Early Goal Directed Therapy for adult meningitis in Malawi

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

by

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August 2014

Table of Contents

List of Tables 9

List of Figures 12

List of Acronyms 15

Appendix: List of included documents 22

Abstract 23

Preface 25

1. Introduction 29

1.1 Background 29

1.2 How is bacterial meningitis diagnosed? 31

1.3 Treatment of bacterial meningitis 39

1.4 How does bacterial meningitis differ between well and low resourced settings? 40

1.5 Project starting points 42

1.6 Aims and Objectives 44

2. Review of the Literature 46

2.1 Introduction 46

2.2 What are the causes of mortality from bacterial meningitis? 48

2.3 Pathological features of ABM and relationship to outcome 51

2.4 Clinical studies and determined predictors of mortality 61

2.5 Why have adjunctive interventions failed in African Adults? 73

2.6 How can Early Goal Directed Therapy (EGDT) be used as an approach to bacterial meningitis in sub-Saharan Africa? 78

2.6.1 What is EGDT? 78

2.6.2 Testing EGDT in clinical trials; the evaluation of complex interventions 78

2.6.3 Is EGDT effective? 81

2.7 What evidence is there to use EGDT for bacterial meningitis? 91

2.8 What evidence exists to support the individual elements of the care bundle in BAM? 92
3 Patients, Materials and Methods 97
3.0 General methodology 97
3.0.1 Introduction 97
3.0.2 Cross-chapter Bacterial meningitis methodology 98
3.1 General Sampling & Laboratory methods 101
3.2 General Data Management and Statistical methods 107
3.2.1 Data Management Methods 107
3.2.2 Epidemiology of bacterial meningitis 108
3.2.3 Predictors of poor outcome using logistic regression 108
3.3 Ethical approvals for studies on human subjects 110
3.4 Methods for the prospective non-randomised clinical trial testing a clinical care bundle for adult meningitis in Malawi (BAM study). 111
3.3.2 Introduction 111
3.3.3 Objectives and research question 111
3.3.4 Study participants, inclusion and exclusion criteria 112
3.3.5 Study site 116
3.3.6 Study design and sample size 116
3.3.7 Care bundle intervention 119
3.3.8 Laboratory investigations including sample storage 126
3.3.9 Endpoints and follow up 128
3.3.10 Verbal assent and informed consent 129
3.3.11 Adverse Events 131
3.3.12 Data analysis and statistics 133
4. Bacterial meningitis in Malawi 2000-2012: 136
4.1 Introduction 136
4.2 Bacterial meningitis surveillance in Malawi 136
4.2.1 Research questions 137
4.2.2 Specific Methods 137

4.2.3 Statistical methods 139
4.3 Results of epidemiology of bacterial meningitis 141
4.3.1 Surveillance trends in culture proven bacterial meningitis..... 141
4.3.2 Estimates of incidence of bacterial meningitis in Malawi..... 146
4.3.3 Seasonality of bacterial meningitis by pathogen 147
4.3.4 CSF findings in bacterial meningitis..... 148
4.4 Clinical predictors of poor outcome from bacterial meningitis 150
4.4.1 Research question 151
4.4.2 Methods details 151
4.5 Results of clinical predictors of outcome from meningitis..... 153
4.5.1 Included clinical studies..... 153
4.5.2 Patient characteristics and clinical and laboratory findings 155
4.5.3 Results of data analysis..... 158
4.5.4 Results of the multivariate analysis to derive clinical prognostic markers of outcome..... 159
4.6 Summary and discussion of results findings from Chapter 4 166
5 Development of a prediction tool for adult bacterial meningitis in sub-Saharan Africa: Malawian Adult Meningitis Score (MAMS)..... 170
5.1 Introduction 170
5.1.1 Overview of severity score development including validation loop processes..... 172
5.2 Methods 174
5.2.1 Objectives and research questions..... 174
5.2.2 Inclusion and exclusion criteria for MAMS 175
5.2.3 Derivation of the MAMS discovery and validation cohorts 176
5.2.4 Synthesis of the MAMS nomogram 178
5.2.5 Methods for dealing with missing data..... 180
5.3 Results..... 182
5.3.1 Final univariate and multivariate analysis results on the discovery dataset 182

5.3.2 Missing data 189
5.3.3 Summary of validation loop processes 196
5.3.4 Final MAMS nomogram and validation data 210
5.4 Summary and Discussion 213
6 Early Goal Directed Therapy for Adult Meningitis in Malawi 220
6.1 Introduction 220
6.2 Methods 221
6.2.1 Research questions and objectives 221
6.2.2 Study endpoints 222
6.2.3 Study design 223
6.3 Results 224
6.3.1 Participant recruitment 224
6.3.2 Causes of meningitis 225
6.3.3 Clinical presentation with ABM 231
6.3.4 Results of the clinical targets achieved. 237
6.3.5 Mortality and morbidity comparisons between Phase 1 and Phase 2. 249
6.3.6 Malawi adult meningitis score (MAMS) in BAM 253
6.3.7 Subgroup analyses 257
6.3.8 Physiology of patients with ABM. 267
6.3.9 Excluded patients 276
6.4 Discussion 277
6.4.1 Causes of culture negative meningitis 277
6.4.2 Is Early Goal Directed Therapy feasible for bacterial meningitis in settings such as Malawi? 280
6.4.3 Does EGDT have an impact on outcome from ABM in Malawi? . 284
7 The relationship between bacterial load, viral co-infection and outcome from bacterial meningitis in Malawian adults 289
7.1 Introduction 289
7.2 Methods 290

7.2.1 Objectives and research question 290
7.2.1 Inclusion and exclusion criteria and outcome measures 291
7.2.2 Laboratory methods DNA extraction and PCR amplification 291
7.2.3 Data analysis and statistics 292
7.3 Results 292
7.3.1 Is bacterial load associated with outcome from bacterial meningitis? 292
7.3.2 Is viral co-infection in the CSF of adults with bacterial meningitis associated with morbidity? 295
7.3.3 CSF and blood inflammatory biomarkers of poor outcome from bacterial meningitis 299
7.4 Discussion 303
7.4.1 Pneumococcal load in CSF of adults with ABM 303
7.4.2 Viral CSF co-infection of adults with bacterial meningitis 305
7.4.3 Laboratory predictors of poor outcome 307
7.4.4 Limitations, biases and contrasts 309
8 Main Discussion 311
8.1 Introduction 311
8.2 The patient journey from symptom onset to hospital outcome: where can things go wrong for adult patients with ABM in SAA? 312
8.3 Persistently high mortality from ABM in African adults 315
8.3.1 Prediction of poor outcome from ABM 316
8.3.2 Host biomarkers of outcome 318
8.3.3 The roles of pathogenic bacteria in determining outcome 322
8.3.4 The role of HIV co-infection in outcome 323
8.3.5 The importance of Herpes viruses co-infection in ABM pathogenesis 325
8.4 The stages of the patient journey with ABM, opportunities for intervention 327
8.4.1 Community onset of symptoms, understanding of disease and urgency of seeking medical help 327

8.4.2 Health centre management and community treatment/referrals for ABM 328
8.4.3 Delay/travel to central hospitals for diagnosis and treatment 330
8.4.4 Role of in-hospital delays and pre-treatment with antibiotics on outcome. 331
8.4.5 Comparisons of admission characteristics for adults with bacterial meningitis 332
8.4.6 Meningitis Diagnostics and the role of culture negative meningitis 333
8.5 Acute in-hospital treatment: Early Goal Directed Therapy in sub-Saharan Africa 334
8.5.1 The feasibility of EGDT for ABM in a resource-poor setting 334
8.5.2 The impact of EGDT on outcome for ABM in Malawi 338
8.5.3 What is the right approach for the acute resuscitation of ABM in adults in sub-Saharan Africa? 340
8.5.4 Future directions for EGDT for ABM in sub-Saharan Africa 342
8.5.5 Ward based care 344
8.5.6 Post hospital care 345
8.6 Future directions for bacterial meningitis studies in sub-Saharan Africa beyond EGDT 347
8.6.1 Utility of severity scoring systems in resource limited settings for ABM 347
8.6.2 Prospects for future therapies 348
8.6.3 Prevention of ABM 349
8.7 Concluding remarks 350
References 355
Appendices 420
Appendix 1.1 BAM study phase 2 clinical care bundle checklist 420
Appendix 1.2 BAM phase 2: Fluid bolus protocol for shocked participants ... 421
Appendix 1.3 BAM phase 2: seizure treatment protocol 423
Appendix 1.4 BAM protocol for management of agitated patients 425

Appendix 1.5 BAM treatment of hypoglycaemia protocol Phase 2 426

Appendix 1.6 BAM nasopharyngeal airway protocol 427

List of Tables

Table 1.1 Summary of CSF values in meningitis 33

Table 1.2 CSF parameters in HIV co-infected African adults 34

Table 1.3 Mortality from bacterial meningitis by region 41

Table 2.1 Available vaccines for bacterial meningitis 71

Table 2.2 Early Goal Directed Therapy studies in emerging countries 89

Table 3.1 Targets for multiplex Real-Time PCR..... 106

Table 3.2 Clinical components of the care bundle 122

Table 3.3 Targets for EGDT in BAM 124

Table 3.4 Definitions of shock from the surviving sepsis guidelines 2008 125

Table 3.5 List of samples taken from BAM patients with informed consent..... 126

Table 3.6 Modified Rankin Scoring system 129

Table 4.1 CSF parameters for proven bacterial meningitis in Malawi..... 149

Table 4.2 Details of studies included in the analysis 154

Table 4.3 Characteristics of patients included in the Malawi meningitis database 157

Table 4.4 Predictors of poor outcome from bacterial meningitis..... 162

Table 5.1 Predictors of mortality in the MAMS discovery cohort 183

Table 5.2 Analysis of the discovery cohort, complete results 186

Table 5.3 Analysis of the discovery data after random imputation 190

Table 5.4 Haemoglobin and outcome in bacterial meningitis 193

Table 5.5 Analysis of variables between discovery and validation cohorts..... 195

Table 5.6 MAMS agreement data for initial nomogram containing significant variables only 198

Table 5.7 MAMS agreement data for initial nomogram containing all tested variables 199

Table 5.8 Summary of MAMS agreement data after missing data analysis 201

Table 5.9 Analysis of Haemoglobin with outcome by group category 202

Table 5.10 Analysis of GCS and outcome by group category 203

Table 5.11 Analysis of CSF WCC and outcome by group category 204

Table 5.12 Analysis of pulse rate with outcome by group category 205

Table 5.13 MAMS agreement data with categorical data 207

Table 5.14 MAMS agreement data with an expanded bacterial culture category 209

Table 5.15 MAMS validation data for the final nomogram 211

Table 5.16 Summarised MAMS validation data for each nomogram 212

Table 5.17 Comparison of MAMS and EMS validation data 213

Table 6.1 Results of PCR for BAM 226

Table 6.2 Comparison of culture and PCR data per pathogen 227

Table 6.3 CSF PCR and culture results with antibiotics 228

Table 6.4 Antibiotic sensitivities in CSF isolates 230

Table 6.5 Frequencies of antibiotic consumption 231

Table 6.6 Demographics of BAM participants 233

Table 6.7 Clinical features of BAM participants 234

Table 6.8 Presenting physical parameters of BAM patients 235

Table 6.9 Laboratory results in BAM patients 236

Table 6.10 Antiretroviral treatment in BAM 237

Table 6.11 Targets achieved in BAM 239

Table 6.12 Targets achieved in all screened participants 242

Table 6.13 Composite target achievement in BAM 245

Table 6.14 Cumulative target achievement in BAM 247

Table 6.15 Mortality and morbidity outcomes in BAM 250

Table 6.16 Adverse events in BAM 252

Table 6.17 Performance of MAMS in BAM 254

Table 6.18 Performance of MAMS in BAM after imputation 256

Table 6.19 BAM outcomes by admission time 258

Table 6.20 Association of BAM outcomes with known predictors of outcome .. 261

Table 6.21 Individual predictors of outcome by study phase 262

Table 6.22 Targets in BAM and outcome by study phase at day 10 265

Table 6.23 Physiology of meningitis over time in BAM 273

Table 7.1 Bacterial load and outcome 294

Table 7.2 Viral co-infection in meningitis 296

Table 7.3 Viral load and outcome in meningitis 297

Table 7.4 Viral load and quality of survival 299

Table 7.5 Associations between CSF parameters and outcome in meningitis . 301

Table 7.6 Association with haematological parameters and outcome in meningitis 302

List of Figures

Figure 1.1 Case fatality rates for bacterial meningitis 1900-2000 31

Figure 1.2 CSF gram stains in meningitis. 35

Figure 1.3 Growth of *Streptococcus pneumoniae* on blood agar..... 36

Figure 1.4 Growth of *N.meningitidis* on chocolate agar *Figure from www.cdc.com* 36

Figure 1.5 Map of the meningitis belt region of Africa 42

Figure 2.1 Host responses to sepsis and outcome **Error! Bookmark not defined.**

Figure 2.2 Host pathogen interactions in sepsis 54

Figure 2.3 Meningitis pathogenesis in African adults 62

Figure 2.4 Factors influencing outcome from meningitis in Malawi 77

Figure 2.5 Developing and evaluating complex interventions 79

Figure 2.6 Effect size of Early Goal Directed Therapy for sepsis 82

Figure 3.1 Comparison of old and new QECH admission areas 100

Figure 3.2 Example of exponential curves for Real-Time PCR 105

Figure 4.1 Meningitis surveillance data by age group and pathogen 2000-2012 143

Figure 4.2 Proportions of CSF culture positive isolates by paediatric age groups per year 144

Figure 4.3 Proportions of CSF culture positive isolates in adults per year..... 146

Figure 4.4 Estimates of incidence of bacterial meningitis in Malawi by age group 147

Figure 4.5 Seasonality of culture positive pneumococcal meningitis 2000-2010 148

Figure 4.6 Selection of cases for the Malawi meningitis database 155

Figure 4.7 Seasonal admission and outcome data for adults with ABM 2000-2009 161

Figure 5.1 Processes for developing severity scoring systems 173

Figure 5.2 Preliminary nomograms from original complete case data 196

Figure 5.3 Receiver Operator Curve (ROC) comparing predictive power of both original nomograms 197

Figure 5.4 Nomogram using significant variables, post missing data completion 200

Figure 5.5 ROC for the nomogram in Figure 5.4 200

Figure 5.6 Nomogram for significant variables, categorised into quartiles..... 206

Figure 5.7 ROC for agreement of the nomogram in Figure 5.6 206

Figure 5.8 Nomogram showing continuous variables, with explosion of bacterial culture 208

Figure 5.9 ROC curve for the nomogram in Figure 5.8 209

Figure 5.10 Final nomogram to predict outcome from ABM in Malawian adults 210

Figure 5.11 ROC showing sensitivity and specificity of the final MAMS nomogram 211

Figure 6.1 BAM trial recruitment summary..... 225

Figure 6.2 Composite proportions of targets achieved by the care bundle..... 246

Figure 6.3 Kaplan-Meir curve of outcome from ABM by study Phase 251

Figure 6.4 Pulse rate over time in patients with ABM..... 269

Figure 6.5 Mean MAP over time in patients with ABM 269

Figure 6.6 Mean GCS over time in patients with ABM 270

Figure 6.7 Mean respiratory rate over time in patients with ABM 271

Figure 6.8 Mean oxygen saturations over time in patients with ABM 272

Figure 6.9 Mean pulse over time, comparing all ABM to those with clinical shock only 274

Figure 6.10 Mean MAP over time, comparing all ABM with those with clinical shock only 274

Figure 6.11 Mean CGS over time comparing all ABM with those with clinical shock only 275

Figure 6.12 Respiratory rate over time comparing all ABM with those with clinical shock only 275

Figure 6.13 Oxygen saturations over time comparing all ABM with those with clinical shock only..... 276

Figure 7.1 CSF bacterial load by outcome..... 294

Figure 7.2 Bacterial load by with outcome by quartile 295

Figure 7.3 Median viral CSF load of EBV by outcome 298

Figure 7.4 EBV viral load with outcome by quartile group..... 298

Figure 8.1 Patient journey with ABM in Malawi 314

Figure 8.2 Published care bundle feasibility..... 337

Figure 8.3 Points for intervention on the patient journey with ABM in Malawi .. 352

List of Acronyms

3TC	Lamivudine
ABM	Acute Bacterial Meningitis
ABx	Antibiotics
ACWY	Meningococcal vaccine directed against serogroups A, C, W135 and Y
AETC	Adult Emergency and Trauma Centre
APACHE	Acute Physiology and Chronic Health Evaluation
ARMAX	AutoRegressive integrated Moving Average model with eXogenous variables
ART	Anti-Retroviral Therapy
AUC	Area Under the Curve
BAM	Bundles for Adult Meningitis
BBB	Blood Brain Barrier
BL	Blood Lactate
BP	Blood Pressure
CCM	Cryptococcal meningitis
CD4	Cluster Differentiation 4 cell
CDC	Centre for Disease Control, Atlanta USA
CFR	Case Fatality Rate
CI	Chief Investigator
CI	Confidence Interval
CMV	Cytomegalovirus
CNS	Central Nervous System
CO_2	Carbon Dioxide

COMREC	College of Medicine Research and Ethics Committee
CONSORT	Consolidated Standard of Reporting of Trials
CrAg	Cryptococcal Antigen
CRF	Case Record Form
CRT	Capillary Refill Time
CSF	Cerebrospinal Fluid
CT	Computed Tomography scan
CURB-65	Confusion Urea Respiratory rate Blood pressure
CVP	Central Venous Pressure
D4T	Stavudine
DNA	Deoxyribonucleic Acid
E.coli	*Escherichia coli*
EBV	Epstein-Barr virus
ED	Emergency Department
EFZ	Efavirenz
EGDT	Early Goal Directed Therapy
EMS	European Meningitis Score
ENT	Ear Nose and Throat clinical service
EPI	Expanded Programme of Immunisation
ESR	Erythrocyte Sedimentation Rate
ETAT	Emergency Triage And Treatment
FBC	Full Blood Count
FDA	Federal Drug Authority

FTD	Fast Track Diagnostics
GAVI	Global Alliance for Vaccines and Immunisations
GCP	Good Clinical Practice for research
GCP	Good Clinical Practice
GCS	Glasgow Coma Score
GLAM	Glycerol for Adult Meningitis trial
GRADE	Grading of Recommendations, Assessment, Development and Evaluation criteria
Hib	*Haemophilus influenzae* type b
HIV-1	Human Immunodeficiency Virus type 1
HSV	Herpes Simplex Virus
ICP	Intracranial Pressure
ICU	Intensive Care Unit
IFNγ	Interferon Gamma
IL	Interleukin (cytokine)
IPD	Invasive pneumococcal disease
IQR	Inter Quartile Range
IRB	Institutional Review Board
IRIS	Immune Reconstitution Inflammatory Syndrome
IRR	Incident Rate Ratio
ISRCTN	International Standard Randomised Controlled Trials Network
IV	Intravenous
IVIG	Intravenous Immunoglobulin
LAT	Latex Antigen Test

LCA	Latent Class Analysis
LFA	Lateral Flow Assay
LIMS	Laboratory Information Management System
LIMS	Laboratory Information Management System
LP	Lumbar Puncture
LSTM	Liverpool School of Tropical Medicine
LytA	Autolysin
MA	Micro Array
MAMS	Malawi Adult Meningitis Score
MAP	Mean Arterial Blood Pressure
MIC	Minimum Inhibitory Concentration
ml	Millilitre
MLST	Multi-Locus Sequence Typing
MLW	Malawi-Liverpool-Wellcome Trust
MMP	Matrix Metalloproteinase
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging scan
MRs	Modified Rankin Score
NK cells	Natural Killer cells
NM	*Neisseria meningitidis*
NP	Nasopharyngeal
NPV	Negative predictive value
NTS	non-Typhoidal Salmonellae
NVP	Nevirapine

OR	Odd's Ratio
P1	BAM Phase 1
P2	BAM Phase 2
P4	Pneumococcal protein derivative 4
PACTR	Pan-African Clinical Trials Registry
PAGe	Pneumococcal African Genome project
PBMC	Peripheral Blood Monocyte Cell
PCR	Polymerase Chain Reaction
PCV-13	13 valent pneumococcal conjugate vaccine
PCV-7	7 valent pneumococcal conjugate vaccine
PDA	Personal Digital Assistant
PDSA	Plan Do Study Act
PI	Principal Investigator
PICU	Paediatric Intensive Care Unit
Ply	Pneumolysin
PMTCT	Prevention of Mother to Child Transmission of HIV
POC	Point of Care
PPV	Positive predictive value
PR	per rectum
PsaA	Pneumococcal surface protein A
QECH	Queen Elizabeth Central Hospital
QI	Quality Improvement
RCT	Randomised Controlled Trial
RLS	Resource Limited Setting

RNA	Ribodeoxynucleic acid
ROC	Receiver Operated Curve
RPM	Revolutions per minute
RR	Relative Risk ratio
RR	Respiratory Rate
RT-PCR	Real-Time Polymerase Chain Reaction
SAA	sub-Saharan Africa
SAE	Serious Adverse Event
SAH	Sub-Arachnoid Haemorrhage
SAM	Steroids for Adult Meningitis trial
SBP	Systolic Blood Pressure
SIADH	Syndrome of Inappropriate Anti-Diuretic Hormone
SIRS	Systemic Inflammatory Response to Sepsis
SNP	Single Nucleotide Polymorphism
SOFA	Sequential Organ Failure Assessment
SPINE	Surveillance Programme for Inpatients and Epidemiology
SpN	*Streptococcus pneumoniae*
SpO_2	Saturation percentage of Oxygen
SSA	sub-Saharan Africa
SSG	Surviving Sepsis Guidelines
STGG	Skim milk-Tryptone-Glucose-Glycerol media
SUE	Serious Unexpected Event
TB	Tuberculosis

TBM	Tuberculous Meningitis
TDF	Tenofovir
TH	Todd-Hewitt Broth
TNF	Tumour Necrosis Factor
TSC	Trial Steering Committee
UOP	Urine Output
USA	United States of America
VAP	Ventilator Associated Pneumonia
VZV	Varicella Zoster Virus
WCC	White Cell Count
WHO	World Health Organisation
WTSI	Wellcome Trust Sanger Institute

Appendix: List of included documents

1. BAM Early Goal Directed Therapy care bundle treatment protocols

1.1 BAM study care bundle prescription checklist

1.2 Fluid resuscitation

1.3 Seizure treatment

1.4 Management of the agitated patient

1.5 Management of hypoglycaemia

1.6 Nasopharyngeal airway support

2. Published manuscripts from this thesis

2.1 High mortality amongst adolescents and adults with bacterial meningitis in sub-Saharan Africa: an analysis of 715 cases from Malawi.

Wall EC, Cartwright K, Scarborough M, Ajdukiewicz KM, Goodson P, Mwambene J, Zijlstra EE, Gordon SB, French N, Faragher B, Heyderman RS, Lalloo DG. PLoS One. 2013 Jul 19;8(7):e69783. doi: 10.1371/journal.pone.0069783.

2.2 Bacterial meningitis in Malawian adults, adolescents, and children during the era of antiretroviral scale-up and Haemophilus influenzae type b vaccination, 2000-2012.

Wall EC, Everett DB, Mukaka M, Bar-Zeev N, Feasey N, Jahn A, Moore M, van Oosterhout JJ, Pensalo P, Baguimira K, Gordon SB, Molyneux EM, Carrol ED, French N, Molyneux ME, Heyderman RS. Clin Infect Dis. 2014 May;58(10):e137-45. doi: 10.1093/cid/ciu057. Epub 2014 Feb 4.

Abstract

Early Goal Directed Therapy for Adult Meningitis in Malawi

Emma C Wall

Introduction: Mortality from acute bacterial meningitis (ABM) in sub-Saharan African adults is 50-60%; twice that of more well-resourced settings. To date, interventions designed to impact on outcome have been ineffective in this setting. This thesis addressed the hypothesis that high mortality is due in part to delayed or inappropriate acute care, and that this may be improved by goal directed resuscitation

Methods: Clinical and laboratory surveillance data from a large central hospital in Malawi were analysed. Clinical predictors of poor outcome from ABM were synthesised into the Malawi Adult Meningitis Score (MAMS). To assess feasibility and outcome, patients with suspected ABM were recruited in the emergency department and observed in year one under routine clinical care (Phase 1), and then managed with a meningitis-specific EGDT clinical care bundle in year two (Phase 2). Laboratory data were tested for outcome associations.

Results: A significant decline in the total number of CSF isolates over 12 years was noted (incident rate ratio 0.93 [95% CI 0.92-0.94], p<0.001), entirely in children under 5 years (IRR =0.87, 95%CI 0.85-0.88, p<0.001) coinciding with Hib vaccination. Adult meningitis remained unchanged (IRR=0.99, 95%CI 0.97-1.0, p=0.135) despite extensive ART provision.
Analysis of 715 historical episodes of adult ABM showed that the mortality was 54% at day 40; HIV seroprevelence was 87%; and treatment delays were marked. Coma, seizures, tachycardia and anaemia but not HIV were significantly associated with mortality.
The MAMS predicted outcome with good agreement, *c* statistic 0.74 (95% CI 0.65 : 0.82), estimated sensitivity was 75%, specificity was 55%.

EGDT was found to be feasible, more clinical targets were met using EGDT, including reduced time to antibiotics (1hr 55 minutes in P1 v 1hr 13 minutes in P2 p<0.001), IV fluids and blood were more frequently given (median volume IV fluid P1 = 750mls, in P2 = 1500mls p<0.001, blood transfusion 0/2 in P1, 2/3 in P2 p=0.4); airway placement was more likely (P1 0%, P2 77% p=0.04). There was no significant difference in outcome at 40 days; death or disability in 29/57 (51%) in P1 and 38/60 (63%) in P2 p=0.19.

Neither high pneumococcal load, nor the presence of EBV co-infection in the CSF were associated with poor outcome. CSF white cell counts were lower in non-survivors, other markers of inflammation were not associated with outcome.

Conclusion: The incidence of bacterial meningitis is not falling in adults in Malawi. Important clinical outcome predictors were identified and synthesised into a prediction tool. EGDT for ABM is feasible in adult patients in Malawi; a larger trial is required to test the impact on outcome. Further work to improve pre-hospital care and identify novel adjunctive treatments is necessary.

