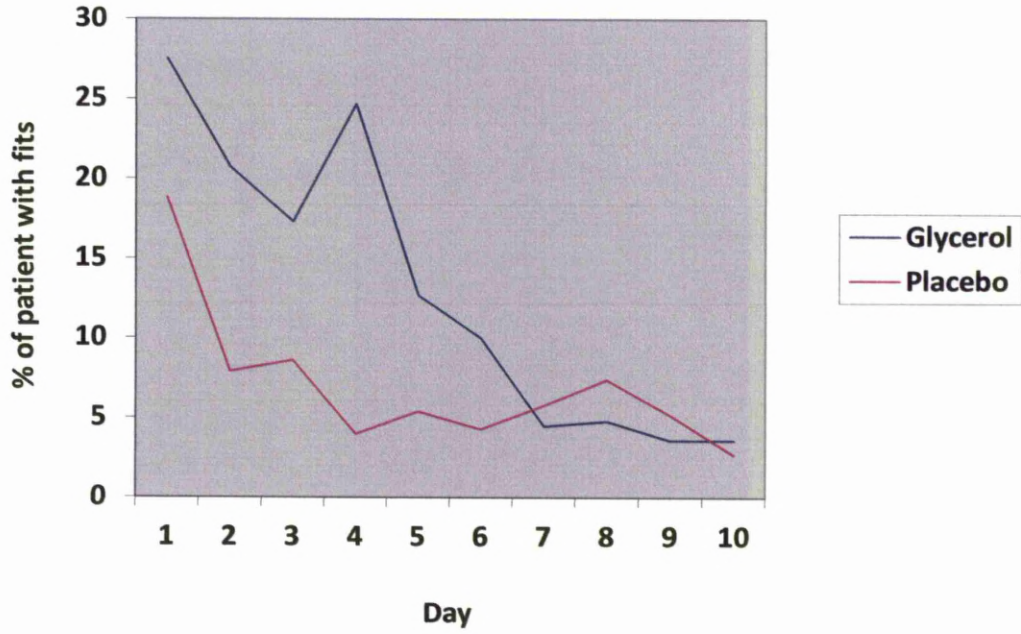


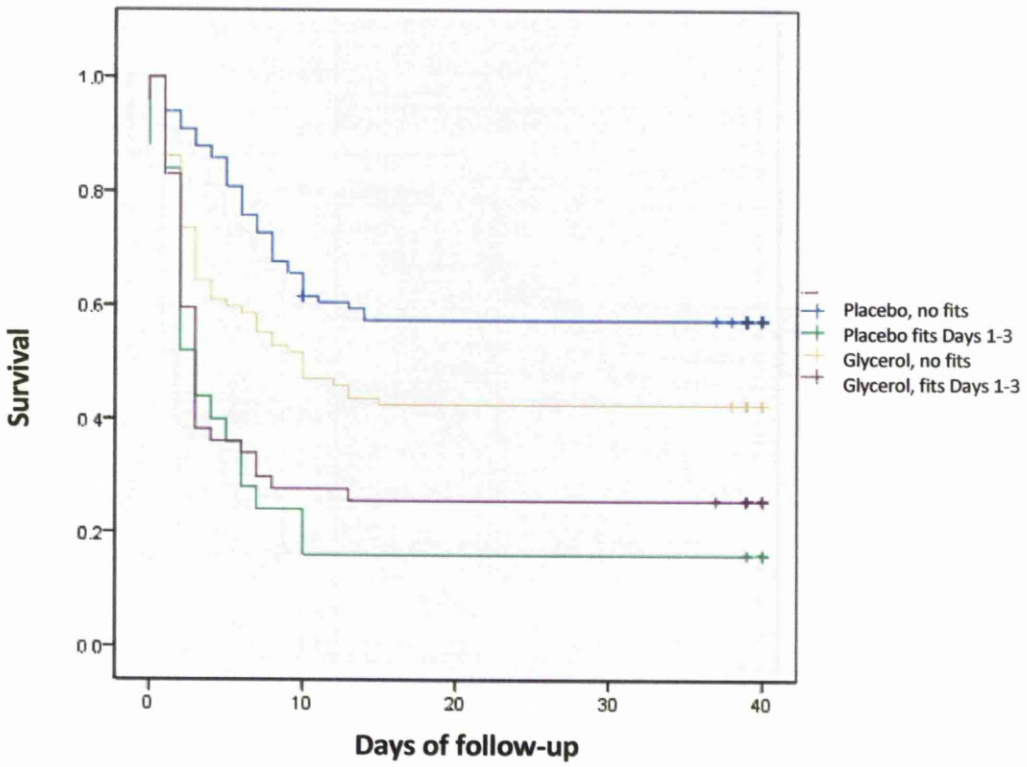
Table 21. Prevalence of fits according to randomisation

	Placebo (%)	Glycerol (%)	p-value
Day 1	22/117 (18.8)	33/120 (27.5)	0.123
Day 2	9/114 (7.9)	25/121 (20.7)	0.006
Day 3	9/105 (8.6)	18/104 (17.3)	0.066
Day 4	4/99 (4.0)	21/85 (24.7)	<0.0001
Day 5	5/93 (5.4)	9/71 (12.7)	0.157
Day 6	4/93 (4.3)	7/70 (10)	0.209
Day 7	5/86 (5.8)	3/67 (4.5)	1.00
Day 8	6/81 (7.4)	3/63 (4.8)	0.732
Day 9	4/77 (5.2)	2/55 (3.6)	1.00
Day 10	2/74 (2.7)	2/55 (3.6)	1.00

There were highly significant differences overall between the four groups: glycerol or placebo, fits Days 1–3 or no fits ($p<0.001$). Glycerol was a significant risk factor

for death (p=0.009) compared with placebo. In the presence of fits (Days 1–3), survival outcome was so poor that there was little difference between the treatment arms. However, in the absence of fits (Days 1–3) survival was better but glycerol appeared harmful in this setting and decreased survival (Figure 9).

Figure 9. Survival to Day 40 by study drug and whether fits occurred on Days 1–3



4.7 Outcome predictors

In order to ascertain features associated with poor outcome, further analyses were performed (Table 18). Mortality at 40 days from randomisation for all patients and

for patients with proven or probable bacterial meningitis was 55%. Mortality for patients with proven bacterial meningitis and with proven pneumococcal meningitis was 55% and 53% respectively.

In patients with proven bacterial meningitis, mortality was not significantly greater for pneumococcal disease than for non-pneumococcal disease (51/96 (53%) versus 11/20 (55%) p=1.0) although the number of patients with non-pneumococcal disease was small.

None of the six patients with meningococcal meningitis died. The highest mortality (100%) was in those with Gram negative rod meningitis – all six patients died; five by Day 10 and one by Day 40.

CHAPTER 5: OPTIC NERVE SHEATH DIAMETER

5.1 Introduction

Raised ICP may be a significant contributory factor to mortality and morbidity from bacterial meningitis [76, 77] therefore early reduction of the ICP should hypothetically improve outcome. At the time of this trial there was little published data on reduction of ICP in adult bacterial meningitis; a small study in intensive care unit demonstrated a worse outcome with higher ICP [79]. If glycerol were to be effective at reducing ICP it would be ideal adjuvant therapy particularly in resource poor settings given that it is cheap, widely available and can be administered orally.

Within the trial, we assessed ICP using CSF opening pressure, ONSD measurements and direct fundoscopy. We aimed to identify the presence of raised ICP and to determine whether ONSD was affected by glycerol therapy and if it correlated with CSF opening pressure at lumbar puncture. In addition, we aimed to demonstrate whether it can be used as a prognostic tool in this context. ONSD >5 mm was considered enlarged and 5 mm or less normal.

5.2 Results

5.2.1 Phase 1: Dose finding and tolerability study

Within the dose-finding preliminary study, 39/45 (87%) patients had ONSD measurements at recruitment, 24/29 (83%) after 48 hours therapy and 14/20 (70%) at Day 10.

Table 22. Phase 1 ONSD results

Median ONSD [mm] (IQR)	**Daily glycerol dose n=45**			
	200 mg (50 mg QDS)	300 mg (75 mg QDS)	400 mg (100 mg QDS)	p value (Chi^2)
ONSD Day 0	4.8 (4.0–5.3)	4.3 (3.8–4.7)	4.6 (4.0–5.1)	0.384
ONSD Day 2	4.9 (4.5–5.3)	4.2 (3.9–5.1)	4.2 (3.3–4.8)	0.316
ONSD Day 10	4.0 (3.8–4.3)	4.1 (3.8–4.9)	3.9 (3.9–3.9)	0.368

There was no difference in ONSD readings between the different doses at baseline, after 2 days therapy or at Day 10 (Table 22). The mean ONSD at recruitment was 4.6 mm (IQR 4.0–5.0). Ten well adults who were either students or members of security or clerical staff and who were assumed to have normal ONSD and ICP were used as the controls. Mean ONSD was 3.5 mm (Table 23).

Table 23. ONSD in controls

	Sex	Age (years)	ONS diameter (mm)
1.	M	37	2.8
2.	F	37	3.4
3.	F	23	2.7
4.	F	36	2.8
5.	F	26	3.2
6.	M	57	4.0
7.	M	27	3.6
8.	M	39	3.6
9.	M	40	3.5
10	M	21	3.7
11.	M	24	4.0
12.	M	27	4.3
Mean		**32.8**	**3.5**

There was no statistical difference between ONSD and CSF OP between the groups either at recruitment or after 2 days therapy (Table 24).

Table 24. Phase 1: ONSD and CSF opening pressure

	Daily glycerol dose						
	200 mg (50 mg QDS)		300 mg (75 mg QDS)		400 mg (100 mg QDS)		p-value
	ONSD ≤5	ONSD >5	ONSD ≤5	ONSD >5	ONSD ≤5	ONSD >5	
CSF OP Day 0 >20 cm	4/7	1/4	5/10	1/2	2/9	0/3	0.940
CSF OP Day 2 >20 cm	2/7	0/1	2/9	0/0	1/3	0/0	0.968

5.2.2 ONSD in intervention study

In the intervention trial, ultrasound measurement of ONSD was made on 196/265 (74%) at recruitment, 123/199 (62%) after 48 hours of therapy, 88/132 (67%) at Day 10 and 75/118 (64%) at Day 40 (Table 25). There was no statistical difference in ONSD between the intervention arm and the placebo arm at baseline or Days 2, 10, and 40.

Table 25. ONSD results: Intervention study

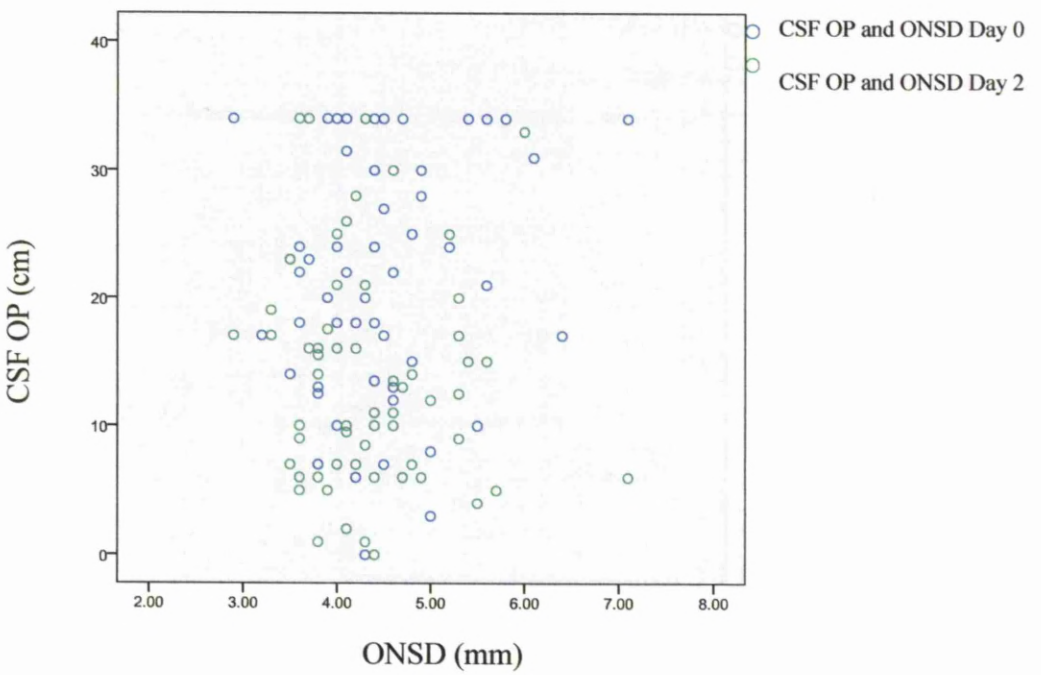
	Glycerol n=128 (95% CI)	**Placebo n=137 (95% CI)**	**p-value**
ONSD Day 0	4.7 (4.5–4.8)	4.4 (4.2–4.6)	0.036
ONSD Day 2	4.5 (4.3–4.7)	4.3 (4.1–4.5)	0.158
ONSD Day 10	4.4 (4.0–4.7)	4.1 (3.9–4.3)	0.158
ONSD Day 40	3.9 (3.8–4.1)	4.0 (3.9–4.2)	0.457

Raised ICP was suspected in 134 because of clinical signs (papilloedema, CSF OP >20 cm, abnormal eye movements or abnormal pupil reactions or GCS <8/15). No patient had a CT brain scan. No correlation was seen in this group between ONSD and CSF opening pressure (Day 0 and Day 2), GCS <8, gaze palsy or abnormal pupil reactions (Table 26 and Figure 10). ONSD was likely to be >5 mm in those with papilloedema at presentation (p=0.002).

Table 26. Correlation between ONSD and signs of raised ICP

	Day 0			After 48 hours therapy		
	ONSD <5	**ONSD >5**	**p-value**	**ONSD <5**	**ONSD >5**	**p-value**
GCS≤8	18/151 (11.9)	11/45 (24.4)	0.054	16/100 (16)	5/23 (21.7)	0.542
Papilloedema on admission	2/105 (1.9)	6/35 (18.2)	0.002			
Gaze palsy	8/90 (8.9)	5/31 (16.1)	0.314			
Abnormal pupil reactions	5/71 (7.0)	1/21 (4.8)	1.0			
CSF OP >20	62/111 (55.9)	24/34 (70.6)	0.163	16/75 (21.3)	4/20 (20)	1.0
GCS <12	47/149 (31.5)	22/45 (48.9)	0.05	41/135 (30.3)	14/43 (32.6)	0.85

Figure 10. ONSD and CSF opening pressure



ONSD >5 mm was not associated with increased mortality. ONSD was not a predictor of outcome (Table 27).

Table 27. ONSD and outcome

	Day 0			Day 2			Day 10		
	ONSD >5 mm	**ONSD ≤5 mm**	**p**	**ONSD >5 mm**	**ONSD ≤5 mm**	**p**	**ONSD >5 mm**	**ONSD ≤5 mm**	**p**
Death by Day 40	24/45 (53.3)	90/147 (61.2)	0.388	8/23 (34.8)	41/98 (41.8)	0.64	0/14	13/73	0.116
Death by Day 10	24/45 (53.3)	77/148 (52.0)	1.0	6/23 (26.1)	32/100 (32.0)	0.803	0/14	3/74	1.0
CSF OP Day 0 >20 cm CSF	24/34 (70.6)	62/111 (55.9)	0.163	11/18 (61.1)	40/76 (52.6)	0.604			
CSF OP at Day 2 >20 cm CSF	5/25 (20.0)	21/83 (25.3)	0.790	4/20 (20)	16/95 (21.3)	1.0	3/11	10/55	0.678
Fits at any time	20/45 (44.4)	55/146 (37.7)	0.486	8/23 (34.8)	29/99 (29.3)	0.621	3/12 (25)	16/73 (21.9)	0.726
Papilloedema	6/35 (18.2)	2/105 (1.9)	0.002	2/15 (13.3)	3/75 (4)	0.2			
Glycerol	30/45 (66.7)	76/151 (50.3)	0.062	12/23 (52.2)	46/100 (46)	0.648	8/14 (57.1)	33/74 (44.6)	0.560

CHAPTER 6: HEARING LOSS AND DISABILITY

6.1 Introduction

Hearing loss is the most common sequelae of bacterial meningitis in children. It occurs in up to 35% of survivors and is more common with pneumococcal than meningococcal infection [161]. One paediatric study suggests that glycerol is of benefit in terms of reducing deafness in survivors (0% vs 7%, p=0.049) [92].

6.2 Methods

Hearing was assessed by live voice speech presentation audiometry [174, 175] and, where possible, by single frequency (1000 Hz) pure tone audiometry at Day 10 and Day 40.

Patients were placed in a quiet environment. The patient was faced away from the person testing and the audiometer was set to 'Pure Tone', with 100 Hz frequency and initially 50 dB intensity. Each ear was tested in turn and the patient asked to indicate verbally or with a hand movement when a tone was heard. The process was repeated in a stepwise manner gradually reducing dB in order to determine the level of intensity (dB) at which the patient was able to hear. This was the threshold level for the given frequency on the audiograph for the corresponding ear.

Each step was repeated at 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz, 8000 Hz and finally repeated at 500 Hz.

Mild hearing loss was defined as 'unable to localise finger rub at approximately 5 cm from the ear, unable to hear whispered voice at 30cm or a hearing threshold above 40 dB'. Severe hearing loss was defined as 'unable to hear spoken voice at 30 cm from the ear or a hearing threshold of >60 dB'. For speech presentation, patients were asked to repeat three words presented to each ear from behind the patient, with occlusion of the contra lateral ear. Words were in the appropriate vernacular, consisted of two equally emphasised syllables and avoided high frequency sounds (e.g. 'f', 's', 'v', 'ch', 'th'). Failure to correctly repeat two or more words was taken to represent hearing impairment for that test. Where possible, tests were conducted in a setting where there was minimum ambient noise.

6.3 Results

Twenty one (21%) of 100 patients who had formal hearing assessment at Day 40 after enrolment had detectable hearing loss. Fourteen patients had moderate-to-severe hearing loss, of whom three had bilateral hearing loss (Table 28). Hearing loss was mild in seven (7%) of these patients and was unilateral in three and bilateral in four. Hearing loss was more common in the control group (5/90 [5.6%] versus 16/75 [21.3%]; p=0.026). It was not possible to formally assess hearing at the time of admission as most patients had impairment of consciousness at presentation and in addition were unable to be transferred to a quiet room for assessment.

Eight/261 patients (3.1%) were reported by their guardians to have some degree of hearing impairment at the time of recruitment, only one of whom was profoundly deaf and unable to communicate by speech.

A total of 22/100 (22%) patients who were assessed at 40 days had a disability defined by the GOS. The proportion of survivors with any disability (determined by GOS, presence of cranial nerve palsy or limb paresis, blindness or deafness) was significantly higher in the control arm compared with the glycerol arm (27/53 [50.9%] vs 12/47 [25.5%]; p=0.013).

Disability was not more common amongst patients with pneumococcal disease (15/38, 39.5%) compared to patients with probable meningitis (20/49, 40.8%) (p=1.0). There was no disability in any of the seven survivors with non-pneumococcal meningitis.

Patterns of hearing loss and of disability amongst survivors are presented in Table 28 and 29.

Table 28. Pattern of hearing loss amongst survivors who were assessed at 40 days

Hearing Loss	Glycerol n=90	Placebo n=75	p-value
Mild unilateral	1	2	
Mild bilateral	1	3	
Moderate unilateral	0	1	
Moderate bilateral	0	1	
Severe unilateral*	1	3	
Severe bilateral	1	2	
Total hearing loss	1	4	
Any hearing loss	5 (5.6%)	16 (21.3%)	0.026

Table 29. Pattern of disability amongst survivors who were assessed at 40 days

	Day 0			Day 40		
	Placebo (%)	Glycerol (%)	p-value	Placebo (%)	Glycerol (%)	p-value
Cranial nerve palsy	16/69 (23.1)	27/85 (31.8)	0.280	7/53 (13.2)	3/47 (6.4)	0.328
Limb paresis	102/125 (81.6)	106/136 (77.9)	0.538	22/53 (41.5)	10/47 (21.3)	0.034
Focal deficit	102/125 (81.6)	108/135 (80.0)	0.756	22/53 (41.5)	10/47 (21.3)	0.034
Debility (GOS 1 or 2)	1/127 (0.8)	2/135 (1.5)	1.0	14/53 (26.4)	8/47 (17.0)	0.335
Blindness	3/49 (6.1)	1/65 (1.5)	0.313	3/49 (6.1)	0/44(0)	0.244
Profound deafness				9/53 (14.3)	3/47 (6.4)	0.13
Any disability				27/53 (50.9)	12/47 (25.5)	0.013

CHAPTER 7: DISCUSSION

7.1 Admission characteristics and background

Over 1500 cases of meningitis were admitted to QECH over the 23 months of the trial. Cryptococcal meningitis was the most common cause of meningitis (373 episodes) followed by bacterial meningitis (230 episodes). There were 799 abnormal CSFs, some of which were lymphocytic (and may have been TBM) or had <100 white cells/μl and the diagnosis was not clear. These will have included cases of viral meningitis and meningoencephalitis.

There are several notable features at presentation that characterise patients within the study. The high prevalence of HIV infection (81%) within our patients is comparable with the (90%) prevalence in the steroid trial in Malawi [14] but higher than the overall reported prevalence in Malawi (14% in 2006). Independent surveillance data suggests however that the prevalence is likely to be over 30% in urban settings within Malawi [186]. HIV testing was not performed in any of the previous paediatric studies of glycerol in meningitis but it was thought unlikely that many of them were HIV infected.

Patients in this trial also presented to hospital much later than other studies – median time to presentation from onset of symptoms was 6 days. Measurement of time may be imprecise in those who live more rurally without the need to record time

Survivors who received glycerol were less likely to have a disability than those who received placebo (p=0.013) and there was a trend towards less hearing loss than in the placebo arm (5/90, 5.6% vs 16/75 21.3% (p=0.026)).

accurately. Delays to presentation in this setting have be seen previously [14]. The reasons for this are unclear and likely to be multifactorial, including difficulties with accessing and cost of secondary care, cultural beliefs including traditional medicines, and poor diagnostic ability within secondary care. Late presentation does not improve outcome and overall mortality in our trial was 56% (53% in pneumococcal disease and 57% in probable meningitis). This is comparable with mortality of 51% (65% in probable meningitis and 50% in pneumococcal) seen in the steroid trial in Malawi [14]. Mortality in Malawi out-with a trial setting is over 60% [13] compared to the much lower mortality in Europe of around 30% [187].

The majority of patients in this trial (69.4%) had received medical care (oral or parenteral antibiotics, anti-malarial treatment or attendance at a traditional healer) prior to randomisation, particularly those who had probable bacterial meningitis. This was similar to findings in the steroid trial where 64% had received pre-admission treatment. Median age of patients in this trial was 32 years (IQR 27–40) which was comparable with the steroid trial where the mean age was 32 years. Mean age of presentation in Europe is 45 years and the difference may be explained by HIV prevalence in the two populations. Most (42.2%) had received antibiotics and just over a quarter (26.4%) anti-malarials. During this study, standard treatment for malaria changed from Fansidar™ (sulfadoxine pyrimethamine) to artesunate combination treatment. This change from a simple to a more complex and lengthier treatment regime may have impacted on the number of patients taking empiric

malaria treatment for non-specific symptoms, including fever, prior to seeking medical attention.

Diagnosis of bacterial meningitis using symptoms and signs alone is difficult. In our trial, the classical triad of fever, neck stiffness and altered level of consciousness was present in only 16% of patients with proven or probable bacterial meningitis and in 24% of those with proven bacterial meningitis. This is much lower than was seen in the steroid trial (51% and 59% respectively) and in the European study in which 44% of patients demonstrated this triad [187]. Given that patients in Malawi present later in the course of their disease, it might have been expected that more patients would have developed these symptoms and signs at presentation. Relying on clinical signs alone is inadequate to diagnose meningitis in this setting and a lumbar puncture is required.

Cryptococcal meningitis tends to present insidiously with a longer history than bacterial meningitis and with headache as the most prominent symptom [35]. It can, however, have a more fulminant presentation with altered mental state and neck stiffness [188]. It was not always possible to diagnose cryptococcal meningitis on CSF microscopy and biochemistry alone. India ink examination of CSF is the only test routinely available for the diagnosis of cryptococcal meningitis in Malawi, as is the case in most health-care settings in sub-Saharan Africa. Cryptococcal antigen testing provides both a high sensitivity and specificity in the diagnosis of cryptococcal meningitis, but at present the kits are too expensive for many developing countries.

Thirty-three patients (12%) in our trial had proven cryptococcal meningitis. Seventeen of these fulfilled CSF criteria for 'probable bacterial meningitis' and were included in this category for the analyses (because they fulfilled the criteria for this definition); three had proven dual CNS infections with other bacteria.

Duration of symptoms also tends to be longer in TBM prior to presentation but has similar clinical features to bacterial meningitis. In resource poor settings, TBM is strongly associated with HIV infection [35, 189]. Distinguishing TBM from bacterial meningitis on clinical grounds is challenging unless retinal tubercles are seen on fundoscopic examination (these are present in the minority). A diagnosis of TBM was made in three patients who were randomised in our study on the basis of turbid CSF at admission. Subsequent CSF analysis revealed lymphocytic CSF with elevated protein levels consistent with a diagnosis of TBM and at the time of this study there were no facilities at QECH for TB culture.

Viral meningitis is usually a more mild disease than bacterial meningitis and is generally self-limiting. Viral meningitis in Malawi is an infrequent diagnosis in those admitted to hospital. In 2000, an estimated 3.6% of adult in-patients with meningitis had a viral aetiology [13]. Confirmation of such a diagnosis is unfeasible outside the context of the research setting.

Several factors may predispose to bacterial meningitis including CNS prosthetic devices, head injury and localised infections. Meningitis associated with prosthetic

devices is comparatively common in the West; no patients in our trial had prosthetic material in the CSF space or history of neurosurgery and none had a history of head injury. Otitis media was seen in eight patients (3%) in our trial compared with <5% in the steroid trial in Malawi. A descriptive study from Europe showed that 25% of patients with proven bacterial meningitis had a concomitant diagnosis of otitis media [187]. Localised infection predisposing to bacterial meningitis is unlikely to be less common in this setting than in Europe; the differences in reported levels of otitis media are doubtless due to less rigorous establishment of such a diagnosis.

CSF biochemistry was more likely to show classical findings associated with bacterial meningitis (low glucose and high protein) in patients with proven bacterial meningitis as compared to patients with probable bacterial meningitis. These findings, combined with the diagnostic challenges outlined above, suggest that a number of 'probable bacterial meningitis' cases may have been due to alternative organisms such as cryptococcus or TB.

History of reduced level of consciousness, reduced GCS on admission and history of confusion were all more likely in proven bacterial meningitis than probable meningitis. This may be a reflection on the more aggressive progression particularly of pneumococcal meningitis. Fever, reduced conscious level and neck stiffness were all more likely in proven than probable meningitis. The triad of all three were only presenting 15% and was more common in proven than probable meningitis.

Pre-hospital treatment led to delays in presentation to hospital. Patients who had received antibiotic or anti-malarial therapy prior to randomisation not only had a significantly longer history than those who did not receive antimicrobial therapy, but this directly affected diagnostic accuracy by reducing the chance of seeing organisms on CSF microscopy and of culturing an organism from CSF (but not blood). Therefore, pre-hospital treatment was most common in those with probable meningitis and this group had a worse outcome than those with proven bacterial meningitis. Twenty six percent received malaria treatment with Fansidar prior to admission suggesting over-diagnosis of malaria and misdiagnosis of early meningitis.

With the high prevalence of HIV infection, there is more cryptococcal infection and TBM in the population. In such a setting the differential for possible and probable bacterial meningitis is therefore much wider than in a lower HIV prevalent setting. In this study, the most relevant risk factor for invasive pneumococcal disease (IPD) was HIV infection. The incidence of IPD in North America, was shown to be 803 per 100,000 person years in HIV infected as compared to 35 per 100,000 person years in the HIV negative population [33]. Other known risk factors for IPD include diabetes, chronic renal/pulmonary/cardiac/ liver disease, solid organ transplant or other forms of immunosuppression and extremes of age (<2 or >65 years). Since this study was completed, the 13-valent conjugate pneumococcal vaccine has been introduced into Malawi following results demonstrating efficacy of the 7-valent conjugate vaccine at reducing IPD [59]. Sixty

nine patients (26%) had a prior AIDS defining event (WHO Stage 3 or 4) and therefore likely had advanced immunosuppression. This figure contrasted with only 39 patients being on ART and nine on cotrimoxazole prophylaxis. This would suggest that the roll-out of ART although deemed effective on a national level, was not reaching all those in need of treatment at the time of the study

Laboratory investigations in our trial were limited to blood culture, full blood count, malaria film and CSF microscopy and culture. Few patients had biochemical tests apart from bedside capillary blood glucose measurement. Blood cultures were positive in 15% of all recruited patients. This is lower than the 32% figure reported from the steroid trial in Malawi and much lower than developed countries where rates of blood culture positivity is over 50% [15, 171].

It is unsurprising that *S. pneumoniae* was the most common organism isolated as this is the most common cause of bacterial meningitis in Malawi and is likely to reflect the population. Only one case of *H. influenzae* was seen (in Phase 1) and this may reflect the success of the immunisation programme.

7.2. The glycerol trial: GLAM Study

Mortality

The overall mortality was 55%. Outcome was slightly better for pneumococcal (53% mortality) than probable meningitis (55% mortality) which is comparable to results from the steroid trial [14] where overall mortality was 51%.

Like the steroid trial in Malawi, the majority of deaths occurred early in the course of admission. Those given glycerol did less well. Mortality for the glycerol arm was 63.2% overall (vs 48.8% in the placebo arm). The reasons for this are not clear. An unlikely explanation is a positive effect in the placebo arm from sugar solution. This worse outcome in the glycerol arm was most marked in pneumococcal meningitis. There was no difference in mortality in probable meningitis between the treatment arms. Given that most of those with pneumococcal disease were also infected with HIV may suggest that glycerol has some effect in those with impaired cellular immunity due to HIV which confers increased mortality.

These findings from the first adult study of glycerol are strikingly different from paediatric studies. Oral glycerol was found to reduce severe or profound hearing loss in children in a small study of infants and children in Finland [92], and the larger multi-centre paediatric study in Latin America [93] recommended glycerol to prevent neurological sequelae. No significant difference was found between glycerol and dexamethasone in improving hearing loss or neurological sequelae in a small randomised double blind study by Sankar *et al* [94] in children with acute bacterial meningitis. Our findings suggest glycerol is not beneficial and appears harmful in adults with bacterial meningitis.

There are differences in our study population; our patients were adults, 81% were HIV positive and glycerol was given for 4 days rather than 2 days.

HIV serostatus was not reported in the paediatric studies. In particular, in the Latin American paediatric study (which did not test routinely for HIV) one must assume that in view of the lower prevalence of HIV within the population (<1%) [UNAIDS 2010], the majority of their patients would have been HIV negative. In addition, many patients had delays to presentation (as is common in sub-Saharan Africa compared with other settings). Even with these population differences, it is challenging to explain why glycerol appeared to be harmful. Hyperglycaemia was rarely found and therefore is not an adequate explanation; there is the unlikely possibility that the sugar solution used for placebo was beneficial in some way. Given that hypoglycaemia was not detected prior to randomisation in any patient makes this postulation implausible.

The causes of meningitis are very different in adults in Malawi compared to Latin America. In the paediatric Latin American study, *H. influenzae* b was the most common causative organism isolated with pneumococcus and meningococcus next most common [93].

The mean weight of a Malawian is 60 kg [190]; therefore the dose of glycerol administered was in the region of 1.25 g/kg/day for 4 days. The Latin American paediatric study administered 1.5 g/kg/day but over 2 days [93].

Sub-group analyses

HIV positive patients, females and also those who took longer time to presentation did less well with glycerol than with sugar solution. There is no obvious or identifiable explanation for these differences in outcome.

ONSD and CSF OP did not predict outcome. GCS <12 did not reach statistical significance in terms of being an outcome predictor. Reduced level of consciousness is usually an indicator of more severe disease and is therefore associated with worse outcome (see below) [191-193]; however, in this setting, poor outcome may have been inextricably associated with so many other factors that this effect was not seen.

Seizures were more common in the glycerol arm than placebo arm and could relate to poor outcome. This may be a coincidental finding in a severely ill population or could be directly due to the glycerol. Such findings have not been reported in humans in the literature. Changes in behaviour and seizures have been seen in mice within 30 min of oral glycerol [194]. The underlying mechanism for this was not known but it was suggested that increased IL-1 β concentrations within the hippocampus associated with increased oxygen species might be the cause.

In this study, mortality was most marked from Days 2–4 during and just after the administration of study drug. The occurrence of fits on Days 1–3 was the strongest risk factor for mortality – glycerol did not appear to either improve or exacerbate the risk. However, if there were no fits on Days 1–3, giving glycerol appears to increase

the risk of death relative to placebo (but not to the level of risk associated with fits). The aetiology is unclear and may be associated with rebound phenomenon but this is not supported by other measures of ICP (ONSD, CSF OP and GCS and fundoscopy). Rebound phenomena may occur in humans following osmotic therapy although evidence for this with glycerol therapy is conflicting [82, 125, 128, 130, 195]. Reversal of the osmotic gradient between blood and CSF or brain interstitium is thought to occur as the drug is eliminated but the significance of this occurring in bacterial meningitis is unclear. This could possibly be related to rebound phenomenon although this theory was not supported by other measurement of possible raised CSF including CSF OP at Day 2, ONSD Day 2 and 10 or neurological examination.

There was no statistical difference in hearing loss between the two arms. There was a trend towards less hearing loss in survivors who received glycerol (p=0.026; 5.6% vs 21.3%). In the paediatric study in Latin America, there was no difference in severe hearing loss between glycerol (12/136, 9%) and placebo arms (12/131, 9%).

There was a trend towards survivors who had received glycerol were less likely to have a disability (cranial nerve palsy, limb paresis, focal neurological deficit, debility GOS 1 or 2, blind, profound deafness) (p=0.013). In the paediatric study in Latin America severe disability between the four arms almost reached statistical significance (p=0.022) (dexamethasone + placebo 10/139, 7%; dexamethasone only 8/134, 5%; glycerol only 7/47, 5%; placebo arm 19/136, 14%).

In our study in adults, which was stopped early by the DSMB, glycerol was associated with significantly increased mortality within 40 days, and worse outcomes in all major secondary analyses except deafness and disability at Day 40. This trial therefore does not support the use of glycerol as adjunctive treatment for bacterial meningitis in adults in Malawi.

Adverse events

In the dose finding and tolerability study preceding the main trial, glycerol was well tolerated. There was no difference in gastrointestinal side effects between the three doses. Impaired glucose tolerance or elevation of glucose due to stress response was seen in 31% of patients. This compared starkly with the main trial were only one patient had an elevated blood glucose and this was transient, not requiring any intervention. Due to concerns with reliability of glucose monitoring in Phase 1, the digital glucometers were changed to more reliable methodology (Glucostix® test) in the main trial. The Glucostix® kits were sourced regularly during the trial and there was no question of their reliability.

Both sugar solution (placebo) and glycerol might be expected to produce hyperglycaemia however there was no difference in hyperglycaemia between the two arms. There was no suggestion of difference in gastrointestinal side effects between the two arms. There was no patient who presented with hypoglycaemia and no Type 2 diabetes was diagnosed during the study. One patient had capillary blood glucose over 11.1 mmol/L whilst receiving study drug.

Two possible adverse events involving the death of two patients could also have been explained as a consequence of their underlying meningitis rather than any effect of study drug. One patient received glycerol and the other placebo.

7.3 ONSD and ICP

ONSD is a non-invasive bedside test which has proven effective at measuring ICP in a number of settings with evidence of reproducibility between different operators [154, 156-158]. If effective in this setting it could be used to diagnose raised ICP which could potentially predict outcome. We aimed to examine if ONSD correlated with CSF OP and whether glycerol affected the ONSD or if ONSD predicted outcome.

In the main trial and Phase 1 there was no difference in ONSD between the treatment arms at randomisation, after 2 days therapy or follow-up at day 10. There was no correlation seen between ONSD and CSF OP.

Papilloedema at presentation was associated with ONSD >5 mm (p=0.002). There was a trend towards GCS <12 at presentation being associated with ONSD >5 mm. ONSD did not predict outcome, was not associated with CSF OP or other signs of raised ICP.

ONS diameter measurement was feasible in this setting of busy medical wards with severely ill patients. Although patients with papilloedema at presentation were likely

to have increased ONSD, ONSD did not predict outcome and was not associated with other clinical signs of raised ICP. It did not add any benefit in terms of management or prediction of outcome and is therefore not a useful tool in this setting in bacterial meningitis.

7.4 Study limitations

There are potential limitations to this study. Although QECH is well supported and has better staffing levels than many hospitals in a similar setting, patient care on medical wards is not as good as that in developed countries. In addition, access to intensive care or high dependency care for such severely ill patients was inadequate. In general, this is a typical southern African hospital and the patient population characteristic of such a setting, therefore this trial is representative of a sub-Saharan African high HIV prevalence setting. The results may not be transferable to a low-HIV prevalent setting where glycerol might be effective. Although residual confounding is a theoretical possibility, the fact that glycerol increases mortality in bacterial meningitis is highly statistically significant, subgroup analysis did not identify any possible benefit, and adjusting for identified confounding factors simply strengthened the association between glycerol and death, makes residual confounding unlikely. The decision made by the DSMB to stop recruitment to the trial early because of a mortality difference between the two arms at interim analysis was supported by subsequent analysis which confirmed this adverse effect.

7.5 Future studies

Intervention to reduce delays to admission could potentially improve outcome. This would include education, particularly around seeking medical attention earlier rather than community administration of antibiotics, empiric malaria treatment or traditional medicine.

7.6 Overall conclusions

Mortality from bacterial meningitis is high in this high HIV prevalent setting. Glycerol has been proposed as a cheap locally relevant adjuvant therapy however this study has shown glycerol to be harmful and its use cannot be recommended in adults in Malawi or other resource-poor regions where bacterial meningitis presents late and often occurs in those who are HIV infected. Other ways of reducing the considerable mortality associated with this disease need to be explored. The introduction of conjugate pneumococcal vaccination combined with the falling prevalence of HIV will hopefully go some way to reduce the incidence of IPD. Timely presentation to hospital, prompt resuscitation and antibiotic therapy are also essential for improved outcome. Glycerol appears harmful as adjuvant therapy in adults with bacterial meningitis in this setting however the outcome of the paediatric study of glycerol adjuvant therapy in Malawi is awaited with great anticipation.

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Appendix 1. Research Grant Application 2004 Meningitis Research Foundation

Midland Way, Thornbury, Bristol BS35 2BS UK Tel +44 (0)1454 280405 FAX +44 (0)1454 281094
www.meningitis.org

Research Grant Application Form

1. Applicants:

	Principal Applicant	**Applicant 2**	**Applicant 3**	**Applicant 4**
Surname	Zijlstra	Njalale	Ajdukiewicz	Scarborough *
Forename(s)	Eduard	Yasin	Katherine	Matthew
Age	50	28	31	41
Title	Professor	Mr	Dr	Dr
Post held	Professor of Medicine, COM, Malawi	Research Clinical Officer, COM, Malawi	Lecturer in Medicine, COM, Malawi SpR in Infectious Diseases, UK	Lecturer in Medicine, COM, Malawi Research Associate, LSTM, UK
Number of hours per week on project	8	48	20	8

The principal applicant is normally the corresponding applicant. If correspondence should be addressed to another applicant, please use an asterisk to indicate this in the table above. If there are more than four applicants, please attach details on a separate page.

2a) Institution/Authority (administering grant if approved):
Liverpool School of Tropical Medicine and Malawi College of Medicine

b) Address at which work is done:
Department of Medicine QECH, College of Medicine, P. Bag 360, Blantyre, Malawi

3. Title of Investigation (not to exceed 116 characters including spaces)
Randomised trial and dose finding study of glycerol adjuvant therapy in adult bacterial meningitis in Malawi.

4. Summary information

a. Proposed start date	b. Duration (in months)	c. Total funding requested
1st April 2005	36	**£ 90390**

(Please choose a realistic starting date: no earlier than July if applying in the spring round or January if applying in the autumn round.)

5. Type of Grant sought:
Small project grant

6. Research Summaries

a) Scientific Abstract (not to exceed 250 words)

Malawi has a high incidence of bacterial meningitis among adults. Currently, mortality exceeds 50%. More effective locally appropriate treatments are an urgent priority.

Glycerol has been shown to reduce mortality by approximately 50% in children with bacterial meningitis in South America. If similarly effective in adults, glycerol would represent a cheap, locally produced adjuvant therapy appropriate for use in this setting. It has been used as a food additive and for medicinal and cosmetic purposes for many years.

The proposed study is a proof of principle placebo controlled trial and dose-finding study of glycerol adjuvant therapy in bacterial meningitis in Blantyre, Malawi. All patients will be treated with antibiotic therapy according to the national guidelines. In addition, patients will receive 25ml bd, 25ml qid or 50ml qid 85% glycerol or water orally or via nasogastric tube. The primary endpoint will be mortality, with measures of morbidity as the secondary endpoint.

One hundred patients per treatment arm and 160 controls (460 patients in total) will be required to provide data on efficacy of glycerol adjuvant therapy at each dosage. At current incidence this is likely to be met in just over two and a half years from the start of the trial.

This study will provide the first information on the efficacy and optimum dosage of glycerol in meningitis in adults. The data will be used in the design of a subsequent larger multi-centre trial in four tropical research centres that will include patients with acute and chronic meningitis.

The current MRF funded trial of dexamethasone in bacterial meningitis in Blantyre will cease recruitment in December 2004. There will therefore be an immediately available and established infrastructure, recruitment mechanism, staffing structure and administrative procedure for a new adjuvant therapy trial.

b) Please state clearly the aims and objectives of your study.

1) To assess the safety and tolerability of glycerol adjuvant therapy in bacterial meningitis.
2) To assess the effectiveness of glycerol in reducing mortality in meningitis in the high HIV context of Malawi.
3) To provide an assessment of optimal dosing schedule of adjuvant glycerol

c) What is your study design?

Open label placebo controlled randomised trial and dose finding study

d) Summary of research proposal for the layperson (not to exceed 250 words). Please include a brief outline of the clinical benefit / potential value of this study in relation to the prevention, diagnosis or treatment of these diseases and their complications. Grant holders are expected to refer to this when discussing outcomes of the project in their final report.

Despite the advent of new antibiotics, bacterial meningitis remains an important cause of death and disability, particularly in resource poor settings. We therefore urgently need additional therapies to improve the outcome.

Used in combination with antibiotics, glycerol, a sweet tasting liquid that is widely used as a food additive, has recently been shown to reduce death rates by about one half in children with bacterial meningitis in South America. If similar results were found amongst adults, glycerol would represent a very significant advance in the treatment of bacterial meningitis. As it is cheap and locally produced, it would be a particularly appropriate additional therapy for use in developing countries where meningitis is most common.

One of the effects of meningitis is that it causes swelling of the brain inside the skull. This is an important factor contributing to the high death rate. Glycerol acts by pulling fluid out of the tissues and therefore reduces such swelling. Theoretically, this should help reduce the death rate seen in meningitis.

In order to test its effectiveness, we plan to treat some of our meningitis patients with glycerol and antibiotic therapy, and others with antibiotic therapy alone. To establish the safest and most effective dose of glycerol, we will compare three different dosage schedules. In all cases we will monitor the patients closely for possible side effects throughout their stay in hospital.

If we demonstrate an advantage from the use of glycerol in our hospital in Malawi, we plan to do a larger study involving four tropical research centres to test the effectiveness of glycerol in several different types of meningitis and in differing populations.

7a) Summary of support requested:

	Project Year 1 £	Project Year 2 £	Project Year 3 £	Project Year 4 £	Project Year 5 £	Total £
Staff	19200	19200	19200			57600
Consumables	7400	7400	6100			20900
Travel and Subsistence	460	460	380			1300
Exceptional items	3310	3340	3940			10590
Equipment						
Grand Total	**30370**	**30400**	**29620**			**90390**

b) Breakdown of costs by financial year (1 April to 31 March):

	Financial Year 1 £	Financial Year 2 £	Financial Year 3 £	Financial Year 4 £	Financial Year 5 £	Total £
Staff	19200	19200	19200			57600
Consumables	7400	7400	6100			20900
Travel and Subsistence	460	460	380			1300
Exceptional items	3310	3340	3940			10590
Equipment						
Grand Total	**30370**	**30400**	**29620**			**90390**

*5 years is the maximum duration for grants, but 5-year grants starting after April of the current year will incur expenditure over 6 financial years.

8. Does this project involve the use of human participants or human tissue? YES

If yes, please attach copies of your submission to the relevant Ethics Committee(s) along with your letter of approval if available. The Foundation will consider applications before ethics committee approval is obtained, but expects a copy of the ethics approval letter within six months of notification of grant. If you answered yes to question 8 and are applying from outside the UK, please attach copies of documents that show compliance with your country's research ethics regulations (along with translation into English, for documents in other languages).

The College of Medicine Research and Ethics Committee (COMREC) consider applications only after the receipt of a processing fee. It is therefore normal local practice to obtain funding prior to submission to COMREC. Attached is a copy of the University of Malawi COMREC guidelines for research proposals and the revised operating procedures of January 2004.