Preface

Declaration

The results presented in this work represent my own work under the supervision of, and in collaboration, with my supervisors Professors David Lalloo and Rob Heyderman, with the support of Professor Theresa Allain (head of department of Medicine) and Dr Mulinda Nyrienda (head of the Adult Emergency Trauma Centre) at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi where this work was carried out. Statistical support was provided by Dr Mavuto Mukaka of Malawi Liverpool Wellcome Trust Clinical Research Programme (MLW) and Philip Gichiru and Dr Brian Faragher of the Liverpool School of Tropical Medicine (LSTM). In Chapter four, epidemiological and seasonal modelling of bacterial meningitis surveillance data was done by Dr Naor Bar-Zeev of MLW, incident rate ratios and graphs were drawn by Dr Mukaka and myself. All other statistical analyses were done by myself. Data analysis for Chapter five was done by myself and Dr Mukaka, I did all the multivariate analyses, Dr Mukaka derived the nomograms, and calculated the validation data. For Chapter six, where MAMS was used, I calculated the scores and the predictive risk for each patient with advice from Dr Mukaka. Data monitoring of the trial was provided by Professor Tim Peto (University of Oxford), Dr Johnstone Kumwenda (John's Hopkins University) and Dr Brian Faragher (LSTM).

I declare that the material presented has not been presented in whole, or in part for any other degree or examination.

The BAM trial was funded by the Wellcome Trust, through the Wellcome Trust clinical PhD fellowship scheme to the University of Liverpool. Individual fellowship grant number 089671/B/09/Z. The trial was carried out at Queen Elizabeth Central Hospital, Blantyre, Malawi.

All laboratory work, with the exception of blood haematology and biochemistry, was performed at the laboratories of MLW. Routine patient blood tests were performed by the hospital laboratory of QECH.

The trial protocol was conceived and authored by myself, with support from David Lalloo and Rob Heyderman. Responsibility for obtaining funding, institutional and ethical approvals, trial registration, recruitment of the study team, running of the trial, data collection and analysis was my own. Support with data collection and collation was provided by the data support team headed by Malango Msukwa at MLW: laboratory support with the molecular studies was provided by Dean Everett, Maaike Aalerts and Mavis Menyere.

Acknowledgements

This trial would not have been possible without generous funding from the Wellcome Trust, through the Clinical PhD Fellowship programme, which provided me with support for a Masters degree in clinical research (MRes) and for the BAM study and associated studies, and for which I am enormously grateful.

This trial would also not have been possible without the support and encouragement of the study team, trial patients and my medical and nursing colleagues at both the QECH Department of Medicine and AETC, and at MLW. I am particularly grateful to Mulinda Nyrienda, Theresa Allain, Vella Kadzu and Neema Mtunthama whose support and guidance was instrumental to the smooth running of the trial. I was very fortunate to make a great many friends in Malawi during the course of this study, and their enthusiasm and stoicism in the practice of medicine was an outstanding example to me of delivery of the highest possible standards in the face of substantial adversity. This practice of high quality research and clinical medicine will stay with me as an example for my entire career.

I would like to thank my supervisors, Professors David Lalloo and Rob Heyderman, for their constant support and advice throughout this project, and whose encouragement led to many additional projects and collaborations from this study. I would also like to thank Professors Stephen Gordon, Liz Molyneux and Malcolm Molyneux whose wisdom and mentorship has greatly assisted me with this project. I would like to thank Dr Mavuto Mukaka and Dr Brian Faragher for all their support with the statistical analyses in this project, and whose talents in teaching and friendship have made statistics enjoyable for the first time for me. I would also like to thank Tim Peto, Brian Faragher and Johnstone Kumwenda for constantly monitoring the study data and providing me with useful, real time feedback for the study. The Wellcome Trust Tropical Centre in Liverpool, provided me with invaluable logistic support during the trial, and I am particularly grateful to Carolyn O'Leary and Kate Jones.

My family, particularly my husband and son have been instrumental from the outset in their support for all aspects of this project, without whose support and encouragement I would not have been able to enjoy and complete this trial. This thesis is dedicated to them.

1. Introduction

In this introduction, an overview of the diagnosis and treatment of acute bacterial meningitis (ABM) in adults will be given. The origins of the research questions addressed in this thesis, arising from previous work in ABM in Malawi and elsewhere will be discussed. A detailed examination of the literature associated with the two main research questions will be presented in Chapter Two.

1.1 Background

Acute bacterial meningitis

Bacterial meningitis is defined as the presence of inflammation in the sub-arachnoid space and pia mata associated with invasion of bacteria into the sub-arachnoid space (Flexner, 1907; Hoffman and Weber, 2009).

Meningitis can be caused by bacteria, mycobacteria, fungi, viruses, malignancies and auto-immune diseases (Jarvis et al., 2010; Dougherty and Jones, 1986). Bacterial meningitis is caused by pathogenic bacteria infecting the meninges lining the brain and the circulating cerebrospinal fluid (CSF) in the sub-arachnoid space. The most common aetiological bacteria are *S.pneumoniae*, *N.meningitidis* and Hib, however bacterial meningitis can be caused by gram negative pathogens including *Escherichia coli*, *Salmonella Typhimurium*, *Salmonella typhi*, *Klebsiella pneumoniae*, and by gram positive pathogens including Group B Streptococci, Group A Streptococci, *Staphylococcus aureus,* and *Listeria monocytogenes* (Brouwer et al., 2010b).

Discrepancies exist in the worldwide epidemiology of bacterial meningitis, with the largest burden of both disease and mortality occurring in sub-Saharan Africa, with young children disproportionately at risk (Molyneux, 2005; Mueller et al., 2012; Pelkonen et al., 2009; Scarborough and Njalale, 2004).

Meningitis epidemiology varies between epidemic meningitis outbreaks of predominately meningococcal disease, and stable sporadic disease which is predominately pneumococcal, with the highest incidence of epidemic cases in the 'meningitis belt' region of West and Central Africa, and the highest incidence of sporadic cases found in sub-Saharan Africa (WHO, 2013b; Irving et al., 2012; Gessner et al., 2010; Molesworth et al., 2003). Malawi is far from the meningitis belt, but has seasonal pneumococcal disease and an estimated incidence of ABM in 2012 of 10/100000 population in adults and 20/100 000 population in children <14 years (Wall et al., 2014a).

Community acquired acute bacterial meningitis (ABM) is one of the most rapidly fatal infectious diseases known, affecting humans of all ages. Untreated, bacterial meningitis caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib) is universally fatal: untreated epidemic meningitis caused by *Neisseria meningitidis* had a mortality rate of 60-80% (Flexner, 1913; Swartz, 2004). In the antibiotic era, the case fatality rate (CFR) has declined substantially, but varies considerably with aetiology and geographical region. The overall CFR is 10-40% in children and 10-60% in adults (van de Beek et al., 2010). Pneumococcal meningitis is associated with the highest mortality rate in both children (20-30%) and adults (25-60%), meningococcal meningitis is associated with the lowest mortality rates (<10%) (Swartz, 2004) (Figure 1.1). In Malawi, the CFR in adults with predominately pneumococcal disease is 54%, in children with a mixture of pneumococcal, Hib and meningococcal meningitis the CFR is 30-40% (Scarborough et al., 2007; Ajdukiewicz et al., 2011; McCormick et al., 2012; Carrol et al., 2007a).

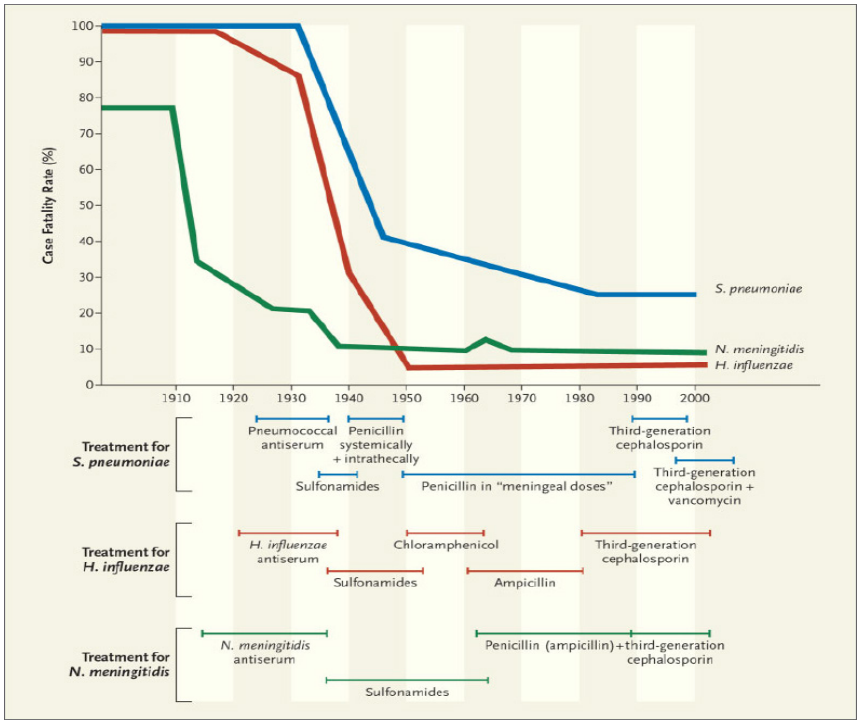

Figure 1.1 Case fatality rates for bacterial meningitis 1900-2000

Reproduced from Swartz 2004. Case Fatality Rates for different meningitis pathogens over time with the introduction of different therapies.

The estimated death rate per 100 000 population world-wide caused by bacterial meningitis in all age groups declined from 8.1/100 000 in 1990 to 6.1/100 000 in 2010, falling from the 21st most common cause of death world-wide to the 29th in 2010 (Lozano et al., 2012). This decline in meningitis mortality has been primarily driven by vaccination campaigns and improved neonatal and infant healthcare (Lozano et al., 2012). Survivors in all age groups have high rates of neurological morbidity including deafness and epilepsy (Ramakrishnan et al., 2009; Weisfelt et al., 2006c; de Louvois et al., 2007; Edmond et al., 2010).

1.2 How is bacterial meningitis diagnosed?

1.2.1 Clinical Diagnosis

The intense inflammation in the sub-arachnoid space causes symptoms of severe headache, fever and may cause other signs of meningism such as neck stiffness,

photophobia, confusion or mental obtundation, seizures and coma (Nijman et al., 2013; Thompson et al., 2006; Durand et al., 1993). These classical signs may present late in the disease, and early symptoms are often similar to a non-specific influenza-like illness with fever, myalgia and mild headache. In the elderly, very young and immunocompromised, classical symptoms and signs may not develop, and a high index of clinical suspicion is required to detect meningitis in these patients (Brouwer et al., 2012; Thompson et al., 2006; Weisfelt et al., 2006f).

However, The classical clinical triad of fever, neck stiffness and altered mental status are not commonly all present in cases of bacterial meningitis (van de Beek et al., 2004)(Attia et al., 1999); the combined sensitivity of all three in European adults is only 44% (van de Beek et al., 2004). The diagnostic accuracy of clinical symptoms and signs to predict bacterial meningitis are low when applied individually to adults, however the lack of all three classical signs has a sensitivity of 99-100% in eliminating bacterial meningitis (Attia et al., 1999).

A meta-analysis of three studies in adults with subsequently proven bacterial meningitis suggests Kernig's sign (pain in the neck elicited when the hip is flexed and the knee extended) has a positive predictive value (PPV) for bacterial meningitis of 60% and a negative predictive value (NPV) of 60%. Neck stiffness alone has a PPV of 41% and a NPV 61% (Thomas et al., 2002, Waghdhare et al., 2010, Brouwer et al., 2012).

Therefore any patient presenting with a suggestive history or clinical feature of possible meningitis must undergo full clinical evaluation for bacterial meningitis, including lumbar puncture if indicated. A high index of suspicion is required to ensure bacterial meningitis is not missed, when this happens it is usually results in fatal consequences (Nigrovic et al., 2012; Heyderman, 2005).

A definitive diagnosis of ABM is dependent on analysis of the CSF using biochemistry, cell microscopy, microbiology and where available, pathogenic DNA detection using PCR.

Lumbar puncture is therefore essential to diagnose bacterial meningitis, as the identification of inflammation in the cerebrospinal fluid (CSF) can provide important data to discriminate between bacterial and other causes of meningitis; where bacteria are identified on culture or Gram stain the diagnosis is conclusive. Typical CSF findings in bacterial meningitis are a CSF pleocytosis with elevated protein and low paired CSF:blood glucose ratio of <0.6 (Deisenhammer et al., 2006). Table 1.1details the typical CSF values for adults with viral and bacterial meningitis in resource rich settings where the prevalence of HIV co-infection in the population is <10%.

Table 1.1 Summary of CSF values in meningitis

Typical CSF values for different types of meningitis in adults, summary statistics from four different studies*

CSF parameter	Normal CSF	Bacterial meningitis (range)	Viral meningitis (range)
Median CSF Protein (g/L)	0.38 (0.1 – 0.58)	4.18 (0.21 – 22.2)	0.77 (0.11 – 4.0)
CSF:Blood Glucose ratio	0.6	<0.4	0.6
Median White cell count (cells/mm^3)	0-5	1000-10000** (<100- >10000)	<300 (<100-<1000)

*Data from: (Dougherty and Jones, 1986; Fitch and van de Beek, 2007; Seehusen et al., 2003; van de Beek et al., 2004)

** wide discrepancy between median cell counts in the studies available, the data given are the range of medians across the studies

However these classical patterns may be substantially different in the presence of HIV infection. In African studies of adults with ABM who are HIV co-infected, the median CSF white cell count (WCC) in patients with culture proven ABM varies between 29 cells/mm^3 to 480 cells/mm^3 compared to >1000 cells/mm^3 in HIV antibody negative patients in Holland (Jarvis et al., 2010; van de Beek et al., 2004; Cohen et al., 2010c; Hakim et al., 2000; Wall et

al., 2013c). The data reporting CSF WCC in adults with proven meningitis from SSA are limited, Table 1.2 summarises the data from four different studies of adult meningitis in the region where CSF WCC were reported, from Zimbabwe, Malawi and South Africa. In each of these studies >90% of patients were HIV co-infected. The CSF WCC values are variable across the studies, but this likely to be due to substantial heterogeneity in recruitment and laboratory measurement. CSF biochemistry parameters where measured are similar to those from resource rich settings (Table 1.1), but the white cell response in all three studies appears to be substantially lower than that recorded in non-HIV studies.

Table 1.2 CSF parameters in HIV co-infected African adults

Bacterial meningitis in HIV co-infected adults in SSA*

Study	Median white cell count (cells/mm^3, range)	Percentage polymorphonuclear cells	Median Protein (g/L)	Median CSF glucose (mmol/L)
Jarvis 2010	29 (5 – 420)	Not reported	5.0 (2.7 - >5.0)	0.3 (<0.3 – 0.4)
Cohen 2010*	260 (32 – 960)	90%	NA	NA
Hakim 2000	100 (no range given)	>50% neutrophils in 87%	> 0.45 in all patients	<2.2 in 80%
Wall 2013*	480 (170 – 1680)	Not reported	Not reported	Not reported

Data from: (Jarvis et al., 2010; Cohen et al., 2010c; Hakim et al., 2000)

*These studies had an entry criteria of cell count >100 cells/mm^3 for entry to the study if CSF culture negative.

The evidence from high HIV prevalence settings shows that the interpretation of CSF white cell count and chemistry data in HIV co-infected adults must take these data into account when diagnosing bacterial meningitis. CSF WCC responses to different pathogens and in different age groups will be explored in Chapter 4.3.4.

1.2.2 Laboratory diagnosis

a) Culture methodology

Traditional microscopy and culture-based microbiological laboratory techniques have been in use to diagnose ABM since the pneumococcus was first identified independently by Louis Pasteur and George Sternberg in 1881(Watson et al., 1993). The pneumococcus is a gram positive diplococcus, the meningococcus is a gram negative diplococcus (Figure 1.2).

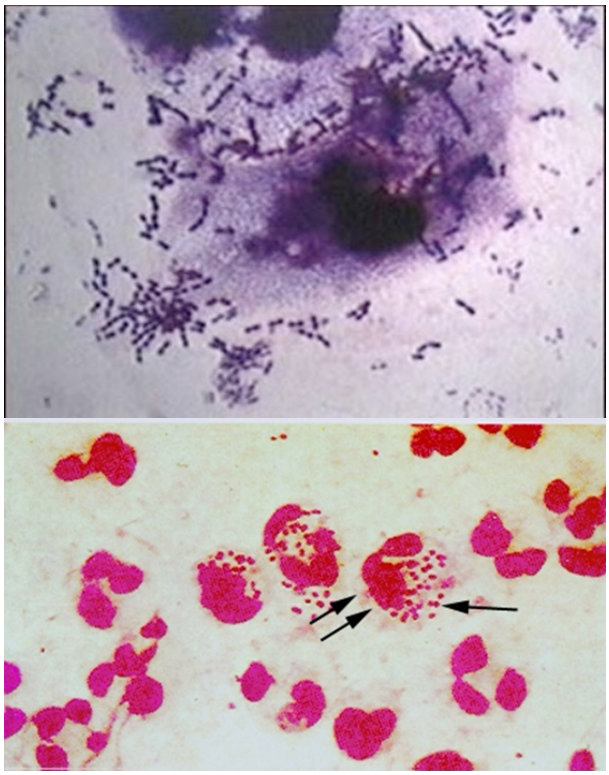

Figure 1.2 CSF gram stains in meningitis.
Gram positive cocci in chains (panel 1) and intracellular gram negative diplococcic in pairs (panel 2).

Optimal culture of the pneumococcus is on blood agar at 37 °C in 5% CO_2, growth is observed as mucoid greyish colonies surrounded by a zone of alpha haemolysis, where the red blood cells have been haemolysed by pneumococcal toxins produced around the colonies (Figure 1.3).

Figure 1.3 Growth of *Streptococcus pneumoniae* on blood agar

S.pneumoniae colonies on blood agar surrounded by a zone of alpha haemolysis.

Image from www.bacteriainphotos.com

Organism identification is done using optochin sensitivity (an antibiotic used only for pneumococcal identification, not clinical use) and where necessary bile sensitivity, where the bacterial colonies are lysed by the addition of bile salts (Werno and Murdoch, 2008). In contrast the meningococcus may grow on blood agar, but is most commonly identified on chocolate agar (Figure 1.4). Colonies are grey and may merge together, they are catalase and oxidase positive and utilise maltose (Rouphael and Stephens, 2012).

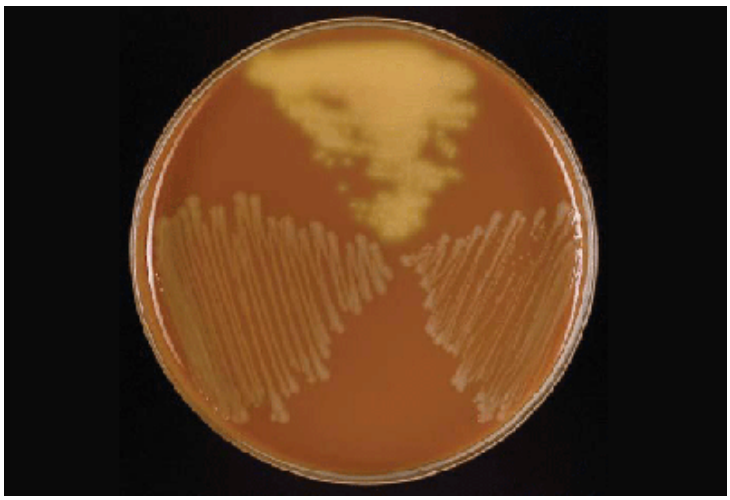

Figure 1.4 Growth of *N.meningitidis* on chocolate agar *Figure from www.cdc.com*

CSF culture is the most widely used methodology used to diagnose bacterial meningitis, however as it is a technique dependent on organism viability; the sensitivity and specificity are affected by the administration of antibiotics to the patient prior to lumbar puncture, and the sample transit time from the patient to the laboratory (when done out of hours). The sensitivity and specificity of culture is estimated to be 81% and 97% respectively in optimal conditions (Wu et al., 2013); data from clinical trials suggest that the real life sensitivity of culture may be lower. Clinical trial data report CSF culture positivity data of between 50-70% for gram stain and 40- 70% for culture, when compared to patients recruited to clinical trials with ABM, with identical clinical and CSF parameters whose CSF is culture negative (de Gans and van de Beek, 2002; Scarborough et al., 2007).

Blood culture, where positive for a pathogen known to cause meningitis, in the presence of evidence of CSF inflammation may be diagnostic for bacterial meningitis, blood culture positivity rates may be pathogen and location specific. Blood culture positivity rates for predominantly pneumococcal meningitis in adults are approximately 30% in Malawi and 60% in Europe (Scarborough et al., 2007; van de Beek et al., 2004).

b) Molecular diagnostic methods

Molecular diagnostics, using polymerase chain reaction (PCR) to amplify and then detect bacterial DNA is a highly sensitive diagnostic tool for pathogen-specific ABM (Corless et al., 2001; Wu et al., 2013; Carrol et al., 2000). PCR does not depend on organism viability, and is not limited by the administration of pre-hospital antibiotics. Very good multiplex PCR tests have been developed for ABM, which can be used on both blood and CSF and detect the presence of Hib, pneumococcus and the meningococcus simultaneously (Corless et al., 2001). The technique is discussed in more detail in Chapter 3.0.2, PCR is now used routinely in the CSF and blood of cases of suspected meningitis where available.

c) Bacterial antigen testing

Urine testing for pneumococcal antigens is most commonly used in bacterial pneumonia, using latex antigen test kits, where the sensitivity approaches 77-88%(Gutierrez et al., 2003). Sensitivity data in CSF are good, with well-resourced settings reporting sensitivities of 95-100% compared to culture (Saha et al., 2005; Samra et al., 2003; Marcos et al., 2001). Sensitivities of the test have not been formally reported from SSA, estimates of sensitivity approach 95% (Moisi et al., 2009). A small additional yield in positive results over culture were reported in patients from Kenya, Nigeria and Burkina Faso (of cases of suspected ABM admitted to hospital, culture identified 13% of pneumococci, with an additional 3% identified by antigen testing compared to Bangladesh and Pakistan (1.1% on culture, additional 5.3% on antigen testing (Moisi et al., 2009).

Meningococcal antigen testing on the CSF is used predominately in the African meningitis belt to identify epidemic cases for diagnostics and surveillance. Kits designed to detect antigens for serogroups A and W135 have sensitivities approaching 87 and 85% respectively (Djibo et al., 2006; Borel et al., 2006). As the kits are serogroup dependent, the antigen test must be used appropriately for the local epidemiology (Sobanski et al., 2001).

d) Cerebral imaging

Brain imaging is commonly performed in suspected bacterial meningitis, to exclude causes of raised intracranial pressure that may preclude safety of lumbar puncture. Little data exist on the diagnostic potential of imaging scans, as a definitive diagnosis is easily made on CSF analysis (Michael et al., 2010). In adults with suspected ABM, a wide variety of pathologies were revealed on admission CT scans, including stroke, space occupying lesions and lepto-meningeal enhancement, but reports of specific CT or MRI findings in adults with ABM that are diagnostic are lacking (Hasbun et al., 2001). The use of acute CT scans has been associated with delay to antibiotics, and possible harm (Michael et al., 2010). The majority of

acute cerebral imaging is done to reassure clinicians that a diagnostic lumbar puncture is safe (Joffe, 2007; Clark et al., 2006). However, CT findings are not necessarily accurate in determining LP safety, and delays to antibiotics for CT may be more harmful than the risk of cerebral coning with lumbar puncture (Brouwer et al., 2012; Joffe, 2007; Heldrich et al., 1986).

1.3 Treatment of bacterial meningitis

All guidelines for the management of suspected bacterial meningitis stress urgency in making the diagnosis and starting parenteral antibiotics. Where evidence of systemic shock is present, the UK and U.S. guidelines also recommend resuscitation (Tunkel et al., 2004; Heyderman, 2005). Further discussion of the evidence base for resuscitation and adjunctive management of cases of suspected meningitis can be found in Chapter 2 Section 2.7.
Adequate treatment of ABM initially requires a broad spectrum antimicrobial to be given at a dose that will penetrate the CSF, achieving above the minimum inhibitory concentration (MIC) of antibiotic required to kill the bacteria. Third generation cephalosporins are the treatment of choice, most guidelines recommend Ceftriaxone at a dose of 2 grams either once or twice per day (BD) (Heyderman, 2005; van de Beek et al., 2012; Tunkel et al., 2004) UK National Institute for Clinical Excellence (NICE) guidelines http://www.nice.org.uk/guidance/CG102/chapter/Key-priorities-for-implementation.
Where cephalosporins are not available or un-affordable, high dose benzyl penicillin with chloramphenicol are given as alternatives (Sloan, 2012; WHO, 2010). Both antibiotics must be given concurrently to reduce the risk of poor responses due to pathogen resistance to one of these antibiotics (Trachtenberg et al., 2007), in epidemic meningococcal disease a single dose of oily chloramphenicol monotherapy may be given (WHO, 2010). Outcomes with this approach are not inferior to those with cephalosporins, a systematic review including 19 trials concluded that the risk difference of death with using either antibiotic was 0% (95% CI -3%-2%) (Prasad et al., 2007). In this systematic review,

ampicillin/chloramphenicol was also not associated with increased risk of deafness or treatment failure, but was associated with decreased risk of persistent CSF culture positivity at 48 hours with ceftriaxone (risk difference -6% (95% CI -11% - 0%) (Prasad et al., 2007). Penicillin resistant bacteria, particularly pneumococci, are an increasing problem world-wide (Klugman et al., 2008). Penicillin is now rarely used to first line to treat meningitis in developed settings, where it is used, either rifampicin or vancomycin are advised to be given concurrently to cover for penicillin resistant pneumococci (Rossoni et al., 2008; Tunkel et al., 2004). Ceftriaxone resistant pneumococci are rarely reported, in one study from France, 4% of isolates had ceftriaxone resistance, but all with a minimum inhibitory concentration (MIC) of <2.0mg/l (Varon, 2009).

Adjunctive treatments given with antibiotics, with the aim of reducing mortality from ABM will be discussed in detail in Chapter 2, Section 5.

1.4 How does bacterial meningitis differ between well and low resourced settings?

The focus of this thesis is on ABM in sub-Saharan Africa (SSA), particularly Malawi. This is because substantial differences in epidemiology and clinical outcome exist between ABM in SSA, compared to better-resourced settings such as Europe, Asia or the United States of America (USA). The estimated incidence of ABM in children in SSA is 70-100/100 000 (Peltola, 2001), compared to 0.1-3/100 000 population in better resourced settings (Thigpen et al., 2011; Azevedo et al., 2013), there are few data from adults for the region and this is addressed in Chapter 4 Section 4.3.1 and Section 4.3.2.