9. Other research grants and grant applications

a) Is this application currently being submitted elsewhere? NO

If yes, to which organisation, and by what date is a decision expected?

b) Has this, or a similar application been submitted elsewhere over the past year? NO

If yes, to which organisation, and what was the result?

c) Do you hold, or are you currently applying for another research grant(s) on a similar or related topic? YES

If yes, please give details, and explain how this does not overlap with your current application to Meningitis Research Foundation.

We are currently recruiting patients to an MRF funded trial of dexamethasone adjuvant therapy in adult bacterial meningitis in Malawi. This trial will cease recruiting in December 2004. The proposed trial of glycerol adjuvant therapy will start in April 2005. The glycerol trial is complimentary to the steroid trial and forms an obvious continuation into the investigation of adjuvant therapies in bacterial meningitis. There will be no temporal or clinical overlap between the two trials although the glycerol trial will benefit from the existing equipment and from a well-established research mechanism and staffing structure currently employed in the steroid trial.

d) Is this application a resubmission of an application previously considered by the Foundation?

NO

If yes, please attach a covering letter briefly stating how this application differs from the original.

e) Have you previously held (a) grant(s) from Meningitis Research Foundation? YES

If yes, please list project titles and start dates along with details of any resulting publications and other outcomes.

Title: Dexamethasone adjuvant therapy and intramuscular ceftriaxone in the management of bacterial meningitis in Malawi.

Start date: October 2001

Publications and presentations:

1) Bacterial meningitis in Sub-Saharan Africa – challenges to a better outcome.
M. Scarborough, Y. Njalale
Tropical Doctor (In press)

2) Application of the Vietnam diagnostic scoring system for TB meningitis to patients in the Queen Elizabeth Central Hospital, Blantyre.
A.Checkley, Y.Njalale, M.Scarborough, E.E.Zijlstra
College of Medicine Research Dissemination Conference, Blantyre.
November 2003

3)Neurological Manifestations of HIV infection
M Scarborough
National Aids Council of Malawi Training Programme 2003

9. Other research grants and grant applications (continued)

f) Please list other current grants you hold, including name of awarding body, title of project, amount awarded and dates of support

12. Full official contact details of all applicants

10. Declaration:

Applicants:

I have read the Terms And Conditions of Grant Aid and agree to abide by them. If a grant is made, I

i. **will take all reasonable actions to ensure that the Foundation's contribution to funding the research is suitably acknowledged in all publications arising from it, and ensure that copies of any such publications are forwarded to the Foundation.**
ii. **will comply with policies on intellectual property rights and commercial exploitation as set out in the Terms and Conditions**
iii. **will inform the Charity of any changes to details set out in the application**

I shall actively be engaged with the project, and in day-to-day control of the project.

To be signed by:	Signature	Name in block capitals	Date
Principal Applicant		EDUARD ZIJLSTRA	26·8·04
Applicant 2		YASIN NJALALE	26·8·04
Applicant 3			
Applicant 4		MATTHEW SCARBOROUGH	26·8·04

11. This application should be submitted by/through (1) the Head of Department and (2) the Officer who will be responsible for administering any grant that may be awarded. Each should sign the following declaration:

I confirm that I have read this application and that, if granted, the work will be accommodated and administered in the Department/Institution in accordance with the terms and conditions of grant aid of Meningitis Research Foundation. The staff grades, salaries and other costs quoted are correct and in accordance with the normal practice of this institution. I confirm also that this institution is subject to external audit.

	Head of Department	**Administrative Authority**
Signature:		
Title:	Professor	REGISTRAR, COLLEGE OF MEDICINE
Name and Initials (Block Capitals):	ZIJLSTRA E.E.	TRIGU C. (MRS)
Institution:	College of Medicine University of Malawi	COLLEGE OF MEDICINE UNIVERSITY OF MALAWI
Address:	Department of Medicine College of Medicine P. Bag 360 Blantyre Malawi	REGISTRARS OFFICE COLLEGE OF MEDICINE P. BAG 360 BLANTYRE MALAWI
Date:	26-8-04	26-8-04

	Principal applicant	Applicant 2	Applicant 3	Applicant 4
Name:	E. Zijlstra	Y. Njalale	K. Ajdukiewicz	M. Scarborough
Department:	Medicine	Medicine	Medicine	Medicine
Institution:	College of Medicine, University of Malawi	College of Medicine, University of Malawi	College of Medicine, University of Malawi	College of Medicine, University of Malawi
Address:	Department of Medicine College of Medicine P. Bag 360 Blantyre Malawi	Department of Medicine College of Medicine P. Bag 360 Blantyre Malawi	Department of Medicine College of Medicine P. Bag 360 Blantyre Malawi	Department of Medicine College of Medicine P. Bag 360 Blantyre Malawi
Telephone No:	+ (265) 01 670202	+ (265) 01 670202	+ (265) 01 670202	+ (265) 01 670202
Fax No:				
Email:	eezijlstra@malawi.net	ynjalale@hotmail.com	katherineaz@doctors.org.uk	mattscar@sdnp.org.mw

13. Proposed Investigation:

1.Title: Randomised trial and dose finding study of glycerol adjuvant therapy in adult bacterial meningitis in Malawi.

2. Purpose:
1. To investigate the feasibility and tolerability of glycerol as an adjuvant therapy in adult bacterial meningitis.
2. To assess the efficacy of glycerol adjuvant therapy in reducing mortality from adult bacterial meningitis in Malawi.
3. To compare three dosage regimens for glycerol adjuvant therapy in adult bacterial meningitis

The study will provide the first information on the efficacy and optimum dosage of glycerol in meningitis in adults. The data will be used to help design, if appropriate, a subsequent larger multi-centre trial in four tropical research centres that will include patients with acute bacterial meningitis and chronic meningitis (e.g. TB and cryptococcal meningitis). It will inform investigators of the potential benefits, constraints, side effects and optimal dosing regime for glycerol adjuvant therapy.

The proposed setting of the current study will differ from other sites in that the local patient population in Malawi is predominantly HIV positive, presentation tends to be late and the vast majority of bacterial meningitis is pneumococcal. The results of this study will therefore not necessarily represent patient populations elsewhere.

3. Background:

Bacterial Meningitis in Malawi.

Bacterial meningitis is a common and serious health problem in sub-Saharan Africa. Data from Malawi suggests that bacterial meningitis in adults is mainly pneumococcal and that the majority of patients are co-infected with HIV. Since the start of the HIV epidemic, the incidence of bacterial meningitis in Malawi has risen by over 800% and is now approximately 40 times the incidence seen in Europe. Currently, mortality from pneumococcal meningitis in this region exceeds 50% and up to 50% of survivors are left with neurological sequelae such as deafness, intellectual impairment or physical disability. More effective locally appropriate therapy in an urgent priority.

GLYCEROL

Glycerol has recently been shown to reduce mortality and morbidity by approximately 50% in children with bacterial meningitis in South America (personal communication H. Peltola). The mechanism of action of glycerol is thought to be through an osmotic reduction of intracranial pressure (ICP). Raised ICP is thought to be a significant contributory factor to mortality and morbidity from all forms of meningitis including bacterial meningitis. Early reduction of the ICP should theoretically reduce the incidence of adverse outcome. No trials of glycerol adjuvant therapy in bacterial meningitis have been reported in adults. If the

findings from the paediatric population can be replicated in adults, glycerol would represent a very significant advance in the treatment of bacterial meningitis.

Glycerol is locally produced, cheap and widely available. It has been used as a food additive and for cosmetic purposes for many years and is widely used amongst sportsmen and sportswomen to improve performance and thermoregulation by hyperhydration prior to endurance events. Medicinally, it has been used as a laxative, in the treatment of acute glaucoma and in the diagnosis of Meniere's disease. In the clinical setting, glycerol may be administered enterally or intravenously. It has been subject to several clinical trials in the treatment of ischaemic stroke. A meta-analysis of these stroke trials, all of which employed intravenous glycerol, suggested short-term advantage from glycerol therapy but no benefit in terms of long-term survival (1). There is one published trial of oral glycerol adjuvant therapy in bacterial meningitis in children (2) that demonstrated significant benefit in terms of neurological sequelae and deafness. No serious adverse events were reported.

Toxicity data for oral glycerol indicates that it is safe in the proposed dosages (3). Side effects are infrequent, mild and are most commonly gastrointestinal in nature. These include nausea, vomiting, bloating and diarrhoea. There are rare reports of neurological side effects such as headache, dizziness, confusion and amnesia (in elderly patients). There is one report of spontaneously reversible hemiparesis in a patient who accidentally ingested 500ml pure glycerol (4).

Antibiotic therapy in Malawi

Bacterial meningitis in Malawi is currently treated according to standard guidelines with parenteral penicillin and chloramphenicol for a minimum of 10 days. Data from the current steroid trial demonstrates that dual resistance to these agents occurs in less than one percent of cases attending QECH. It is therefore unlikely that there will be a change in the national prescribing policy within the timeframe of the proposed trial. If such a change were to be introduced, it would be unlikely to affect the interpretation of the results from the proposed trial unless mortality fell considerably in which case the power of the study would be reduced.

Dexamethasone adjuvant therapy

Dexamethasone is not currently used in the treatment of bacterial meningitis in Malawi and its use remains controversial. A recent trial in Europe demonstrated benefit from dexamethasone in adults in a predominantly HIV negative population (5). A trial in the paediatric population in Malawi showed no benefit (6). An ongoing trial of dexamethasone adjuvant therapy in adults in Malawi is due to complete enrolment by December 2004. If the results from the steroid trial show significant benefit of dexamethasone, the trial steering group will decide whether the proposed trial should seek to include an additional treatment arm of both glycerol and steroids. The rationale for this is that it may be considered unethical to withhold a potentially beneficial intervention outside the context of a rigorous

clinical trial. There is no reported interaction between glycerol and steroids but this possibility cannot be excluded on the basis of data currently available.

Study design and rationale

The proposed study is an open label randomised clinical trial and dose finding study to be conducted in two phases. The first phase aims at identifying immediate logistic or tolerability problems associated with glycerol administration. The second phase will consist of an open label clinical trial to assess the effect of glycerol adjuvant therapy at three different doses on the clinical outcome of bacterial meningitis at one month. The dosages to be used for this trial will be 25ml BD, 25ml QDS or 50ml QDS unless, in the opinion of the trial steering committee, results from phase one indicate that alternative dosages should be used. The higher dose of 50ml QDS is selected as it represents the accepted dosage used by sportsmen prior to endurance events and most closely replicates the 6ml/kg/day dosage used in the paediatric study.

If, as the paediatric studies suggest, mortality were reduced by up to 50%, the use of glycerol adjuvant would represent an important therapeutic advance in bacterial meningitis. Glycerol is easy to administer, cheap and widely available making it a potentially highly appropriate therapy for resource poor settings.

It is anticipated that the results from this study will precede a larger multi-centre trial in four tropical research centres aimed at investigating the use of glycerol in bacterial, tuberculous and cryptococcal meningitis.

4. Plan:

Phase one – April 2005 – May 2005

Feasibility study of 24 patients to assess tolerability of enterally administered glycerol adjuvant therapy at a dose of 25ml BD, 50ml BD or 50ml QDS during the first four days of antibiotic therapy.

Phase two – June 2005 – December 2006

Open label placebo controlled trial of glycerol adjuvant therapy at a dose of 25ml BD, 25ml QDS or 50ml QDS or placebo (sterile water) during the first four days of antibiotic therapy.

5. Methods:

Study place

Adult medical wards, Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi.

Study population; entry and exclusion criteria.

All patients admitted to the adult medical wards at QECH with headache, fever and neck stiffness have a lumbar puncture. Any patient with CSF findings suggestive of bacterial meningitis (or in the case of unconscious patients, their guardian on their behalf) will be invited to participate. This will be defined as:

- Cloudy CSF in a patient requiring immediate treatment

- *or* greater than 100 white cells/μl in the CSF with predominant neutrophils,
- *or* gram-stain showing bacteria.

Exclusion criteria will be pregnant females, type II diabetics, patients with heart failure or patients whose CSF results indicate infection by cryptococcus or mycobacteria by direct staining or antigen detection procedures.

Treatment allocation.
Phase I – Twenty four patients will be recruited for assessment of glycerol tolerability. Eight patients each will receive glycerol at a dose of 25ml BD, 25ml QDS or 50ml QDS for four days.
Phase II - Patients will be randomised by computer generated permuted blocks to receive glycerol adjuvant therapy at a dose of 25ml BD, 25 ml QDS, 50ml QDS glycerol or 50ml water QDS for four days. If, in the opinion of the trial monitor and steering committee, phase I demonstrates significant patient intolerance to one (or more) of the dosage regimens, this will be modified prior to the start of recruitment to phase II. Recruitment for phase II will continue until 460 patients have been recruited or until the stopping rules have been achieved.

Administration of drugs.
In patients who are fully conscious, glycerol/placebo will be administered orally. In semi-conscious and unconscious patients, glycerol/placebo will be administered through a nasogastric tube (NGT). The NGT will be placed and secured by an experienced nurse. Its position will be checked using the two standard techniques of auscultation and litmus test of the aspirate prior to the administration of any medication via the NGT. Glycerol or placebo will be administered for the first four days of treatment.

Protocol changes and other therapy.
If, in the view of the attending physician there is a clear clinical need to withdraw the patient from the trial or adjust the dosage of glycerol, this will be taken as a secondary endpoint.
All other treatments, and treatment of complications such as fits will be as for any case of bacterial meningitis in this clinical setting.

Sample size and study period.
Phase I will take two months to complete.
For phase II, power calculations based on α 0.05 β 0.8 with current mortality of 50% indicate that 100 patients per treatment arm and 160 controls will be required to detect an absolute risk reduction of 20%. At current incidence, it is likely that this will be within thirty two months of recruitment to phase II. Recruitment to may cease earlier than this if interim analysis performed at twelve, eighteen and twenty four months indicates that the stopping rules have been reached.

Estimates of patient recruitment rates are considered entirely realistic based upon the current recruitment rate into the steroid trial. An additional two months will allow for recruitment variation, contingencies and data analysis.

Interim analyses and stopping rules

The trial monitor will be asked to review outcome data at twelve, eighteen and twenty four months of recruitment. In light of these analyses, the monitor will advise the Chairman of the steering committee if, in his view, the randomized comparisons in the trial have shown both
a) proof beyond reasonable doubt that for all, or for some, types of patient one particular treatment is clearly indicated or clearly contra-indicated in terms of a net difference in mortality
and
b) evidence that might reasonably be expected to influence the patient management of many clinicians who are already aware of the results of other studies.
The steering committee can then decide whether to modify intake to the study.

Appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but the criteria that have been provisionally agreed are that the trial will have uneven stopping rules to avoid stopping on a random high:

a) Recruitment to be halted if a treatment arm has evidence of harm exceeding conventional levels of chance alone ($p<0.05$)
b) Recruitment to be halted if clear evidence of benefit with significance level of p<approx.0.002 (3SD)

The trial monitor may decide to stop the trial in other circumstances if it is his view that continuing is not justified on ethical grounds. In exceptional circumstances, the independent monitor would, after taking further ethical advice, seek to extend the trial if there were clear ethical reasons so to do.

Budget and costings

The budget is estimated from expenditure incurred during the steroid trial.
Staff increments are not calculated into the budget, as the influence of exchange rate fluctuation would render these meaningless in terms of local salary. Clinical staff employed by the trial will, at the start, receive approximately 10% more than the equivalent grade working for the Ministry of Health. Adjustments to salaries will be made at least annually to take into account currency fluctuation and changes in local wage scales. Nursing staff are recruited preferentially from the retired population to ensure that Ministry of Health resources are not compromised. Fewer nurses will be required than were employed in the steroid trial, as new patients will now be assessed on the medical wards rather than in a geographically distinct admissions unit. There will therefore now only be two areas (male and female wards) in which meningitis patients will require cover by trial staff.
Laboratory and consumable costs are likely to be lower than those incurred during the steroid trial as the proposed study will employ antibiotic therapy as per the national guidelines (rather than ceftriaxone) and will only process a second CSF if clinically indicated.
The immediate availability of items purchased for use in the steroid trial will considerably reduce the cost of trial establishment and equipment.
It is requested that the Liverpool School of Tropical Medicine and the College of Medicine, Malawi jointly administer any new award arising from this application.

This arrangement has proven a reliable, effective and secure fund management mechanism during the steroid trial.

Data collection

Historical data
Demographics
Presentation including duration of symptoms
Prior medication and consultation for current illness
Past medical history including AIDS defining conditions and neurological deficits.
Drug history and social history

Physical examination
Glasgow coma score
Focal neurological deficits
Hearing assessment where possible
Ophthalmoscopy
Co-existing physical signs
Physical examination to be repeated daily
Formal neurological examination to be repeated at day 10 and at day 40

Investigations

On admission:
Full blood count (FBC) and random blood sugar
Urea and electrolytes (U&E), liver function tests (LFT's)
Blood culture
CSF opening pressure on admission
CSF microscopy, culture, sensitivity and protein and glucose estimation
CSF cryptococcal antigen testing

During treatment:
Daily random blood sugar
U&E and LFT's on day 4 and as clinically indicated thereafter
CSF opening pressure on day 4
CSF microscopy, culture and sensitivity on day 4 if clinically indicated

Patients capable of giving valid consent on completion of therapy will be pre-counselled for HIV testing so that HIV positivity can be controlled for in the trial analysis. Those giving consent will be tested and be given post-test counselling with their HIV test result as per WHO PROTEST guidelines. Where a patient accepted testing but actively insisted they did not want to know the results their wishes will be respected. Stored, anonymised samples may be submitted for further microbiological and biochemical analysis relevant to the understanding of the pathophysiology and treatment of bacterial meningitis.

Neurological deficit of survivors at discharge and at one month will be assessed by audiometry and by Glasgow Outcome Score. A fixed transport fee for the patient

and a guardian on attendance will facilitate one-month follow up appointments. Defaulters will be visited at home within two weeks of their missed appointments wherever possible.

Endpoints.

Phase I

Tolerability of glycerol adjuvant therapy and serious adverse events

Phase II

Primary endpoint
1) Death.
Secondary endpoints
1) Physician decision to alter treatment based on the occurrence of complications
2) Death or residual neurological deficit (Glasgow Outcome Score and hearing loss) at discharge, and one month after completing antibiotic therapy.
3) Time to death, time to discharge

Analysis

Phase I

Data monitoring and ethics committee to review all cases and provide informed decision as to dosages to be employed in phase II

Phase II

Essential data will be double entered onto a computer database. Analysis will be performed on an intention-to-treat basis. A further analysis will be performed after excluding those who do not have bacterial meningitis, according to the following pre-defined laboratory criteria:

- a patient entered only on the basis of a cloudy CSF where white cells are less than 10 cells/μl
- *or* the red cell: white cell ratio >500:1.
- *or* cryptococcus or mycobacterial species identified without another organism being isolated.

At endpoint, uncorrected odds ratios will be calculated, as well as odds ratios corrected for potential confounding factors by logistic regression. Baseline prognostic features will be compared between the different groups. Analysis will be performed with the STATA or SPSS statistical package.

Ethical considerations.
All patients will sign consent having had written and verbal explanations of the trial. Where patients are deemed to be incapable of giving valid informed consent next-of-kin or guardians will be asked to give consent on their behalf.

Patients will be offered pre- and post- test counselling for HIV tests in accordance with local guidelines (see previous sections for details). The justification for requesting HIV testing is that HIV infection, and AIDS (which for WHO case

definition requires an HIV test) is an important potential confounding factor which will need to be controlled for in the analysis of those who consent. It is helpful to know HIV status in critically ill patients, and therefore testing should benefit individuals who consent to testing when unexpected clinical complications arise.

The treatment being investigated is appropriate in the Malawian context and, if proved effective, could be deployed locally.

Possible constraints.
The major foreseeable constraint would be if the mortality from meningitis drops significantly due to unrelated advances in management. This would reduce the power of the study and possibly therefore extend the time frame.

6. Detailed justification for support:

Personnel

Nurses: To provide nursing cover for the trial participants at all times it is estimated that 6 nurses must be employed, after allowing for sick leave and other periods of absence. The adult medical wards cannot support this trial without maintaining the standing force of research nurses. The trial, as designed, is a net contributor to staff levels rather than any drain on resource.

Clinical officers: The clinical officers will coordinate patient recruitment and ensure optimal clinical care, data and sample collection and outpatient follow-up. Together with the PI, the clinical officers provide nightly and week-end cover for new recruits and medical emergencies. Experience from the steroid trial currently underway demonstrates that this is not sustainable with a core staff of less than three clinicians with prescribing rights, knowledge of the trial and experience in the management of acutely ill patients with bacterial meningitis.

Technical staff: Data entry, daily logistic and administrative aspects of the study require a committed full time research assistant. The laboratory work generated by the trial cannot be accommodated by the hospital laboratory service. The Laboratory technician employed will use the facilities at the Wellcome Trust laboratories, which are fully equipped to deal with the requirements of this trial. There is a significant workload generated by such a trial including the processing of CSF's and blood cultures, separating and storing specimens and maintaining laboratory equipment used by the trial.

Messenger: The messenger will be required to facilitate patient recruitment, to transport patients to and from the wards, to deliver urgent specimens to the laboratory and to ensure the timely availability of results. He will be responsible for organising defaulter tracing and for maintaining ward stocks of consumables and equipment.

Equipment and consumables

No large equipment purchase will be necessary, as the ongoing steroid trial has

provided for these. Items such as ward furniture, filing cabinets, audiometers, refrigerators (but not freezers), glucometers, sphygmomanometers and computer hardware will be available. A fully established follow-up clinic is currently operational and will continue to be available for the proposed trial.
The provision for ward and laboratory consumables will ensure a continuous and reliable supply of items necessary for the investigation and management of patients with bacterial meningitis. We have been fortunate in the past to receive intermittent donations of ward equipment which, although unpredictable, have helped considerably in reducing expenditure.

Transport

Patients: Provision must be made to cover patient transport for effective follow up. Patients who attend for their one-month follow up appointment will therefore receive a fixed fee to ensure maximal compliance. In the case of those who default from follow up appointments, a hospital car, borrowed car or hire car will be used to trace the patients to their homes. Such mechanisms have proven highly successful in ensuring completeness of follow-up data in the steroid trial.

Staff: Out-of-hours staff transport costs will be minimised by the use of an on-call room at the hospital. Costs associated with international travel will be avoided by ensured the deployment of a trial monitor and steering committee chairman resident within Malawi.