In addition to a greatly increased burden of disease, the mortality rates from ABM in both adults and children are strikingly higher in SSA than anywhere else in the world (Table 1.3) (Scarborough and Njalale, 2004; van de Beek et al., 2004; McCormick et al., 2012; Edmond et al., 2010). Few studies have been done in adults with ABM in SSA, and limited data are available to determine why the mortality rates in this region are so dramatically different to all

other regions of the world. In this thesis the study was focussed on addressing ABM, which is a significant clinical problem, in the highest incidence region of the world with the highest rates of mortality and disabling sequelae.

Table 1.3 Mortality from bacterial meningitis by region

Regional Case Fatality rates (CFR) for bacterial meningitis in adults			
Study	**Region**	**Number of cases**	**CFR**
Ajdukiewicz 2011 **(Ajdukiewicz et al., 2011)**	Malawi	261	56%
Thigpen 2011 **(Thigpen et al., 2011)**	USA	3188	14.6%
Georges 2009 **(Georges et al., 2009)**	France	82	20.7%
Ishihara 2009 **(Ishihara et al., 2009)**	Japan	71	23%
Manga 2008 **(Manga et al., 2008)**	Senegal	73	70%
Lazzarini 2008 **(Lazzarini et al., 2008)**	Italy	289	6.9%
Scarborough 2007 **(Scarborough et al., 2007)**	Malawi	465	54%
Nguyen 2007 **(Nguyen et al., 2007)**	Vietnam	435	11%
De Gans 2002 **(de Gans and van de Beek, 2002)**	Europe	301	11%
Gordon 2000 **(Gordon et al., 2000)**	Malawi	64	65%
Durand 1993 **(Durand et al., 1993)**	USA	296	25%

1.5 Project starting points

1.5.1 Summary of available data from bacterial meningitis in African adults

The highest burden of bacterial meningitis in adults is found in the meningitis belt region of Africa, stretching from West Africa across central Saharan Africa to Ethiopia (Figure 1.5), where large epidemics occur most years, caused primarily by *N.meningitidis* serogroups A, Y and X (Irving et al., 2012).

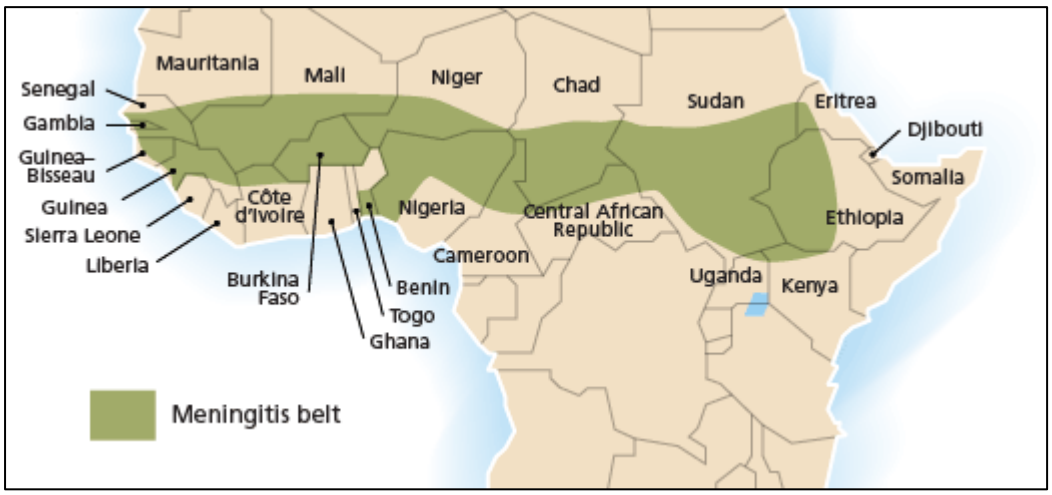

Figure 1.5 Map of the meningitis belt region of Africa

Figure reproduced from www.meningivax.org

The study and control of meningitis in this region has been primarily related to epidemic management, including case identification and notification, mass vaccinations and surveillance. Treatment of epidemic meningitis in this region is commonly with oily chloramphenicol, given intramuscularly (Wali et al., 1979; WHO, 2013a). The adult case fatality rate in most meningococcal epidemics is 10% (Artenstein and LaForce, 2012). Epidemics of *S.pneumoniae* serotype one have also been described in the West African parts of the meningitis belt region, with a substantially higher mortality of 50-70% in adults (Traore et al., 2009; Gessner et al., 2010).

The recent introduction of a serogroup A vaccine in Chad was associated with remarkable epidemic control, with a 94% reduction in case notifications of meningitis in regions receiving the vaccine from matched neighbouring regions where the vaccine was not given (Daugla et

al., 2014). Within this region, large collaborative research programmes exist studying epidemic disease, but no studies of clinical management, beyond the use of depot intramuscular antibiotics for outbreak control have been done. The effect of HIV co-infection in adults with meningitis in this region has also not been studied extensively, one study from central Africa showed 50-80% of cases of culture proven ABM over 5 years were co-infected with HIV, with an estimated population prevalence of 10-15% (Mbelesso et al., 2006), another study from the same centre demonstrated higher rates of ABM in HIV co-infected adults, 77% of a prospective cohort of adults with a meningitic illness were HIV co-infected, (Bekondi et al., 2006).

Other centres in SSA beyond the meningitis belt have noted an increase in cases of bacterial meningitis since the start of the HIV epidemic, associated with high mortality (Walker et al., 2012; Williams et al., 1986; Wiersinga et al., 2004; Jarvis et al., 2010), but the data are primarily from community and laboratory surveillance. The only published data on clinical management, trials to test adjunctive therapies to reduce mortality, and laboratory work to investigate the causes of high mortality from ABM in adults have been done in Malawi.

Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, in collaboration with the Malawi Liverpool Wellcome Trust Clinical Research programme (MLW) has been the site for extensive research in both bacterial meningitis and invasive pneumococcal and salmonella infections in both adults and children for over 20 years. Two major clinical trials testing adjunctive therapies for ABM, and a pneumococcal vaccine trial were all undertaken in adults admitted to QECH between 2000-2010 (Scarborough et al., 2007; Ajdukiewicz et al., 2011; French et al., 2010), further studies of the epidemiology and biomarkers of mortality from ABM were also undertaken (Gordon et al., 2000; Kelly et al., 2012; Goonetilleke et al., 2010). Mortality in all studies was in excess of 50% and neither adjunctive therapy tested caused an improvement in mortality. The causes of such high mortality remained unclear,

although an association of mortality with anaemia and altered mental status was described (Scarborough et al., 2007).

1.5.2 Origin of the research questions

Two main questions arose from these previous studies. Firstly, why is the mortality from ABM in adults in SSA so much higher than other settings, and secondly why did interventions with proven efficacy in other regions fail to impact on these poor mortality rates? The data presented in this thesis attempts to answer these questions by analysing data from surveillance of ABM in Malawi undertaken by MLW to determine the epidemiology of ABM in both adults and children, by analysing the data from the previous clinical trials, to determine the causes of poor outcome in adults, and by designing and implementing a clinical care bundle of resuscitation which was tested for feasibility in adults with ABM.

1.6 Aims and Objectives

1.6.1 Overall aim of the project

The aims of the project were to determine the epidemiology and clinical predictors of ABM in Malawi, and to test if delivery of Early Goal Directed Therapy (EGDT) for acute bacterial meningitis in adults was feasible and safe in QECH.

1.6.2 Specific study objectives

a) To explore 12 years of surveillance data for ABM to examine trends in the causes and incidence rates of ABM over time in Blantyre
b) Create a meningitis clinical database consisting of data from all appropriate studies of ABM in Malawi in the last 20 years and analyse the data for clinical predictors of poor outcome.
c) Use the data from the previous objective to design a severity scoring tool to predict outcome from ABM in Malawian adults

d) Test the concept of Africa-appropriate EGDT for adults with ABM in a clinical feasibility trial in the emergency department of QECH.

At the end of the work presented in this thesis, we hoped to achieve an impact on the unacceptably high rates of mortality and morbidity from ABM in Malawi, and a greater understanding of the causes of this high mortality. The project ended with the aim to go forward to design further intervention studies based on the data generated.

2. Review of the Literature

2.1 Introduction

This review will address two questions fundamental to this PhD thesis.

1. What are the causes of mortality from bacterial meningitis, and why is mortality so high in sub-Saharan Africa?
2. How can Early Goal Directed Therapy (EGDT) be used as an approach to bacterial meningitis in sub-Saharan Africa?

2.1.1 Outline of the search strategies used

The search strategies used were designed to be fully inclusive of all relevant literature to the field, and followed the search strategies developed by the Cochrane Collaboration (Higgins JPT, 2009). The National Library of Medicine (PubMed) and Medline databases were searched using the following search strategies:

1. Meningitis, bacterial
2. meningit*tw
3. death
4. mortal*tw
5. outcome
6. predictor
7. therapy
8. treatment

Searches for question one were: (1 OR 2) AND (3 OR 4 OR 5 OR 6 OR 7 OR 8)

The search strategy for question two was:

1. Meningitis, bacterial

2. meningit*tw
3. sepsis
4. shock
5. care bundle
6. goal directed therapy
7. outcome
8. survival

Searches for question two were: (1 OR 2 OR 3) AND (4 OR 5 OR 6 OR 7 OR 8).

Search results were stratified according to human or animal work, human search results were further stratified into adults and children. Increased weight was applied to publications from prospectively collected human adult data and systematic reviews, data from paediatric studies and animal work was considered when adult human data were lacking.

The first sections (2.2-3) of this review explores primarily human data available for pneumococcal meningitis, summarising work on pathogenesis with the focus on data that details mortality, with reference to animal work where relevant. Known predictors or associations with poor outcome will be discussed, followed by a summary of the evidence for tested and prospective adjunctive interventions.

The rationale for the intervention tested in this thesis is discussed in the second section (2.6), with a review of the literature supporting early goal directed therapy in other conditions, and the reasons for testing it in bacterial meningitis. Finally, the evidence for each of the interventions comprising goal directed therapy in the Africa-appropriate care bundle tested will be summarised.

2.2 What are the causes of mortality from bacterial meningitis?

2.2.1 Pathogenesis of ABM

S. pneumoniae is a human commensal. In well-resourced settings, it is found in the nasopharynx in of up to 50% of healthy children under age 5 years, and up to 10% of adults (Huang et al., 2004; Bogaert et al., 2004). In SSA, rates of colonisation are much higher in both adults and children, with up to 90% of children under age 5 years having demonstrable pneumococcal carriage (Huang et al., 2004; Egere et al., 2012; Anthony et al., 2012). In adults, carriage is higher in HIV-infected adults (38%) compared to HIV uninfected adults (13%) (Glennie et al., 2013).

Bacterial carriage in the nasopharynx is a dynamic state, triggering immunological responses in the host, which are impaired in HIV-infected adults (Ferreira et al., 2013; Glennie et al., 2011). The host responses are mediated by humoral CD4 cells and mucosal T-regulatory cells, which maintain carriage but prevent invasive disease (Pido-Lopez et al., 2011; Zhang et al., 2011). HIV co-infection causes substantial alterations to the normal dynamic carriage state in adults (Glennie et al., 2013). The switch from carriage to invasive phenotype that occurs in the bacteria is not fully described, but may be due to alterations in the biome in the nasopharynx, caused by factors including viral infections which up-regulate receptors used by the pneumococci to bind to the epithelial membrane, and factors that cause decreased muco-cilliary clearance (McCullers, 2006; Wolter et al., 2014b; Trivedi et al., 2011). It is also possible that a temperature change, caused by either environmental factors or viral infection may alter expression of virulence markers and immune evasion in the pneumococcus, as seen in the meningococcus (Loh et al., 2013), or alterations in the mucosal biome may lead to emergence of a pathogenic phenotype (Hajishengallis et al., 2012). Environmental factors which have been shown to influence carriage include humidity, high winds and ambient temperature (Mueller et al., 2008; Kinlin et al., 2009).

Once the pneumococcus has bound to the epithelial cells lining the nasopharynx, the bacteria transmigrates the epithelial layers to the basement membrane through many mechanisms, including cell transport via down-regulation of several receptors to permit bacterial entry and squeezing through tight junctions to enter the bloodstream (Mook-Kanamori et al., 2011). Once in the circulation, the pneumococcus must evade the host responses to enter the CNS either through the cribriform plate, or through the systemic circulation.

An estimated two thirds of patients with ABM have concurrent proven bacteraemia in Europe (van de Beek et al., 2004) only 30% have proven bacteraemia in Malawi (Scarborough et al., 2007).

Mucosal carriage leads to immunological tolerance through low level production of serum IgG to pneumococcal proteins, so it is possible that this tolerance permits very low levels of bacteraemia to avoid host killing after transmigration into the blood (Ferreira et al., 2013; Mook-Kanamori et al., 2011). Further host evasion in the blood occurs through encapsulation, to evade complement mediated killing (Mook-Kanamori et al., 2011).

Once bacteria reach the CNS, they must cross the blood brain barrier (BBB) either through very tight junctions between brain endothelial cells and capillary blood vessels and astrocytes in the case of pneumococci (Abbott, 2002), or through binding to host endothelial cells and entering through arachnoid granulations using type four pili in the case of meningococci (Miller et al., 2014; Brissac et al., 2012). Once within the CSF space, the bacteria can multiply rapidly, and are circulated throughout the CSF triggering an intense host inflammatory response (Mook-Kanamori et al., 2011). Bacterial cell wall proteins and toxins bind to pathogen recognition receptors in the walls of brain endothelial cells lining the blood brain barrier (BBB), triggering both innate CNS inflammatory responses, and neutrophil recruitment through the BBB endothelial cells from the cerebral venous plexus, leading to breakdown of the BBB and further inflammation (Mook-Kanamori et al., 2011). Neutrophils that phagocytose pneumococci then undergo autolysis, releasing further

inflammatory products into the CSF, leading to degradation of cellular integrity of cells in the surface layers of the brain (Neal and Gasque, 2013). Pneumococcal toxins, the most studied of which is pneumolysin (PLY), then penetrate the CNS tissues through all six cortical layers, causing synaptic disruption and resulting brain dysfunction (Wippel et al., 2013). Activated pro-coagulant factors in the blood cause microhaemorrhage and thrombi within the CNS, further disrupting brain cell function (Pryde et al., 2013).

The net physical result of this intense inflammation within the CNS is clinical presentation with severe headache, altered consciousness, neck stiffness, seizures and in some patients, evidence of a systemic inflammatory response to sepsis (SIRS), (Weisfelt et al., 2007b). Studies in both animals and humans demonstrate that intracranial pressure rises in response to the inflammation (Choi et al., 2005; Goh and Minns, 1993; Lindvall et al., 2004; Lorenzl et al., 1996; Minns and Engleman, 1988; Park and Chang, 2000), which if it is not controlled, will eventually lead to brain cell death, (Glimaker et al., 2014; Tunkel, 2004).

Why some patients who experience this process survive and others die is not clear. The level of consciousness on admission clearly influences outcome: the Glasgow Coma Score (GCS) at presentation has the strongest association with outcome of all the clinical variables that have been studied. (Dzupova et al., 2009; Roine et al., 2008; van de Beek et al., 2004; Wall et al., 2013c; Durand et al., 1993). Seizures, a marker of severe CNS dysfunction, are also a strong predictor of poor outcome (Wall et al., 2013c; Aronin et al., 1998; Durand et al., 1993; Zoons et al., 2008). Despite having good data about the clinical predictors of outcome in meningitis, their relationship to CSF inflammation and biomarkers of poor outcome is very limited. Such data as are available into the individual underlying causes for non-survival are discussed in the following section.

2.3 Pathological features of ABM and relationship to outcome

2.3.1 CSF WCC and outcome

White cells, predominately neutrophils, are found in the CSF during ABM in response to the presence of the pathogen. The host response is predominantly dependent on adequate numbers of neutrophils present to phagocytose and remove bacteria from the CSF (Neal and Gasque, 2013).

HIV uninfected adults with pneumococcal meningitis in Europe and Vietnam have a substantially higher CSF WCC than the Malawian patients. The mean cell count in Europe in data from the dexamethasone trial was 7753 cells/mm^3 (SD 14736), and 2970 cells/mm^3 (IQR 1-64000) in adults in Vietnam where 10% were HIV co-infected, compared to a median cell count of 480 cells/mm^3 (IQR 170 – 1680) in adults in Malawi, where 90% were HIV co-infected (van de Beek et al., 2004; Wall et al., 2013c; Nguyen et al., 2007).

Studies from several settings show that a low CSF WCC count is associated with poor outcome from ABM (Weisfelt et al., 2006d; Bruyn et al., 1988; Roine et al., 2014b; Wall et al., 2014b)

In the trials of dexamethasone done in Europe and Vietnam, a small proportion of patients presented with ABM had a low CSF WCC with a corresponding increase in mortality in these patients. In Vietnam, the overall trial CFR was 11.2%, but the mortality rate was 28% in patients with CSF WCC <100 cells/mm^3 (personal communication Dr Mai, OUCRU Vietnam, (Nguyen et al., 2007). In the Dutch data, where a CSF WCC of <1000 cells/mm^3 was shown to be significantly associated with mortality (van de Beek et al., 2004), the Odd's Ratio of mortality with a CSF cell count of <100 cells/mm^3 was 3.4, $p<0.001$ (personal communication Dr Brouwer).

In Malawian data from the SAM and GLAM trials, the median CSF WCC was substantially below the 1000 cells/mm^3 threshold, and low CSF WCC were also associated with poor outcome (Wall et al., 2014b; Scarborough et al., 2007; Ajdukiewicz et al., 2011).

Why are low CSF WCC associated with poor outcome? While the inflammatory products released during neutrophil apoptosis in the CSF trigger further CNS inflammation, the lack of, or severe reduction in the neutrophil response appears to have more adverse effects in allowing further replication of pneumococci and release of toxins through autolysis (Mook-Kanamori et al., 2011). No data exist examining the functional ability of CSF macrophages in response to infection with ABM in HIV infection. Laboratory data exploring the effects of HIV on macrophage function demonstrate that the presence of HIV related proteins may alter the functional ability of macrophages (Olivetta et al., 2014; Chihara et al., 2012; Cobos-Jimenez et al., 2011).

HIV infection of the CNS is well recognised, and is associated with specific neuro-cognitive decline(Garvey et al., 2014; Ances and Ellis, 2007). HIV affects the functioning of microglial cells, and may alter the intrinsic reaction of the CNS to pathogen, and cause increasing neuronal cell apoptosis (Geffin and McCarthy, 2013; Li et al., 2013).

All CSF WCC have transmigrated from the blood, and were produced in the bone marrow, and as such are affected by systematic conditions that may cause bone marrow suppression such as HIV infection. Conditions that are associated with invasive pneumococcal disease, including ABM, are associated with weakened immunity and bone marrow suppression including HIV-1 infection, diabetes, alcoholism and malignancy (van de Beek et al., 2004; Harboe et al., 2009; Domingo et al., 2009; Wall et al., 2013c; Muhammad et al., 2013).

Ethnic neutropaenia is commonly observed in adults and children from SSA, and does not appear to alter neutrophil function (Haddy et al., 1999). However it is possibly associated

with increased HIV transmission (Ramsuran et al., 2011). The effect of ethnic neutropaenia on the inflammatory response in the CSF to ABM is not clear, further data are required.

2.3.2 Cytokines, chemokines and outcome

Cytokines, and a subset of cytokines called chemokines are molecules with a wide variety of functions in inter-cellular and intracellular signalling in the immune system (Borish and Steinke, 2003). Chemokines specifically cause neutrophil migration towards sites of infection and inflammation, and have been associated with increased blood-brain-barrier permeability (Commins et al., 2010; Stamatovic et al., 2005). In the presence of infection, cytokines and chemokines are produced by inflammatory cells in response to inflammation, co-ordinating the response by both stimulating further inflammatory activity, and by limiting the extent of that activity. In systemic sepsis, poor outcome has been linked to inadequate pro-inflammatory cytokine activity, without appropriate counter-balancing anti-inflammatory activity (Emonts et al., 2007; Chung et al., 2009; Angus and van der Poll, 2013) (Figure 2.1).

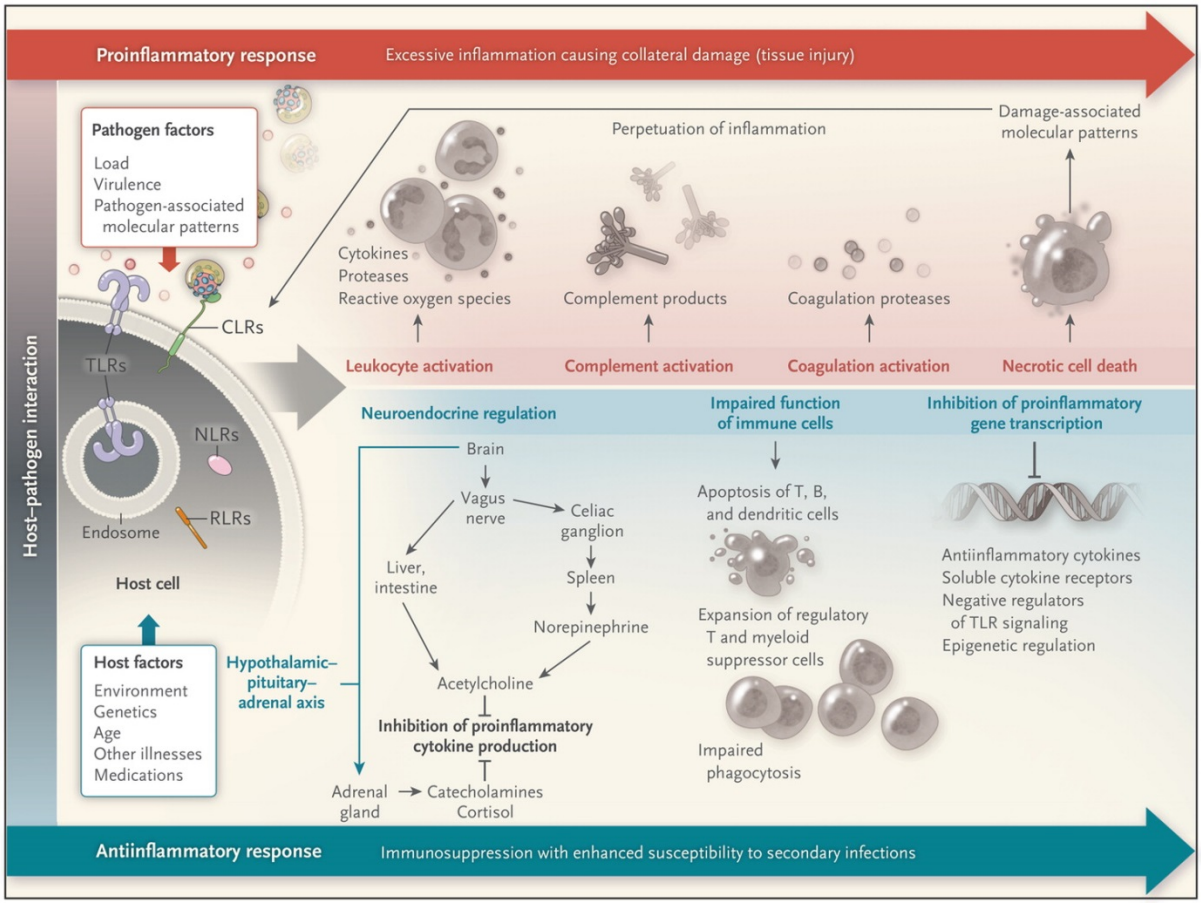

Figure 2.1 Host pathogen interactions in sepsis

Abbreviations: TLR, toll-like receptors; CLRs, C-type lectin receptors; RLRs, retinoic acid inducible gene 1–like receptors; NLRs, nucleotide-binding oligomerization domain–like receptors.

Figure reproduced from: (Angus and van der Poll, 2013)

In sepsis, two host parallel responses, one pro-inflammatory and one anti-inflammatory, are activated, and the balance between the two responses determines outcome (2.1) (Kasten et al., 2010; van der Poll and Opal, 2008; Angus and van der Poll, 2013). Excessive inflammation causes collateral tissue damage, such as acute kidney injury, and excessive anti-inflammation causes weak responses to the primary infection and increases susceptibility to secondary infections (Angus and van der Poll, 2013). Conditions such as HIV, diabetes, alcoholism and advanced age all may lead to a weakened pro-inflammatory response.

Similar responses are activated in the CSF compartment in bacterial meningitis as in the peripheral blood to sepsis; both failure of, and over-activity of the cytokine response to ABM within the CNS are implicated in excessive inflammation and neurological damage seen in patients with poor outcome (Nau and Eiffert, 2005).

The presence of bacteria in the CSF triggers a wide range of innate and adaptive immune responses. Cytokines and chemokines have been measured in the CSF of animal models of meningitis and humans with ABM (Coutinho et al., 2013; Geldhoff et al., 2013; Grandgirard et al., 2013). Pro-inflammatory cytokines are produced by perivascular endothelial cells in the BBB, meningeal macrophages and astrocytes, and play a significant role recruiting cells from the peripheral circulation into the CSF and therefore intensifying the inflammatory process (Szelenyi, 2001; Gerber and Nau, 2010). These recruited cells then produce further cytokines, determining if the cytokine originated from the CNS or the peripheral cells is therefore difficult.

Cytokine responses are markedly raised in the CSF of patients with pneumococcal meningitis, and levels of response relate to outcome, with increased pro-inflammatory cytokines seen in poor outcome groups. In children in Malawi, high levels of TNFα, IL1β,IL6, and IL10 were associated with a poor outcome in HIV co-infected children with pneumococcal meningitis, but were not significant for HIV uninfected children (Carrol et al., 2007a). In the same children, chemokine responses were again more enhanced in children with pneumococcal meningitis and HIV co-infection compared to HIV un-infected children (Carrol et al., 2007b), the only chemokine associated with outcome in that study after correction for multiple variables was CCL2 (Carrol et al., 2007b). This chemokine specifically recruits monocytes and memory T cells to sites of inflammation, and has been shown to with correlate with the degree of brain injury in HIV infection (Ragin et al., 2006). Although HIV infection alone can induce a measurable cytokine response in the CSF, patients with ABM

had substantially higher chemokine levels than in HIV infected control patients (Carrol et al., 2007b).

In children with Hib meningitis, CSF high levels of activity of TNFα and IL-1β have been associated with seizures and neurological damage: the effect of the cytokine activity appeared to be attenuated by adjunctive dexamethasone (Mustafa et al., 1989c; Mustafa et al., 1989d; Arditi et al., 1990; Mertsola et al., 1991; Ichiyama et al., 1997). One study in children with predominantly Hib meningitis showed that TNFα was elevated in the CSF, high levels were associated again with poor outcome (Leppert et al., 2000). However these studies were done in the pre-Hib vaccine era, the results are important, but less relevant to adult pneumococcal and meningococcal meningitis.

Few studies have examined host CSF response and outcome from ABM in adults. The inflammasome is a multi-molecular structure built in inflammatory cells, triggered by activation of toll-like receptors by inflammatory particles (Lamkanfi and Dixit, 2014). The inflammasome then activates caspases, leading to cytokine production and the innate immune response (Martinon et al., 2002). A study from Holland showed high levels of inflammasome associated cytokines IL1β and IL18 to be associated with a poor outcome phenotype, but no other cytokines were measured in that study, and cytokine response was not correlated with either immune status or CSF WCC (Geldhoff et al., 2013).

Interestingly, CSF cytokine responses only varied minimally in follow up CSF samples taken at 48 hours in patients randomised to either dexamethasone or placebo in Vietnam, dexamethasone was shown to have a mild immunosuppressive effect only. (Mai et al., 2009). The relationship of cytokine levels to outcome was not examined. In a small number of adults and children in West Africa, higher levels of pro-inflammatory cytokines were observed in patients with a poor outcome, including TNFα, IL-1β and IL-6 (Grandgirard et al., 2013). The majority of patients in that study had meningococcal (n=22), or pneumococcal

meningitis (n=14). However these data are difficult to interpret fully as there are significant differences in cytokine response between pathogen, and these two pathogens have substantially different mortality rates, although there is a probable association between pneumococcal meningitis, high CSF cytokine levels and poor outcome. HIV data were not included in the analysis.

In Malawian adults recruited to the SAM trial, cytokines TNF α, IL1β, IFN γ, IL6, IL8, IL10 and IL12 were also present at very high levels, but no clinically relevant differences in CSF cytokine level by outcome were seen after correcting for HIV status (Wall et al., 2014b). In the same study, low CSF WCC was associated with poor outcome, but CSF WCC did not correlate with any of the CSF cytokine results.

Dexamethasone therapy, given with the first dose of antibiotic, has been shown in animal models to attenuate the pro-inflammatory cytokine response, particularly TNFα and IL-1β (Mertsola et al., 1989; Mustafa et al., 1989a; Paris et al., 1997). This mechanism was postulated as the explanation for the beneficial effect of dexamethasone treatment in several clinical trials (de Gans and van de Beek, 2002; Lebel et al., 1988; van de Beek et al., 2010). The lack of relationship between cytokine levels and outcome in the SAM trial may partly explain why dexamethasone had no impact on outcome in that trial.

2.3.3 Bacterial load and outcome

The CSF compartment is a sterile site with limited acute immunological responses (Geffin and McCarthy, 2013). Therefore bacterial multiplication can occur rapidly, with a doubling rate of approximately 20 minutes, and immunological responses may take time to achieve control of the infection (Ernst et al., 1983). Bacterial products, particularly cell wall lipopolysaccharides (LPS) and toxins such as pneumolysin, have been shown to be potently immunogenic in the CSF of animals with experimental meningitis (Leib et al., 2000; Mustafa et al., 1989b; Mustafa et al., 1989e; Wippel et al., 2013). Therefore, if bacterial loads are

high, the quantity of bacterial product is likely also to be high, and require an increased immunological pro-inflammatory response to obtain control. The increased levels of inflammation are hypothesised to be associated with increasing neurological damage and poor outcome (Nau and Eiffert, 2005).

Bacterial load, measured in DNA copy number/ml of CSF using Real-Time PCR (RT-PCR) has been examined in both adults and children with pneumococcal meningitis in Malawi, and in the UK with meningococcal meningitis, and children in Finland with pneumococcal meningitis (Carrol et al., 2007a; Roine et al., 2009; Darton et al., 2009; Hackett et al., 2002; Wall et al., 2014b). The three paediatric studies demonstrated a relationship between higher bacterial loads and poor outcome (Carrol et al., 2007a; Roine et al., 2009; Hackett et al., 2002), as did the adult UK study of meningococcal meningitis, although in that study the differences in bacterial load by outcome were more marked in the blood than in the CSF (Darton et al., 2009; Darton et al., 2011). The only study of bacterial load in adults with pneumococcal meningitis was done in Malawi and showed no difference between the bacterial load and outcome (Wall et al., 2014b). Why there are differences in the relationship between bacterial load and outcome between adult and paediatric patients in the same setting is intriguing. CSF WCCs were low in the adult study (median 760 cells/mm^3, IQR 181-2600), and the rate of HIV co-infection was very high (82%). In the Malawian paediatric study, the HIV co-infection rate was 62%, neither the median CSF WCC nor the relationship between CSF WCC and bacterial load was reported. The relationship between bacterial loads, CSF WCC and outcome will be further explored in Chapter 7 Section 7.3.1 and 7.3.3.

2.4.4 Bacterial toxins and proteins and outcome

Bacterial toxins are produced by the pathogen to facilitate bacterial growth and to inhibit the immunological response to infection (Friedland et al., 1993; Lacroix et al., 1998). Different

toxins have different mechanisms of action, but toxins may also damage host cells including neurones as well as immune cells through indiscriminate action, and therefore may be associated with poor outcome. Pneumolysin (PLY) is the best characterised of pneumococcal toxins; one of the roles of PLY is to create pores in cell membranes of cells of the host, and disrupt immunological killing (Hirst et al., 2004).

Animal models of meningitis using both wild type and PLY deficient strains of pneumococci show a mild disease only in those infected with the non-PLY producing strains, and markedly more severe disease in the wild type infections (Wippel et al., 2013). In humans, failure to clear PLY from the CSF at 48 hours, despite reductions in the bacterial load is associated with poor outcome in a small number of adults with pneumococcal meningitis in Malawi (Wall et al., 2012). High levels of PLY in the CNS are highly neuro-toxic, and cause considerable synaptic damage and dysfunction at all layers of the cerebral cortex (Wippel et al., 2013). Definitive studies of the role of pneumolysin and outcome in humans with pneumococcal meningitis are lacking, but it is likely that PLY production and clearance plays a significant role in outcome, removal of toxin from the CSF is likely to limit toxin-mediated damage (Gerber and Nau, 2010; Marriott et al., 2008). Further work on both meningococcal and pneumococcal toxins in humans is required to understand their roles in pathogenesis further.

2.3.5 Viral co-infection

i) Influenza and bacterial meningitis

Viral co-infections may increase the risk of meningitis by causing bacteria carried in the nasopharynx to become invasive (Section 2.2.1). Viral infection, particularly influenza is well-recognised to lead to pneumococcal pneumonia, through inhibiting the host response to the invasive infection, allowing increasing bacterial loads in the nasopharynx which are then aspirated, and pre-disposing the lung to pneumococcal adherence and invasion (Wolter et al., 2014a; McCullers, 2006). Epidemiological studies have shown that the seasonality of

influenza is temporally associated with peaks of invasive pneumococcal disease in South Africa, The Netherlands and the USA (Dangor et al., 2014; Jansen et al., 2008; Kim et al., 1996). No clinical data currently exist to show if influenza infection is directly associated with pneumococcal meningitis, mathematical models from France suggest that pneumococcal meningitis seasonality may be temporarily associated with peak influenza transmission (Opatowski et al., 2013). A study in mice showed that inflammation from influenza A virus in the middle ear, pre-disposed animals to pneumococcal otitis media (Short et al., 2013). Otitis media was the source of ABM in 34% of adults with ABM, (Almirante et al., 1995). Children in the USA, in the pre-Hib vaccine era, had a history of upper respiratory symptoms preceding the onset of ABM in 60% of cases, active viral infection was found in 40% of children with proven ABM (Krasinski et al., 1987).

A direct causal association between influenza infection and pneumococcal meningitis remains to be proven, but given the epidemiological relationship, causation is possible.

ii) Herpes viruses and bacterial meningitis and CSF viral co-infection

EBV is thought to infect up to 90% of the world's population and is found widely in the serum of children and adults in SSA (Schaftenaar et al., 2014; Vetsika and Callan, 2004). Primary infection in children is often asymptomatic, but leads to lifelong latency in B memory cells (Vetsika and Callan, 2004).

Active infection commonly found in HIV co-infected individuals in SSA (Petrara et al., 2013), and it is associated with HIV associated opportunistic infections as well as a rare cause of viral encephalitis in children (Hung et al., 2000; Siddiqi et al., 2014). Several common malignancies including Burkitt's lymphoma and non-Hodgkin's lymphoma are caused by chronic EBV infection (Kabyemera et al., 2013; Petrara et al., 2013; Mutalima et al., 2008; Molyneux et al., 2012).

In HIV co-infected patients with meningitis in Malawi recruited to the GLAM trial, high levels of EBV infection in the CSF were found, and these were associated with mortality in HIV co-infected patients (Kelly et al., 2012). A small number of patients had dual EBV and CMV infection, all of these died (Kelly et al., 2012; Ajdukiewicz et al., 2011). However when the CSF of patients with ABM in Europe (none with HIV co-infection) were tested for the same viruses, no DNA from either EBV or CMV was found (Brouwer et al., 2013).

EBV and CMV were both isolated from the Malawian meningitis patients, however other herpes viruses including HSV-1 and Varicella Zoster virus were not isolated (Kelly et al., 2012), but were found in patients with aseptic meningitis (Benjamin et al., 2013). Children in Angola with bacterial meningitis had several herpes viruses including HSV and EBV were found in the CSF, no formal association between viral co-infection outcome from ABM was shown, but the numbers were small (Pelkonen et al., 2013).

While a relationship exists between EBV and viral illnesses and malignancies, it is not clear from the Malawian study if the EBV found in the CSF was involved in active meningitis pathogenesis, or was a marker of advanced immunosuppression (Kelly et al., 2012), no data are currently available from other studies to explore this further. The relationship between EBV and CMV and outcome will be explored further in Chapter 7 Section 7.3.2.

2.4 Clinical studies and determined predictors of mortality

Many studies have been done in well-resourced settings exploring clinical parameters and prediction of poor outcome at the bedside of adults with bacterial meningitis (Dzupova et al., 2009; Durand et al., 1993; van de Beek et al., 2004; Aronin et al., 1998; Chang et al., 2008; Georges et al., 2009; Ishihara et al., 2009; Lu et al., 2001b; Merkelbach et al., 1999; Zoons et al., 2008). One severity score has been derived from these data to predict outcome in Europe (Weisfelt et al., 2008). These studies all show that presentation with altered mental status, seizures, older age, presence of co-morbidities such as diabetes or alcoholism,

pneumococcal disease and low CSF WCC are associated with poor outcome (van de Beek et al., 2004; Dzupova et al., 2009; Weisfelt et al., 2006b; Lepur and Barsic, 2007; Nguyen et al., 2007). However the mortality in these studies ranges between 10-30% and does not approach the high mortality rates in adults in SSA with ABM, where case fatality rates (CFR) of >50% are commonly recorded (Gessner et al., 2010; Scarborough et al., 2007). A study, which is the focus of Chapter 4 Section 4.5 details the clinical predictors of poor outcome from ABM in Malawian adults, show that these differ from the European data and include presentation with coma and altered mental status, anaemia, seizures, tachycardia and low CSF WCC (Wall et al., 2013a). In addition, hypoxaemia was associated with poor outcome in a small number of patients on univariate analysis, data were too few to include in the multivariate analysis and the causes of hypoxaemia were unclear (Wall et al., 2013a). Hypoxia and outcome will be examined in Chapter 6 Section 6.3.8.

In summary, pathogenesis of bacterial meningitis is a complex interaction between host and pathogen, with the fatal outcome due to synaptic and cellular dysfunction, microvascular and microcellular thrombi and breakdown of the blood brain barrier, leading to rising intracranial pressure and brain death (Section 2.2.1). These processes are summarised in Figure 2.3.

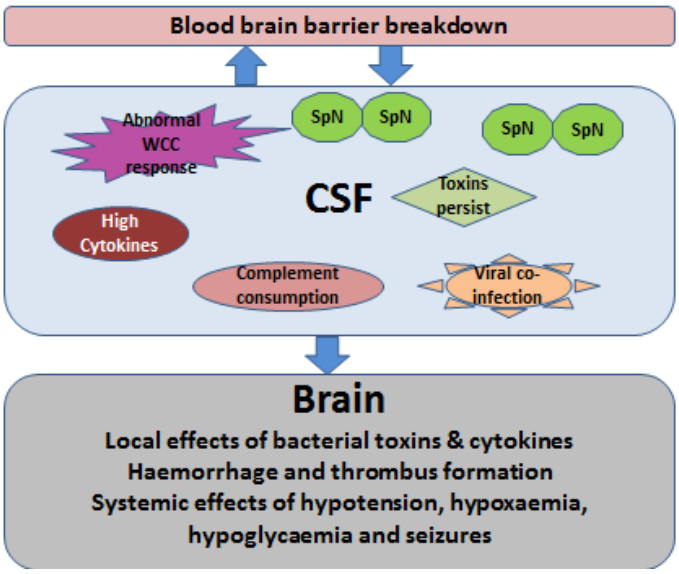

Figure 2.1 Meningitis pathogenesis in African adults

2.4.1 Previously tested interventions in bacterial meningitis

Despite the overall declining mortality rates from ABM over the last 100 years (Swartz, 2004), many interventions have been developed as adjuncts to antibiotics in attempts to reduce or eliminate mortality. The next section details different treatments that have been tested to impact on mortality from ABM, with a discussion of the different responses seen in different settings. The focus will be on meningitis in adults.

a) Dexamethasone

The most widely tested adjunctive intervention to antibiotics in acute bacterial meningitis (ABM) has been dexamethasone. The rationale for testing dexamethasone was the hypothesis that neurological damage in ABM is mediated by excesses of the host inflammatory response, and that by reducing inflammation within the CSF compartment, intracranial pressure would be reduced and host-mediated inflammatory damage would be attenuated (Martos et al., 1995; Pecco et al., 1991; Townsend and Scheld, 1996; Tunkel and Scheld, 1989). Animal models of ABM using dexamethasone showed mixed results, with some studies showing benefit (Tauber et al., 1985; Scheld et al., 1980; Mustafa et al., 1989d; Kadurugamuwa et al., 1989; Nolan et al., 1978), and others showing problems, including delayed antibiotic penetration to the CSF with dexamethasone (Paris et al., 1994), increased cerebral oxidative stress (Barichello et al., 2011) or failure to prevent deafness (Coimbra et al., 2007).

Large, well conducted studies in well-resourced settings demonstrated significant mortality benefit from adjunctive dexamethasone in both adults and children (de Gans and van de Beek, 2002; Lebel et al., 1988; van de Beek et al., 2007; Nguyen et al., 2007). These studies were done predominantly in the pre-Hib and pre-pneumococcal vaccines era with the majority of disease being caused by Hib in children, and by the pneumococcus in adults.

As a result of these studies, many national guidelines for the treatment of ABM were changed to include dexamethasone, given at high dose with the first dose of antibiotics (Brouwer et al., 2010a; Weisfelt et al., 2007a; Heyderman, 2005).

The beneficial effect of dexamethasone in adults appeared to be greatest in adults with pneumococcal meningitis (de Gans and van de Beek, 2002) and in *Streptococcus suis* meningitis in Vietnam (Nguyen et al., 2007). Children with both pneumococcal and Hib meningitis appeared to benefit in terms of mortality reduction and lower rates of deafness in survivors (Lebel et al., 1988; Kilpi et al., 1995; Peltola and Roine, 2009).
However, when dexamethasone was tested in Malawi in both adults and children, no reduction in either endpoint of mortality or deafness was observed (Scarborough et al., 2007; Molyneux et al., 2002). A large subsequent meta-analysis of individual patient data shows that the effect of dexamethasone is confined to patients with ABM in well-resourced settings and that it is not an effective intervention in less well-resourced countries (van de Beek et al., 2010). Why, when the causative pathogens are the same, an adjunctive intervention has been found to have varying effects by geographical location is intriguing and will be discussed further in section 2.5.

b) Glycerol

Glycerol is a hyper-osmolar agent that is taken orally, and enters the bloodstream, exerting an osmotic effect on surrounding tissues when concentrated in capillary beds. Within the CNS, this effect is thought to draw excessive water from the CNS following an acute inflammatory insult, and reduce intracranial pressure (Frank et al., 1981). Glycerol has an excellent safety profile, low cost and easy administration (Righetti et al., 2004) and as such has been tested for efficacy in ABM, first in children and subsequently in adults. The overall quality of the evidence, (tested using the GRADE criteria (Guyatt et al., 2011) http://www.gradeworkinggroup.org/intro.htm) examining the effects of Glycerol in ABM is weak (Wall et al., 2013b). Paediatric studies of glycerol have shown marginal efficacy in reducing deafness in survivors of childhood meningitis in Finland and South America, with even more limited effects noted in small, less well conducted studies in India (Kilpi et al., 1995; Peltola et al., 2007; Singhi et al., 2008), no effect was noted in a trial in Malawi (Molyneux et al., 2014).

An adult trial in Malawi was stopped by the data monitoring committee when excessive deaths were noted in the group randomised to receive glycerol, adjusted OR for poor outcome with glycerol was 2.4 (95% CI 1.3-4.2) p=0.003 (Ajdukiewicz et al., 2011). A meta-analysis of all trials testing glycerol for ABM noted overall no effect from glycerol in children, and harm in adults (Wall et al., 2013b). Concerns were raised over the adult trial in the use of 50% dextrose as the agent in the placebo arm of the trial, due to possible osmotic effects of dextrose, however the baseline mortality in the placebo group was the same as observed in the dexamethasone trial, and harmful effect of glycerol appeared to be independent of this (Brouwer and van de Beek, 2011). Glycerol is therefore not advocated for adult meningitis. Other osmotic therapies in clinical use, such as mannitol and hypertonic saline have been used on an ad-hoc basis but have not been formally tested for bacterial meningitis, and have mixed effects in other conditions associated with raised intracranial pressure (Foundation, 2000; Ichai et al., 2009; Okoromah and Afolabi, 2004).

c) Hypothermia

In animal models of experimental meningitis, brain cooling through hypothermia was shown to attenuate markers of inflammation in the brains of animals with *E. coli* meningitis (Park et al., 2001; Rowin et al., 2001) and is used in intensive care for adults surviving cardiac arrest, undergoing aneurysm clipping and for children with head injury and other neurosurgical conditions, although evidence to support its use is limited (Milani et al., 2011; Sydenham et al., 2009; Corry, 2012). Preliminary observational evidence suggested that hypothermia may be a useful adjunct to adults admitted to intensive care with ABM (Lepur et al., 2011), however a large European randomised controlled trial revealed harm in the group randomised to brain cooling and the trial was stopped early (Mourvillier et al., 2013).

d) Intracranial Pressure Management

A major result of the acute inflammation associated with ABM is raised intracranial pressure (ICP) (Haarman et al., 2008; Goh and Minns, 1993; Moller et al., 2000). No randomised

controlled trials of management of raised ICP in meningitis currently exist, but observational data suggest that in patients on intensive care with meningitis and documented raised ICP, aggressive management strategies including lumbar and ventricular drains may be helpful in reducing ICP and possibly improving outcome (Abulhasan et al., 2013; Glimaker et al., 2014; Lindvall et al., 2004). However given the inherent biases of observational data, and the failure of glycerol to improve outcome when reducing ICP in adults with ABM, this management approach must be used with caution until data from randomised trials are available. None of the strategies described so far would be feasible in resource-limited settings without access to neurosurgical support and intensive care.

e) **Antibiotic strategies**

Antibiotic strategies that have been tested in animal models include bacteriostatic antibiotics compared to bactericidal antibiotics, intra-ventricular antibiotics and combination antibiotic therapy. Animal models of experimental bacterial meningitis have shown improved outcomes in animals treated with bacteriostatic as opposed to bactericidal antibiotics (Bottcher et al., 2004; Egermann et al., 2009; Mook-Kanamori et al., 2009). This observation has been explained by preservation of the bacterial cell wall by the bacteriostatic antibiotics, with limited release of highly inflammatory cell wall compartments and intracellular bacterial products into the CSF, therefore leading to a less aggressive host response and hence less serious neurological damage (Pankey and Sabath, 2004). In these models, antibiotics were given via the intra-ventricular route as well as the parenteral route. However, when CSF penetration of these antibiotics was examined following intramuscular administration of antibiotics, only antimicrobial therapy that achieved a bactericidal effect in the CSF was associated with clearance of bacteria from the CSF; animals treated at bacteriostatic doses failed to clear infection from the CSF at 5 days (Scheld and Sande, 1983). Children with penicillin resistant, chloramphenicol sensitive pneumococcal meningitis were more likely to fail treatment and have a poor outcome when treated with chloramphenicol compared to cephalosporin treatment (Friedland and Klugman, 1992).

Clinical trials of bacteriostatic versus bactericidal antibiotics have not been undertaken in humans to date. Effective antibiotic therapy is considered to be high dose beta-lactam antibiotics, which when given at the correct dose and early in the disease course are effective in killing all the common meningitis pathogens in the CSF (Scarborough et al., 2007; Girgis et al., 1988). Most bacteriostatic antibiotics have not been tested clinically in ABM, and may not penetrate the CSF adequately (Scheld and Sande, 1983). Bacteriostatic antibiotics are effective in treating either gram positive or gram negative localised or systemic infections, but rarely both (Pankey and Sabath, 2004). As ABM may be caused by one of several pathogens from across the gram stain spectrum, bacteriostatic agents alone are never going to be appropriate first line therapy for ABM (Finberg et al., 2004). Further concerns about the use of bacteriostatic antibiotics to treat ABM in humans include negative interactions with bactericidal antibiotics in-vivo (Ostergaard et al., 2003; Scheld et al., 1980), and the potential slow rate of in-vivo clearance of pathogen from the CSF when such antibiotics are used, which may be harmful (Ribes et al., 2005; Scheld and Sande, 1983; Lepper and Dowling, 1951; Chowdhury and Tunkel, 2000; Finberg et al., 2004).

In summary, third generation cephalosporins as the first line antibiotic treatment of choice for ABM have good evidence of efficacy, resistance rates are currently low, and their use is supported by published guidelines (WHO, 2010; Tunkel et al., 2004; Heyderman, 2005; Fitch and van de Beek, 2007). Bacteriocidal antibiotics remain useful treatments as adjunctive therapies in the treatment of penicillin-resistant pneumococcal meningitis, but are not used as primary treatment (Tunkel et al., 2004; Rossoni et al., 2008).

f) Conventional drugs as adjuncts

Statins

Statin therapy has been proposed as an adjunct to several infectious diseases including sepsis and dengue fever, as a powerful anti-inflammatory effect from the use of statins has been observed in animal models, and in patients taking statins for other reasons (Quist-

Paulsen, 2010; Rosch et al., 2010). Despite significant heterogeneity, a systematic review shows that patients who are taking statins incidentally as outpatients may have better outcomes from infectious diseases than those not taking statins (Ma et al., 2012). One animal model has shown that statin therapy in adjunct to antibiotics for experimental meningitis effectively reduces the CSF WCC compared to animals treated with antibiotics alone (Winkler et al., 2009). Given that a low CSF WCC has been shown to be a marker of poor outcome, it is unlikely that human trials of statin therapy for ABM will be granted ethical approval in the near future without substantial further data .

Anti-pyretics

Anti-pyretic therapies have been tested twice in children in sub-Saharan Africa, and neither trial showed any difference in mortality from receipt of high dose paracetamol with antibiotics on admission with ABM (Molyneux et al., 2014; Pelkonen et al., 2011). No studies have tested this approach in adults.

g) Future directions

P4 is a peptide derived from a pneumococcal surface protein (PsaA) that has been developed by the Centres for Disease Control (CDC) and shown to enhance opsonisation of pneumococci and hence enhance macrophage phagocytosis. P4 has been tested in macrophage cell lines and in animal models of pneumonia and sepsis in combination with intravenous immunoglobulin (IVIG) as an adjunct to antibiotics. In these models, animal mortality from pneumococcal sepsis was substantially lower than with antibiotics alone, and P4 was shown to independently improve mortality when given without antibiotic treatment (Bangert et al., 2012; Rajam et al., 2010; Bangert et al., 2013). CSF penetration of P4 is not known, and its adjunctive effect on pneumococcal meningitis is unclear. When tested in macrophages from healthy HIV un-infected Malawians, a much less substantial effect on macrophage killing was noted compared to macrophages from healthy Europeans (Bangert

et al., 2013). P4 is now in development for phase one clinical trials, and represents a promising adjunct for pneumococcal sepsis and meningitis in the future.

As discussed in a previous section, intense cytokine responses in the CSF of patients with meningitis may be associated with worse neurological damage and higher rates of non-survival. However no drugs blocking particular cytokines have been tested in humans with ABM to date. In animal models, IFNγ knockout mice had better outcomes from experimental pneumococcal meningitis than wild type (Too et al., 2014), IFNγ given as an adjunct to amphotericin for cryptococcal meningitis improved clearance of fungus from the CSF, but no effect on outcome was seen (Jarvis et al., 2012). There are no clinical data to help determine whether augmenting or blocking the action of IFNγ would be a useful therapeutic approach in pneumococcal meningitis

Another compound inflammatory molecule associated with poor outcome in pneumococcal meningitis is matrix metalloproteinase nine (MMP9) (Roine et al., 2014a). MMPs are caspases produced as part of the innate immune response and are involved in apoptosis, MMP9 is involved in the degradation of protein from the extracellular cell matrix, and high levels are associated with poor outcome (Leib et al., 2000; Roine et al., 2014a; Tsai et al., 2008). Mice who received a compound blocking MMP9 had better outcomes from experimental pneumococcal meningitis compared to controls (Liechti et al., 2014), however no clinical trials of MMP9 blocking treatment have been undertaken in ABM. Dexamethasone blocks MMP9 production in TBM, and therefore may partially contribute to the beneficial effect of dexamethasone in some studies ABM (Green et al., 2009).

Citicoline is an essential component in the synthesis of phospholipids in cell membranes (Morton et al., 2013). Bacterial inhibition of this compound has been shown to increase neuronal apoptosis in an animal model of pneumococcal meningitis (Zweigner et al., 2004). Citicoline has been shown to reduce the size of the area of ischaemia in stroke (Bustamante

et al., 2012), and it is hypothesised that it may be an effective adjunct in bacterial meningitis. However only one animal study has been performed to date, Citicoline prevented cell death and attenuated hippocampal apoptosis in animals with pneumococcal meningitis (Zweigner et al., 2004). Clinical human data are lacking.