Exceptional items:
Sample handling, storage and transport are arranged through the Wellcome Trust laboratories. Insufficient samples will be generated by this trial to justify the purchase of a –80 freezer or a dry shipper.
The use of telephones is essential for on-call commitments and for defaulter tracing activities. Small two-way radio sets have been used most effectively in the steroid trial in order to communicate information between colleagues within the hospital. The sets currently in use are unlikely to provide a reliable service for another three years.
Dissemination costs include the teaching materials, travel and subsistence for three local meningitis workshops each year. This is necessary to ensure that the findings of the trial are fed back to the clinicians who are mainly responsible for the delivery of care to patients with meningitis. Although we acknowledge that The Foundation will not normally consider fees or travel associated with conference attendances, the trial falls outside the jurisdiction of any organisation capable of meeting such costs. In order to fulfil the scientific and ethical obligation of appropriate dissemination of results, we request that consideration be given to this item should the need arise.
The College of Medicine in Malawi raises a 10% levy on all research conducted locally. The funds are used to cover incidental costs incurred by the College that are otherwise not recoverable. This levy has been the subject of recent debate and is currently being audited. Ethical approval for all research activities is dependent upon provision for this payment.

Refs:
1. Glycerol for acute stroke (Cochrane Review)

Righetti E, Celani MG, Cantisani T, Sterzi R, Boysen G, Ricci S
The Cochrane Library, Issue 3, 2004

2. Oral glycerol and intravenous dexamethasone in preventing neurologic and audiologic sequelae of childhood bacterial meningitis. The Finnish Study Group
Kilpi T, Peltola H, Jauhiainen T, Kallio,M.J
Pediatr.Infect.Dis.J., 14 (4); 270-278, 1995

3. Joint FAO/WHO Expert Committee on Food Additives, Geneva.
WHO Technical Report Series No. 599, 1976

4 Reversible Neurologic manifestation after glycerol: a short report.
Singh R, Lehl SS, Sachdev A, Sood A, Malhotra HS
Neurology India 49 (3); 320 – 321, 2001

5 Dexamethasone in adults with bacterial meningitis
de Gans,J.; van de,Beek D et al.
NEJM 347 (20); 1549-1556, 2112

6. Dexamethasone treatment in childhood bacterial meningitis in Malawi.
Molyneux E, Walsh A, Forsyth H, Tembo M, Mwenechanya J, Kayira K, Bwanaisa L, Njobvu A, Rogerson S, Malenga G.
Lancet 360 (9328); 211-21, 2002

14. Details of support requested broken down by project year (summarised in Section 7)

Details of posts by name	Grade	Start Point	Incre-mental date	Start Salary	Londo n weight	Other	Superan and Nat Ins	Total costs Yr 1
Research staff 1 Principal Investigator		N/A	N/A	N/A	N/A	N/A	N/A	N/A
2 Clinical Officer 1	CO		1.4.06	2200	N/A	N/A	N/A	2200
3 Clinical Officer 2	CO		1.4.06	2200	N/A	N/A	N/A	2200
Technical staff 1 Laboratory technician			1.4.06	2200	N/A	N/A	N/A	2200
(MLSO) 2 Research assistant			1.4.06	900	N/A	N/A	N/A	900
Other staff 1 Nurses (6)	H		1.4.06	10800	N/A	N/A	N/A	10800
2 Messenger			1.4.06	900	N/A	N/A	N/A	900
Annual Costs of above posts	Effort on project per cent months		Year 1	Year 2	Year 3	Year 4	Year 5	Total
Research staff 1 Principal Investigator	% 40	months 24	N/A	N/A	N/A			
2 Clinical Officer 1	100	24	2200	2200	2200			
3 Clinical Officer 2	100	24	2200	2200	2200			
Technical staff 1 Laboratory technician	% 100	months 24	2200	2200	2200			
(MLSO) 2 Research assistant	100	24	900	900	900			
Other staff 1 Nurses (6)	% 100	months 24	10800	10800	10800			
2 Messenger	100	24	900	900	900			
Grand Total:			**19200**	**19200**	**19200**			**57600**

14. Continued

Consumables please specify	Year 1 £	Year 2 £	Year 3 £	Year 4 £	Year 5 £	Total £
Animals - purchase 1) Intended source of supply 2) Species and microbiological quality required 3) Number required 4) Purchase price per animal Animals - maintenance						
Subtotal annual costs £						
Consumables etc. (continued) Please specify	Year 1 £	Year 2 £	Year 3 £	Year 4 £	Year 5 £	Total £
Laboratory costs (haematology, microbiology and biochemistry)	3900	3900	3350			11150
Ward consumables (Syringes, needles, gloves, dressings etc)	2600	2600	2200			7400
Glycerol	700	700	350			1750
Sundries (Bank charges, customs clearance, phones, etc)	200	200	200			600
Total Annual Costs	7400	7400	6100			20900

14. Continued

Travel and subsistence, destination and purpose	Number of journeys/ days	Mode of transport	Fare/ milage	Subsistence	Fees	Total £
Within the UK Overseas Patient follow up Defaulter tracing	N/A 240 journeys 140 journeys	 Public transport Hospital vehicle	 2.50 per /patient 5.00 per patient			 600 700
Total Annual Costs £	Year 1 460	Year 2 460	Year 3 380	Year 4	Year 5	1300

The Foundation will not normally meet costs associated with travel to conferences. See Guidance.

Exceptional Items						Total
Sample storage, handling and transport						900
Phones and two way radios for on-call commitments						270
Dissemination costs including meningitis workshops and conference fees.						1200
10% administrative levy payable to the College of Medicine						8220
Total Annual Costs	Year 1 3310	Year 2 3340	Year 3 3940	Year 4	Year 5	 10590

14. Continued

Equipment Description of items and country of manufacture	Expiry date of quotation	Likely Delivery Date	Basic Price £	Import Duty	Total £
1 Refrigerators 2 Ward furniture and storage 3 Filing cabinets 4 Glucometers 5 Sphymomanoteters 6 Audiometers 7	Items in place from steroid trial				
Annual cost of above items	Year 1	Year 2	Year 3	Year 4	Year 5
1 2 3 4 5 6 7					
Total Annual Costs					

Medical / scientific equipment purchased with charitable funds, when donated to designated non-profit making (hospital or research) institutions to be used for medical research is zero rated for VAT. VAT should therefore be excluded when applying for equipment costs from the UK and Republic of Ireland.

15. Curriculum Vitae of applicant (please complete a copy of this page for each applicant and for any named staff for whom salary support is requested in section 14)

Name: Matthew Scarborough

Age and Date of birth: 41; 25.12.1962

Degree etc. (subject, class, university, and date):
BSc Anatomy. 1st Class. Queen's University, Belfast. 1988
MB, BCh, BAO. Distinction. Queen's University, Belfast. 1990
MRCP. Royal College of Physicians, London. 1998
Diploma in Tropical Medicine and Hygiene. University of Liverpool 1998

Posts held (with dates):
Lecturer in Medicine, University of Malawi. November 2001 – December 2004
Specialist Registrar in Internal Medicine, Oxford Deanery. October 1999 – September 2001
Specialist Registrar in Infectious Diseases, London. March 1998 – September 1999

Do you have assured salary support for the period of the grant?

Yes

Do you have links (eg. consultancies, equity holdings) to companies or other organisations that are relevant in any way to the current proposal? **No**

If yes, please give details.

Recent Publications; also papers in press:

Bacterial meningitis in sub-Saharan Africa; challenges to a better outcome
M Scarborough, Y Njalale
Tropical Doctor (In press)

Clinical Features and Outcome of Cryptococcal Meningitis in Zambia
Postgraduate Medical Journal. 2001 Dec;77(914):769-73.
P Mwaba, J Pobee, M Scarborough, S Portsmouth, A Zumla

Varicella Zoster Virus associated neurological disease in HIV-Infected patients
Int J STD & AIDS *2001; 12: 79 - 83*
M Brown, M Scarborough, N Brink, H Manji, R Miller

Lymphocytic Interstitial Pneumonitis in an HIV infected adult: response to antiretroviral therapy.
Int J STD & AIDS *2000; 11: 119-122*
M Scarborough, S Lishman, P Shaw, A Fakoya, R F Miller

15. Curriculum Vitae of applicant (please complete a copy of this page for each applicant and for any named staff for whom salary support is requested in section 14)

Name: Yasin Njalale

Age and Date of birth: 24; 18.7.1980

Degree etc. (subject, class, university, and date):
Diploma in Clinical Medicine, Malamulo College of Health Sciences, Malawi 2001

Posts held (with dates):
Intern Clinical Officer, QECH. April 2002 – March 2003
Research Clinical Officer, College of Medicine, Malawi. April 2002 – present
Honorary Registrar, Department of Medicine, QECH. March 2004 - present

Do you have assured salary support for the period of the grant?

Salary applied for in current proposal

Do you have links (eg. consultancies, equity holdings) to companies or other organisations that are relevant in any way to the current proposal? **No**

If yes, please give details.

Recent Publications; also papers in press:

Bacterial meningitis in Sub-Saharan Africa – challenges to a better outcome.
M. Scarborough, Y. Njalale
Tropical Doctor (In press)

Application of the Vietnam diagnostic scoring system for TB meningitis to patients in the Queen Elizabeth Central Hospital, Blantyre.
A.Checkley, Y.Njalale, M.Scarborough, E.E.Zijlstra
College of Medicine Research Dissemination Conference, Blantyre. November 2003

15. Curriculum Vitae of applicant (please complete a copy of this page for each applicant and for any named staff for whom salary support is requested in section 14)

Name: Eduard Evert Zijlstra

Age and date of birth: 50 years; 19 April 1954

Degree etc. (subject, class, university, and date):

- Medical licensure ("artsexamen"), Medical Faculty Rotterdam, 27 July 1979
- Registered as internist, University Hospital Rotterdam, 1 April 1986
- MSc Clin Trop Med, London School of Hygiene and Tropical Medicine, 19 October 1988
- DTM&H, London School of Hygiene and Tropical Medicine, 14 July 1988
- PhD, University of Amsterdam, 2 February 1995
- MRCP (UK), 14 July 1989; FRCP (UK), 1 May 2002
- MRCPath, 27 June 2002

Posts held (with dates):

- Academic Specialist:, University Hospital Rotterdam, March 1986 - September 1987, and January 1989 - July 1989.
- Programme Coordinator:Kala-azar programme in the Sudan, Médecins sans Frontières - Holland, July 1989 - October 1991.
- University Lecturer/ Academic Specialist, Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, University of Amsterdam, November 1991 - March 1995.
- Assistant Professor, Institute of Endemic Diseases, University of Khartoum, Sudan, April 1995 to August 1998.
- Associate Professor of Medicine, College of Medicine, University of Malawi, August 1998 – October 2003. Head of the Department of Medicine from August 1999.
- Professor of Medicine, College of Medicine, University of Malawi, 31 October 2003.

Do you have assured salary support for the period of the grant?

Yes

If neither of the above is true, please explain the circumstances below.

Do you have links (eg. consultancies, equity holdings) to companies or other organisations that are relevant in any way to the current proposal? No

If yes, please give details.

Recent Publications; also papers in press:

James J, Hofland HWC, Borgstein ES, Kumiponjera D, Komolafe OO, Zijlstra EE. The prevalence of HIV infection among burn patients in a burns unit in Malawi and its influence on outcome.
Burns 2003; 29: 55-60.

Zijlstra EE, Musa AM, Khalil EAG, El Hassan IM, El-Hassan AM.
Post-kala-azar dermal leishmaniasis (Review).
Lancet Infectious Diseases 2003; 3: 87-98.

Lewis D, Joaki G, Peters R, Schijffelen M, Walsh A, Kublin J, Kumwenda J, Kampondeni S, Molyneux M, Zijlstra E.
Mycobacteraemia in adults admitted to hospital in Blantyre, Malawi.
International Journal for Tuberculosis and Lung Disease 2002; 6: 1067-74.

Peters RPH, Zijlstra EE, Schijffelen MJ, Walsh AL, Joaki G, Kumwenda JJ, Kublin JG, Molyneux ME, Lewis DK.
The importance of bloodstream infections as cause of fever in a population with high HIV prevalence in Blantyre, Malawi -- identification of clinical predictors and implications for management: a prospective study.
Tropical Medicine & International Health, 2004; 9: 928-934.

15. Curriculum Vitae of applicant (please complete a copy of this page for each applicant and for any named staff for whom salary support is requested in section 14)

Name: Katherine Mary Brzechwa Ajdukiewicz

Age and Date of birth: 31, 23 August 1973

Degree etc. (subject, class, university, and date):
MBChB Dundee 1997, MRCP (UK) 2000

Posts held (with dates):
August 2002 – present Specialist Registrar Infectious Disease/Medicine, Newcastle upon Tyne
August 2001- July 2002 Specialist Registrar (LAT) ID/Medicine, Dundee
August 2000 – July 2001 SHO Infectious disease/Microbiology, Newcastle upon Tyne
August 1998 – July 2000 SHO Medicine, Gateshead

Do you have assured salary support for the period of the grant?

YES Salary applied for in current proposal ☐

If neither of the above is true, please explain the circumstances below.

Do you have links (eg. consultancies, equity holdings) to companies or other organisations that are relevant in any way to the current proposal? **NO**

If yes, please give details.

Recent Publications; also papers in press:

Cost-minimization analysis and audit of antibiotic management of bone and joint infections with ambulatory teicoplanin, in-patient care or outpatient oral linezolid therapy.
D Nathwani, G Barlow, K Ajdukiewicz, K Gray, J Morrison, B Cleft, A France, P Davey
Journal of Antimicrobial Chemotherapy 51; 391-396: 2003

An African HIV cohort in a provincial infectious diseases unit
H Hadi, J Smith, K Ajdukiewicz, M Schmid, E Ong, M Snow
Federation of Infection Societies 2003

Differences in adult and paediatric meningococcal sero-group and mortality in Northern England
M Schmid, K Ajdukiewicz, B Fultton, T Flood, D Price, H Thaker, M Snow, E Ong, V Hollyoak
Federation of Infection Societies 2002

Outcome of meningococcal disease in adults and children within Northern NHS region 1998-2000
Federation of Infection Societies 2001

16. Experiments involving animals

Applicants must have regard to animal welfare and advances in the refinement, replacement and reduction of animal use. Meningitis Research Foundation will not support research involving live animals unless there is no alternative, and it is essential to the outcome of the research. If the proposed research project

involves research on live animals, their use must be minimised and optimised. The number of animals requested must be fully justified. Meningitis Research Foundation emphasises the importance of refining procedures to minimise any pain or distress caused.

Please refer to the Foundation's policy on the use of live animals before answering these questions:

16.1 Do the experiments you propose involve the use of protected animals in regulated procedures under the Animals (Scientific Procedures) Act 1986? (For the information of non-UK applicants, this includes all vertebrates as well as octopus.) **NO**

If yes, which species and how many animals?

Are any of the procedures of substantial severity? **Yes** ☐ **No** ☐

16.2 Has a project licence, under the terms of the Animals (Scientific Procedures) Act 1986, been granted which authorises the proposed experiments? Yes ☐ **No** ☐

If yes, please state the name and address of the licensee, Home Office reference and date of issue and attach a copy of the front page of the project licence.

If not, has it been applied for? **Yes** ☐ **No** ☐

Does each individual carrying out work on animals have a personal licence?
Yes ☐ **No** ☐

If you are applying from outside the UK, please explain what efforts have been made to comply with local procedures regulating the use of animals in scientific experiments.

16.3 Have all those involved in the care and use of animals before, during and after the experiments, received appropriate training in animal care and in the procedures involved? Has this training included attendance at the relevant courses?

16.4 Does your institution have an Ethics (or Animal Care and Use) Committee for animal experiments? If so, have the proposed experiments received its approval?

If not, what steps have been taken to gain its approval?

If no such committee exists, what alternative measures have you taken?

16.5 Will the animals be conscious for all or part of the experiments? If so, explain why this is necessary, what, if any, discomfort they are likely to experience and how it is ameliorated.

16.6 If the animals are to be anaesthetized, will they be allowed to regain consciousness? Unless the animals are to be the subject of survival studies, explain why this is being allowed.

16.7 Have the appropriate power calculations been performed to determine the number of animals required? If so, please give the calculations.

16.8 Does the proposed experimentation on live animals duplicate any other research which has already taken place, or which is known to be currently taking place in any research establishment?

16.9 Will you be engaging any other establishment to carry out experiments on live animals as part of this research project? If so, please provide full details.

17. Scientific Integrity

The Foundation expects the highest standards of integrity to be adhered to by researchers whom it funds. Institutions in the UK and Republic of Ireland are required to have in place their own published standards of good research practice and formal written procedures for the investigation of allegations of scientific misconduct. These must comply with the Association of Medical Research Charities' Guidelines on Good Research Practice (**http://www.amrc.org.uk/aboutus/Good Research Practice.PDF**).

Please attach a copy of your Institution's policy on good research practice/scientific integrity. If you are applying from outside the UK and Republic of Ireland, please outline your institution's procedure on a separate sheet or attach a copy of the relevant document from your institution (in English).

18. Dissemination

The Foundation has a responsibility to ensure that all useful knowledge acquired from research it funds is disseminated to the public and to others able to utilise or benefit from it. Annual and final reports are required which specifically indicate the relevance of research progress and outcomes to meningitis and associated infections. Grantholders are expected to seek publication of findings in peer reviewed journals as soon as possible, even where results prove negative. The Foundation must be notified in advance of publication, and acknowledged in all publications and presentations arising from research it funds.

Please outline proposed arrangements for dissemination.

The results of the ongoing or completed parts of the trial will be presented at the College of Medicine Research Dissemination Conference in Blantyre, Malawi in November 2005, 2006 and following completion of the trial. We are currently organising three regional Meningitis workshops annually in Malawi to discuss research findings and medical updates in meningitis in each of the county's three main regional hospitals. In addition, the findings will be published in the Malawi Medical Journal and more widely in the

international medical press and at international conferences where appropriate.

19. Monitoring and Evaluation

The Foundation's Trustees have a responsibility to ensure that work of the highest quality is produced. The Foundation therefore requires that the Research Institution ensures that all funded work is adequately supervised at all times, monitored and evaluated. The results of the research must be subject to proper evaluation before they are published.

Please indicate what arrangements are in place for monitoring and evaluation.

The College of Medicine Research and Ethics Committee requires quarterly reports and an annual presentation on all ongoing research activities within its remit. This is attended by academics, clinicians, lay representatives and medical ethicists appointed by the College to ensure that research is of a high quality and that it conforms to the Universities research code of conduct.

An experienced trialist and senior scientist in Malawi will serve as trial monitor and will be asked to visit the trial and review the data every three months. The trial monitor will review the standard operating procedures prior to recruitment and have open access to all patient data throughout the recruitment period. Any possible or probable adverse events will be recorded and reported to a senior physician unconnected with the trial who will decide whether the trial monitor should be asked to review the case immediately.

The steering committee will provide overall supervision of the trial, provide advice to the investigators, host institution and the DMEC on matters relating to the conduct of the trial, notify the investigators of emerging evidence from other trials which may be relevant, and ensure adherence to the study protocol and MRC guidelines for clinical trials. It will oversee the progress of the trial and serve to protect the interests of the trial participants and sponsors.

The analysis plan and the evaluation of final results will be reviewed by the trial steering committee in order to ensure that the results truly represent the findings from the study.

Trial Monitor: Professor Anthony Harries, Foundation Professor of Medicine, University of Malawi

Steering Committee Chairman: Dr. David Lalloo, Clinical Director, Liverpool School of Tropical Medicine

20. Collaboration on a research project

Any individuals named as collaborators (NOT as applicants) in this research application should complete a copy of this form.

Name of principal applicant:

Principal applicant's department and institution:

Name of collaborator:

Full address of collaborator, including department and institution:

Title of research grant application:

Extent and nature of collaboration:

I confirm that I am willing to collaborate as stated above on this research project:

Signed:

Appendix 2. Research Grant additional funding application 2006

Costings

7a) Summary of support requested:

	Project Year 1 £	Project Year 2 £	Project Year 3 £	Project Year 4 £	Project Year 5 £	Total £
Staff	23800	25750	28000			
Consumables	21400	21400	21400			
Travel and Subsistence	460	460	460			
Exceptional items	9120	900	2400			
Equipment						
Grand Total	54780	48510	52260			**155550**

b) Breakdown of costs by financial year (1 April to 31 March):

	Financial Year 1 £	Financial Year 2 £	Financial Year 3 £	Financial Year 4 £	Financial Year 5 £	Financial Year 6* £	Total £
Staff	23800	25750	28000				77550
Consumables	21400	21400	21400				64200
Travel and Subsistence	460	460	460				1380
Exceptional items	9120	900	2400				12420
Equipment							
Grand Total	54780	48510	52260				**155550**

14. Details of support requested broken down by project year (summarised in Section 7)

Details of posts by name	Grade	Start Point	Incremental date	Start Salary	London weight	Other	Superan and Nat Ins	Total costs Yr 1
Research staff								
Principal Investigator		N/A	N/A	N/A	N/A	N/A	N/A	
Clinical Officer 1								
Clinical Officer 2	CO		1.3.7	2700	N/A	N/A	N/A	
	CO		1.3.7	2700	N/A	N/A	N/A	
Technical staff								
Laboratory technician (MLSO)			1.3.7	3000	N/A	N/A	N/A	
Research assistant			1.3.7	1200	N/A	N/A	N/A	
Other staff								
Nurses (5)	H		1.3.7	10500	N/A	N/A	N/A	
Principal nurse	I		1.3.7	2500	N/A	N/A	N/A	
Messenger			1.3.7	1200	N/A	N/A	N/A	
Annual Costs of above posts	Effort on project per Cent months		Year 1	Year 2	Year 3	Year 4	Year 5	Total
Research staff	%	months						
Principal Investigator	40	36	N/A	N/A	N/A			
Clinical Officer								
Clinical Officer	100	36	2700	3000	3300			
	100	36	2700	3000	3300			
Technical staff	%	months						
Laboratory technician (MLSO)	100	36	3000	3300	3600			
Research assistant	100	36	1200	1350	1400			
Other staff	%	months						
Nurses (5)	100	36	10500	11100	12000			
Principal nurse	100	36	2500	2700	3000			
2 Messenger	100	36	1200	1300	1400			
Grand Total:			23800	25750	28000			**77550**

14. Continued

Consumables please specify	Year 1 £	Year 2 £	Year 3 £	Year 4 £	Year 5 £	Total £
Animals - purchase 1) Intended source of supply 2) Species and microbiological quality required 3) Number required 4) Purchase price per animal 5) Animals - maintenance						
Subtotal annual costs £						
Consumables etc. (continued) Please specify	Year 1 £	Year 2 £	Year 3 £	Year 4 £	Year 5 £	Total £
Laboratory costs (haematology, microbiology and biochemistry)	4000	4000	4000			
Ward consumables (Syringes, needles, gloves, dressings etc)	5700	5700	5700			
BM Stix	3000	3000	3000			
Ceftriaxone	7700	7700	7700			
Glycerol	700	700	700			
Sundries (Bank charges, customs clearance, phones, etc)	300	300	300			
Total Annual Costs	21400	21400	21400			**64200**

14. Continued

Travel and subsistence, destination and purpose	Number of journeys/ days	Mode of Transport	Fare/ mileage	Subsistence	Fees	Total £
Within the UK Overseas Patient follow up Defaulter tracing	N/A 240 journeys 140 journeys	 Public transport Hospital/PI 's vehicle	 2.50 per /patient 5.00 per patient			 600 700
Total Annual Costs £	Year 1 460	Year 2 460	Year 3 460	Year 4	Year 5	**1380**

The Foundation will not normally meet costs associated with travel to conferences. See Guidance.