Further future directions for the treatment of ABM beyond these topics discussed include the use of newer anti-seizure agents for patients at risk of seizures, other mechanisms for reduction of intracranial pressure, newer adjuncts and newer antibiotics. However as vaccination reduces the burden of ABM worldwide, particularly in resource rich settings, it is a significant concern that less attention will be given to the development of further therapies to improve outcome from adults and children with ABM.

h) Prevention of bacterial meningitis

Where introduced to the paediatric population, vaccination against the most common pathogens causing paediatric meningitis has resulted in substantial decreases in the burden of disease, irrespective of the development status of that country (McIntyre et al., 2012).

Currently available vaccines for ABM include the following (Table 2.1).

Hib meningitis is rare in adults, but was the most common cause of ABM in children (Chandran et al., 2005). the highly effective vaccine has resulted in substantial decreases in disease burden in children, with possible herd effects reducing sporadic diseases in adults (Adam et al., 2010; Ulanova et al., 2011; Adegbola et al., 2005; Cohen et al., 2010d; Cisse et al., 2010; Daza et al., 2006; Giufre et al., 2011).

The pneumococcus is the commonest contemporary cause of bacterial meningitis in both adults and children (Brouwer et al., 2010b; van de Beek, 2012).

Table 2.1 Available vaccines for bacterial meningitis

Vaccines for bacterial meningitis				
Pathogen	**Serogroup/serotypes covered**			
H.influenzae	Type b			
N.meningitidis	C	ACWY	A	B (newly licensed)
S.pneumoniae	23-valent polysaccharide	PCV-7 (4,6B,9V,14,18C,19F, and 23F)	PCV-10 (1, 4, 5, 6B, 7F, 9V, 14, 18C)	
			PCV-13 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F)	

The first widely used vaccine developed to prevent IPD and pneumococcal pneumonia was the 23 valent polysaccharide vaccine. Efficacy of this vaccine was limited in immunocompromised individuals, particularly HIV co-infected adults, and its use has now been replaced with the newer, serotype specific conjugate vaccines that are more effective in IPD prevention (Nielsen et al., 1998; Opravil et al., 1991; van Hoek et al., 2012; French et al., 2000; French et al., 2010).

The overall incidence of pneumococcal meningitis has decreased following the use of PCV 7 in children, although increases in disease caused by non-vaccine serotypes has been observed (Hsu et al., 2009b). Invasive adult pneumococcal disease has declined in several centres after childhood vaccination has been introduced, through herd immunity and reduction in transmission through lower carriage rates in children (Muhammad et al., 2013; Griffin et al., 2013). Data show that this effect of herd immunity from PCV-7 has reached adults in the USA with HIV co-infection, where the burden of IPD in this community has decreased from 7.81 cases/1000 person-years to 3.69 cases/1000 person-years (-53%; 95% confidence interval: -65% to -36%, $p< 0.001$) (Burgos et al., 2012b). Another study noted the same phenomenon in the USA (Flannery et al., 2006; Cohen et al., 2010a), although also noted serotype replacement occurring. No data on IPD in HIV positive adults in SSA

following the introduction of PCV-7 or PCV-13 are yet available, but IPD in all age groups has decreased over the last 10 years in Malawi prior to the introduction of PCV-13 into the paediatric schedule in 2012 (Everett et al., 2011), but the incidence of adult pneumococcal meningitis has not changed (Wall et al., 2014a). Data on the indirect effects of the pneumococcal vaccine on HIV co-infected adults in SSA are awaited. A trial testing the effect of PCV-7 on the recurrent IPD in HIV co-infected adults in Malawi showed that the vaccine significantly reduced the risk of recurrent IPD, but meningitis cases were few and no effect on reduction of pneumococcal ABM can be extrapolated (French et al., 2010). No data currently are published to show if vaccination in African adults can prevent primary IPD.

In SSA, IPD is commonly caused by serotypes one and five, which are not included in PCV-7. Introduction of PCV-13 into the paediatric vaccination schedule in SSA has started, supported by the Global Alliance for Vaccines and Immunisation (GAVI), (http://www.gavialliance.org/about/ghd/), surveillance data on the effects of this vaccine on IPD from the continent are awaited.

However despite dramatic reductions in disease in both children and adults following introduction of PCV-7, overall incidence of IPD over time are starting to increase, caused by non-vaccine serotype disease, 'serotype replacement' (Byington et al., 2010; Elston et al., 2012; Flannery et al., 2006). It is not clear yet if this phenomenon will extend to PCV-13, but given that only 13 serotypes are covered of a possible 90-100, it may be problematic in the future (Feldman and Anderson, 2014). This subject is beyond the scope of this review, but is an important reason for continuous surveillance of IPD and ongoing development of vaccines that are protective against all serotypes.

With regards to meningococcal prevention through vaccination, fewer data exist for adults compared to children. Meningococcal meningitis in the UK has substantially decreased in children, adults and adolescents following vaccination for *N. meningitidis* serogroup c (Martin et al., 2014; Okike et al., 2014). Meningococcal meningitis incidence has been substantially reduced in the meningitis belt region of Africa where meningococcal serogroup A vaccination has been introduced (Daugla et al., 2014) and roll out of this vaccine for epidemic control is now supported by GAVI. Meningococcal meningitis beyond the meningitis belt is a sporadic and relatively rare disease in SSA, meningococcal vaccines are not currently a priority for expansion in the region (http://www.gavialliance.org/search/?SearchText=meningitis). The newly licensed serogroup B meningococcal vaccination is likely to be effective in preventing serogroup B disease (Elena Santolaya et al., 2013; Gossger et al., 2012), however as this serogroup is rare in Africa it is unlikely to be relevant on the continent.

The incidence of IPD and pneumococcal ABM may be substantially reduced in African adults from herd immunity from paediatric vaccination campaigns, but with the limitations of the current vaccines and the serotype replacement phenomenon, it is unlikely that this strategy will result in eradication of this disease, and on-going research into improvement in detection and treatment remains a priority.

2.5 Why have adjunctive interventions failed in African Adults?

It is notable that two major interventions, dexamethasone and glycerol, failed to reduce the CFR from ABM in SSA compared to potential beneficial effects in other countries. It is unclear in particular why glycerol was found to be harmful when it had benign effects elsewhere. The substantial differences exist between the populations in SSA and Europe might contribute to differential effects of these interventions.

2.5.1 Delay

Adults with pneumococcal meningitis in Malawi have different demographics compared to those studied in other settings outside SSA; they are younger (median age 33 years compared to 50 years in Europe), are commonly HIV co-infected, present late to hospital and they experience in-hospital delays to lumbar puncture and antibiotic treatment (Desmond et al., 2013; Wall et al., 2013a). Time from symptom onset to lumbar puncture in the two main trials in Malawi was 3-5 days, compared to <48-72 hours for most European studies (Scarborough et al., 2007; Ajdukiewicz et al., 2011; Lepur and Barsic, 2007; Thompson et al., 2006; de Gans and van de Beek, 2002).

Prompt recognition of ABM and rapid access to specialised treatment was instrumental in reducing mortality in paediatric ABM in the UK from 30-40% to <10% (Nadel et al., 1998; Harnden et al., 2006). Part of this strategy was rapid in-hospital antibiotics, which have been associated with decreasing mortality in both adults and children (Lepur and Barsic, 2007; Hsu et al., 2009a). In Malawi the median wait in hospital for parenteral antibiotics was 3-5 hours (Wall et al., 2013c).

2.5.2 Disease severity

An alternative cause for higher mortality rates in SSA is the possibility that disease severity was higher in patients in this region. A severity score designed by the Dutch group to predict outcome from ABM, derived from the data from the dexamethasone trial was of low predictive quality when applied to Malawian data, underlying the differences between these two populations (Weisfelt et al., 2008; Schut et al., 2012). An objective, direct comparison of disease severity on admission between the two populations is not possible as the inefficacy of the score reveals, a comparison will require more sensitive tools than those currently reported.

2.5.3 Disease severity: role of the pathogen

In SSA, pneumococcal disease is caused disproportionately by serotype 1, compared to other settings where serotype 1 disease is rare (Martin et al., 2014; Mueller et al., 2012; Everett et al., 2012; van Hoek et al., 2012). In Europe, IPD caused by serotype one has been shown to be associated with lower disease severity and mortality than other serotypes (Burgos et al., 2012a). In contrast, in Malawi, pneumococcal meningitis caused by serotype one may be associated with marginally higher mortality than other serotypes (CFR serotype one was 59%, all other serotypes CRF was 50.1%) (Wall et al., 2013c). Mortality from epidemic pneumococcal meningitis in West Africa caused by serotype one reaches 40-60% (Gessner et al., 2010; Leimkugel et al., 2005). The differences in serotype causing disease in different settings may be due to either additional virulence potential or increased transmissibility potential within populations in Africa (Ritchie et al., 2012). Data from the PAGE project investigating the genetics of African serotype one disease will reveal more http://www.pagegenomes.org/.

Data from studies of pneumococcal meningitis in either the meningitis belt or in SSA may therefore not be directly comparable to data from other regions.

2.5.4 Disease severity: host

African adults are at higher risk of IPD than adults in other settings, with increased risk in those who are HIV co-infected (Walker et al., 2012; Scott et al., 2000; Nunes et al., 2011; French et al., 2010; Gilks et al., 1996).

Eighty seven percent of the patients in the Malawian meningitis data were HIV-antibody positive compared to 0% from the European series (Wall et al., 2013c; van de Beek et al., 2004). Median GCS on presentation in Malawi was 12/15, compared to 11/15 in Europe, the seizure frequency was 44.9% compared to 15% in Europe and the rates of tachycardia >120 beats per minute were similar (Wall et al., 2013c; van de Beek et al., 2004).

The presence of low CSF WCC responses in African adults to meningitis pathogens (Wall et al., 2014b), combined with high rates of HIV co-infection, severe anaemia (Wall et al., 2013c), poverty (Desmond et al., 2013) malnutrition (Zachariah et al., 2009) and high levels of carriage (Glennie et al., 2013) may all be important in determining disease severity in the host. HIV co-infection alters serological responses to pneumococci (Glennie et al., 2011; Glennie et al., 2013), macrophages in healthy Malawian adults may be less able to handle infection with pneumococci compared to healthy European adults, a factor that may be compounded by HIV infection and exposure to biofuels (Bangert et al., 2013; Fullerton et al., 2009).

High rates of HIV co-infection and particularly aggressive pathogens may mean that the effect of adjunctive treatments designed to reduce intracranial inflammation were insufficient to overcome disease severity in the absence of high quality resuscitation and medical care.

2.5.5 Disease severity: Poor clinical care

The admission units (Room 6 and ward 4B) at QECH (described in Chapter 3 Section 3.3.5) possibly provided inadequate basic medical care for the patients with ABM studied in Malawi. Severe anaemia is common in adult and paediatric Malawian inpatients, and is associated with high mortality from a number of conditions, access to blood transfusion is limited, particularly in adults (Lewis et al., 2005). Seizures can be controlled in resource-rich settings with effective drugs, in Malawi access to these drugs is profoundly limited, and the options for seizure treatment are few (Ogutu et al., 2002).

In addition, patients admitted with severe ABM into clinical trials in other settings had the option for admission to intensive care, and support for failing organ systems was provided. These facilities are lacking for the majority of patients in Malawi and without access to this supportive care this may have further impacted on mortality.

A summary of the factors influencing outcome for patients with ABM in Malawi is shown in Figure 2.4. It is possible that patients in Malawi presented too late to hospital, compounded

by delays in hospital for the effects of either glycerol or dexamethasone to be effective, as opposed to intrinsic inefficacy in this population compared to other centre.

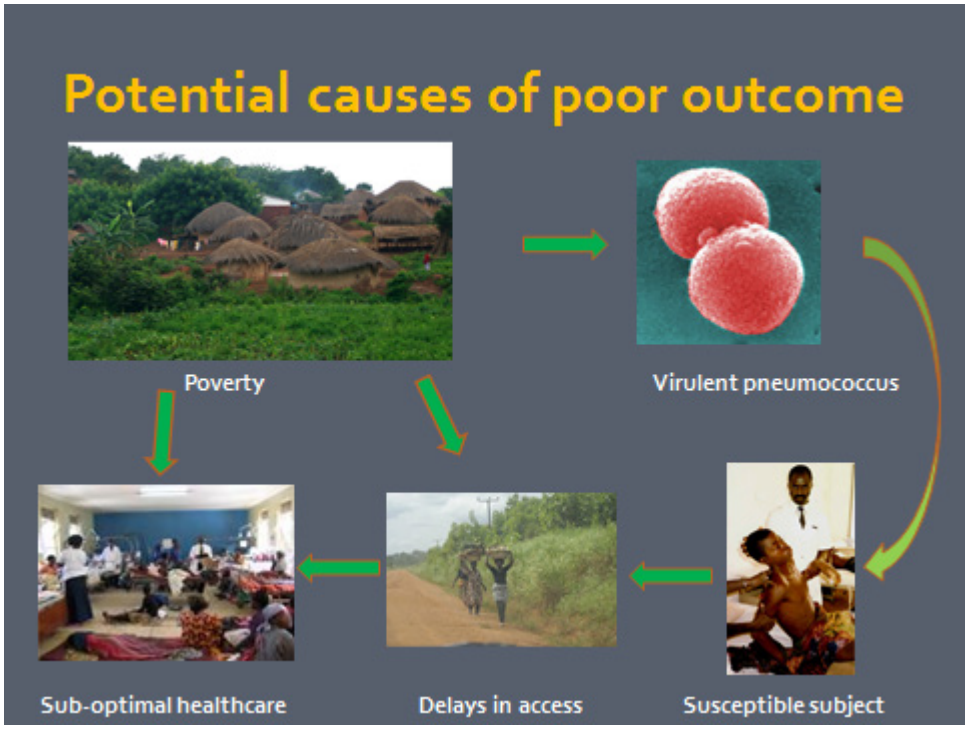

Figure 2.2 Factors influencing outcome from meningitis in Malawi

In summary, adults in presenting with suspected ABM in Malawi are different to those presenting in more well-resource settings. Access to resuscitation on admission and intensive care support are lacking. From the factors shown in Figure 2.4 that are susceptible to intervention, improving delays in access and optimising in-hospital recognition and treatment of ABM is a clear priority towards improving treatment and possibly outcome from ABM. The next section outlines the rationale for the intervention tested in this PhD thesis, Early Goal Directed Therapy for adult meningitis in Malawi.

2.6 How can Early Goal Directed Therapy (EGDT) be used as an approach to bacterial meningitis in sub-Saharan Africa?

2.6.1 What is EGDT?

Early Goal Directed Therapy is a term for protocolised resuscitation care, delivered to set clinical targets within an acute time period, first defined by in the study by Emanuel Rivers of goal directed therapy for septic shock in 2001 (Rivers et al., 2001) . EGDT was originally developed for sepsis, but has been used for several other clinical conditions such as post-operative care, ventilator associated pneumonia, central venous catheter line management and post cardiac arrest protocols amongst others (Wip and Napolitano, 2009; Cecconi et al., 2013; Marwick and Davey, 2009; Gaieski et al., 2009). The concept of EGDT has led to development of clinical care, protocolised into a package called a 'care bundle'. Clinical care bundles can be applied, as developed for acute resuscitation, but are also now used for many interventions in critical care. This review will primarily cover the rationale for EGDT for acute resuscitation in adult medicine.

Valid care bundles require clinically effective components, each with individual evidence of benefit (Barochia et al., 2010). One meta-analysis of ten care bundles for sepsis found that the overall benefit of the care bundle is greater than that would be expected by the evidence supporting the individual components (Barochia et al., 2010).

2.6.2 Testing EGDT in clinical trials; the evaluation of complex interventions

The evaluation of complex interventions is difficult (Campbell et al., 2007; Council, 2008). When one single intervention is tested against a control, well established methodologies exist to calculate the appropriate sample size and the size of the effect of the intervention compared to control on the study endpoints; in a well-designed study with adequate numbers, the effect of the intervention is clear (Oakley et al., 2006). When designing a

complex intervention incorporating several individual elements such as EGDT, both design and methodological difficulties must be met in parallel with the analysis plan of the data (Campbell et al., 2000).

The Medical Research Council published guidelines for evaluating complex interventions, including but not limited to EGDT in 2000, updated in 2008 (Campbell et al., 2000; Campbell et al., 2007). These guidelines defines a complex intervention as having any one of the following (Council, 2008):

i. Number of interacting components within the experimental and control interventions
ii. Number and difficulty of behaviours required by those delivering or receiving the intervention
iii. Number of groups or organisational levels targeted by the intervention
iv. Number and variability of outcomes
v. Degree of flexibility or tailoring of the intervention permitted

EGDT encompasses all of these complexities, particularly with the number of interacting components within the care bundle and controls, and the flexibility and tailoring of the care bundle to the clinical needs of each participant.

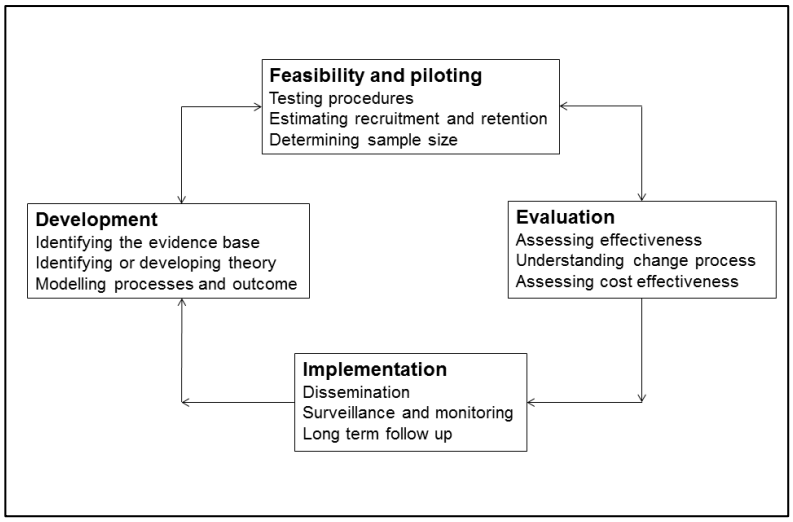

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Figure 2.3 Developing and evaluating complex interventions

Figure adapted from MRC 2008 guidelines (Council, 2008).

In the analysis of EGDT, given the complex nature of the care bundle and the degree of flexibility involved on an individual patient level, it is not possible to determine if one individual element of the care bundle was the cause of a good or negative outcomeInstead, the entire care bundle must be treated statistically as one intervention and the evaluation designed as such. Larger sample sizes are often required to ensure that all variability within the care bundle can be taken into consideration (Council, 2008; Campbell et al., 2000). Given the complexity in study design and analysis, pilot studies are recommended by the MRC guidelines, to ensure that issues of intervention delivery and acceptability are tested before larger studies planned (Figure 2.5) (Power et al., 2004; Eldridge et al., 2005).

Randomised controlled trials provide the best methodology for evaluating EGDT, as parallel recruitment and delivery of the EGDT or the control hospital based care minimise allocation and observer biases (Oakley et al., 2006). The Consolidated Standard of Reporting of Trials (CONSORT) statement has been updated to account for complex interventions beyond pharmacological interventions (Boutron et al., 2008). In RCTs testing EGDT, blinding is usually not possible due to the complex nature of the intervention, and neither the treating investigator nor the patient can be blinded (Oakley et al., 2006). Single site RCTs that recruit in parallel are therefore subject to the potential for cross-contamination between study teams; delivery of EGDT to one patient and routine care to another by members of the same team means that it is difficult to determine if the 'routine care' was not influenced by the presence of the study team and surrounding education and resources (Eldridge et al., 2005; Campbell et al., 2007). Cluster randomised trials are the ideal design for testing EGDT, as randomisation by centre rather than patient ensures that all patients recruited to one centre can be managed in the same way and a true comparison between routine care and EGDT can be made, providing that the centres are equally matched for resources and patient attendance (Campbell et al., 2007).

Due to the cost and complexity of using RCTs to test EGDT, most studies of EGDT have been observational in nature, commonly in a before/after study design. This design enables the team to monitor routine care in the first defined time period, and then deliver the EGDT in the second time period, and then make comparisons between the two groups. However this design is subject to many biases, including observer bias, allocation bias and confounding of the results over time by external changes within the study environment not controlled by the study team. The MRC does not recommend observational studies for evaluating complex interventions unless randomisation in a parallel design is not practical or feasible for local reasons (Council, 2008). However observational data to provide feasibility and pilot information before embarking on a RCT are recommended by the guidelines (Figure 2.5) (Council, 2008). The quality of evidence provided from observational studies of EGDT is weaker than those from RCTs, with the strongest evidence provided by cluster randomised RCTs (Campbell et al., 2007).

2.6.3 Is EGDT effective?

2.6.3.1 Sepsis

EGDT has been most extensively tested in severe sepsis with shock (Rivers et al., 2001; El Solh et al., 2008; Levy et al., 2012; Nguyen et al., 2011; Wang et al., 2013; Yan, 2010; Yealy et al., 2014). A landmark trial published in 2001 described a significant reduction in mortality from sepsis with the use of EGDT compared to routine care. This study was performed in one large hospital over three years (Rivers et al., 2001), patients were randomised to receive either EGDT or routine care on admission to the emergency department. Mortality from severe sepsis was reported as 30.5% in the EGDT group, compared to 46.5% in the standard care group, a relative risk reduction in all patients of 0.58 (95% CI 0.39 – 0.87) (Rivers et al., 2001). The key protocol components in this study were early appropriate antibiotics and an aggressive fluid resuscitation strategy that included central venous pressure and venous blood lactate monitoring, with specific targets set for both CVP and

venous lactate measurements. In addition blood was given to target a haematocrit of >30% (Hb >10g/dL).

The success of the study led, with the support of the Institute for Healthcare Improvement, a US based non-government organisation (www. http://www.ihi.org/Pages/default.aspx), to development of surviving sepsis guidelines, which were widely published and implemented in order to attempt to improve the management of sepsis world-wide (Dellinger et al., 2004). Given the success of the study, other centres attempted to replicate the results of the six hour resuscitation protocol with varying effect, most of these studies were observational before/after studies compared to randomised controlled trials (Barochia et al., 2010). A meta-analysis of data from these studies showed that early antibiotics and the overall use of a care bundle were associated with substantial mortality reduction; other care bundle elements were not individually assessed due to heterogeneity between the studies (Barochia et al., 2010) (Figure 2.6).

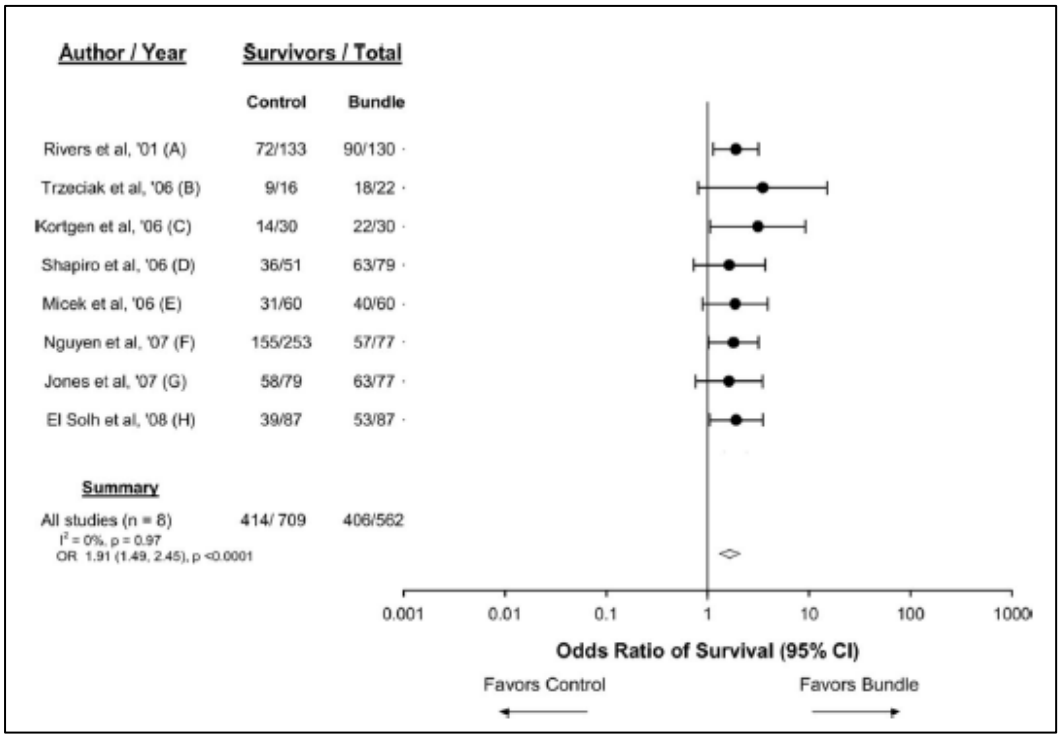


Figure 2.4 Effect size of Early Goal Directed Therapy for sepsis

Forest plot of eight studies testing EGDT for sepsis. Rivers et al was a randomised controlled clinical trial, the others all before/after observational studies. Figure reproduced from Barochia 2010.

A separate review of bundled care for sepsis included 21 studies, and found again that six hour resuscitation bundles were effective in reducing mortality, and that 24 hour bundles were less effective than six hour bundles, but still resulted in mortality reduction over routine care (OR 1.646, 95% CI 1.036 - 2.614, $p < 0.035$) (Chamberlain et al., 2011). This larger but statistically weaker meta-analysis included only results from observational studies and did not include data from the Rivers trial. Data from the other studies included in Barochia 2010, and additional smaller studies were included. As the data included are purely observational, the quality of the review and the conclusions drawn are limited (Chamberlain et al., 2011). Data for both meta-analyses were predominately from open label before/after studies, although Barochia and colleagues did include the Rivers 2001 trial data, and therefore definitive conclusions from these reviews are limited. Future meta-analyses that include data from newer EGDT parallel randomised controlled trials, of which there have been three published to date (Rivers et al., 2001; Yealy et al., 2014; Yan, 2010) are awaited.

A RCT in China tested EGDT as a sepsis bundle in a parallel design against routine hospital care across eight hospitals. 303 patients with severe sepsis were included, the 28 day mortality rate was 24.8% in the EGDT group compared to 43.5% in the routine care group $p=0.01$ (Yan, 2010). This study is published in Chinese language only, no translation is available currently, so the quality of randomisation and detail of any biases cannot be fully assessed (Yan, 2010).

In 2014, a large multicentre randomised trial throughout 30 hospitals in the USA was published that compared the Rivers protocol against either a less intensive EGDT protocol or standard hospital care (PROCESS trial)(Yealy et al., 2014). Overall case fatality rates were 18-21% and did not differ between the three arms of the study, despite increased CVP

monitoring, fluid resuscitation and vaso-active agents and blood being given in the goal directed protocol arms. The Rivers protocol arm of the PROCESS trial had set targets based on central venous monitoring of lactate and venous oxygen saturation, the hospital sepsis protocol arm had more simplified targets based on monitoring of blood pressure response to intravenous fluids and inotropes delivered through a peripheral catheter, and routine care in the third arm was dictated by the treating physician. (Yealy et al., 2014). All hospitals enrolled in the study had to have used surviving sepsis guidelines in the past, but did not have hospital-specific protocols in place at the start of the study.

When the Yealy and Rivers studies were compared, patients in the Rivers study were slightly older with more comorbidity, had lower central venous saturation measurements on admission, and may have had more persistent shock (Yealy et al., 2014; Rivers et al., 2001), but otherwise patients between the two studies were equally matched. The authors speculate that the significantly reduced mortality across 13 years between the two studies may be due to overall improvements in recognition and management of patients with sepsis, particularly early recognition in the community and improvements made in intensive care with regards to critical organ support (Yealy et al., 2014). Results from two further randomised controlled trials of EGDT in sepsis are awaited ARISE NCT00975793, and ProMISE ISRCTN36307479.

In summary, EGDT when tested in hospitals where it had not been previously used, appears to be highly effective in reducing mortality from sepsis, with the potential of national campaigns to improve clinical management resulting in influences on routine care and overall reduced mortality from sepsis since the original Rivers trial (Huang et al., 2013). The PROCESS study shows that in the USA, mortality from sepsis has reduced substantially over 10 years since the surviving sepsis campaign was introduced (Yealy et al., 2014). Data from further RCTs are awaited to see if these successes have been replicated in other settings, or if the impact of the SSG has led to overall improvements in sepsis management, without the need for further EGDT testing (Yealy et al., 2014). The success of EGDT for

sepsis has led to goal directed therapy strategy being used for other areas of medicine, including intensive care medicine and post-operative survival patients (see following section).

2.6.3.2 Post-operative care

The majority of patients undergoing surgery experience only minor complications (Cecconi et al., 2013). However for high risk patients, the post-operative period is associated with increased risk of complications including sepsis, shock and organ failure. The principles of EGDT, when applied to the fluid and oxygen management of these patients have been shown to improve outcome (Futier et al., 2010; Nisanevich et al., 2005). Subsequent systematic reviews have shown these benefits to be mainly limited to the very high risk patients, care bundles reducing both complications (OR 0.45, [95% CI 0.34 to 0.60]; $p<0.00001$) and death in high risk patients (OR 0.20, [95% CI 0.09 to 0.41]; $p<0.0001$) (Arulkumaran et al., 2014; Cecconi et al., 2013).

2.6.3.3 Bundles used in Intensive Care Units (ICU)

Clinical care bundles tested in intensive care unit include reduction of ventilator associated pneumonia (VAP) and catheter associated blood stream infections and tight glycaemic control, with an overall relative risk reduction of harm by 25% (Marwick and Davey, 2009). One interesting facet of these bundles, is that, in contrast to sepsis, where one protocol was has been used in different studies, several studies in VAP have tested different elements over time, and a consensus has been reached to include the most efficacious elements of the VAP bundles only (Wip and Napolitano, 2009). Overall ICU bundles have met with considerable success in reducing complications in patients associated with prolonged ICU stay, with a number needed to treat between 3-11 for different ICU bundles (Marwick and Davey, 2009). From these studies a considerable literature has been generated on optimising compliance and sustainability of a care bundle once in use (Lipshutz et al., 2008; Levy et al., 2004; Noritomi et al., 2014; Rangachari et al., 2014).

2.6.3.4 Care bundles in other conditions

The care bundle approach has been tested in a small number of studies in sub-arachnoid haemorrhage (Molyneux, 2006; Mutoh et al., 2007; Mutoh et al., 2009; Mutoh et al., 2014; Wartenberg and Mayer, 2006), in one study the proportion of patients with a mRS of zero at 3 months was 52% in those who received the bundle versus 36% who received routine care; p=0.026 (Mutoh et al., 2014). Care bundles for kidney injury prevention are being trialled, no detailed data are available (Ronco, 2004). A full discussion of the benefits and risks of these bundles is beyond the scope of this chapter, but given the expanding literature on the subject of goal directed therapy, particularly when improved outcomes and cost-efficacy are demonstrated, more bundles designed to improve clinical care are likely to be developed (Ebm et al., 2014).

2.6.3.5 Quality improvement initiatives to ensure care bundle success and sustainability

Care bundles now form part of quality improvement (QI) initiatives that have been taken on in many countries after the success of care bundles in improving outcomes. The success of a care bundle is dependent on the QI processes, and sustainability of care bundles amongst medical, nursing and ancillary staff within a hospital is required to ensure the high standards of care delivered by the bundle are maintained (Rangachari et al., 2014; Robb et al., 2010; Chamberlain et al., 2011). The body of evidence exploring reasons behind care bundle failures has grown to inform bundle implementation and success. QI initiatives revolve around the loop of PDSA, 'Plan, Do, Study, Act' (Lipshutz et al., 2008), where each stage is important to ensure that problems are identified early, and effective changes are made to sustain the initiative.

One study examined issues around the implementation and sustainability of three different care bundles within one hospital, and found that nurse-led care bundles in critical care were sustained by both regular audit of and publicity about check list use for the four components

of the care bundle, and dissemination of data showing improving outcomes from the use of the bundle (Lipshutz et al., 2008). However the implementation in the same hospital of EGDT for sepsis was much more problematic with issues arising in both the nursing and medical teams relating to clinician autonomy and additional nursing burdens involved in the care bundle (Lipshutz et al., 2008).

In a hospital in Brazil, QI initiatives and improved communication within the emergency department led to a substantial increase in adults with sepsis receiving bundled care, from 13% to 62%, associated with decreased all cause hospital mortality and substantial cost savings (Noritomi et al., 2014). Care bundles to reduce central line infections have been shown to be effective, but problems have also been encountered in bundle implementation, particularly over agreement within a multidisciplinary team to adhere to a care bundle, and sustainability of adherence over time (Rangachari et al., 2014; Levy et al., 2004). Improved communication between the teams in ICU and PICU in one hospital led to increasing compliance with the bundle, a decrease in line infections and considerable estimated cost savings (Rangachari et al., 2014).

Concerns have been raised about the feasibility of all the elements in the Rivers protocol (Levy et al., 2012), and full compliance with the bundle was found to be difficult in some studies (Levy et al., 2004; Otero et al., 2006). The surviving sepsis guidelines were revised to reflect this in 2013, with a divide in the timeframe for the goals, with antibiotic and fluid resuscitation to be completed within three hours, and targeted vasopressor treatment and oxygenation within six hours (Barochia et al., 2013; Dellinger et al., 2013).

To implement and sustain a care bundle, several factors have been shown to be important: high quality planning involving all the entire multidisciplinary team, regular feedback and support, involving the whole team in discussions, celebrating successes and pilot testing with step-wise implementation (Lipshutz et al., 2008) (Levy et al., 2004; Noritomi et al., 2014;

Rangachari et al., 2014). The principles of QI for care bundles were taken forward and used to set up and sustain the care bundle tested in this study.

2.6.3.6 Cost efficacy of care bundles

Clinical care bundles have been primarily tested to examine efficacy in reducing serious outcomes such as death and disability. However, given the overall evidence of the benefit of this approach, data are now required to test if this approach is cost-effective. One study from Brazil, not only showed that a sepsis bundle reduced mortality from sepsis, but was associated with an estimated decreased cost of $11800 per patient treated with the bundle compared to routine care (mean difference -11,815; 95% CI -18,604 to -5,338) (Noritomi et al., 2014). Further data on cost-efficacy of care bundles are required.

2.6.3.7 Care bundles in resource-limited settings

Clinical care bundles have been well described and tested in resource rich hospitals in developed and emerging economies. However goal directed therapy has not been formally tested for sepsis in sub-Saharan Africa in either adults or children.

A large, well conducted paediatric study of fluid resuscitation for unwell African children showed that fluid boluses compared to maintenance fluid therapy were associated with higher risk of death (Maitland et al., 2011a). This study did not formally test the clinical care bundle concept, but the mortality observed was lower than expected when the sample size calculation was done. Prior to randomisation, the study team introduced significant QI to the hospitals involved in the trial, including early antibiotics, prompt recognition of critical illness and triage of children according to degree of illness severity. An extrapolation of the lower mortality rates observed during the trial due to introduction of elements of sepsis care bundles is possible (Myburgh, 2011). It is therefore possible that the introduction of these QI processes into African hospitals may have an effect on mortality, formal testing is required.

EGDT for sepsis has been tested successfully in Asia and South America, in relatively well-resourced hospitals (Kuan et al., 2013; Nguyen et al., 2011; Noritomi et al., 2014; Na et al., 2012; Yan, 2010; Wang et al., 2013; Chen et al., 2008) (Table 2.2).

Table 2.2 Early Goal Directed Therapy studies in emerging countries

Studies of EGDT in emerging settings

Study	Location	Trial design	Intervention	Outcome	Statistically significant? p value
Kuan 2013	Singapore	Before/after	Sepsis bundle compared to routine care	2/18 deaths in the bundle group, 29/99 deaths in the observational group	0.15
Nguyen 2011	8 centres in Asia (ATLAS)	Before/after	SSG bundle compared to modified SSG bundle with lactate clearance	The ratio of the relative risk reduction in mortality with bundle = 1.94 (95% CI 1.45 to 39.1)	No p value given
Na 2012	8 centres in Asia (ATLAS)	Before/after	Sepsis bundle compared to modified sepsis bundle	27/193 (24.5%) deaths in 'bundle completed', 119/364 (37.2%) deaths in bundle not completed group	0.04
Noritomi 2014	10 hospitals in Brazil	Before/after	Sepsis bundle compared to routine care	112/203 (55%) before group, 41/161 (26%) in full bundle compliance group	<0.001
2010	8 hospitals in China	Multicentre RCT	Sepsis bundle compared to routine care	Routine hospital care mortality rate 42.5%, sepsis bundle mortality rate 24.8%	<0.01
Wang 2013	China	Before/after single site, ICU care	Sepsis bundle compared to routine care	Before group mortality 44.8%, after group mortality 31.6%	P<0.05
Chen 2008	China	Before/after single site, ICU care	Sepsis bundle compared to routine care	Before group mortality 72.5%, after group 55.1%	P=0.01

However there is substantial heterogeneity between these studies and the quality of the data is variable. Using EGDT to improve survival is highly applicable to resource limited hospitals. In 2001, the Department of Paediatrics in Queen Elizabeth Central Hospital (QECH) developed Emergency Triage and Treatment (ETAT) in their clinic to prioritise the care of sick children, and have shown an overall reduction in mortality amongst emergency admissions from 18% to 9% (Molyneux et al., 2006). Rapid recognition and management of acute illness in adults is part of the 2004 WHO guidelines on the management of unwell adults in resource limited settings 'Integrated management of adolescent and adult illnesses' (WHO, 2004). Although neither EGDT nor ETAT are specifically mentioned in this publication, the principles that underline both of these methodologies are recommended in the guidelines.

No formal trials of any element of goal directed therapy for sepsis in adults have been done to date in sub-Saharan Africa. A team in Uganda compared two cohorts of patients, three years apart, who had different volumes of fluid given, and extrapolated that mortality differences between the two cohorts were due in part to the increased volumes of fluids given to the later cohort (Jacob et al., 2012). However this observational study was subject to several biases in data capture, particularly due to the time differences between the two cohorts, and as such the conclusion that increased fluid administration for sepsis improves mortality cannot be supported by the data presented.

This group are now planning a clinical trial testing a fluid resuscitation strategy with or without dopamine for sepsis within the context of a randomised controlled trial, although they are not formally testing EGDT within that trial entitled 'Adaptive Randomization Trial of Early Management Interventions for Sepsis in Uganda (ARTEMIS-Uganda)'. This trial is not cluster randomised, but randomised on an individual level across four centres, which leaves the study team again open to allocation bias and potential contamination between the two groups by members of the study team. Another trial is planned in Zambia and Zimbabwe,

also testing fluid resuscitation strategies with the addition of vasopressor support, this trial has also yet to start recruitment, and the group's early data have yet to be published. These trials have yet to be registered on the clinical trial registries.

2.7 What evidence is there to use EGDT for bacterial meningitis?

As discussed in Section 2.2.4, several interventions have been tested for bacterial meningitis in adults and children. However the clinical care bundle concept has not been tested formally for meningitis in either group. As an acute, severe infection, with critical time pressure, bacterial meningitis is potentially an ideal disease to utilise the delivery of optimised clinical care in the form of goal directed therapy. The sepsis bundle approach has been used for children with meningococcal septicaemia (Kjaergaard et al., 2006), and a care bundle has been developed by neurosurgeons to reduce the incidence of meningitis in patients with ventricular drains in-situ (Leverstein-van Hall et al., 2010), but has not been tested prospectively to determine if this approach is safe or feasible for bacterial meningitis in adults.

Applying EGDT to bacterial meningitis in Africa

Given the success with which EGDT has reduced mortality and improved clinical care for sepsis in resource-rich settings, applying this approach to a critical illness in a profoundly resource limited environment should be appropriate. EGDT is associated with cost savings and is dependent upon nurse-led care (Lipshutz et al., 2008; Barochia et al., 2010; Tromp et al., 2010), two factors which support testing feasibility and efficacy of EGDT in a profoundly resource-limited hospital environment in Africa.

Acute bacterial meningitis is a condition where early recognition and treatment is deemed essential to optimise outcome (Heyderman, 2005; Prasad et al., 2004; Harnden et al., 2006;

Auburtin et al., 2006; Fitch and van de Beek, 2007; Gjini et al., 2006a), and therefore potentially an ideal condition to test EGDT.

The evidence behind each intervention chosen for inclusion in the care bundle is discussed in the following section.

2.8 What evidence exists to support the individual elements of the care bundle in BAM?

All elements of the bundle have strong evidence to support their use in ABM, and are based on the surviving sepsis guidelines (Dellinger et al., 2008b; Dellinger et al., 2013). All have been adapted to be feasible in a low income setting. The surviving sepsis guidelines were used as a backbone to design the care bundle, as they are the most well evidence-based guidelines for care bundles in critical illness available. The UK meningitis treatment guidelines were also used (Heyderman et al., 2003). The care bundle elements were based around the ABCDE approach to resuscitation, advocated by the UK and US resuscitation council guidelines (Council, 2010).

2.8.1 Airway and Oxygenation

Airway management has been shown to reduce the risk of airway obstruction in coma (Foundation, 2000; Roberts et al., 2005; Stocker and Biro, 2005), and is essential for a patient with seizures who is at risk of airway obstruction (Roberts et al., 2005). However endotracheal-tubes and formal airway protection require the patient to be sedated and ventilated, such facilities are essentially unavailable in Malawi where access to intensive care is severely limited (Branson et al., 2014; Stocker and Biro, 2005). Naso-pharyngeal airways are the safest option where invasive ventilation is not available, as this allows supports the airway to allow oxygen can pass, although no protection against aspiration is offered (Roberts et al., 2005).

Cerebral oxygenation and perfusion are essential in ABM (Moller et al., 2002). Oxygen saturations of less than 93% on univariate analysis were shown to be associated with an increased risk of death in an analysis of mortality from meningitis in Malawi (Wall et al., 2013c). Patients with ABM may have lower cerebral oxygen metabolism compared to controls (Moller et al., 2002), and adequate oxygen delivery to the brain is critical if intracranial pressure is increased (Glimaker et al., 2014). Oxygen delivery to tissues in critically ill patients has been shown to decrease significantly when the haemoglobin is <7g/dL, and transfusion to a haematocrit of >30% forms a component of the surviving sepsis guidelines (Shander, 2004; Dellinger et al., 2008a). Oxygen was only available in QECH via concentrators that take ambient air and concentrate the oxygen to a maximum 28%. All patients who had hypoxaemia were given oxygen via a concentrator and nasal prongs rather than facemask oxygen, due to resource limitations. Where a nasopharyngeal airway was required, the nasal prongs were inserted into the airway to deliver oxygen.

The WHO guidelines on blood transfusion state that a transfusion should be given when tissue hypoxia is compromised by anaemia (WHO, 2009). Blood was not independently provided for the study, and the team were dependent on hospital supplies, which were often critically low. Therefore we could only justify the use of blood acutely in this study when the patients met the in-hospital criteria for adult transfusion (<5-6g/dL).

2.8.2 Intracranial pressure management

Extensive studies have attempted to manage the raised intracranial pressure associated with bacterial meningitis using either lumbar drains or intra-ventricular drains (Murad et al., 2008; Glimaker et al., 2014; Abulhasan et al., 2013). Repeat lumbar punctures have been used successfully in cryptococcal meningitis where a clear relationship between raised CSF pressure and outcome have been documented, with improvements in pressure and possibly outcome by serial LP (Wijewardana et al., 2011; Bicanic et al., 2009). However this approach has not been tested in bacterial meningitis and other approaches to the

management of raised intracranial pressure are not suitable for resource limited hospitals without access to both critical care and an experienced neurosurgeon (Murad et al., 2008; Abulhasan et al., 2013). In addition, serial lumbar punctures would require ongoing clinical ward management and as such would not be suitable for inclusion in an acute clinical care bundle.

In the literature from sub-arachnoid haemorrhages (SAH) and brain trauma, a 30°C head tilt has been shown to optimise perfusion and minimise oedema in brain injury (Molyneux, 2006). As SAH is also a condition with acute inflammation with the sub-arachnoid space, parallels between the effects of SAH and ABM on raised intracranial pressure can be drawn. This simple intervention of a head tilt was adapted and applied to all patients in BAM with a GCS <11. Intracranial pressure monitoring to test the efficacy of the head tilt was not available.

2.8.3 Tissue perfusion

Fluid resuscitation in ABM is more controversial, as no RCTs have been performed to test different resuscitation strategies. Fluid restriction in children was originally thought to be a useful treatment in paediatric meningitis, as the syndrome of inappropriate anti-diuretic hormone (SIADH) was a recognised consequence of ABM, and associated with poor outcome (Mendoza, 1976). Studies testing fluid restriction in ABM showed this strategy is associated with harm in meningitis (Duke et al., 2002), and for children, parenteral fluids treatment to maintain euvolaemia are currently advocated (Maconochie et al., 2008; Oates-Whitehead et al., 2005). Extensive data shows that initial aggressive resuscitation in adult sepsis is associated with survival (Dellinger et al., 2008a). However, bolus resuscitation was harmful for children with shock in East Africa compared to maintenance fluid treatment (Maitland et al., 2011a). This study included children with both malaria and sepsis, very few children with meningitis were included, and no sub-analysis of fluid treatment and meningitis was done. Policies on fluid resuscitation for shocked children have been extensively re-considered following this trial.

No consensus exists regarding fluid resuscitation in adults with meningitis. UK guidelines state that resuscitation should be given if the patient is shocked, but this statement is backed with little data (Heyderman et al., 2003). Therefore if adults met the surviving sepsis guidelines criteria for clinical shock, they were resuscitated with fluid as per the surviving sepsis guidelines, but if no clinical evidence of shock was seen, the patient received maintenance IV fluids at 125ml/hour for the duration of the intervention

2.8.4 Early Antibiotics

Early antibiotics in meningitis have been shown to have a significant association with survival in several studies, when given by community practitioners pre-hospital, or rapidly in-hospital (Harnden et al., 2006; Auburtin et al., 2006; Durand et al., 1993; Koster-Rasmussen et al., 2008). However, no randomised clinical trials of delayed versus rapid treatment have been done as this has been considered unethical; all data are observational and hence subject to bias (Radetsky, 1992). . A delay of >3 hours from hospital admission to parenteral antibiotics was detrimental in pneumococcal meningitis (Auburtin et al., 2006). In another study in Denmark, delay to in-hospital antibiotics and poor outcome were even more closely linked, with time to antibiotics of > two hours independently associated with poor outcome (Koster-Rasmussen et al., 2008). The evidenced based recommendation from the surviving sepsis guidelines, and included in the majority of trial protocols included within those guidelines, is for parenteral antibiotics to be delivered within one hour from admission to the emergency department (Dellinger et al., 2008b; Dellinger et al., 2013). From these data a target of one hour from admission to parenteral antibiotics was set in the Adult Emergency and Trauma Centre at Queen Elizabeth Central Hospital in Blantyre, with the expectation that in attempting to meet this target, all patients would receive antibiotics within two hours.

2.8.5 Summary

Adult patients with bacterial meningitis in Malawi have important differences to patients presenting with ABM elsewhere, and predictors of mortality in this setting are different. Interventions designed in resource- rich countries have been ineffective in reducing mortality from ABM in Malawi. Improvement in in-hospital care, particularly access to early disease recognition and parenteral antibiotic therapy has impacted on mortality from ABM elsewhere. Early Goal Directed Therapy offers a well validated methodology to introduce rapid disease recognition and targeted treatment protocols to the treatment of ABM in Malawi. The methodology for the trial testing this approach in Queen Elizabeth Central Hospital are found in Chapter 3 Section 3.4, and the results in Chapter 6 Section 6.3.4.

3 Patients, Materials and Methods

3.0 General methodology

3.0.1 Introduction

The clinical burden and mortality rates from bacterial meningitis are substantially higher in sub-Saharan Africa compared to Europe. In the last 10 years, numerous public health interventions such as the roll out of ART and co-trimoxazole prophylaxis, intensification of malaria control, improvements in child nutrition have been introduced into Malawi that have been associated with considerable improvements in under-5 childhood mortality and substantial decreases in the mortality rate of HIV-infected adults. However, it is less certain how these interventions have impacted on the burden and mortality rates of ABM in adults. In addition, two adjunctive interventions (dexamethasone and glycerol) that have demonstrated efficacy in reducing mortality and morbidity from acute bacterial meningitis (ABM) in other settings have been ineffective in Malawian adults with ABM, the Steroids for Adult Meningitis (SAM) and Glycerol for Adult Meningitis (GLAM) trials (Scarborough et al., 2007; Ajdukiewicz et al., 2011). It is unclear why these trials failed to show benefit. A better understanding the of the disease burden and causes of high mortality was required before embarking on the design of any further clinical interventions to reduce mortality, including the intervention tested in this thesis.

Firstly, two analyses of historical meningitis surveillance and clinical data from MLW were undertaken, to understand better the disease epidemiology and clinical phenotype ABM in adults in Malawi. The first analysis was designed to examine epidemiological trends in bacterial meningitis in adults and children using laboratory surveillance data, and the second to identify clinical markers of poor prognosis in adults using data from all clinical trials where

adults with bacterial meningitis had been recruited. Details for the specific statistical methods and research questions can be found in Chapter 4 Section 4.4.2.

Secondly, these data were then taken forward to synthesise a clinical scoring system to attempt to predict outcome from ABM in adults in Malawi, the detailed methodology for this can be found in Chapter 5 Section 5.2.

Data generated from these analyses were used to inform the design and analysis of the clinical trial of early goal directed therapy for adult meningitis that is the focus of this PhD thesis, of which the detailed methods can be found in this Chapter, Section 3.4.

The results from the data generated by the clinical trial were used to examine for predictors of poor survival versus healthy survival in the BAM cohort patients and compared to the mortality predictors in the historical cohort. In addition real-time PCR was used to detect causes of culture negative meningitis and bacterial and viral co-infection loads were estimated using this technology. The specific methods for this are in this chapter, Section 3.1.

Methods used across these different chapters including definitions of bacterial meningitis cases, database creation, laboratory and statistical methods are discussed in Section 3,0.2 and 3.1-2. Specific methods for the early goal directed therapy trial are discussed in in Section 3.4.

3.0.2 Cross-chapter Bacterial meningitis methodology

3.0.2.1 Definitions of bacterial meningitis and generic inclusion and exclusion criteria

Bacterial meningitis is defined as either the presence of a bacteria known to cause meningitis in the CSF on stain, bacterial culture or DNA detected by PCR, or evidence of severe inflammation in the CSF including an elevated WCC of over 100 cells/mm^3 with a marked neutrophilia (>50%) in the presence of a presenting clinical phenotype (Brouwer et al., 2012). HIV infection may substantially alter the CSF WCC response in bacterial

meningitis, and classical cut off of >100cells/mm^3 may not be accurate for HIV infected adults (Jarvis et al., 2010). Where CSF chemistry is available, in the presence of an elevated CSF WCC and presenting clinical phenotype, a CSF protein of >0.5 g/L and a CSF:Blood glucose ratio of <0.4 are considered to be indicative of bacterial meningitis (van de Beek et al., 2004; Tamune et al., 2013; White et al., 2012). In this thesis, cases of bacterial meningitis where an organism was found to be present on Gram's stain, culture or PCR were defined as 'proven' cases, and cases where bacterial meningitis was highly likely with a clinical presentation and typical CSF findings in the absence of a proven bacterial pathogen were defined as 'probable' cases. The CSF WCC is critical in determining the probability of bacterial meningitis in probable cases, where the WCC is not sufficiently elevated or is represented by predominately lymphocytes and no prior antibiotics have been given, the chances of the meningitis being caused by another pathogen are significant (White et al., 2012). The CSF WCC cut off to determine probable cases for the historical clinical cases and the BAM study differed slightly. The specific case definitions and reasons for choosing that definition for each study are therefore included in the methods section for that chapter.

3.0.2.2 Patients and hospital setting

Queen Elizabeth Central Hospital is a tertiary referral hospital for Southern Malawi, and the District General Hospital for Blantyre city. The hospital serves a population of approximately 1 million people, and an estimated 10-12 000 adults are admitted to medicine each year (Sanjoaquin et al., 2013). Patients over the age of 14 years are admitted to the adult wards, and therefore considered as adolescent adults for the purposes of this thesis. Prior to 2011, all admissions to adult medicine were screened and triaged in an area of the outpatient department of the hospital called Room 6, where they were given an initial diagnosis. Patients to be admitted were then transferred to a medical admissions ward called 4B where they were seen by a nurse and clinician and treatment was initiated (Figure 3.1). All patients with bacterial meningitis included in the historical data 2000-2011 were admitted to QECH

through this system. Patients admitted after November 2011 were assessed in the Adult Emergency and Trauma Centre (AETC) where they were reviewed by emergency-trained clinicians and nurses and given treatment prior to transfer to the medical wards. Further methodological explanation of this system is given in Section 2.