Exceptional Items						Total
Sample storage, handling and transport						2500
Phones and two way radios for on-call commitments						500
Dissemination costs including meningitis workshops and conference fees.						1200
10% administrative levy payable to the College of Medicine						8220
Total Annual Costs	Year 1 9120	Year 2 900	Year 3 2400	Year 4	Year 5	**12420**

14. Continued

Equipment Description of items and country of manufacture	Expiry date of quotation	Likely Delivery Date	Basic Price £	Import Duty	Total £
1 Refrigerators 2 Ward furniture and storage 3 Filing cabinets 4 Sphymomanoteters 5 Audiometers	Items in place from steroid trial On loan from UK physician				
Annual cost of above items	Year 1	Year 2	Year 3	Year 4	Year 5
1 2 3 4 5 6 7					
Total Annual Costs					

Appendix 3. Proforma

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

QECH Glycerol Trial ISRCTN70121840 Phase 2: Version 6
- 1 -

1st Name ..... **2nd Name**.....

Front sheet

Admission date: / / admdate
Admission time: admtime

Sex: **Male / Female**
Age: (exclude if <16yrs)
DOB: / /

Into each of the boxes below insert a number: YES=1; NO=0; DON'T KNOW=9
Into each of the circles (○) place a tick when indicated
Write an explanation/description on lines.

Inclusion criteria

CSF criteria at recruitment (one or more of the following):

Cloudy / hazy CSF in a patient requiring immediate treatment.....	☐	cloudy
>100 white cells/µl of CSF, >50% neutrophils.....	☐	wcc100
Gram-stain showing bacteria in CSF.....	☐	orgs

AND Clinical criteria (1 or more of the following)

Headache.....	☐	headache
Neck pain/stiffness.....	☐	neckpain
↓consciousness.....	☐	rloc
Photophobia.....	☐	photobia
Confusion.....	☐	conf
Fits.....	☐	fits
Rash.....	☐	rash
Fever.....	☐	fever

Exclusion criteria

Pregnancy...LMP:_____/ postmenopausal / no sexual contact /
pregnancy test done / other_____.....

Age <16yrs.....	☐
BM>20mmol/L.....	☐
Type 2 Diabetes.....	☐
Heart failure.....	☐

In order to recruit, the answer must be "NO" to all exclusion criteria.

Is the patient suitable for enrolment? ..... ☐
(at least 1 clinical and 1 CSF criteria; no exclusion criteria)

Consent obtained..... ☐

Randomization Date: / / randdate
Randomization Time: randtime
(i.e. point at which trial number allocated)

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**.....

Admission samples: **Date: / / Time:**

CSF 1: O 4ml CSF for Microbiology (WT)

Whole blood: O 4ml in Plain tube for U&Es, LFTs (QECH)

(20ml minimum) O 2ml in EDTA for FBC (QECH or WT if QECH unable to do)

O MPs (QECH)

O 1ml EDTA for HIV test (Jimmy, Tiyanjane)

O 8ml Blood culture (WT)

O 4ml in EDTA for storage* (WT)

O Random blood glucose using BM sticks

O Other.....

For patients recruited over the weekend/ on holidays: place blood for HIV test in the fridge on 4A back bay. Samples can be taken to Jimmy on the next working day.

Who has taken blood admission samples? (write your name here): _____

***Label admission sample like this 'GLAM XXX A' where 'X' is the trial number of the patient**

Outcome

Completed 10 days IV antibiotics .....Date / / ..... ☐ complete

Number of doses given: Ceftriaxone..... _____ dosecfr

Cefotaxime..... _____ dosecxm

Benzylpenicillin..... _____ dosepen

Chloramphenicol..... _____ dosechlor

Other_____

If <10days, reason for stopping: cryptococcal / TBM / died / other_____ stopabx

Absconded..... Date: / / ..... ☐ absc

Discharge..... Date: / / ..... ☐ disch

Died.....Date: / / ..... ☐ died

Glycerol/placebo discontinued..... ...Date: / / ..... ☐ glycstop

Reason: cryptococcal / TBM / other_____

Total number of doses glycerol/placebo given: _____ numbgly

Record complete.....Date / /

If incomplete, details 1) _____ O (Tick when rectified)

2) _____ O

3) _____ O

1st Name ..... 2nd Name.....Contact details sheet

Contact details

Residential address.

Residential location:

Residential Tel:

(Please record precise details and directions below or overleaf)

Personal contact 1	Personal contact 2
Name:	Name:
Relation:	Relation:
Address:	Address:
Tel:	Tel:
Location:	Location:

Continue over the page if necessary

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... 2nd Name..... Date: / /
Time:

Source of history: Patient / Spouse / Parent / Sibling / Friend / Other_____

P/C and duration

Headache _____ hours / days	☐	headache
Neck stiffness_____ hours / days	☐	neck
Confusion_____ hours / days	☐	confn
Fever_____ hours / days	☐	fevr
Photophobia_____ hours / days	☐	photo
↓consciousness_____ hours / days	☐	redloc
Fits_____ hours / days	☐	fitshpc
Rash_____ hours / days	☐	rashhpc
Ear ache or pain_____days.....	☐	earache
Night sweats_____ days	☐	nitswts
Cough_____ days	☐	cough
SOB_____ days	☐	sob
Chest pain_____ days	☐	cpain
Abdo pain_____ days	☐	apain
Diarrhoea_____ days	☐	diarr
Vomiting_____ days	☐	vomit
Frequency_____ days	☐	
Dysuria_____ days	☐	

Other_____
Other_____
Other_____

HPC

Date of onset: / / Time of onset:
Duration of illness:_____days;_____hrs
Symptoms / Events.. _____

TRIAL NO:_____

1st Name ..... 2nd Name..... Date: / /
Drug history Time:

Have antibiotics been taken during this illness before admission?..... ☐ preabx
Which ones?_____ abxwhich
For how long in days?_____ abxdurn
Start date?_____ abxtime
Were antimalarials taken for or during this illness?..... ☐ premala
SP / Quinine / other_____ malatype
Start date?_____ malatime
Have any other medications been taken in the past month?..... ☐ anytype
Which ones?_____ medtype
When?_____ medwhen
ARV start date_____/_____/_____ artstart
Was any treatment or advice obtained from a traditional healer for this illness? ..... ☐ tradtl
Details_____ tradinfo

Coexisting conditions

Acute diarrhoea ..... ☐ acutedi
Known epilepsy ..... ☐ epil
TB ..... ☐ tb
Ear pain or ache..... ☐ earp
Ear discharge ..... ☐ eardisch
HIV positive..... ☐ hiv
Pregnancy* ..... ☐ preg
Type 2 diabetes* ..... ☐ type2dm
Heart failure* ..... ☐ chf
Other_____ *if yes, do not administer glycerol

Has the patient had (before this admission) any:

Deafness L / R ..... Date / / ..... ☐ deafhr
Gaze palsy/squint..... Date / / ..... ☐ squint
Paralysis..... Date / / ..... ☐ paralys
Mental disorders..... Date / / ..... ☐ mental
Other _____ other

Functioning ability before this illness

4=Fully independent with no disability..... ☐ function
3=Disabled but independent, able to work..... ☐
2=Disabled: dependent on others for daily support..... ☐
1=Minimally responsive..... ☐

PHASE TWO ISRCTN70121840		TRIAL NO:__________

1st Name .... 2nd Name....................................... **Date: / /**
Time:

Past Medical History (PMH)

Meningitis (admitted).. ... . Date/s: ____________	☐	mgts
Shingles .... Date/s:____________.	☐	vzv
TB... Date/s:____________.....................	☐	tb
Pneumonia (admitted). .... Date/s:____________	☐	pneum
Oral candida..	☐	candoral
Oesophageal candidiasis	☐	candoes
Chronic diarrhoea>1/12.	☐	chronicd
Weight loss...	☐	wtloss
Kaposi's sarcoma..	☐	kspmh
Chronic fevers>1/12...	☐	chrtemp
Peripheral neuropathy..	☐	pn

Social History

Married (now)	☐
Divorce or separated (ever)	☐
Widowed (ever)	☐
Currently employed?..	☐
Any children?	☐

No. of children alive__________

No. of children died__________

Literate / Illiterate Education level reached:__________

Lives: Urban / Rural

1st Name ..... 2nd Name ..... Date: / /
Time:

O/E: Is the patient fully orientated in: Time ..... Y / N time
Place ..... Y / N place
Person ..... Y / N person

Eye opening:
O4=Spontaneous
O3=To speech
O2=To pain
O1=None

Verbal:
O5=Orientated speech
O4=Confused speech
O3=Inapprop; no conversation
O2=Incomprehensible
O1=None

Motor/ pain response:
O6=Obeys commands
O5=Localises
O4=Withdraws
O3=Flexes to pain
O2=Extends to pain
O1=No response

GCS total: gcs1 / 15

Wt: _____kg (estimated / measured) wt

Oxygen saturation (SpO2) _____% spo2

Neck stiffness ..... neckstif
Photophobia ..... phbiace
Kernig's +ve ..... kernig
Lymphadenopathy: ..... lnodes
If present: Cervical / Axillary / Inguinal
Oral thrush ..... thrush
KS_____ ..... ks
Fungal skin disease ..... fungal
Shingles scars ..... vzvscar
Oral hairy leukoplakia ..... ohl
Wasting ..... wasted

Heart sounds: Any abnormality .....
If abnormal, explain_____

Chest: Crackles_____ .....
Effusion_____ .....
Bronchial breathing_____ .....
Rhonchi_____ .....
Any other abnormality_____ .....

Abdomen:
Tenderness_____ .....
Hepatomegaly_____ .....
Splenomegaly_____cm .....
Ascites .....
Any other abnormality .....

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**..... **Date:** / / **Time:**

Neurological system:

PERLA OYes ONo – details_____ perla

Vision RIGHT	**Vision LEFT**	**Fundus RIGHT**	**Fundus LEFT**	visionr/l fundusr/l
O normal	O normal	Onormal	Onormal	
O counting fingers	O counting fingers	Opapilloedema	Opapilloedema	
O hand movement	O hand movement	Ounable to assess	Ounable to assess	
O not able to see light	O not able to see light	Oother_____	Oother_____	
O unable to assess	O unable to assess			

Optic nerve sheath diameter 1 **RIGHT / LEFT** _____mm optnve1

Eye movements
Any abnormality..... ☐ eyemove
If abnormal, indicate which: RIGHT III / IV / VI
LEFT III / IV / VI

Other cranial nerves - any abnormality of:

CN V –corneal ..... ☐ CNV
If abnormal, indicate: RIGHT / LEFT

CN VII ..... ☐ CNVII
If abnormal, indicate: RIGHT / LEFT

Gag reflex... (if unable to assess, write 9 – don't know) ..... ☐ Gag

CN XII ..... ☐ CNXII
If abnormal, indicate: RIGHT / LEFT

Any other abnormality_____..... ☐ otherCN

Hearing assessment **RIGHT**_____db **LEFT**_____db rightdb
[Circle minimum sound heard] leftdb
RIGHT Finger rub / Whisper / Speech / Shout / Totally deaf / Unable to assess
LEFT Finger rub / Whisper / Speech / Shout / Totally deaf / Unable to assess hearingr/l

1st Name ..... 2nd Name..... Date: / /
Time:

Neurological system cont'd:

Arms

	RIGHT	LEFT
Tone	↓ / Normal / ↑	↓ / Normal / ↑
Power	0 / 1 / 2 / 3 / 4 / 5 / unable to assess	0 / 1 / 2 / 3 / 4 / 5 / unable to assess
Reflexes	↓ / Normal / ↑	↓ / Normal / ↑

Legs

	RIGHT	LEFT
Tone	↓ / Normal / ↑	↓ / Normal / ↑
Power	0 / 1 / 2 / 3 / 4 / 5 / unable to assess	0 / 1 / 2 / 3 / 4 / 5 / unable to assess
Reflexes	↓ / Normal / ↑	↓ / Normal / ↑
Plantars	↓ / ↑ / equivocal	↓ / ↑ / equivocal

Summary of neurological findings: Normal / Abnormal

neuro

1st Name ..... 2nd Name..... **Investigations sheet A**

Investigations:

FBC Date: / / **Biochem Date:** / /

Hb_____g/dl	**Urea**_____mg/dl	Total prot_____g/dL
MCV_____fl	Creatinine_____mg/dl	Albumin_____g/dL
MCHC_____g/dl	Sodium_____mEq/l	**Tot Bili**_____mg/dL
WCC_____10^9/l	Potassium_____mEq/l	Direct Bil_____mg/dL
PLT_____10^9/l	Chloride_____mEq/l	Alk phos_____U/L
	RBS_____mmol/l	**AST**/SGOT_____U/L
MPs		**ALT**/SGPT_____U/L
Negative / + / ++ / +++ / ++++ mps		GGT_____U/L

HIV test

Positive (known on admission) / Positive (tested by us) / Negative / Not done hivtest

Post-test counselling: Signature: _____ date / /

Pregnancy test

Positive / Negative / Not done

Sputum AFB (if indicated)

Sputum 1 Positive / Negative / Not done afb1/2/3

Sputum 2 Positive / Negative / Not done

Sputum 3 Positive / Negative / Not done

CXR

Not done / Date of CXR: / /

Any abnormality? ..... ☐

If abnormal explain_____

Blood culture result (Lab No.....) bcgrewth

Pneumococcus..... O
Meningococcus..... O
H influenzae..... O
Group A Strep / Strep. pyogenes ..... O
Cryptococcus ..... O
Other_____..... O
No growth..... O

Sensitivities

Penicillin..... O
Chloramphenicol..... O
Tetracycline..... O
Co-trimoxazole..... O
Gentamicin ..... O
Erythromycin ..... O
Cefaclor ..... O
Ceftriaxone..... O
Cloxacillin..... O
Other_____ ..... O

1st Name ..... 2nd Name..... **Investigations sheet B**

CSF 1 (Lab No.....) **Date of LP: / / .**

Opening pressure_____cm op1

CSF appearance: Clear / Turbid or hazy / Bloodstained

WCC: _____mm^{-3} **Protein:** + / ++ / +++ / ++++ wcc1 / poly%1

%Polys_____% **Glucose:** Neg / Trace / + / ++ / +++ prot1 / gluc1

RBC: _____mm^{-3}

Gm stain: No organisms / Gm+ diplococci / Gm- diplococci / Gm- rods gmstain1

India Ink: Positive / Negative india1

CRAG (CSF): Positive / Negative / Not done crag1

CSF culture csfcult1

Pneumococcus ..... O
Meningococcus ..... O
H influenzae ..... O
Group A Strep / Strep. pyogenes ..... O
Cryptococcus ..... O
Other_____ ..... O
No growth ..... O

Sensitivities

Penicillin ..... O
Chloramphenicol ..... O
Tetracycline ..... O
Co-trimoxazole ..... O
Gentamicin ..... O
Erythromycin ..... O
Cefaclor ..... O
Ceftriaxone ..... O
Cloxacillin ..... O
Other_____ ..... O

PHASE ONE
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**..... **Investigations sheet C**

For most patients, there will be a second LP by consent after 2 complete days of treatment. Take samples in the morning to reach the lab by 10:30 (Mon-Fri), 11.30 (Sat & Sun).

Place a yellow sticker on the form, label CSF and EDTA blood 'GLAM XXX B' where X is the patient trial number, place these into a plastic specimen bag and take to the WT lab.

Second lumbar puncture O Consent obtained
O Not done _____
Clinical indication for LP_____

DAY 2 SAMPLES: **Date:** / / **Second LP/bloods:**

CSF 2: O 4ml for Microbiology (WT) **Time taken:**_____ lp2time

Whole blood: O 4ml EDTA blood (WT) **Time frozen:**_____ lp2freez
O Blood in plain tube for U&Es, LFTs (QECH)

Optic nerve sheath diameter 2 **RIGHT / LEFT** _____mm optnve2

CSF 2 (Lab No.....)

Opening pressure_____cm op2

CSF appearance: Clear / Turbid or hazy / Bloodstained

WCC: _____mm^{-3} **Protein:** + / ++ / +++ /++++ wcc2 / poly%2

%Polys_____% **Glucose:** Neg / Trace / + / ++ / +++ prot2 / gluc2

RBC: _____mm^{-3}

Gm stain: No organisms / Gm+ diplococci / Gm- diplococci / Gm- rods / not done gmstain2

India Ink: Positive / Negative india2

CRAG (CSF): Positive / Negative / Not done crag2

CSF culture csfcult2
Pneumococcus..... O
Meningococcus..... O
H influenzae..... O
Group A Strep / Strep. pyogenes ..... O
Cryptococcus ..... O
Other_____..... O
No growth..... O

Sensitivities
Penicillin..... O
Chloramphenicol..... O
Tetracycline..... O
Co-trimoxazole..... O
Gentamicin ..... O
Erythromycin ..... O
Cefaclor ..... O
Ceftriaxone..... O
Cloxacillin..... O
Other_____ ..... O

1st Name ..... 2nd Name..... **Investigations sheet D**

Date sample taken: / /

Urea_____mg/dl		Total prot_____g/dL	urea2
Creatinine _____mg/dl		Albumin_____g/dL	
Sodium_____mEq/l		Tot Bili_____mg/dL	totbil2
Potassium _____mEq/l		Direct Bil_____mg/dL	
Chloride_____mEq/l		Alk phos_____U/L	
		AST/SGOT_____U/L	ast2
RBS_____mmol/l		ALT/SGPT_____U/L	alt2
		GGT_____U/L	

1st Name ..... 2nd Name..... Day 10 Sheet
Date / /

Day 10 neurology summary

Deafness L / R ..... Date / / ..... ☐ Deafl/r10
Gaze palsy/squint..... Date / / ..... ☐ Squint10
Paralysis..... Date / / ..... ☐ Paraly10
Mental disorders..... Date / / ..... ☐ Mental10
Other _____ other10

Functioning ability Day 10

4=Fully independent with no disability..... ☐ functn10
3=Disabled but independent, able to work..... ☐
2=Disabled: dependent on others for daily support..... ☐
1=Minimally responsive..... ☐

Day 10 examination

Is the patient fully orientated in: Time..... Y / N time10
Place ..... Y / N place10
Person..... Y / N person10

PERLA OYes ONo – details_____ perla10

Vision RIGHT	Vision LEFT	Fundus RIGHT	Fundus LEFT	
Onormal	O normal	Onormal	Onormal	visr10
Ocounting fingers	O counting fingers	Opapilloedema	Opapilloedema	visl10
Ohand movement	O hand movement	Ounable to assess	Ounable to assess	fundr10
Onot able to see light	O not able to see light	Oother_____	Oother_____	fundl10
Ounable to assess	O unable to assess			

Optic nerve sheath diameter 3 RIGHT / LEFT _____mm opthve3

Eye movements
Any abnormality..... ☐ eyemov10
If abnormal, indicate which: RIGHT III / IV / VI
LEFT III / IV / VI

Other cranial nerves - any abnormality of:

CN V –corneal ..... ☐ CNV10
If abnormal, indicate: RIGHT / LEFT
CN VII ..... ☐ CNVII0
If abnormal, indicate: RIGHT / LEFT
Gag reflex..... ☐ gag10
CN XII ..... ☐ Cnxii10
If abnormal, indicate: RIGHT / LEFT
Any other abnormality_____..... ☐ OthCN10

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**..... **Day 10 Sheet**
Date / /

Hearing assessment **RIGHT**_____db **LEFT**_____db

[Circle minimum sound heard]

RIGHT Finger rub / Whisper / Speech / Shout / Totally deaf / unable to assess

LEFT Finger rub / Whisper / Speech / Shout / Totally deaf / unable to assess

Arms

	RIGHT	LEFT
Tone	↓ / Normal / ↑	↓ / Normal / ↑
Power	0 / 1 / 2 / 3 / 4 / 5 / unable to assess	0 / 1 / 2 / 3 / 4 / 5 / unable to assess
Reflexes	↓ / Normal / ↑	↓ / Normal / ↑

Legs

	RIGHT	LEFT
Tone	↓ / Normal / ↑	↓ / Normal / ↑
Power	0 / 1 / 2 / 3 / 4 / 5 / unable to assess	0 / 1 / 2 / 3 / 4 / 5 / unable to assess
Reflexes	↓ / Normal / ↑	↓ / Normal / ↑
Plantars	↓ / ↑ / equivocal	↓ / ↑ / equivocal

Summary of neurological findings: Normal / Abnormal neuro10

Ensure **Outcome** is completed on Page 2

○**Remains for further treatment** (PTO for further details)

Does this patient need post-test counselling, cotrimoxazole prophylaxis and ARVs?