3a **3b**

Figure 3.1 Comparison of old and new QECH admission areas

3a: Waiting area and Room 6 clinic rooms. 3b: Entrance to AETC

Once admitted to an adult medical ward, all patients were assigned a bed space, which may have been either an actual bed or mattress on the floor in the ward or corridor, depending on the number of admitted patients. Two to three nurses were commonly available on shift to nurse up to 100 patients at any one time, and therefore all patients required an attending guardian to assist with their needs. Guardians were commonly close family members and provide services not performed by the nurses including giving food and water, changing dressings, toileting, cleaning and other care needs (Hoffman et al., 2012). Guardians were also commonly asked if they felt able to provide informed consent for entry into clinical studies where the patient was not able to give consent for medical reasons such as altered Glasgow coma score (Section 3.3.10). Therapeutic drug supplies were often erratic on the medical wards and the medical and nurses work in a profoundly resource constrained environment with limited access to basic necessities such as clean running water, hand soap, intravenous fluids amongst others. Conditions on the medical wards did not change substantially during the period studied (2000-2013).

3.1 General Sampling & Laboratory methods

3.1.1 Sample collection and storage

Since 2000, all adults (14-years or older) and children presenting to QECH with a clinical suspicion of meningitis have routinely undergone lumbar puncture (LP) (Everett et al., 2011). Diagnostic CSF was obtained by LP, a procedure where a needle is passed through the skin and subcutaneous tissues into the CSF space between the vertebral bodies, usually at the L4 space (Kneen et al., 2002). The CSF was drained into a sterile container and where possible transported immediately to the laboratory. Where CSF was taken at night, the sample was stored at room temperature until analysis the following morning.

Clinical indications for LP did not change in the hospital between 2000 to the present time. Clinical studies at QECH recruited patients who had undergone a clinically required LP, and whose CSF WCC met specific inclusion criteria.

3.1.2 Microbiology

All diagnostic testing at the MLW Clinical Research Programme laboratory was quality controlled as part of internationally recognised quality control programmes. A Gram's stain was performed if CSF white cell count (WCC) was >10/mm^3; India ink stain was performed on all adult CSF samples. Samples were cultured on sheep blood and chocolate agar for 48 hours under aerobic and microaerophilic conditions. Bacteria and fungi were identified using standard methods (Barrow and Feltham, 1993). Antibiotic susceptibilities of all bacterial isolates were determined by the disc diffusion method (Oxoid, UK) using standard guidelines (2001). CSF biochemistry was determined using a Beckman Coulter CX5 Synchron Pro analyser (Beckman Dickinson USA) from 2009 onwards.

3.1.3 Molecular diagnostics

Molecular diagnostic methods including PCR, pneumococcal antigen screening and CSF lactate were not available routinely for the diagnosis of ABM. These methods were used during the BAM trial in a sub-set of included patients, results are found in Chapters 6 Section 6.3.2 and 7 Section 7.3.1-2

Routine CSF Cryptococcal Antigen (CrAg) testing using latex agglutination strips (IMMY, USA) became available during 2013, funded by a cryptococcal meningitis clinical trial, ACTA ISRCTN45035509 http://www.sgul.ac.uk/research/centres/ii/projects/cryptococcal-meningitis-group/clinical-trials-in-progress.

3.1.3.1 DNA extraction for PCR

To extract bacterial DNA and viral DNA and RNA, the following protocol was followed using standard pneumococcal extraction methodology (Corless et al., 2001; Greiner et al., 2001). CSF stored at -80°C was thawed (no additional preservatives were used). 100-200µl of CSF was removed from each sample depending on the volume of CSF available, and subjected to a lysozyme-buffer digestion protocol to break down the thick encapsulated pneumococcal wall. 180ul of digestion buffer was used per sample. The buffer consisted of 20mM Tris.HCl pH 8.0, 2mM EDTA, 1.2% Triton-X-100, 100ul of 10x lysozyme and 10ul of 10x lysostaphin (obtained from Sigma Aldrich USA) (Greiner et al., 2001; Rello et al., 2009). The sample was incubated for 30 minutes at 37°C, and then 40ul of Proteinase K and 200ul of 100% ethanol were added. The sample was then incubated for 1 hour at 56°C. DNA was extracted manually using Qiagen mini-blood and tissue kits (Qiagen, Germany) following the manufacturer's protocol as follows:

1) Add 200µl ethanol to each sample and mix
2) Pipet the mixture including any precipitate into the DNeasy Mini spin column, placed in a 2ml collection tube.
3) Centrifuge at 6000g/8000 RPM for 1 minute. Discard flow through and collection tube

4) Place the DNeasy mini spin column in a new 2ml collection tube.
5) Add 500µl of buffer AW2
6) Centrifuge for 3 minutes at 20000g or 14000 RPM. Discard flow through and collection tube
7) Place DNeasy mini spin colum in a clean 1.5ml or 2ml microcentrifuge tube
8) Pipet 200 µl buffer AE directly onto the DNeasy membrane
9) Incubate at room temperature for 1 min and then centrifuge at >6000g (8000RPM) for 1 minute to elute.
10) Repeat elution step 6-8 to maximise DNA yield.

Extracts for bacterial PCR were stored at -80°C until PCR could be performed.

3.1.3.2 Methods for Real-Time PCR

Real-Time PCR (RT-PCR) was performed on all samples and controls using the Viaa7 Real-Time PCR system (Applied Biosystems, USA). Primers and probes for RT-PCR were supplied in diagnostic kits from Fast Track Diagnostics (FTD) for bacterial and viral meningitis (Table 3.1). Samples were thawed and the PCR plate set up. The reaction mixture consisted of primer/probe mix for the pathogens tested per kit (either SpN, NM and Hib or CMV and EBV), buffer (Life Technologies) and AgPath-ID enzyme (Life technologies) and was set up as follows. The complete protocol can be downloaded from FTD at http://www.fast-trackdiagnostics.com/

1. Take a 96 well plate which is compatible with the Viaa7.
2. Pipette 15 µl of the BacMeng PP with the “2xRT PCR buffer” and the “25x RT-PCR enzyme mix” in the wells.
3. Add 10 µl of the extracted samples, the extracted negative control and the positive control (which is not extracted).
4. Each run must include a negative and a positive control

5. Mix briefly by pipetting up and down.
6. Close the plate with the ABI optical adhesive film.
7. Centrifuge briefly at 4000 RPM for 1 minute.
8. Put the plate in the Viaa7 with the correct wells identified on the Viaa7 software for each sample.
9. Set up the PCR programme for the following:

   50°C for 15 minutes hold

   95°C for 10 minutes hold

   40 cycles of: 95°C for 8 sec alternating with 60°C for 34 sec.

The number of wells used was calculated for each sample to be run in duplicate for each multiplex kit tested. Therefore four wells per sample were used, two for the bacterial meningitis kit and two for the EBV/CMV kit. All samples were duplicated so that the mean of Ct values and copy numbers could be used.

The multiplex PCR kits tested for the following pathogens in individual kits: Bacterial meningitis (Hib, *N. meningitidis*, *S. pneumoniae*), viral meningitis (EBV, CMV). Cycle threshold (Ct) values were determined from the PCR reaction for each sample. Ct values of >38 were discarded and deemed non-significant. Only results where a true exponential curve with a Ct value of <38 were included as true results (
Figure 3.2). This was because a Ct value above this threshold indicates the copy number is <10 copies/ml of CSF, at which point the sensitivity of the assay is significantly reduced and the result is not reliable (Greiner et al., 2001).

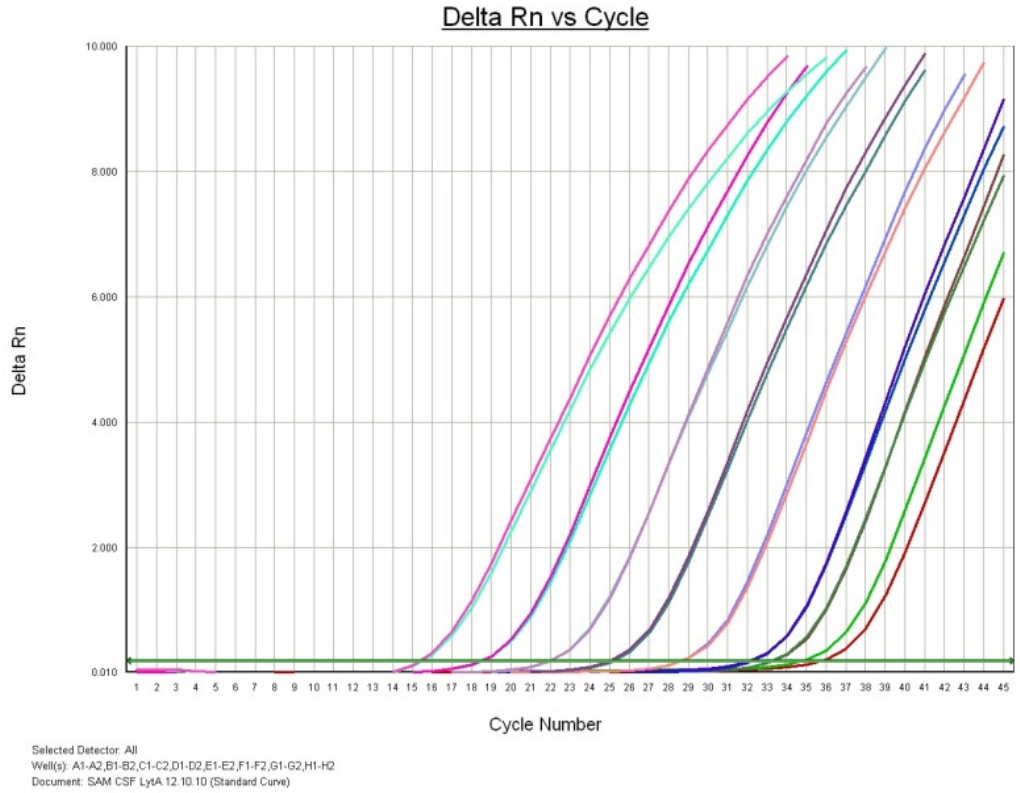


Figure 3.2 Example of exponential curves for Real-Time PCR

Cycle Threshold line shown as the horizontal green line. Exponential lines represent individual samples.

Positive and negative controls supplied in the kits by FTD were run with each plate. Positive internal controls consisted of *Streptococcus suis* DNA, to a predetermined Ct value of 33, the material in the negative controls was not released by FTD. Negative controls were extracted with the samples to test the integrity of the extraction procedure; positive controls to test the integrity of each reaction were added to the lysis buffer for each sample. Where doubt existed over the validity of the results, the sample was re-extracted with a new negative control. The estimated bacterial loads, expressed in copies/ml, were extrapolated from the Ct values denoted by the positive control following pathogen specific calculations provided by FTD (Table 3.1). Viral quantification was done by estimations based on the Cycle threshold (Ct) value compared against a standard curve of known quantification standards provided by FTD in each kit (Kelly et al., 2012).

Table 3.1 Targets for multiplex Real-Time PCR

Gene targets and quantification methods provided by FTD.

X denotes the final copy number, y denotes the Ct value measured in the sample.

Pathogen	Gene target	Quantification formula
N. meningitidis	ctrA	$X=e^{((y-43.424)/-1.433)}$
S. pneumoniae	LytA	$X=e^{((y-44.054)/-1.429)}$
H. influenza	oompP2	$X=e^{((y-45.995)/1.491)}$
Epstein Barr Virus	Proprietary information from FTD not supplied	Via standard curves
Cytomegalovirus	Proprietary information from FTD not supplied	Via standard curves

3.1.4 Sample storage

a) Patient samples

All diagnostic CSF taken for historical patients enrolled in clinical research was rapidly centrifuged, a CSF WCC was done on the pellet, and biochemistry testing performed on the supernatant. Remaining CSF supernatant from samples that met the initial CSF WCC inclusion criteria for the diagnosis of bacterial meningitis, taken in working hours for research studies was immediately frozen at -80°C (Scarborough et al., 2007; Ajdukiewicz et al., 2011). BAM CSF samples for research were taken by the research team in a separate container and frozen whole at -80°C immediately, irrespective of CSF WCC. When full CSF results were available and an inclusion/exclusion decision had been made, samples from patients who were excluded were discarded.

b) Microbiological isolates

All culture positive CSF isolates of bacteria of interest to ongoing surveillance work were beaded on glycerol [REF] and stored at -80°C as part of the MLW microbiology surveillance programme from 2001.

3.2 General Data Management and Statistical methods

3.2.1 Data Management Methods

3.2.1.1 Laboratory database

All culture positive isolates were logged by date, isolate and patient sex and age. These data were logged in laboratory books, and entered manually into a computer spreadsheet for analysis between 2000-2010. In 2010, the Laboratory Information Management System (LIMS) was introduced to the MLW laboratory, replacing the paper system with an electronic sample management system. These electronic data were imported directly from the laboratory system to the MLW data department in an Excel spreadsheet (Microsoft Office 2007). The data from the manual and electronic databases were merged prior to analysis. Details of the data cleaning process can be found in Chapter 4, Section 4.4.2.

3.2.1.2 Clinical database

All clinical trials and observational studies (published and unpublished) undertaken at the Department of Medicine, College of Medicine, QECH between 1990-to the current time, where adults with ABM were recruited prospectively and data were freely available were identified. Published studies were found using an online literature search of the medical literature database PubMed from the National Institute of Health in the United States. Un-published studies were identified by consultation with the previous and current MLW directors, Professors Malcolm Molyneux and Rob Heyderman, and with consultation of the

heads of the Department of Medicine, Professors Ed Zijlstra and Theresa Allain. Free access for each study database was obtained from the principal investigator of the individual study included. Selected variables were standardised with matching names, and re-coded for unification. Data from these variables were then merged from each individual database into a single database with the help of Philip Gichiru, statistician at LSTM using IBM SPSS version 17 for Windows. The clinical database was cleaned, removing cases where no mortality data at day 10 or day 40 were available. Further cases that did not meet the criteria for the diagnosis of bacterial meningitis (CSF WCC >100 cells/mm^3 in HIV negative, or >5 cells/mm^3 in HIV positive, or proven evidence of ABM). Details of the full inclusion criteria for this database can be found in Chapter 4, Section 4.4.2. The database was cross-checked for coding errors using basic frequency analysis, and missing data identified. Where possible, the contributing clinician was contacted for details on coding and missing data.

3.2.2 Epidemiology of bacterial meningitis

The laboratory database was cleaned, removing duplicate cases and all cases whose isolate was not consistent with bacterial meningitis. A separate database was kept of all culture negative samples. The database was first interrogated for pathogen seasonality, incidence. All isolates were then divided into age groups, the incidence, trends and frequency of each pathogen over time per age group were calculated. Detailed methods including data cleaning can be found in Chapter 4, Section 4.2.2. All analysis was undertaken using Stata version 10 (Statcorp USA), tables and charts were generated using Microsoft Excel.

3.2.3 Predictors of poor outcome using logistic regression

The clinical database was interrogated using univariate and multivariate logistic regression to test for variables that independently predicted poor outcome at 10 days and 40 days post admission with bacterial meningitis. Data were analysed using IBM SPSS/PASW version 17-20. For continuous variables, parametric (Student t) and non-parametric (Mann-Whitney U)

tests were used to compare survivors and non-survivors; Fisher exact / chi-square tests were used to compare categorical variables. Backwards stepwise logistic regression methods were used to estimate the influence of different variables on clinical outcome; each model was compared at each step with the previous model and only variables that remained significant at the 90% level were retained in the analysis for the next step. The first analysis plan denoted that only variables reaching statistical significance at or greater than the 95% level univariately were to be included in multivariate analyses. However variables that were predicted to be significant from previously published data that were found to be non-significant on univariate analysis were subsequently included in the multivariate analysis to test the strength of the negative association. The strength of relationships was expressed using odds ratios with 95% confidence intervals. All statistical tests were two-tailed, and a p value of <0.05 was used to denote statistical significance.

This approach was used to analyse the data from the historical clinical database (Chapter 4), to synthesise the predictive outcome score (Chapter 5), to compare data in Phase 1 and Phase 2 of BAM (Chapter 6) and to identify predictors or poor survival outcomes from BAM (Chapter 7). Selection methods for the variables used in the multivariate models for each chapter can be found in the relevant methods sections of those chapters.

3.2.4 Statistical methods for specific chapters

a) **Derivation and validation of a score to predict poor outcome from bacterial meningitis**

The clinical database was divided randomly into separate derivation and validation cohorts. The BAM Phase 1 data was added to the validation cohort to obtain a case ratio of 2:1 between the two cohorts. The severity score was derived first by using methods detailed in section 1.4.3 to determine predictors of poor outcome using logistic regression. Variables were then put forward into a nomogram to calculate a total score with a predictive index. The Nomogram was then applied to the validation cohort and sensitivity and specificity, plus

predictive index of the nomogram was calculated. Detailed methods are available in Chapter 5, Section 5.2.

b) **Methods to analyse the BAM study before/after design**

Standard approaches to clinical trial analysis were applied to the BAM data to compare Phase 1 and Phase 2 data for each endpoint. These include Kaplan-Meier curves, Fisher's exact and Chi square tests, logistic regression and composite analysis of individual elements of the clinical care bundle to test for the proportion of targets achieved. Full statistical methods are detailed in this Chapter, Section 3.3.12.

3.3 Ethical approvals for studies on human subjects

3.3.1 Ethical approval for historical data analysis

Data collection for surveillance at QECH by MLW was approved by the College of Medicine, University of Malawi, Research and Ethics committee (COMREC) in 2000. All constituent studies for the mortality analysis had been approved by COMREC and by the Liverpool School of Tropical Medicine (LSTM) Research Ethics committee and conformed to institutional guidelines. All participants in the included clinical trials and studies gave written informed consent, or this was given by a named guardian if the participant was under 18 years of age. No additional ethical approval was required for all historical analyses including the synthesis of the meningitis severity score.

3.3.2 Ethical approval for the BAM study

Ethical approval for the main clinical study (BAM) and the sub-studies tested in this PhD thesis was granted by LSTM Research Ethics committee (approval number 10.70), and COMREC approval number P.09/10/980. The laboratory analyses of the BAM data were approved under the umbrella approval of the BAM trial.

3.4 Methods for the prospective non-randomised clinical trial testing a clinical care bundle for adult meningitis in Malawi (BAM study).

3.3.2 Introduction

Goal directed therapy combines a set of clinical interventions, each with a clinical evidence base, and delivered as a protocolised package of care (Rivers et al., 2001; Dellinger et al., 2013). The evidence behind goal directed therapy for sepsis demonstrates that the effect size of the care bundle is greater than predicted from the evidence behind the individual interventions (Barochia et al., 2010). In this section the methodology for this study testing a clinical care bundle for meningitis will be outlined. In addition the study design and ethical considerations related to study design and informed consent in emergency evidence based clinical intervention studies will be discussed.

3.3.3 Objectives and research question

Research questions

1. Is the delivery of a clinical care bundle of resuscitation over a 6 hour time period for adults with suspected bacterial meningitis feasible and safe in a central teaching hospital in Malawi?

2. Can early goal directed therapy impact on mortality and neurological morbidity from adult bacterial meningitis in Malawi?

2.1.2 Broad main objective

Assessment of the efficacy of early goal directed therapy to deliver target directed resuscitation in a resource limited setting for adults with suspected bacterial meningitis using a clinical care bundle.

Specific objectives

1. To assess the feasibility of achieving each clinical target by using the clinical bundle, and perform a composite assessment of all targets achieved by the care bundle.

2. To assess the relative change in mortality due to ABM in adults receiving the bundle compared to standard hospital care

3. To estimate if the bundle has an impact on the incidence of acute seizures and neurological disability in survivors of ABM

3.3.4 Study participants, inclusion and exclusion criteria

This study recruited adults (defined in Malawi as aged 14 or over, the age at which admission to adult medicine is permitted) presenting to the AETC with clinically suspected bacterial meningitis. All adults presenting to the AETC were screened by a study nurse and patients meeting the screening inclusion criteria for initial entry into the study were enrolled, subject to verbal assent. These screening inclusion criteria were as follows:

Inclusion criteria at screening

- Adults ≥14 years age

AND

- Fever/history of fever plus at least one or more of the following presenting features:

1. Coma/altered mental status
2. Severe Headache
3. Nuchal rigidity
4. Seizures
5. Confusion

These screening inclusion criteria were chosen based on clinical inclusion criteria for previous meningitis trials at QECH, and on the high sensitivity of these clinical presenting

features for the diagnosis of ABM (Brouwer et al., 2012; Thomas et al., 2002). The diagnosis of suspected ABM from these screening inclusion criteria led the study team to perform a lumbar puncture and obtain CSF samples for a definitive diagnosis.

A formal inclusion or exclusion decision was made following CSF analysis.

CSF inclusion criteria:

Cases of proven and probable bacterial meningitis were included in the main study analysis.

Proven case:

- Presence of a positive Gram's stain or culture or PCR positivity for any bacteria known to cause meningitis in the CSF irrespective of cell count

Probable case:

- CSF WCC >50 cells/mm^3 (predominantly neutrophils), PCR and culture negative with an acute history and biochemical evidence of meningitis (raised CSF lactate, CSF: Blood glucose ratio of <0.4, raised CSF protein >0.5g/L).
- Clumped CSF WCC with >50% neutrophils (if available) with negative PCR/ Culture and corresponding biochemical evidence of CSF inflammation (see above) and an acute clinical history
- Positive Gram's stain or culture/PCR positivity for any bacteria known to cause meningitis in the blood with corresponding evidence of inflammation (elevated WCC, raised lactate, CSF: Blood glucose ratio of <0.4, raised CSF protein >0.5g/L) in the CSF.
- CSF WCC >50 cells/mm^3 (predominantly lymphocytes only where pre-hospital antibiotic use is demonstrated), PCR and culture negative with an acute history and biochemical evidence of meningitis (raised lactate, CSF : Blood glucose ratio of <0.4, raised CSF protein >0.5).

These thresholds were chosen for the following reasons. It is clear that HIV infection in adults from sub-Saharan Africa may alter the CSF WCC response in bacterial meningitis (Jarvis et al., 2010). A classical cut-off for CSF WCC in ABM is 100 cells/mm^3(Brouwer et al., 2012), but from anecdotal data from excluded patients collected by the SAM and GLAM trials whose CSF WCC cut of was 100 cells/mm^3 suggested that many ABM patients with HIV infection may have had CSF WCC that were considerably lower than this. For this study the WCC cut off was adjusted to 50 cells/mm^3. Where clumped cells were reported and no formal count was given, it was assumed that the cell count was over the 50 cells/mm^3 cut off, and these cases were included as probable cases. CSF WCC clumping has been reported with diagnostic accuracy of 92-98% sensitivity for diagnosis of bacterial meningitis in children compared to other causes of meningeal inflammation (Michelow et al., 2000).

It is well recognised that ABM in adults causes a very high CSF protein and low CSF glucose, data from HIV infected adults with ABM are lacking. The cut off of >0.5g/L for protein and CSF:Blood ratio of <0.4 were chosen based on literature from better resourced settings (Tamune et al., 2013; van de Beek et al., 2004; White et al., 2012).

In addition to the proven and probable categories, a further category of possible cases of bacterial meningitis was created. These included all participants with non-inflammatory CSF < 50 WBC/mm^3 CSF and culture/gram/PCR negative, in whom the other inclusion criteria were fulfilled including: raised lactate (>4mmol/L), CSF: Blood glucose ratio of <0.4, raised CSF protein, with an acute history and high clinical suspicion of ABM with no evidence of fungal meningitis. In these patients a possibility remained that these patients may have had ABM caused by a pathogen not tested for by PCR such as non-typhoidal *Salmonellae* or *E. coli*. These cases were excluded from the main study analysis due to a probability of acute TB meningitis as a causative agent as specific TB culture was not available, but clinical data were retained and these cases were subject to a separate mortality analysis as 'possible cases'.

Exclusion criteria

Study patients were excluded at two time points in the study, either at screening or on analysis of the CSF after 48 hours. Patients were excluded at screening if they had at least one of the following criteria:

1. Pre-admission diagnosed terminal illness (e.g. metastatic malignancy)
2. Age < 14 years
3. Severe head trauma or recent head injury
4. Other clear source of illness such as intra-abdominal abscess

Study patients were subsequently excluded after review of the CSF results if they met the following criteria:

1. CSF positivity on microscopy, culture or antigen testing for an organism known to cause chronic meningitis (fungal/tuberculosis/parasitic meningitis). In cases where CSF cryptococcal antigen (CrAg) testing was positive, patients were retained in the study until the fungal and bacterial culture results were known. Cases in which the CSF CrAg was positive and there was proven evidence of bacterial meningitis in the CSF were included in the main study. All other cases where the CSF CrAg was positive were excluded as cryptococcal meningitis.
2. Cases in which the CSF was culture/microscopy/PCR negative, but the CSF WCC was greater than 50 cells, with >80% lymphocytes and no pre-admission antibiotics were given were excluded.
3. Subjects with non-inflammatory CSF < 50 WBCs and culture/gram/PCR negative were excluded from the main ABM analysis and an alternative diagnosis assigned after clinical review.

3.3.5 **Study site**

This study was conducted in the new Adult Emergency and Trauma Centre (AETC) at Queen Elizabeth central hospital (QECH). The AETC was designed to undertake assessment of all emergencies in adults, and act as an admission centre for adult medicine and surgery to replace the old admission systems for both specialities. Room 6 and ward 4B were closed in November 2011 when AETC opened, two months before recruitment started to this study. Therefore, the AETC management, clinical and nursing teams were all new to the AETC environment and training in procedures such as triage and clinical assessment were on-going as study recruitment started.

The first phase of this study, where only observation of clinical activity was undertaken, was necessary to enable a comparison between care provided in the AETC in phase 1 and any efficacy of the care bundle in phase 2. Comparison between historical Room 6- 4B data and the care bundle would not have been applicable or relevant as the AETC was predicted to provide substantially improved care by providing direct access to good quality facilities and trained staff and reducing clinical delays compared to the old system. The Ministry of Health of Malawi is auditing outcomes under both systems, but no data are publically available.

3.3.6 **Study design and sample size**

This study was designed as a before/after study in line with the recommendations by the Medical Research Council on trial design for complex interventions on a single site (Campbell et al., 2000, Council, 2008). The study was divided into two consecutive phases, the ‘before’ group named phase 1 (observational controls) and the ‘after’ group, phase 2 (implementation of bundle).