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**..... **Day 1- 10 inpatient sheet**

Day 1 (Day recruited to GLAM)
Date: / /
Ward round notes:

GCS..... ☐
Confusion ..... ☐
Fits in the past 24 hours.. ☐
Diarrhoea ..... ☐
Nausea..... ☐
Vomiting..... ☐
Dizziness..... ☐
Anuria ..... ☐
Jaundice..... ☐
Chest clear..... ☐

○ CXR if signs of aspiration. CXR findings_____

Day 2
Date: / /
Ward round notes:

GCS..... ☐
Confusion ..... ☐
Fits in the past 24 hours.. ☐
Diarrhoea ..... ☐
Nausea..... ☐
Vomiting..... ☐
Dizziness..... ☐
Anuria ..... ☐
Jaundice..... ☐
Chest clear..... ☐

○ CXR if signs of aspiration. CXR findings_____

Day 3
Date: / /
Ward round notes:

GCS..... ☐
Confusion ..... ☐
Fits in the past 24 hours.. ☐
Diarrhoea ..... ☐
Nausea..... ☐
Vomiting..... ☐
Dizziness..... ☐
Anuria ..... ☐
Jaundice..... ☐
Chest clear..... ☐

○ CXR if signs of aspiration. CXR findings_____

Total number of glycerol doses given: _____

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**..... **Day 1- 10 inpatient sheet**

Day 4
Date: / /
Ward round notes:

GCS..... ☐
Confusion ..... ☐
Fits in the past 24 hours.. ☐
Diarrhoea ..... ☐
Nausea..... ☐
Vomiting..... ☐
Dizziness..... ☐
Anuria ..... ☐
Jaundice..... ☐
Chest clear..... ☐

○ CXR if signs of aspiration. CXR findings_____

Day 5
Date: / /
Ward round notes:

GCS..... ☐
Confusion ..... ☐
Fits in the past 24 hours.. ☐
Diarrhoea ..... ☐
Nausea..... ☐
Vomiting..... ☐
Dizziness..... ☐
Anuria ..... ☐
Jaundice..... ☐
Chest clear..... ☐

○ CXR if signs of aspiration. CXR findings_____

Day 6
Date: / /
Ward round notes:

GCS..... ☐
Confusion ..... ☐
Fits in the past 24 hours.. ☐
Diarrhoea ..... ☐
Nausea..... ☐
Vomiting..... ☐
Dizziness..... ☐
Anuria ..... ☐
Jaundice..... ☐
Chest clear..... ☐

○ CXR if signs of aspiration. CXR findings_____

Total number of glycerol doses given: _____

PHASE TWO
ISRCTN70121840

TRIAL NO:___________

1st Name .... **2nd Name............................** **Day 1- 10 inpatient sheet**

Day ....
Date: / /
Ward round notes:

GCS.......................... ☐
Confusion .... ☐
Fits in the past 24 hours.. ☐
Diarrhoea .... ☐
Nausea..................... ☐
Vomiting.................... ☐
Dizziness................... ☐
Anuria .... ☐
Jaundice.................... ☐
Chest clear................ ☐

○ CXR if signs of aspiration. CXR findings________________________________

Day ....
Date: / /
Ward round notes:

GCS.......................... ☐
Confusion .... ☐
Fits in the past 24 hours.. ☐
Diarrhoea .... ☐
Nausea..................... ☐
Vomiting.................... ☐
Dizziness................... ☐
Anuria .... ☐
Jaundice.................... ☐
Chest clear................ ☐

○ CXR if signs of aspiration. CXR findings________________________________

Day ....
Date: / /
Ward round notes:

GCS.......................... ☐
Confusion .... ☐
Fits in the past 24 hours.. ☐
Diarrhoea .... ☐
Nausea..................... ☐
Vomiting.................... ☐
Dizziness................... ☐
Anuria .... ☐
Jaundice.................... ☐
Chest clear................ ☐

○ CXR if signs of aspiration. CXR findings________________________________

Total number of glycerol doses given: _______

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**..... **Day 1- 10 inpatient sheet**

Day .....
Date: / /
Ward round notes:

GCS.....
Confusion .....
Fits in the past 24 hours..
Diarrhoea .....
Nausea.....
Vomiting.....
Dizziness.....
Anuria .....
Jaundice.....
Chest clear.....

○ CXR if signs of aspiration. CXR findings_____

Day .....
Date: / /
Ward round notes:

GCS.....
Confusion .....
Fits in the past 24 hours..
Diarrhoea .....
Nausea.....
Vomiting.....
Dizziness.....
Anuria .....
Jaundice.....
Chest clear.....

○ CXR if signs of aspiration. CXR findings_____

Total number of glycerol doses given: _____

Post day 10 inpatient Sheet

1st Name ..... 2nd Name.....

(Please use blank sheets to continue if needed. Date each entry)

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**..... **Day 40 Sheet**

Date of follow up: / / date40
Number of days after recruitment:____ day

Follow up visit at:
O Hospital (planned clinic FU) planned
O Hospital (remains an inpatient) iphosp
O Home following tracing traced
O Not followed up: O 1. Patient died. Date of death: / / nofu
O 2. Lost to follow up
O 3. Withdrew consent
O 4. Other _____

Day 40 neurology summary

Deafness L / R	Date / /	☐	Deafl/r40
Gaze palsy/squint.....	Date / /	☐	Squint40
Paralysis.....	Date / /	☐	Paraly40
Mental disorders.....	Date / /	☐	Mental40
Other _____			other40

Functioning ability Day 40

4=Fully independent with no disability.....	☐	functn40
3=Disabled but independent, able to work.....	☐	
2=Disabled: dependent on others for daily support.....	☐	
1=Minimally responsive.....	☐	

Day 40 examination

Is the patient fully orientated in: Time..... Y / N time40
Place ..... Y / N place40
Person..... Y / N person40

PERLA OYes ONo – details_____ perla40

Vision RIGHT	**Vision LEFT**	**Fundus RIGHT**	**Fundus LEFT**	
Onormal	O normal	Onormal	Onormal	visr40
Ocounting fingers	O counting fingers	Opapilloedema	Opapilloedema	visl40
Ohand movement	O hand movement	Ounable to assess	Ounable to assess	fundr40
Onot able to see light	O not able to see light	Oother_____	Oother_____	fundl40
Ounable to assess	O unable to assess			

Optic nerve sheath diameter 4 RIGHT / LEFT _____mm optnve4

1st Name ..... 2nd Name..... Day 40 Sheet
Date / /

Eye movements
Any abnormality..... ☐ eyemov40

If abnormal, indicate which: RIGHT III / IV / VI
LEFT III / IV / VI

Other cranial nerves - any abnormality of:

CN V –corneal ..... ☐ CNV40
If abnormal, indicate: RIGHT / LEFT

CN VII ..... ☐ CNVII40
If abnormal, indicate: RIGHT / LEFT

Gag reflex..... ☐ gag40

CN XII ..... ☐ Cnxii40
If abnormal, indicate: RIGHT / LEFT

Any other abnormality_____..... ☐ OthrCN40

Hearing assessment RIGHT_____db LEFT_____db
[Circle minimum sound heard]
RIGHT Finger rub / Whisper / Speech / Shout / Totally deaf / unable to assess
LEFT Finger rub / Whisper / Speech / Shout / Totally deaf / unable to assess

PHASE TWO
ISRCTN70121840

TRIAL NO:_____

1st Name ..... **2nd Name**..... **Day 40 Sheet**
Date / /

Arms

	RIGHT	LEFT
Tone	↓ / Normal / ↑	↓ / Normal / ↑
Power	0 / 1 / 2 / 3 / 4 / 5 / unable to assess	0 / 1 / 2 / 3 / 4 / 5 / unable to assess
Reflexes	↓ / Normal / ↑	↓ / Normal / ↑

Legs

	RIGHT	LEFT
Tone	↓ / Normal / ↑	↓ / Normal / ↑
Power	0 / 1 / 2 / 3 / 4 / 5 / unable to assess	0 / 1 / 2 / 3 / 4 / 5 / unable to assess
Reflexes	↓ / Normal / ↑	↓ / Normal / ↑
Plantars	↓ / ↑ / equivocal	↓ / ↑ / equivocal

Summary of neurological findings: Normal / Abnormal

neuro40

Is this patient on cotrimoxazole prophylaxis and booked for/started ARVs?

Transport fee: Paid: MK_____

Received by: _____

Witnessed by. _____

GLAM Trial ISRCTN70121840 Phase 2 version 1

GLAM TRIAL: DAILY OBSERVATIONS AND TREATMENT CHART

TRIAL NO:_____

1st Name ..... 2nd Name.....

	DAY 1				DAY 2				DAY 3				DAY 4				DAY 5			
DATE																				
Treatment times	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700
Glycerol/placebo QDS																				
Glycerol/placebo witnessed by:																				
Ceftriaxone 2g IV BD																				
Temperature (°C)																				
Pulse (bpm)																				
BP (mmHg)																				
Resp rate (/min)																				
NG tube in place?																				
Position checked																				
Blood sugar (mmol/L)																				
Urine output (mls)																				

NGT inserted Date: / / Check position: O Litmus test O Auscultation

If any other antibiotic has been given in hospital before recruitment to GLAM, write it onto this chart and tick when the dose/s were given.
Initial each administration of a drug. *Glycerol is for 16 doses only*. Continue ceftriaxone until stopped by doctor/clinical officer
Observations BD; blood sugar BD while taking glycerol, then once a day.

GLAM TRIAL: DAILY OBSERVATIONS AND TREATMENT CHART

TRIAL NO:_____

1st Name ..... 2nd Name. .....

	DAY 6				DAY 7				DAY 8				DAY 9				DAY 10			
DATE																				
Treatment times	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700
Glycerol/placebo QDS																				
Glycerol/placebo witnessed by:																				
Ceftriaxone 2g IV BD																				
Temperature (°C)																				
Pulse (bpm)																				
BP (mmHg)																				
Resp rate (/min)																				
NG tube in place?																				
Position checked																				
Blood sugar (mmol/L)																				
Urine output (mls)																				

NGT inserted Date: / / Check position: O Litmus test O Auscultation

If any other antibiotic has been given in hospital before recruitment to GLAM, write it onto this chart and tick when the dose/s were given.
Initial each administration of a drug. ***Glycerol is for 16 doses only.*** Continue ceftriaxone until stopped by doctor/clinical officer
Observations BD; blood sugar BD while taking glycerol, then once a day.

GLAM Trial ISRCTN70121840 Phase 2 version 1

TRIAL NO:_____

GLAM TRIAL: DAILY OBSERVATIONS AND TREATMENT CHART

1st Name ..... 2nd Name.....

	DAY ___				DAY ___				DAY ___				DAY ___				DAY ___			
DATE																				
Treatment times	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700	0730	1100	1400	1700
Glycerol/placebo QDS																				
Glycerol/placebo witnessed by:																				
Ceftriaxone 2g IV BD																				
Temperature (°C)																				
Pulse (bpm)																				
BP (mmHg)																				
Resp rate (/min)																				
NG tube in place?																				
Position checked																				
Blood sugar (mmol/L)																				
Urine output (mls)																				

NGT inserted Date: / / Check position: O Litmus test O Auscultation

If any other antibiotic has been given in hospital before recruitment to GLAM, write it onto this chart and tick when the dose/s were given.
Initial each administration of a drug. ***Glycerol is for 16 doses only.*** Continue ceftriaxone until stopped by doctor/clinical officer
Observations BD; blood sugar BD while taking glycerol, then once a day

Appendix 4. Patient information sheet Chichewa

PATIENT INFORMATION SHEET

THIS SHEET CAN BE GIVEN TO THE PATIENT/GUARDIAN TO READ AND KEEP

GLYCEROL ADJUVANT THERAPY IN ADULT MENINGITIS IN MALAWI

Kuchokera mukuyeza kwathu ndiponso kutengedwa madzi a pa msana zikusonyeza kuti Inu / m'bale wanu akudwala matenda oumitsa khosi. Tikufuna kuti tione ngati mankhwala atsopano wotchedwa Glycerol atha kuchepetsa imfa ndiponso zilema zina za pathupi zobwera ndi nthendayi. Kuti izi zitheke tikupempha kuti inu / mbale wanu alowe mukafukufukuyu pogwiritsa nthito "glycerol" ndi madzi a sugar. Makhwalawa adzakhala okumwa, koma ngati inuyo kapena mbale wanuyo akulephera kumwa ndiye kuti tidzayika ka tube kakang'ono ka plastic kudzela mphuno mpaka kufikira m'mimba, kuti mankhwalawa aperekedwe.

Panopa palibe umboni woti Glycerol ndi wa phindu kwa odwala matendawa kapena kuti gulu la wodwala ena angathandizike kuposera enawo.

Odwala onse adzalandira mankhwala (antibiotics) a nthendayi monga mwa nthawi zonse muno m' Malawi.

Pofuna kudziwa mulingo wa Glycerol mu madzi a pa msana ndiponso kuwona ngati mankhwalawa angathe kuchepetsa ululu (pressure) mu mutu, tidzakupemphani kuti tidzatenge madzi a pa msana a chiwiri patapita masiku awiri (maola makhumi anayi ndi mphambu zisanu ndi zitatu) mukulandira mankhwala.

Tidzakhala tikuyesa zoyesa zina ndi zina pofufuza matendawa, mu madzi a pa nsana ndi magazi, zokhuzana ndi m'mene mukupezera, choyambisa matenda, komanso mankhwala ake. Zitha kukhala kwenikweni zofuna kudziwa mtundu wa tizilombo tomwe tayambitsa, komanso kuti thupi likuchita bwanji polimbana ndi tizilomboto ndiponso mulingo wa mankhwala (antibiotic) mu madzi a munsana. Tidzafunanso tiyeze inu / m'bale wanu ngati ali ndi kachilombo koyambitsa matenda a Edzi, ndi cholinga chakuti ife madokotala tikuthandizeni ndi chithandizo choyenera. Odwala

a mukafukufukuyu, omwe apezeka kuti ali ndi kachilombo koyambitsa Edzi adzakhala ndi mwayi oyamba kulandira mankhwala aja a ARV akayamba kupeza bwino.

Wodwala onse ali ndi ufulu wofuna kulowa mukafukufuku kapena kukana popanda kusokoneza chisamaliro chawo pachipatala. Ngati wodwala sangathe kuvomereza yekha m'bale wake kapena womuyang'anira angathe kutero m'malo mwake.

Inu / m'bale wanu, ndipo wodwala wina aliyense ndi womasuka kusiya kupanga nawo kafukufuku nthawi yina yili yonse, ndipo izi sizikhuzana ndi ufulu wanu wolandila thandizo molingana ndi ndondomeko zovomelezeka za za chipatala.

Appendix 5. Patient Information sheet English

PATIENT INFORMATION SHEET

Glycerol adjuvant therapy in adult bacterial meningitis in Malawi.

The results from the examination and /or the brain fluid test (lumbar puncture) suggest that you / your relative have been diagnosed with bacterial meningitis.
We wish to see if a new treatment called glycerol reduces the mortality and disability. In order to do this we are asking if you / your relative would like to participate in a trial comparing glycerol with an inactive agent (sugar water). Half of the patients enrolled will receive glycerol and half will receive sugar water. This will be swallowed, but if you / your relative is unable to swallow then we will place a small plastic tube (naso-gastric tube) via the nose and into the stomach so that the glycerol or sugar water can be given. We currently have no evidence that glycerol is of benefit in meningitis or if either group of patients will be at a disadvantage compared to the other.

In order to treat the meningitis effectively, we will be using antibiotic (ceftriaxone) which is effective medication used for meningitis treatment in Malawi.

In order to measure the glycerol level in the brain fluid and to see if the glycerol reduces the pressure around the brain, we would also like to ask you if we could perform a second lumbar puncture after 48 hours of treatment.

We will want to see you / your relative one month (40 days) after starting treatment to follow you / them up. We will assist with transport costs.

Samples of blood and of brain fluid will also be subject to a range of other tests relevant to you / your relative's progress and to the cause, effect and treatment of meningitis in general. This may include looking more closely at the type of infection which has caused the meningitis, how the body has responded to the infection and what antibiotic levels are in the brain fluid. We will also test you / your relative's blood for HIV because this will help us when treating you / your relative. Anyone in the trial who tests positive for HIV will be eligible for free HIV treatment (ARVs) when they are better.

All patients are free to choose whether they wish to take part in the trial without jeopardy to their care in hospital.

Where patients are unable to give their consent, guardians or relatives will be invited to act on their behalf. You / your relative, and any patients entering the trial are free

to withdraw at any time without affecting your right to hospital treatment according to the standard clinical guidelines.

Sept 2006

Appendix 6. Consent form Chichewa

Patient's Name .....

KUVOMELEZA KULOWA MUKAFUKUFUKU

Ine _____ wodwala* / woyang'anira wodwala* _____ ndawerenga ndipo ndamvetsetsa zonse zalembedwa pamwambazi choncho ndavomereza kulowa mu kafukufuku wa mankhwala wotchedwa Glycerol ndi mankhwala ena pochiza matenda owumitsa khosi.

Ine, wodwala*/woyang'anira wodwala* ndavomereza mwaufulu wanga kutengedwa kachiwiri madzi a pa msana ngati mbali ya kafukufukuyu. Ndondomeko ndiponso zovuta zina andilongosolera momveka bwino ndipo ndikudziwa kuti zotsatira za zoyesazi zidzakhala zoyenera mukafukufuku yekhayu.

Ine odwala/kapena woyang'anira wodwala, ndikuvomereza kuti ndiyikidwe kapena ayikidwe ka chubu (Naso-gastric tube) ngati kudya ndi kumeza kuli kovuta.

Ine odwala/kapena woyang'anira wodwala ndikuvomera kuti magazi amene ndatengedwa akayezemo zina ndi zina monga mlingo wa glycerol ndiponso kachilombo koyambitsa matenda a Edzi.

Ine ndikudzindikira kuti mugwirizanowu ungathe kuthetsedwa nthawi ina yili yonse popanda kuphwanya ufulu wanga wopitiliza kulandira mankhwala.

Tsiku :

Woyang'anira wodwala :_____

Dzina _____

(* futani moyenera)

Appendix 7. Consent form English

Patient's Name .....

GLAM Trial - Glycerol adjuvant therapy in adult bacterial meningitis in Malawi.

Consent to enter the clinical trial

I,_____ the patient* / legal guardian of* _____, have read and understood the above statement and agree to the enrolment in the clinical trial of glycerol with antibiotics in the treatment of bacterial meningitis.

I, the patient / legal guardian, consent fully and freely to a second lumbar puncture as part of the above clinical trial. The procedure and its possible side effects have been satisfactorily explained and I am aware that the information obtained from this test will be of relevance only to the clinical trial outlined above.

I, the patient / legal guardian, consent to placement of a naso-gastric tube if swallowing is not possible.

I, the patient / legal guardian, consent to blood samples being taken for various tests including measuring glycerol levels and HIV testing.

I understand that this consent may be withdrawn at any time without affecting my right to continuing treatment.

Date:

Verbal consent witnessed by legal guardian:_____

Name:_____

(*Delete as appropriate)

Appendix 8. Serious adverse event proforma

Study Number	

GLAM STUDY **Serious Adverse Event (SAE) Report Form**

Form completed by		*(initials)*
Date of presentation		/ /
Date of SUSAR onset		/ /
Date of SUSAR awareness		/ /
Date of last treatment		/ /

SYMPTOMS AND SIGNS
Description of adverse (signs symptoms, time course and treatment if any)

Relevant diagnostic tests/laboratory data

Check all appropriate:

☐	Patient died date:	☐☐ /☐☐ /☐☐
☐	Life threatening illness	Fatal
☐	Required hospitalisation	*(.....Days)*
☐	Resulted in prolongation of hospitalisation	
☐	Resulted into permanent disability/incapability	
☐	Other SUSAR	*(specify.....)*

ACTION TAKEN

☐	No action
☐	Other treatment given *(specify)* Supportive care given, see above
☐	Study drugs discontinued N/A
☐	Other action *(specify)* CT brain scan was performed. Unfortunately results are not available.

OUTCOME

☐	Completely recovered (on / /)
☐	Condition improving
☐	Condition unchanged

☐ Condition deteriorating
☐ Death *(attach autopsy findings if done)* Autopsy not available in Malawi

CONCLUSION BY INVESTIGATOR

Diagnosis *(if made)*.....

Adverse event occurred Yes ☐ No ☐
Event defined as: *(tick one)*

☐ Adverse Event (AE)
☐ Adverse Reaction (AR)
☐ Serious Adverse Event/Serious Adverse Reaction (SAE/SAR)
☐ Unexpected Adverse Reaction (UAR)
☐ Suspected Unexpected Serious Adverse Reaction/Event (SUSAR/SUSAE)

Intensity ☐☐ *1 = Mild, 2 = Moderate 3 = Severe*
Causality ☐☐ *1 = Not Related, 2 = Remote, 3 = Possible 4 = Probable*

Reporting *(tick if reported to the following bodies)*

DMC ☐
Ethics committee MW ☐
Ethics committee LP ☐

Information Source

Signature..... Date ☐☐/☐☐/☐☐

Appendix 9. Nurse/Clinical Officer contract

UNIVERSITY OF MALAWI
COLLEGE OF MEDICINE

Principal

Prof. R.L. Broadhead, MBBS, FRCP, FRCPCH, DCH

Our Ref.:

Your Ref.:

College of Medicin
Private Bag 36
Chichi
Blantyre
MALAW
Telephone: 67724
67729
Fax: 67470
Telex: 4374

MEDICINE DEPARTMENT

The GLAM Trial (glycerol in adult meningitis)
Official title: "Randomised controlled trial of glycerol adjuvant therapy in adult bacterial meningitis in Malawi".

Job description and terms and conditions of: Clinical Research Nurse

Job description

1) To provide daily nursing care to all patients enrolled into the GLAM study and to any other patient assigned to the care of trial nurses by medical consultants, lecturers or trial clinical officer.
2) To ensure strict adherence to the trial protocol for administration of medications, completion of data collection and collection of laboratory results.
3) To carry out duties at the request of the investigators, trial clinical officers, medical consultants and lecturers.
4) To keep other members of the trial staff informed of progress of each patient at all times with organised and agreed meeting times including with any shift change.
5) To attend trial meetings on a monthly basis or as requested by the investigators regardless of off-duty or leave arrangements. Special dispensation may be granted by prior arrangement with the investigators.
6) To initially provide nursing care during the day and at weekends according to a pre-defined timetable, taking into account annual leave and public holidays.

Once the full team of nursing staff (6 nurses) are employed, this will comprise nursing cover at night using a rolling rota.

Appointment

Employees are hired with the understanding of the special nature of the GLAM study with the commitment to uphold the highest standards in thoroughly performing their tasks, as assigned by the Principal Investigator, and in protesting confidential information.

All project employees will, on the first appointment, be engaged on probation. The probationary period will be for 2 months. During this period, in the event of unsatisfactory performance, the probationary appointment may be extended or terminated at the discretion of the Principal Investigator. After the probationary period, a notice period of one month will be served if a staff member wishes to resign.

All staff must be registered with the relevant professional body e.g. Malawi Council of Nurses, before practicing at QECH.

Salary and wages

The commencing salary shall be based on the level and seniority of the position and the profession, as well as the qualifications necessary to carry out the functions of the project. The *basic salary includes leave and overtime grants*, which will therefore not be separately paid.
Tax liability will be calculated according to standard levies by the College of Medicine finance office.
All issues related to salaries will be dealt with by the College of Medicine unless trial staff are otherwise advised – this includes any application for an advance payment from a salary.

Hours

The workday is from 7.30AM to 5.00PM, Monday through Friday and at weekends (with 2 days off per week), with 40 minutes lunch break and two 20-minute tea breaks. Breaks can be rearranged at the discretion of the Principal Investigator, if necessary, based on the workload.
[Once the full team of nursing staff (6 nurses) are employed, this will comprise nursing cover at night using a rolling rota. This will include a stretch of seven nights on duty.]

Pay Day

The GLAM Study pay day shall be the 25th of each month (as per COM current practice) unless the 25th falls on a Saturday, Sunday or Malawi Public Holiday, in which case pay day shall be the last working day preceding the 25th.

Death of an Employee

In the event of the death of a project employee, the deceased employee's next-of-kin will receive the following on behalf of the deceased:
One month's salary in addition to the remaining month's salary.

If possible, GLAM Study will provide transport for employees to attend the funeral.

Name of next of kin:_____

Workman's Compensation

In the event of an employee being injured while on duty in laboratories, clinic, office or authorized field activities, the GLAM Study shall comply with and be guided by the Laws of Malawi and act in accordance with the provisions of the law regarding compensation.

Termination of contract

A. A permanent employee wishing to resign his appointment shall give one months notice in writing to the project of his intention to resign or pay the GLAM Study three months salary in lieu of notice. Likewise, the GLAM study may discharge a permanent employee giving him one month's notice or paying him three months salary in lieu of notice. Any part of holiday period may be included in the period of due notice.
B. Serious misconduct will result in immediate dismissal of the employee. An employee is guilty of serious misconduct if he/she:
    a. Is under the influence of intoxicating liquor or habit forming drugs during normal working hours of attendance or during such other hours as he may be required to be on duty
    b. Commits any of the following acts of misconduct:
        i. Breach of communication of privileged information.
        ii. Extortion, bribery and corruption.
        iii. Theft, theft by false pretences and receiving stolen property knowing it to have been stolen.
        iv. Fraud, forgery, uttering a forged instrument knowing it to have been forged.
C. Two warning letters that will contain all relevant information will precede dismissal for less serious neglect of duties or "less serious misconduct". Warning letters will be presented by the Principal Investigator and discussed with the employee. The employee has the right to respond to warning letters and any letters will be placed in the personal file.

A project employee is guilty of less serious misconduct if he/she:

a) Absents himself from his post during normal working hours of attendance, without permission from his immediate boss or without valid excuse
b) Performs his duties negligently.
c) Fails to perform any duties assigned to him/her or to obey any instructions given to him by persons having authority to give such instructions and
d) Displays insubordination by word or by conduct.

Leave

Annual Paid Leave

All GLAM Study employees are eligible for annual paid leave and leave must be taken at a time approved by the Principal Investigator, based on the project activities. To be considered for approval, leave must be requested at least one month prior to the start of the requested

leave time. Annual leave will be calculated as 2 days earned per month. Annual leave will only be given after it is earned.
Staff members must ensure that a trial colleague covers any allocated shifts in their absence.

Sick leave

In the event of sickness, all reasonable effort should be made to arrange cover for the shifts affected. Where, due to unforeseen circumstances there is a significant staff shortage, staff members may be expected to work additional shifts until such time as the crisis can be resolved.

Compassionate Leave

In the case of emergencies or urgent private affairs, an employee can apply for compassionate leave.
The granting of paid compassionate leave of up to 10 working days per year is at the discretion of the Principal Investigator and each case will be considered on its own merit. When such leave is granted, the reason will be recorded and kept on file. When requests are received after the 10 days have been exhausted or when no prior permission has been obtained, days of compassionate leave will be deducted from the employee's annual leave.

Disability/Extended illness

Employees are eligible for 30 days paid leave for disability or extended illness, provided the leave is medically certified and approved by the Principal Investigator. Employees taking such leave for 30-60 days will receive half-pay and employees taking such leave for more than 60 days will receive no pay.

Maternity

A female employee of the project shall be granted paid maternity leave up to 90 consecutive days for the purpose of confinement before or after delivery as the case may be, after she has worked for a minimum of 6 months for the project,. However, no maternity leave shall be granted unless three years have elapsed from the date of birth of the last surviving child.

Project vehicles

Employees are not provided with project vehicles for transport and from their houses or for personal use.

Grievances

All grievances should be addressed to the Principal Investigator.

Amendments

The GLAM Trial shall, from time to time, delete, substitute or add to these terms and conditions of service and shall notify the staff.

The Principal Investigator of the GLAM Trial is:
Dr Katherine Ajdukiewicz or Dr Katharine Cartwright (on behalf of KA)
Department of Medicine

The GLAM Trial (glycerol in adult meningitis)
Official title: "Randomised controlled trial of glycerol adjuvant therapy in adult bacterial meningitis in Malawi".
A research study of the Department of Medicine of the College of Medicine

College of Medicine
Private Bag 360
BLANTYRE 3

EMPLOYMENT AGREEMENT

Agreement made on this day .....
Between the GLAM Study of the Department of Medicine of the College of Medicine and:
.....
.....
(Hereinafter) called the "employee" whereby it is agreed as follows:

1. The employee is appointed to the position of Clinical Research Nurse. The total salary before deductions is MK 57,200 per month.
2. The salary includes housing allowance.
3. Duty allowance of K500 per night worked will be paid each month.
4. No pension will be paid at the end of the contract.
5. A performance-related bonus may be paid on completion of the project.
6. The **AGREEMENT** is for the duration of **twelve months or until the project is completed**.
7. The **AGREEMENT** is subject to the offer of appointment and the Conditions of Service of the GLAM Trial, which are constructed as and understood to be part of the **AGREEMENT.**
8. By signing this contract, the employee is confirming that he/she has resigned from the Ministry of Health and as such, is no longer receiving a Ministry of Health salary.

This contract replaces all previously signed contracts.

Date:_____

As witness our hands the day and year above written

_____

(On behalf of the project)

_____

(Employee)

(Witness)

Date probation period ends: _____ **Signed:** _____

Appendix 10. Trial Steering Group

Trial Steering Group Members:

Dr N French (Chairman), Director Karonga Prevention Study, Chilumba and College of Medicine, Blantyre, Malawi

Professor M Molyneux, Director Malawi-Liverpool-Wellcome Trust Programme.

Dr M Scarborough, Nuffield Department of Medicine, John Radcliffe Hospital, Oxford

Dr D Lalloo, Liverpool School of Tropical Medicine, Liverpool

Observers:

Dr K Ajdukiewicz, Principal Investigator and Lecturer in Medicine, Blantyre, Malawi

Dr J Kumwenda, DSMB chairman

The TSG provided overall supervision of the study, and in particular, provided independent advice through the chairman of the group to the Investigator, the MRF and the Host institutions. The group incorporated individuals with relevant complementary experience and skills to advise on the running of a randomised controlled trial of glycerol adjunctive therapy in adult bacterial meningitis in Africa.

Terms of reference:

1. To review and approve the study protocol, recruitment and follow-up procedures prior to the commencement of the study. This ensured participants were managed

in the study in a way consistent with the advice of the independent ethical review boards and prevailing international standards.

2. To monitor the progress of the study which ensured recruitment targets were achieved and efforts to maximise recruitment undertaken.
3. To identify and invite a Data and Safety Monitoring Board (DSMB) to convene.
4. To consider and act upon advice provided by the DSMB. The DSMB was invited to carry out an interim analysis after 100 deaths. Stopping rules for use in the case of serious adverse events were agreed and established before the study began. The TSG had responsibility for stopping the study in discussion with the Investigator and on the recommendation of the DSMB.
5. To provide advice and guidance to the investigator if significant changes in knowledge or clinical practice took place during the course of the study which will alter the trial equipoise, performance of the clinical trial or modify the likelihood of achieving a result.
6. To provide an annual, structured report to the MRF –this took the form endorsing a report submitted by the principal investigator.
7. To ensure as far as possible, the investigator was conducting the trial in an honest, ethical and credible way and did not inappropriately manipulate or falsify the data or results
8. To advise the MRF on the need for a study extension in the event of a meaningful result being unlikely at the end of the currently planned study timescale.

Appendix 11. Data Safety Monitoring Board

Data and Safety Monitoring Board:

Professor E Molyneux, College of Medicine, Blantyre, Malawi

Professor R Auty, Consultant in Pharmaceutical Medicine, Salient Consulting Ltd, UK.

Dr J Kumwenda, College of Medicine, Blantyre, Malawi.

Role of the DSMB:

1. To review safety data and unblinded data during the study and report any safety or ethical issues, which may lead to premature cessation of the trial.
2. To report to the TSG the results of unblinded analyses relevant to safety and ethics.
3. To undertake an unblinded interim analysis after 100 deaths and report to the TSG.
4. To review the integrity of data collection and handling by study team members.
5. To inspect labelling of glycerol and placebo and randomisation procedures to ensure it is done in a manner that maintains blinding and is safe and ethically sound.
6. To perform appropriate analyses in the event of sub-optimal recruitment or end point ascertainment and assess the consequences for achieving a valid and

meaningful result.

7. If, in the view of the TSG, an extension to the study would produce a definitive result, to recommend to the MRF for the study to continue.

Appendix 12. Ethics approval: COMREC

GLAM Trial
Department of Medicine
College of Medicine
P/Bag 360, Chichiri
Blantyre, BT3
Malawi
ISRCTN70121840

3rd January 2007

Prof E Borgstein
Chairman
COMREC

Dear Professor Borgstein,

RE: P.04/05/363 – Randomised trial of glycerol adjuvant therapy in adult bacterial meningitis in Malawi by Dr K Ajdukiewicz

Thank you for your letter dated 20th December 2006 regarding storage of samples for bacterial PCR. Quantitative PCR to measure bacterial load within blood or CSF is only carried out at dedicated specialist centres within the UK. These are specific Health Protection Agency (Public Health) laboratories which produce quality controlled results.

It would be extremely difficult to carry out the tests here due to the lack of local expertise and equipment. The results may not be accepted as appropriately verified. I understand that one of the paediatric studies within QECH also transported samples back to the UK for similar tests due to the same reasons. This paediatric study has shown that there is a correlation between bacterial load and outcome, particularly in the context of HIV[1].

I hope that COMREC agree that the only way we will be able to adequately measure bacterial load and thus determine if this is the cause of high mortality in our adult patients, is to transport samples to a specialist laboratory in the UK for testing.

Yours sincerely,

Dr K Ajdukiewicz
Principal Investigator

[1] Carrol ED et al. High pneumococcal DNA loads are associated with mortality in Malawian children with invasive pneumococcal disease. **Pediatr Infect Dis J** 2007; in press

UNIVERSITY OF MALAWI

Principal
Prof. R.L. Broadhead, MBBS. FRCP, FRCPCH, DCH

Our Ref.. COMREC/16
Your Ref.: P.04/05/363

College of Medicine
Private Bag 360
Chichiri
Blantyre 3
Malawi
Telephone: 677 245
677 291
Fax: 674 700
Telex: 43744

10th January, 2007

Dr Katherin Ajdukiewicz
Medicine Department
P/Bag 360
Blantyre 3

Dear Dr K. Ajdukiewicz,

RE: P.04/05/363– Randomised trial of glycerol adjuvant therapy in adult bacterial meningitis in Malawi

Thank you for your letter of response dated 3rd January, 2007. Thank you for your explanation and the justification. You may go ahead with your plans as requested.

Yours sincerely,

Prof E. Borgstein
CHIARMAN – COMREC

EB/tck

UNIVERSITY OF MALAWI

Principal

Prof. R.L. Broadhead, MBBS, FRCP, FRCPCH, DCH

Our Ref.: MC/COMREC/16

College of Medicine
Private Bag 360
Chichiri
Blantyre 3
Malawi
Telephone: 01 671 911/01 674 377
Fax: 01 674 700/01 674 740
Telex: 43744

6th February, 2007

Dr Katherine Ajdukiewicz
Paediatrics Department
P/Bag 360
Chichiri
Blantyre 3

Dear Dr Ajdukiewicz,

P.04/05/363 – Randomised trial of glycerol adjuvant therapy in adult bacterial meningitis in Malawi (GLAM)

I write to inform you that COMREC reviewed the progress report which you submitted at its meeting of 31st January, 2007. I am pleased to inform you that COMREC approved the continuation of the study for another 12 months with effect from 1st January, 2007.

This renewal is subject to continued adherence to the College of Medicine requirements for all COMREC approved research studies

Yours sincerely,

Prof. E. Borgstein
CHAIRMAN – COMREC

Appendix 13. Ethics approval: LSTM

LIVERPOOL
SCHOOL OF
TROPICAL
MEDICINE (Affiliated to the University of Liverpool)

Pembroke Place
Liverpool L3 5QA
Telephone: 0151 708 9393
Fax: 0151 705 3370
http://www.liv.ac.uk/lstm

27 February 2006

Dr Katherine MB Ajdukiewicz
& Dr D Lalloo

Dear Dr Ajdukiewicz

The research protocol **Randomised controlled trial of glycerol adjuvant therapy in adult bacterial meningitis in Malawi** re-submitted on 4 January 2006 Reference No **05.64A** was considered by the Research Ethics Committee on **12 January 2006**.

Thank you for your letters sent via e-mail on 6 February 2006 and 16 February 2006 with the information requested by the committee. The protocol now has formal ethical approval from the LSTM Research Ethics Committee.

Conditions of Approval

- The approval is for a fixed period of three years or for the duration of the grant, renewable annually thereafter.
- The committee may suspend or withdraw ethical approval where it is felt appropriate.
- In accordance with International Committee on Harmonisation of Good Clinical Practice (ICH GCP) Guidelines, annual update must be provided to the committee. Failure to do so could result in suspension of the study without further notice.
- A copy of the final report should be sent to the committee
- Any serious adverse events must be reported to the committee.

Any proposed amendments to the protocols must be notified to the LSTM Research Ethics Committee for approval before implementation. (Full application is not necessary at this stage)

The Research Support Office (RSO) maintains a Database of Local Research Committees in the countries where collaborative work is being carried out. Could you, therefore, feed back to me (via Sharda Mistry in the RSO) as much information as possible on the local Committees/Review Bodies that will review (or have reviewed) this protocol. The following details would be much appreciated:

- Name
- Address
- Contact numbers or individuals (tel / fax / e-mail)
- A copy of the appropriate form or some details on the submission mechanism (including charges)
- Any details you are able to obtain on
  a) number on the committee
  b) how many lay representatives sit on the committee?

Yours sincerely

Dr T O'Dempsey
Acting-Chair, Research Ethics Committee

Glycerol adjuvant therapy in adults with bacterial meningitis in a high HIV seroprevalence setting in Malawi: a double-blind, randomised controlled trial

Katherine M B Ajdukiewicz, Katharine E Cartwright, Matthew Scarborough, James B Mwambene, Patrick Goodson, Malcolm E Molyneux, Eduard E Zijlstra, Neil French, Christopher J M Whitty, David G Lalloo

Summary

Background Southern Africa has a high incidence of bacterial meningitis in adults, often associated with HIV co-infection. Mortality exceeds 50%, even with appropriate antibiotic therapy, and is not improved with corticosteroids. Glycerol adjuvant therapy reduces long-term morbidity in bacterial meningitis in children, and its use is being promoted. We aimed to assess the effectiveness of glycerol as an adjuvant therapy for adults with bacterial meningitis in Africa.

Methods The study was done in two phases. First, in an open-label dose-finding study, 45 adult patients with symptoms, signs, and cerebrospinal fluid findings consistent with bacterial meningitis received either 50 mL, 75 mL, or 100 mL of glycerol four times a day for 4 days. We then did a randomised, double-blind, placebo-controlled trial of oral glycerol in adults with bacterial meningitis. Patients with clinical and cerebrospinal fluid findings suggestive of bacterial meningitis were randomly assigned in blocks of 12 by use of a random number list produced by an independent statistician to receive either glycerol or an equivalent volume of sugar solution. Glycerol and placebo were indistinguishable by colour or taste. The primary outcome was mortality at 40 days, with secondary outcomes including disability and mortality restricted to pneumococcal disease. All patients were analysed for the primary outcome excluding those who were lost to follow-up. This trial is registered at controlled-trials.com, number ISRCTN70121840.

Findings 75 mL glycerol four times a day was the highest tolerated dose, and was used for the main study. 265 patients were assigned treatment: 137 glycerol and 128 placebo. The trial was stopped early on the advice of the data and safety monitoring board after a planned interim analysis. By day 40, 61 (49%) of 125 patients in the placebo group and 86 (63%) of 136 in the glycerol group had died (adjusted odds ratio 2·4, 95% CI 1·3–4·2, p=0·003). There was no benefit from glycerol for death and disability by day 40, and glycerol did not improve death and disability by day 40 or death at day 40 in patients with proven bacterial disease or pneumococcal disease. Two serious adverse events occurred that were possibly due to the study drug.

Interpretation Oral glycerol therapy cannot be recommended as an adjuvant therapy in adults with bacterial meningitis in resource-poor settings with a high HIV prevalence.

Funding Meningitis Research Foundation.

Lancet Infect Dis 2011; 11: 293–300

Published Online February 18, 2011 DOI:10.1016/S1473-3099(10)70317-0

See Comment page 257

Department of Medicine, College of Medicine, Chichiri, Blantyre, Malawi (K M B Ajdukiewicz MRCP, K E Cartwright MRCP, M Scarborough PhD, J B Mwambene Dip Med Sci, P Goodson Dip Med Sci, M E Molyneux Dip Med Sci, E E Zijlstra PhD); Monsall Unit, Department of Infectious Diseases and Tropical Medicine, North Manchester General Hospital, Delaunays Road, Manchester, UK (K M B Ajdukiewicz); Microbiology, Leicester Royal Infirmary, Infirmary Square, Leicester, UK (K E Cartwright); Microbiology, John Radcliffe Hospital, Headington, Oxford, UK (M Scarborough); Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK (M E Molyneux, D G Lalloo FRCP); Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands (E E Zijlstra); Karonga Prevention Study, Chilumba, Malawi (N French FRCP); Department of Clinical Research, London School of Tropical Medicine and Hygiene, Keppel St, London, UK (N French, C J M Whitty FRCP)

Correspondence to: Katherine Ajdukiewicz, Monsall Unit, Department of Infectious Diseases and Tropical Medicine, North Manchester General Hospital, Delaunays Road, Manchester M8 5RB, UK katherineaz@doctors.org.uk

Introduction

Meningitis is a common cause of adult in-patient death in Malawi. The incidence of bacterial meningitis has risen substantially in Malawi, as it has in other southern African countries, since the start of the HIV epidemic.[1] Even with effective antibiotics in-patient case fatality exceeds 50%, and up to half of survivors are left with neurological sequelae and disability.[2,3] Measures to reduce mortality are urgently needed.[1]

Corticosteroids, which can be an effective adjunctive treatment in settings with a low prevalence of HIV,[4] are not effective where most meningitis is HIV-related, including Malawi.[2,5,6] Raised intracranial pressure impairs cerebral blood flow, and might be a significant contributory factor to mortality and morbidity from all forms of meningitis; therefore, early reduction of intracranial pressure might improve outcome. Glycerol, an orally administered hyperosmolar liquid widely used as a food additive, has been suggested as a promising adjuvant treatment. In children with bacterial meningitis, glycerol reduces neurological sequelae but not case fatality when given either alone or in combination with dexamethasone,[7,8] although there is some controversy surrounding the design of a Latin American study that found a benefit for glycerol.[9] Glycerol has previously been used in stroke, head injury, and glaucoma to reduce increased tissue pressure.[10–12] A meta-analysis of intravenous glycerol in ischaemic stroke suggested that glycerol conferred a short-term advantage, but no benefit in terms of long-term survival.[13]

No trials of glycerol adjuvant therapy in bacterial meningitis have been reported in adults. If the paediatric findings were replicated in adults, glycerol would represent a significant advance in the treatment of

bacterial meningitis, particularly in resource-poor settings with a high prevalence of HIV, such as southern Africa, where the burden of disease is high and adjuvant steroid therapy has no clinical advantage.

Glycerol has been used as a food additive and for cosmetic purposes for many years. Toxicity data for oral glycerol indicate that it is safe. Side-effects are infrequent, usually mild, and are mostly gastrointestinal in origin.[20,21] There are rare reports of neurological side-effects, such as headache, dizziness, confusion, and amnesia (in elderly patients),[22,23] and one report of spontaneously reversible hemiparesis in a patient who ingested 500 mL of pure glycerol as a single dose.[24] Glycerol is stable in hot climates, widely available, and inexpensive, and would therefore be ideal for use in resource-poor settings.

Queen Elizabeth Central Hospital (QECH) is a large hospital in Blantyre, Malawi, and serves a catchment population in excess of 1 million people. According to UNAIDS, HIV prevalence in adults in Malawi aged 15–49 years in 2006 was 12%.[25] Admissions to QECH exceed 10000 per year, and 70% of admitted patients are infected with HIV.[26] Meningitis is one of the commonest causes of admission, with *Streptococcus pneumoniae* the most common form of bacterial meningitis.[1] Mortality from bacterial meningitis exceeds 50%, and adjunctive steroid therapy in adults does not reduce mortality, so it is not routinely used.[3]

We designed a randomised, double-blind, controlled trial to test whether glycerol is an effective and safe adjuvant treatment for adults with bacterial meningitis in a setting with high HIV seroprevalence and high mortality from meningitis.

Methods

Patients

The trial started on Sept 10, 2006, and recruitment was halted on Aug 23, 2008. There were no facilities for the invasive monitoring of patients at the time of the trial.

Adult patients admitted to the QECH with signs and symptoms of meningitis were screened by clinicians on the wards. All patients had a lumbar puncture and received intravenous ceftriaxone. Patients were then referred to the trial staff for assessment of eligibility. Trial inclusion criteria were clinical suspicion of meningitis (headache, neck pain or stiffness, reduced level of consciousness, photophobia, confusion, fits, rash, or fever) plus cerebrospinal fluid evidence of bacterial meningitis (>100 white cells per μL with predominant neutrophils or Gram-stain showing bacteria), or cloudy cerebrospinal fluid if microscopy was delayed (eg, at night). Exclusion criteria were age less than 16 years, cryptococcal meningitis (India ink stain or cryptococcal antigen positive on cerebrospinal fluid), less than 100 white cells per μL or lymphocytic meningitis, pregnancy, heart failure, and known type-2 diabetes. A capillary whole blood glucose (BM glucose) greater than 12 mmol/L was added as a criterion for exclusion for phase 2 of the study on the advice of the data and safety monitoring board.

Ethics approval was granted by the College of Medicine Research Committee, Malawi, and the Liverpool School of Tropical Medicine, UK. Written informed consent was obtained from all study participants.

Randomisation and masking

A randomisation number list in blocks of 12 was produced by an independent statistician using Stata version 9.0. Numbers and allocation were placed into sealed envelopes. Envelopes were opened sequentially by an independent person not involved in the clinical care or assessment of trial participants, who labelled pre-prepared containers of glycerol and placebo with a unique study number. Study drug packs were then placed in consecutive order within a secure room. If a patient fulfilled the study inclusion criteria, verbal and written informed consent was obtained from the patient or, if the patient was unconscious or confused, from the patient's legal guardian. Those unable to read gave witnessed consent. Allocation of the next available study number constituted entering the trial, and analysis was based on study allocation irrespective of subsequent treatment. All envelopes were accounted for. Allocation was masked from clinicians and patients. Treatment with glycerol or placebo was given by trial staff. After the addition of Orange SOBO (a locally produced orange squash) into glycerol and placebo, both liquids were a pale orange colour and were more palatable and indistinguishable in appearance and taste, as assessed by medical and clerical staff within the Department of Medicine, QECH.

Procedures

The study was done in two phases. The first was an open-label study to assess the ease of giving glycerol and the tolerability of a range of doses by patients to find the highest tolerated dose for use in the second phase—the double-blind, randomised controlled trial.

45 adult patients with symptoms, signs, and cerebrospinal fluid findings consistent with bacterial meningitis infection who fulfilled inclusion criteria were recruited. After consent was obtained, 15 patients each received 50 mL, 75 mL, or 100 mL of glycerol four times a day for 4 days. Paediatric studies used 6 mL/kg per day:[4] this corresponded to 300–360 mL glycerol daily for the average Malawian weighing 50–60 kg. Glycerol was diluted with water at a ratio of 5:4 so that the consistency was indistinguishable from that of 50% sugar solution (the proposed placebo for phase 2). Patients therefore received diluted glycerol 90 mL, 135 mL, or 180 mL four times a day. Clinical details of all cases, including possible or probable adverse events due to glycerol, were recorded.

The second phase of the study was the main trial, in which patients were randomly assigned to receive the highest tolerated dose of glycerol or equivalent volume of

placebo. All patients with probable meningitis were treated with intravenous ceftriaxone twice a day for at least 10 days, or until treatment was changed according to the results of culture and sensitivity testing. If cryptococcal infection was diagnosed after recruitment to the study, patients were treated with oral fluconazole (in accordance with national guidelines), and the study drug was discontinued. Those not enrolled in the study received standard care in accordance with national guidelines.

Glycerol or placebo was given orally or via nasogastric tube immediately after enrolment at a dose of 135 mL (75 mg glycerol mixed with water or 135 mL 50% sugar solution) four times a day for 4 days (16 doses). This was the highest tolerated dose in the dose-finding study. The dose was repeated after antiemetics if vomiting occurred within 30 min of administration. A nasogastric tube was routinely inserted in those with a score on the Glasgow coma scale (GCS) of less than 8, or in any patient unable to swallow for any other reason. Position of the tube was checked daily by auscultating over the epigastrium while injecting air through the nasogastric tube and by ensuring that tube aspirate was acidic (turned blue litmus paper pink).

After 48 h or at least eight doses of glycerol therapy, a second lumbar puncture was done. After 10 days of antibiotic therapy a full neurological examination including hearing assessment was done. Hearing tests were done with a Kamplex Diagnostic Audiometer AD12. All patients were treated in hospital for at least 10 days, with daily clinical assessment. Patients alive at discharge were asked to return at day 40 for follow-up assessment; those who did not attend were traced at home. At day 40, patients had a full neurological examination including hearing assessment using audiometry, and the Glasgow outcome scale was used as a measure of neurological disability.

The primary endpoint was death by day 40. Secondary endpoints were death or disability (Glasgow outcome score—disabled and partially or completely dependent) by day 40;[17] death by day 10; hearing loss in those not dead or disabled by day 40; time to death; cerebrospinal fluid opening pressure 2 days after treatment; and serious adverse events (SAEs). A-priori subgroup analyses included patients with proven or probable bacterial meningitis alone and proven pneumococcal meningitis.

Adverse events and SAEs were recorded. SAEs were classified as definitely, possibly, or probably related to study drug, as defined by the International Conference on Harmonisation Guidelines.

All patients were tested for HIV. Consent with appropriate counselling was sought at recruitment to the study, or retrospectively after recovery for those initially unconscious or confused. Patients' guardians were aware that HIV testing would be done, but they were not informed of results. Relatives and guardians were encouraged to attend for voluntary HIV testing, regardless of patients' results. Patients were counselled about their results before discharge from hospital. Those found to be HIV positive were started on cotrimoxazole prophylaxis and referred to the antiretroviral therapy clinic.

Cerebrospinal fluid and blood for culture were analysed by the Malawi–Liverpool–Wellcome Trust (MLW) laboratory. Cerebrospinal fluid was processed by use of standard laboratory techniques for cell count, differential white-cell count (when >20 cells per μL present) and Gram stain. Cerebrospinal fluid was cultured on sheep blood and chocolate agar for 48 h and subcultured for

	50 mL glycerol four times a day (n=15)	75 mL glycerol four times a day (n=15)	100 mL glycerol four times a day (n=15)
Died	10 (67%)	8 (53%)	12 (80%)
Gastrointestinal side-effects*	7 (47%)	8 (53%)	8 (53%)
Number with random capillary blood glucose >12·2 mmol/L†	5 (33%)	3 (20%)	6 (40%)
Number with proven bacterial meningitis	4 (27%)	6 (40%)	7 (47%)

Data are n (%). *Nausea, vomiting, diarrhoea. †World Health organisation definition for diabetes.[19]

Table 1: Results of the dose-finding study

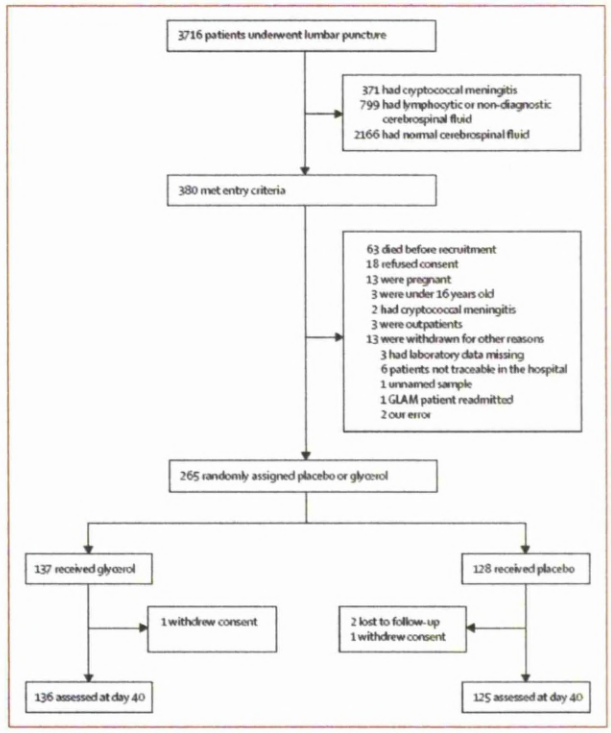

Figure 1: Trial profile

	Placebo	Glycerol
Number recruited	128	137
HIV seropositive*	104/124 (84%)	111/134 (83%)
Prior AIDS defining illness stage modified WHO 3 or 4	33/128 (26%)	36/137 (26%)
Median (IQR) age (years)	32 (27-38)	32 (27-40)
Female	67/128 (52%)	72/137 (53%)
Initial Glasgow coma scale <12	48/128 (37·5%)	39/137 (29·3%)
Initial Glasgow coma scale <8	14/128 (11%)	9/137 (6·6%)
Median (IQR) cerebrospinal fluid white cell count (white cells per μL)	400 (200-1040)	395 (200-1120)
Proven bacterial meningitis	54/128 (42%)	64/137 (47%)
Pneumococcal disease	52/128 (41%)	46/137 (34%)
Median (IQR) cerebrospinal fluid opening pressure (cm CSF)	28 (18-34)	21·5 (12·5-34)
Fits or history of fits in last 2 weeks	51/128 (40%)	49/137 (36%)
Prior antibiotic use	59/128 (46%)	53/137 (39%)
On antiretrovirals	16/128 (13%)	23/137 (17%)
Median (IQR) duration of symptoms (days)	6 (3-8)	5 (3-7)

Data are n (%) unless otherwise stated. *HIV status not known in three patients who received placebo and four patients who received glycerol.

Table 2: Baseline characteristics in the main trial

identification as appropriate. Blood was cultured with the BacT alert system. Organisms were identified by use of conventional culture-based microbiological techniques. Cerebrospinal fluid cryptococcal antigen agglutination tests (Pastorex Crypto Plus Biorad, Marnes-la-Coquette, France) were done by the MLW laboratory on a subset of patients. Blood for malaria film, full blood count, and biochemistry was analysed by the QECH laboratory. HIV serological testing was done in duplicate for every patient by use of Uni-Gold Recombigen HIV rapid test (Trinity Biotech, Wicklow, Ireland) and Determine HIV-1/2 (Abbott, USA). BM glucose was measured with Glucostix (Bayer Diagnostics, Basingstoke, UK) at least twice a day while the patient was taking study drug.

Statistical analysis

The sample size required to detect a reduction in mortality from 56% (previous mortality in observational studies) to 40%, with α=0·05 and β=0·9, was 216 patients per group (calculated using Stata 8.0). Data were double entered into Microsoft Access and verified before being analysed with Stata 8.0. The analytical plan was finalised before unmasking.

All patients were analysed for the primary outcome excluding those who were lost to follow-up. A per-protocol analysis (restricted to those not withdrawn from glycerol or placebo for reasons other than death) was also planned for the mortality-defined end-points. Logistic regression was used to construct a model to estimate the primary and secondary outcomes (with the exception of hearing loss and cerebrospinal fluid opening pressure at 2 days), and odds ratios (ORs) calculated unadjusted and adjusted for the following prespecified potential confounding factors: HIV serostatus, antiretroviral use, prior AIDS-defining illness (modified WHO grades 3 or 4),[28] age, sex, prior antibiotic use, score on the GCS on admission, fits before admission, duration of symptoms, pretreatment with antibiotics, and organism isolated from cerebrospinal fluid or blood. The secondary analysis of time to death was analysed with Cox's proportional hazard ratios, unstratified and stratified by the same potential confounding factors. Statistical significance for the primary outcome was defined as p less than 0·05.

An unblinded interim analysis by the data safety monitoring board had been prespecified after 100 deaths. Indicative criteria for stopping included recruitment to be halted if a treatment group showed evidence of harm exceeding conventional levels of chance alone (p<0·01), and recruitment to be halted if there was clear evidence of benefit with a significance level of p less than 0·001.

The trial is registered with controlled-trials.com, number ISRCTN70121840.

Role of the funding source

The sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The dose-finding study ran from March, 2006, to July, 2006. 45 patients were randomly assigned to receive one of three doses of glycerol (table 1). About 50% of patients in each group experienced nausea, vomiting, or diarrhoea. Vomiting did not occur in any patient receiving glycerol via nasogastric tube. No clear relation existed between the dose of glycerol and frequency of side-effects. 14 (31%) patients had transiently increased blood glucose concentrations greater than 12·2 mmol/L after taking study drug. Peak hyperglycaemia ranged from 12·2–20·4 mmol/L. One patient had a single reading of 40 mmol/L at recruitment, but died before receiving study drug. No patients needed treatment with insulin. Five patients had hyperglycaemia (12·2–20·4 mmol/L) after glycerol had been discontinued, suggesting type 2 diabetes or a stress response was the cause. Patients had difficulty swallowing the largest volume given. Eight of 15 patients in the 50 mL group, seven of 15 in the 75 mL group, and ten of 15 in the 100 mL group died in hospital. On the basis of tolerability, the 75 mL dose was used for the subsequent trial.

The trial started on Sept 10, 2006, and recruitment was halted on Aug 23, 2008. 3716 patients were screened, 380 met the inclusion criteria, and 265 were randomly assigned to placebo or glycerol (figure 1). Baseline characteristics of the two groups were similar (table 2). Follow-up of survivors to day 40 was 98% and 99% in the placebo and glycerol groups, respectively.

The study was stopped following the planned interim analysis after 100 deaths, with 7 months of the trial still to

	Placebo	Glycerol	Odds ratio (95% CI, p)	Adjusted odds ratio (95% CI, p)*
Died before day 40	61/125 (49%)	86/136 (63%)	1·8 (1·1–3·0) p=0·02	2·4 (1·3–4·2, p=0·003)
Died or disability before day 40†	75/124 (60%)	93/135 (69%)	1·4 (0·87–2·4) p=0·2	1·7 (0·97–3·1, p=0·07)
Died by day 10	53/126 (42%)	80/136 (59%)	2·0 (1·2–3·2) p=0·007	2·7 (1·5–4·8, p=0·001)
Per-protocol analysis death to day 40	57/106 (54%)	77/118 (65%)	1·6 (0·9–2·8) p=0·08	2·2 (1·2–4·1) p=0·01
Died by day 40 restricted to proven bacterial disease	21/53 (40%)	43/63 (68%)	3·3 (1·5–7·0) p=0·002	5·5 (1·9–15·4, p=0·0011)
Died by day 40 restricted to pneumococcal disease	20/51 (39%)	31/45 (69%)	3·4 (1·5–8·0) p=0·004	8·2 (2·4–28·5, p=0·0006)

Data are n (%) unless otherwise stated. *Prespecified factors: HIV status, age, organism in blood or cerebrospinal fluid, antiretroviral treatment, pre-treatment antibiotics, fits prior to admission, Glasgow coma score, duration of symptoms, sex, prior AIDS-defining events. †No day 40 data for two patients.

Table 3: Primary and secondary outcome data

run and 61% of the predetermined recruitment completed. The data safety monitoring board advised stopping recruitment on the grounds of futility. Patients already recruited to the trial were followed up until completion of follow-up or the primary endpoint was reached.

40 days after enrolment, the proportion of patients who died was significantly larger in the glycerol group than in the placebo group: this was the same for death and disability. Overall, 61 (49%) of 125 patients in the placebo group and 86 (63%) of 136 in the glycerol group died by day 40 (odds ratio [OR] 1·8, 95% CI 1·1–3·0; p=0·02). The OR adjusted for prespecified potential confounding factors was 2·4 (95%CI 1·3–4·2, p=0·003). Glycerol did not improve death and disability by day 40, or death at day 40 in proven bacterial disease or pneumococcal disease (table 3). Among survivors tested by audiometry, 14 of 41 patients in the placebo group and four of 31 patients in the glycerol group were deaf at day 40 (p=0·02). Time to death is shown in figure 2. The unadjusted hazard ratio (HR) for death was 1·7 (95% CI 1·2–2·4), and the adjusted HR was 2·0 (95% CI 1·4–2·9), with glycerol seeming to be detrimental.

138 (52%) of 265 patients had a second lumbar puncture. The median opening pressure of cerebrospinal fluid 2 days after randomisation was 15 cm CSF (IQR 9–21) in the placebo group, and 11·5 cm CSF (IQR 6–19) in the glycerol group (Willcoxon rank sum difference p=0·08). Median BM glucose on day 2 in both groups was 6 mmol/L (range 4–11 mmol/L).

There were two deaths thought possibly to be due to study drug in view of the rapid and unexpected deterioration in the patients' clinical condition. A 70-year-old HIV-positive woman was admitted with 1 week's history of headache, fever, and confusion, and on admission had a GCS score of 13. She was assigned glycerol. Her level of consciousness improved, but on day 2 she developed seizures refractory to anticonvulsant therapy and she became hypertensive (170/90 mmHg from a baseline of 120/80 mmHg). *Salmonella typhimurium* was cultured from cerebrospinal fluid. A second lumbar puncture was not done, and the study drug was discontinued on day 3. She died on day 12. The second

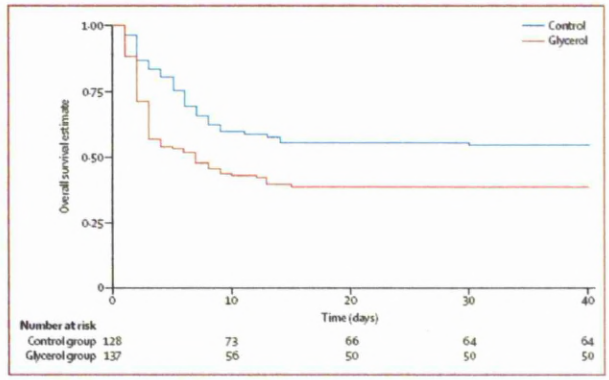

Figure 2: Kaplan-Meier survival estimates for glycerol vs control

case was a 35-year-old HIV-positive woman with a 1-day history of illness and a GCS score of 12 on admission. She was assigned placebo. She improved rapidly to GCS score of 15 by day 2. *Streptococcus pneumoniae* was cultured from blood and cerebrospinal fluid. Discharge was planned on day 10 when she developed generalised weakness and vomiting without fever. She maintained a normal blood pressure and good urine output. On day 11 her level of consciousness fell (GCS 6), although no focal neurological deficit was identified. She died on day 13. The most likely diagnosis for both patients was, on clinical criteria, a major cerebrovascular event secondary to meningitis. However, a brain scan was not possible in either case in this setting.

Glycerol was well tolerated compared with placebo. 79 (65%) of 121 patients who were conscious and able to communicate had gastrointestinal adverse events (nausea, vomiting, diarrhoea) in the placebo group, compared with 85 (66%) of 129 patients who were conscious and able to communicate in the glycerol group. Seizure during the initial 10 days after randomisation occurred in 37 (31%) of 121 patients in the placebo group and 64 (50%) of 129 in the glycerol group (p=0·002);

almost half of these occurred while taking study drug. 72 (27%) of 265 patients had a seizure within the first 2 days of admission; 25 (19%) of 128 in the placebo group and 47/137 (34%) in the glycerol group (p=0·006, post-hoc analysis). No other symptoms or signs differed between the two groups after randomisation. A total of 135 (51%) patients presented with a GCS score of less than 14, 23 of whom had a GCS score of less than 8 (14 in the placebo group, and nine in the glycerol group).

Discussion

In our study in adults, which was stopped early by the data safety monitoring board due to futility, glycerol was associated with significantly higher mortality within 40 days than was placebo. Glycerol was also associated with worse outcomes in all major secondary analyses, except deafness, at day 40. This trial therefore does not support the use of glycerol as adjunctive treatment for bacterial meningitis in adults in Malawi.

These findings from the first adult study of glycerol are markedly different from those of studies in children (panel). In a small study of infants and children in Finland, oral glycerol reduced severe or profound hearing loss.[7] A larger multicentre paediatric study in Latin America[8] suggested that glycerol prevented neurological sequelae, although several methodological concerns were subsequently raised about this study.[9] A small, randomised, double-blind study in India by Sankar and colleagues[10] comparing dexamethasone and oral glycerol adjuvant therapies in children with acute bacterial meningitis did not find any significant difference in hearing loss and neurological sequelae between the groups.[11] Animal models of pneumococcal meningitis have not shown a beneficial effect from glycerol.[12] Our findings suggest glycerol confers no benefit and might be harmful. However, there are major differences in our study population and those of other studies: our patients were adults, 84% were HIV positive, and glycerol was given for 4 days rather than 2 days. HIV serostatus was not reported in the paediatric studies, although it is highly likely that most participants were HIV negative. Additionally, access to health care is often delayed in sub-Saharan Africa compared with other settings; most of our patients had symptoms for 5 days or more, compared with over 85% presenting within 48 h of the onset of symptoms in previous studies. Despite these population differences, why glycerol seemed harmful is hard to explain. Hyperglycaemia—which adversely affects outcome compared with normoglycaemia after stroke, acute myocardial infarct, pneumonia, and in those acutely ill on intensive care[13–17]—is not a plausible explanation, because it was rarely detected in either of the treatment groups in our study. One possibility is that the sugar solution used as placebo for blinding was beneficial, but this seems unlikely; there was no evidence of hypoglycaemia in any patient before giving the study drug.

That a higher proportion of patients had convulsions in the glycerol group than in the placebo group is concerning, and might relate to the poor outcome. This could be a coincidental finding associated with the severely ill population in this study, or could be directly related to an effect of glycerol. This has not been previously documented in humans. A study in mice found that oral glycerol could produce changes in behaviour and seizures within 30 min.[18] The exact cause of these effects was not clear, but an increase in oxygen species might be the cause, and the effects were associated with increased interleukin 1β concentrations in the hippocampus. In human studies, rebound phenomena can occur with osmotherapy, particularly mannitol,[19] but evidence for its occurrence with glycerol therapy is mixed. Some studies do not show a rebound increase in intracranial pressure with either oral or intravenous glycerol (compared with mannitol),[15,39,40] others show substantial rises in intracranial pressure with continuous intravenous and intermittent oral administration.[41,42] Rebound increase of intracranial pressure might be attributable to a reversal of the concentration gradient of the osmotic agent between blood and cerebrospinal fluid (or brain interstitial fluid) as the drug is eliminated.[43] Although this increase in pressure is not generally thought to be clinically significant, its relevance is unclear in the context of bacterial meningitis. Reliable clinical interpretation of the changes in cerebrospinal fluid opening pressures is difficult, in part because of early mortality.

There are potential limitations to this study. Although this hospital is better staffed and supported than many in a similar setting, general medical care is suboptimal compared with that offered in developed countries. Additionally, there was limited intensive or high dependency care available for such critically ill patients with severe sepsis. Overall, this is a typical southern

Panel: Research in context

Systematic review

We searched PubMed using the terms "glycerol", "adjuvant therapy", and "bacterial meningitis". There are no randomised controlled trials using glycerol adjuvant therapy in meningitis in adults. There is one trial in children in Latin America where adjuvant dexamethasone or glycerol with placebo was administered. Glycerol did not improve mortality or deafness, but did reduce severe n eurological sequelae. Hearing loss was reduced in a small study in children in Finland, but no advantage was seen from glycerol in another small paediatric study in India.

Interpretation

Our trial is in adults, most of whom were HIV positive, and showed evidence of increased mortality with glycerol. It therefore makes it unlikely that glycerol is beneficial in adults or in those who are HIV positive, but does not exclude the possibility of benefit in children.

African hospital, and the population of patients and trial setting is likely to be representative of other sub-Saharan African settings with a high prevalence of HIV. The results do not exclude the possibility that glycerol might be effective in a setting with low HIV prevalence in which patients present earlier and where HIV infection is less common. Residual confounding is a possibility, but the finding is highly statistically significant, no subgroup analysis suggested benefit, and adjusting for identified confounding factors only strengthened the association, making residual confounding unlikely. The mortality difference seen at interim analysis by the data safety monitoring board strengthened with follow-up of enrolled patients, and the adverse effect persisted after adjusting for confounding, thus supporting the decision to stop early.

In adults in Malawi or other resource-poor regions where bacterial meningitis presents late and commonly occurs in people who are infected with HIV, glycerol cannot be recommended as adjunctive therapy. Other ways of reducing the substantial mortality associated with this disease need to be explored. Glycerol might be harmful as adjunctive therapy in adults with bacterial meningitis in sub-Saharan African settings with a high prevalence of HIV.

Contributors

KMBA helped design the study, and contributed to the literature review, data collection, writing, data analysis, and interpretation. KEC contributed to data collection and writing. MS helped design and write the study. JBM and PG contributed to data collection. MEM helped do the trial, interpretation, and writing. EEZ contributed to data collection and writing. NF contributed to design, data interpretation, and writing. CJMW and DGL contributed to design, data analysis, interpretation, and writing.

Conflicts of interest

The authors declared no conflicts of interest.

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