3.4.5.1 Admission and recruitment process

All adults admitted to AETC in both phases with suspected ABM were screened and included using identical inclusion criteria to ensure the participants in both phases were

matched. To minimise confounding, active recruitment, prospective data collection and follow up of all study subjects were identical in both phases.

3.4.5.2 Out of hours recruitment

One main difference in recruitment between the two phases was present. Funding for an additional study nurse became available at the start of Phase 2b, facilitating 24 hour recruitment between Sunday 5pm and Saturday 8am. Phase 1 recruitment was between Monday to Friday 8am -5pm. Patients admitted out of hours in Phase 1 were recruited from the wards following notification from the admitting medical team and the laboratory, acute deaths out of hours on the wards and in the AETC in patients meeting the CSF inclusion criteria were noted.

3.4.5.3 Recruitment start and end for each phase

The study timing was planned to ensure data collection was running at peak capacity during the cold dry season when it has been noted that more meningitis cases may present to the hospital (Gordon et al., 2000). Patient recruitment to phase 2b started at the same time point as phase 1, but one year later to minimise the effect of seasonal variation in the presentation of ABM. Phase 1 ran between the 2nd of January 2012 and data collection ended on the 31st of October 2012. Phase 2a ran between November and December 2012, and Phase 2b ran between the 2nd of January 2013 and the 31st of October 2013.

3.4.5.4 **Division of phase 2 into pilot and active phases**

Phase 2 was divided into 2a and 2b. 2a was a short pilot phase that involved training the study team in the care bundle delivery, where the team were introduced to each bundle element sequentially. They underwent bedside assessments of their skill in delivering each care bundle element. Phase 2a was followed immediately by phase 2b, the active bundle. This element of the study design was chosen due to the complexity of the care bundle to

ensure that all study team members were familiar with each target and intervention before full active data collection started in Phase 2b.

3.4.5.5 Sample size considerations

A formal sample size calculation was not performed for this study. No formal studies on care bundles for meningitis in adults or children have been done prior to this study from which to estimate a potential effect size. Care bundle studies on adults with sepsis in high resource settings, which have been used as a model for the design of this study design do not provide directly translatable estimates of effect size that could be used for this study. A pragmatic plan to recruit equal numbers to each phase with a target of 100 patients per phase was therefore made instead. It was decided from the outset that this study would act as a feasibility and safety pilot study to assess the care bundle, and it would be under-powered to detect any significant difference in mortality between the two phases.

3.4.5.6 Drug prescription and medical care in the AETC

Medical care in phase 1 was entirely prescribed by the AETC and medical clinical teams, and delivered by the BAM study nurses. In Phase 2, the BAM study clinician and nursing team instituted the care bundle protocol, and gave any additional treatments prescribed by the AETC or admitting medical clinical teams, such as oral fluconazole for suspected cryptococcal meningitis.

3.4.5.7 Follow up

The study team performed daily follow up of all screened patients on the medical wards from Monday-Friday until a decision for inclusion or exclusion on CSF grounds was made. Once patients were excluded, follow up ceased. Patients and guardians whose CSF results met the inclusion criteria were invited to remain in the study, and those who agreed gave written informed consent. Included patient follow up on the ward was daily for 10 days or until discharge if that was earlier. The patients were then followed weekly by phone call or text

message for 6 weeks and invited to a follow up appointment at 6 weeks. If they were unable to attend or unreachable by phone, community based follow up was done. This was only achieved in the second phase of the study after it became apparent that a significant number of patients were lost using telephone based follow up only in phase 1.

It was made clear to the inpatient team that the study team was not medically responsible for the study patient once they were admitted to the ward. At the day 40 outpatient appointment, further medical referrals were made by the study team as necessary, such as to antiretroviral clinic, if these had not been done at discharge by the medical team.

3.4.5.8 Trial Registration

This study was registered online with two clinical trials registries. The Pan-African Clinical Trials Registry (PACTR) number 201111000338157, and the International Standard Randomised Controlled Trials Network (ISRCTN) 96218197. The details of the study were published on both registries websites, and kept updated as the study progressed.

3.3.7 Care bundle intervention

The clinical care bundle intervention tested in this study was designed using two concomitant approaches. Firstly, all published data and guidelines on clinical care bundles in the literature for infectious diseases, critical care and emergency medicine, plus general emergency resuscitation guidelines for acute medical and surgical emergencies were reviewed. The details of these searches can be found in Chapter 2 Section 2.1. The data from the analysis of patients with meningitis in Malawi to determine which predictors of poor outcome would be amenable to treatment with a care bundle was additionally reviewed (Results Chapter 4 Section 4.5.3-4). The data from this analysis was combined with the published data and guidelines to create a simple clinical care bundle that would meet the resuscitation needs of the screened patients within the resource constraints of the clinical environment in Malawi. The care bundle was additionally designed to be a nurse-led

intervention that enables a nurse or clinical officer following a detailed protocol to identify physiological abnormalities from baseline observations and use the protocol to correct these safely over a 6 hour time period. The protocol gives clear clinical targets that should be achieved by 6 hours where possible.

At the end of the 6 hour time period, every effort should have been made to optimise the clinical care given with the bundle to normalise physiological abnormalities and stabilise the patient prior to ward transfer.

3.3.7.1 **Published guidelines on the approach to critically ill medical patients**

The clinical care bundle of early goal directed therapy tested in this study was designed primarily based on the 'ABCD' approach to resuscitation which has been developed and validated by the American and UK resuscitation councils (Council, 2010). These guidelines indicate the priority order of emergency life-saving interventions that should be given, according to clinical need to patients presenting with acute illness. The most urgent is considered the airway "A", followed by breathing "B" where oxygenation must be optimised, and are followed by "C" circulation and "D" disability. The ABCD system however is designed primarily for patients in cardiac arrest and not presenting with a severe medical condition such as bacterial meningitis. As discussed in chapter 2, the components of the care bundle advocated by the Surviving Sepsis campaign were revised, and those which were applicable to the needs of the Malawian patient with ABM were determined, based on the clinical predictors of poor outcome from the previous analysis.

3.3.7.2 **Clinical predictors of poor outcome from bacterial meningitis in Malawi**

The results of the analysis detailed in section 1.5 of this chapter were reviewed. The detailed results are presented in Chapter 4 Section 4.5, however coma, altered mental status, hypoxia, seizures and anaemia were all independently associated with poor outcome. For

each individual predictor of poor outcome, the care bundles from the ABCD and the surviving sepsis guidelines were reviewed, along with the literature from appropriate parallel specialities such as neurosurgery and adapted for a resource limited environment. The literature supporting each care bundle element is discussed in detail in Chapter 2, Section 2.8. The care bundle tested was designed to optimise clinical care for each predictor with the aim to restore normal physiology within a 6 hour observation period.

3.3.7.3 **Final care bundle**

The care bundle consisted of eight components, all of which were clinically available in the AETC and all of which are recognised existing clinical treatments which have supportive evidence of efficacy. The individual components of the bundle can be seen in Table 3.2 below, the ABCD order as determined by the Resuscitation Council guidelines.

A

Airway support is deemed essential by all guidelines for adults with a GCS of less than 8/15 at which point airway reflexes are minimal. Altered mental state is associated with raised intracranial pressure in meningitis, and the head tilt is designed to reduce ICP while optimising oxygenation and minimising aspiration risk, as shown in the neurosurgical literature.

B

Anaemia and hypoxaemia were identified as predictors of poor outcome; these were addressed in the care bundle by the application of oxygen via a concentrator for hypoxic patients, and the rapid transfusion of blood where available to those who were determined to be profoundly anaemic on a Hemocue test.

C

Tachycardia was shown to be independently associated with mortality; very few patients were hypotensive in the severity analysis. However hypotension leads to under-perfusion of cerebral tissues and therefore fluid resuscitation was given to patients with a clinical

definition of shock as determined by the surviving sepsis campaign (Table 3.4) to optimise cerebral perfusion.

D

Seizures at presentation and prior to admission to hospital were associated with poor outcome in the severity analysis, and are associated with an altered mental state. Therefore acute treatment of seizures and the underlying causes of seizures such as hypoxia and hypoglycaemia were addressed in the care bundle using intravenous glucose and facial oxygen where indicated.

In addition, early antibiotic therapy is associated with improved outcomes from meningitis elsewhere in the world, and in Malawi, trends towards higher mortality from meningitis when antibiotic treatment is delayed were observed in a small number of patients in the pilot cohort. We attempted to give the first dose of ceftriaxone within one hour of arrival in AETC in this care bundle.

Table 3.2 Clinical components of the care bundle

Bundle components
1. Naso-pharyngeal airway if GCS <8 2. Head up/bed tilt 30° if GCS <11 3. IV access, 2g ceftriaxone stat 4. Oxygen via concentrator if SpO2 <93% 5. IV fluid (Ringer's Lactate) bolus 20ml/kg if clinical shock, 125ml/hr if no clinical shock (Table 3.4) 6. Transfusion of packed red cells if haemoglobin <6.0g/dL 7. Correction of hypoglycaemia with oral dextrose and drinks/food if GCS >11, or with iv dextrose 10-50% via a bolus/infusion if GCS <11 8. Prompt treatment seizures *(IV diazepam/lorazepam plus phenobarbitone)*

This was due to difficulties in assessing staff training when no formalised resuscitation training was available that is appropriate to resource limited settings. During phase 2a, 6 weeks of time were devoted to care bundle training. The senior study nurse led the training of the other nurses at the bedside and in a series of practical seminars. Each of 6 weeks were devoted to one particular bundle element, and staff debriefed after recruiting a patient and following that protocol to discuss how to optimise the delivery of each care bundle element. During this time active study patient recruitment continued with a target of one patient per day. The study team recruited that patient as a team and delivered the care bundle intervention as dictated by the study protocol. The PI was involved in the initial staff training and in the daily de-brief meetings where what had been done well and which targets had not been met were discussed. The team then decided each day how to take the training forward to the next day. By the end of the 6 week period the team were entirely comfortable to deliver the care bundle to an individual patient without the support of their peers, but with 24 access to the PI by telephone.

3.3.7.4 **Care bundle delivery and timing**

The intervention care bundle was delivered using defined targets as detailed in Table 3.3 within the AETC. The duration of the intervention was 6 hours from initial screening, by which point all attempts to meet all targets using the protocols had been undertaken. Each subject received hourly observations including Glasgow Coma Score (GCS), blood pressure and pulse measurements. A lumbar puncture for the diagnosis of ABM was performed at the earliest opportunity. After each hourly observation, the study nurse reviewed the care bundle progress and added or stopped elements as per protocol. For example if the GCS dropped below 11 at hour 4, a head tilt was given.

Table 3.3 Targets for EGDT in BAM

Clinical targets for the care bundle intervention

Parameter	Target	Intervention	Test of intervention
Timing of clinical assessment	Medical review <1 hour of arrival	Training in recognition of the symptoms and signs of meningitis and rapid triage	Timed and signed flow chart
Antibiotic therapy	1st dose within 1 hour of arrival	Education of importance of antibiotics, sepsis flow chart	Timed drug chart
Glucose	BM >4	S/L , NG or IV dextrose	Repeat BM
Oxygenation	Sp02 >94%	Nasal flow 02 from concentrator Packed red cell Transfusion if significant anaemia (Hb 6.0g/dL)	Regular Sp02 monitoring
Perfusion	CRT<2 sec, BP >90 syst, MAP >70, no postural hypotension (lying-sitting)/ UOP>0.5ml/kg (if catheterised	IV fluid bolus 20ml/kg Ringer's lactate where available.	Repeated fluid balance assessment at 1hour then repeated
Seizures	No seizures	Acute prompt treatment of seizures with IV/PR benzodiazepines and IV phenytoin if seizures persist	Seizure chart

At the end of the 6 hour study period, the bundle was stopped, and the subject was transferred to the medical ward for ongoing routine care as deemed appropriate by the admitting clinician. A flowchart of the study process is available in the appendix. Patients were discharged to the most appropriate clinical care area in both phases of the study. If invasive ventilation or inotropic support was required, they were transferred to intensive care. Patients requiring on-going oxygen or control of persistent seizures they were transferred to the high dependency unit on the medical ward. All other patients were transferred to the main male or female medical wards.

Clinical shock was defined by abnormalities in the following general variables defined in Table 3.4. If shock was present then the fluid bolus protocol was instituted as per protocol (appendix 1.3). All protocols are in the appendix .

Table 3.4 Definitions of shock from the surviving sepsis guidelines 2008

Organ variables for the definition of clinical shock (Dellinger et al., 2008b)

General variables	Hemodynamic	Organ dysfunction	Tissue perfusion
Heart rate >100 bpm	Arterial hypotension (SBP <90 mm Hg	Acute oliguria*	Decreased capillary refill (>3 seconds)
Tachypnoea RR>25	MAP <70 mm Hg	Ileus (absent bowel sounds)	Skin mottling
Altered mental status	SBP decrease >40 mm Hg	Blood lactate >4 mmol/L	

*(urine output <0.5 mL/Kg/hr **or** <45 ml/hr for at least 2 hrs (if catheterised)/ no urine production for 12 hours despite adequate fluid resuscitation)

3.3.7.5 **Staff training**

Staff training formed an important component of bundle delivery during Phase 2a and was on-going through Phase 2b, but did not form a specific bundle element. This was due to difficulties in assessing and including the effects of staff training in the data analysis.

3.3.8 **Laboratory investigations including sample storage**

All patients underwent the following investigations. All research samples were taken from surplus blood and CSF at the same draw as for clinical testing, with the exception of day 10 and day 40 blood taken for microarray studies (Table 3.5).

Lactate was measured at the bedside in both CSF and blood using a point of care test (Lactate Pro, Habdirect). Point of care (POC) lactate tests are comparable to formal laboratory measured lactate (Karon et al., 2007).Glucose was measured using a POC bedside glucometer (Accu-chek, Aviva, USA). Haemoglobin, Creatinine, Sodium were measured in the QECH hospital laboratory. HIV tests were performed either by the hospital laboratory or on the hospital wards as part of the Government funded Provider Initiated Testing (PITC) and Counselling service for HIV.

Table 3.5 List of samples taken from BAM patients with informed consent

Research and diagnostic samples taken	
Tissue type	**Justification**
Cerebrospinal fluid (CSF)	
Microbiology (microscopy, protein, glucose, bacterial/fugal/TB culture)	Clinical diagnostics (Heyderman, 2005)
Real-Time PCR	Clinical diagnostics and potential marker of poor outcome (Carrol et al., 2007a)
RNA expression storage	CSF host RNA expression patterns in ABM

Blood	**Justification**
Lactate	Marker of septicaemia (Jones et al., 2007a)
Glucose	Risk factor for disease severity and seizures (Schut et al., 2009b)
Haemoglobin	Marker of severity and to determine transfusion requirements
Sodium (1ml)	Risk factor for seizures and disease severity (Brouwer et al., 2007)
Creatinine (1ml)	To determine if acute renal failure is present (Dellinger et al., 2008b)
Full Blood Count (2ml)	Clinical diagnostics
HIV antibodies when consent given (1ml)	Clinical diagnostics
Malaria thick film (1ml)	Clinical diagnostics.
Blood culture (10ml)	Clinical diagnostics.
Real-Time PCR (1ml)	Clinical diagnostics and potential marker of poor outcome.
Storage in RNA preservation tubes (2-3ml)	Additional blood samples were requested from included participants at day 10 discharge and day 40 follow up for RNA expression studies of bacterial meningitis.
Nasal swab	Culture for *S. pneumoniae* and genotypic exploration of micro-evolution of *S. pneumoniae*

3.3.9 **Endpoints and follow up**

The following endpoints were used for this study.

Primary

1. Measurement of the proportion of each clinical target achieved by each care bundle element at 6 hours
2. Total proportion of all clinical targets combined together, achieved by the bundle at 6 hours.

Secondary (measured at day 10 and day 40)

1. Death
2. Persistent seizures requiring treatment
3. Significant neurological disability
4. Functional ability measured using the modified Rankin Score (mRS)
5. Efficacy of the care bundle on sepsis outcomes of death at 48 hours, and tissue perfusion at 6 hours

Death was measured as a composite secondary endpoint (days to death/last known alive) using the following methods:

Day 10 (acute) survival measured by either a date of discharge (alive) or date of death recorded up to 10 days of inpatient antibiotic therapy.

Day 40 (convalescent) - Final outcome recorded 'alive or dead' from follow up or response to mobile telephone to 6 weeks.

A history of seizures and functional ability since discharge was taken at day 40, and endpoints 2-4 were measured formally using a clinical history, and the mRS. This score is well validated and determines outcome based on the following parameters (Table 3.6).

Table 3.6 Modified Rankin Scoring system

mRS (Rankin, 1957; Bonita and Beaglehole, 1988)	
Activity	**Score**
Normal, able to work all activities of daily living	0
No significant disability despite symptoms of illness; able to carry out all usual duties and activities	1
Slight disability; unable to carry out all activities, but able to look after own affairs without assistance	2
Moderate disability; requiring some help, but able to walk without assistance	3
Moderately severe disability; unable to walk without assistance or unable to attend to own bodily needs without assistance	4
Severe disability; bedridden, incontinent and requiring constant nursing care and attention	5

3.3.10 **Verbal assent and informed consent**

A two stage, delayed formal informed consent to enable the rapid onset of the bundle delivery was used. The consent process was GCP compliant, and consisted of two steps: verbal assent by the participant or their guardian on admission and recruitment, and written informed consent from the participant or guardian when the subject was stabilised on the ward some days later. This was planned for two main reasons. Firstly, the process of verbal assent to gain basic agreement for participation was designed to facilitate rapid intervention. Verbal assent, followed by formal informed consent for on-going participation at a later date is a standardised practice in trials of emergency interventions, is recommended by the FDA and has been used in sub-Saharan Africa (Maitland et al., 2011b; consent, 1996). Secondly, potential study participants were commonly critically unwell on arrival at the hospital, with impaired consciousness, and did not fulfil the requirements for standard GCP informed

consent. This has been a problem for all studies that investigate critical illness testing rapid delivery of the intervention to a severely unwell participant.

a) Verbal assent

When the study participant was identified as a potential study subject at triage in AETC, the recruiting study nurse gave verbal details of the study, and requested verbal consent from the patient or accompanying guardian for inclusion 'verbal assent'. If the answer was affirmative, the subject was recruited and monitoring/bundle was started immediately. An assent form was signed at this point by the study nurse and the patient or carer.

b) Deferred verbal assent

A small number of participants presented who were personally unable to give verbal assent to enter the study and did not have an attending guardian who was able to give verbal assent. In phase 1, those patients were found on the ward the subsequent day and informed consent was taken from the patient or guardian. If those patients had died, minimal mortality information was retained from the notes in the CRF to avoid selection bias. In Phase 2, the process was changed (with formal permission from COMREC) to ensure high quality clinical care was given rapidly to patients presenting without a guardian. Participants were enrolled and received the care bundle within the time targets without any assent or consent process. Delayed verbal assent was then taken from an attending guardian at the earliest opportunity. At any point in both phases if deferred verbal assent or informed consent was denied we immediately de-recruited the participants and deleted all study data for that person.

c) Delayed formal informed consent

Once the subject was stabilised and able to talk formal written informed consent was requested for subjects meeting the CSF inclusion criteria. If a patient took time to become stabilised or was unable neurologically to give written consent, this was requested from the accompanying guardian. Excluded subjects were formally told they did not have bacterial

meningitis and were excluded from the study without going through a consent process. All retained samples were discarded for excluded subjects, unless informed consent was sought for their use as control samples. If no guardian was present to give informed consent when patient was comatose, the subject was included on the basis of best interests, until a time when a carer was located to request consent.

d) Death before informed consent

Due to the severe and acute nature of bacterial meningitis, a number of participants died after giving verbal assent but before informed consent could be obtained. Attempts were made to contact the guardians of the dead study participant to request informed consent. Where this was not possible, the verbal assent form was deemed adequate consent for data retention by COMREC.

e) Informed consent for research samples

No samples of tissue were analysed, excepting those necessary for acute clinical management, until written informed consent was given. If consent was denied, all records of that subject were deleted from the main trial database and samples discarded.

3.3.11 **Adverse Events**

a) Data monitoring

A formal data monitoring committee was not considered to be required for this study, as the interventions being tested had individually been proven to be effective, and there was no blinded/randomised intervention. However, Professor Tim Peto (Professor of Medicine, University of Oxford) and Professor Johnstone Kumwenda (Dean of clinical medicine, College of Medicine) acted as an independent study monitors to review cumulative monthly aggregated reports on all outcomes and SAEs. In addition the study statistician, Dr Brian Faragher also reviewed the monthly outcome report and provided comments and feedback.

b) Process of detecting and reporting SAEs

Any suspected or unexpected serious adverse events (SAE/SUE) were discussed immediately with the PI, study nurses and clinical officer and a record form was completed. A serious adverse event was classified as an unexpected negative outcome, harm to a trial participant, or an occurrence that results in a complaint to the trial team from a participant or their carer, or from within the AETC team. The standard definitions of SAE and SUEs can be found at http://www.mhra.gov.uk/Howweregulate/Medicines/Licensingofmedicines/Clinicaltrials/Safetyreporting-SUSARsandASRs/

These standard definitions were adapted for this study, as patients with suspected meningitis in Malawi are already critically unwell and at high risk of death or adverse outcome. Therefore COMREC deemed it unnecessary to report all adverse neurological outcomes formally, but all deaths and potential harms from the study intervention were reported.

An SAE was required in phase 2 the event of any of the following during the care bundle delivery:

- Death
- Drop in GCS of >2 points
- New oxygen requirement
- Presence of pulmonary or suspected cerebral oedema
- New seizure
- Respiratory depression
- Any other concern from a study team member that harm had come to the trial participant

In the follow up phase all deaths were recorded as SAEs in the acute phase in-hospital. Out of hospital deaths were not regularly reported as SAEs.

All SAEs and SUEs were discussed by the principal investigator with the chief investigators and details given to the trial monitor with aggregated mortality data. COMREC were given

summary details of mortality at the end of each phase. The study protocol dictated that if the CI, IRB COMREC or the study monitor considered it necessary, recruitment would be paused, and a meeting of the trial steering committee (TSC) convened to discuss the SAE. Several SAEs relating to deaths during the care bundle period in phase 2b were sent to the study monitors, but no meeting of the TSC was deemed necessary during the conduct of the trial.

3.3.12 **Data analysis and statistics**

The study analysis was designed to interrogate the data primarily by intention to treat, and then per-protocol.

a) Data management

Data was collected on a paper case record form (CRF) and transferred by the study team to an electronic data capture system 'REDCap' (source). In addition PDAs were planned to be used for ward based data capture with linkage to the main study computer.
Originally the study was designed to have pure electronic data capture. However during the start of Phase 1, dual paper and electronic data capture to test the system were run. The team noticed that data that had been entered from the CFR into the electronic system was subsequently missing. The cause of the missing data was extensively investigated, including screening for viruses and cross checking the PDAs, no apparent cause was found, but the problem continued. Following re-training of the team in the use of RedCap the MLW Data manager discovered that the team were not saving entered data in the proper manner as originally outlined in the staff training and this was likely the cause of the missing data. The decision was taken to continue with dual paper and electronic data capture to ensure a robust backup system was in place and to abandon use of the PDAs. The study team for the

entire study entered the data onto paper CRFs and then transferred this to the electronic system.

The MLW data team regularly cross checked the electronic data against the paper CRF for transcription errors and accuracy, after which the data were exported from REDCap to IBM SPSS/PASW version 20 and GraphPad5™ software for analysis. The data analysis plan was lodged with the COMREC before commencement of the study .

b) Statistical methods

The following statistical tests were used to compare the intervention (Phase 2) and control groups (Phase 1).

- Composite analysis of each proportion of target achieved
- Simple mortality proportions
- Kaplan-Meir survival analyses to establish the pattern of the death rate
- Chi squared tests and Fisher's exact test to analyse the significance of differences between the two phases fixed endpoints.
- Logistic regression was used to give an estimate of the size of the change in outcomes and to control for confounding.
- Direct comparison of meningitis severity scores calculated for each phase, developed in Chapter 5 to assess the degree of illness severity in each patient on admission in each phase. These scores will be compared using parametric tests and logistic regression.
  All statistical tests were two tailed and a significance value of <0.05 determined statistical significance. 95% confidence intervals are presented for Odds Ratios.

c) Sub-analyses were undertaken of the following groups

1. Patients presenting where death was predicted by the admitting clinician or occurred within the bundle implementation time period (6-8 hrs).
2. Subjects with normal CSF and an alternative diagnosis such as sepsis by outcome at 48 hours.
3. Subjects with abnormal CSF but no formal evidence of proven or probable bacterial meningitis on CSF analysis (e.g. 'possible cases of ABM', CCM and TBM patients).

Patients who presented with bacterial meningitis in-extremis or with symptoms or signs suggesting death within 4-6 hours were included in the intention to treat analysis, but were subjected to a separate sub analysis and excluded from the per-protocol analysis.
All data were analysed using IBM SPSS version 20 (IBM, USA), figures were generated using this software with the addition of GraphPad PRISM (GraphPad USA) version 5 and version 6 software.

4. Bacterial meningitis in Malawi 2000-2012: Laboratory surveillance and Clinical predictors of mortality in adults and adolescents.

4.1 Introduction

This chapter summarises work done in support of the BAM trial, exploring the significant data collected in MLW over the last 12 years, both through the microbiological surveillance programme and by analysis of data from important clinical studies undertaken during this time.

Section 1 details the changing epidemiology of ABM in both adults and children over twelve years through an analysis of data from microbiological surveillance. This is followed by Section 2, which is an analysis of the clinical data collected over the same time period, testing for possible causes of the very high mortality seen from ABM in Malawian adults and adolescents. By putting both the surveillance and clinical data in context, the results presented in this chapter provide important background information for the design and analysis of the BAM trial, presented in chapter 6.

4.2 Bacterial meningitis surveillance in Malawi

There are little surveillance data for bacterial meningitis from resource poor regions, particularly SAA and estimates of incidence are often based on poor quality data (Peltola, 2001). MLW was been conducting routine surveillance for invasive bacterial pathogens in the blood and CSF of adults and children presenting to QECH for 15 years (Everett et al., 2011; Gordon et al., 2008; Gordon et al., 2001; French et al., 2010), over which time numerous public health interventions have been introduced which may or may not impact on bacterial meningitis incidence.

These include roll-out of free antiretroviral therapy (ART) and co-trimoxazole prophylaxis, the introduction of Hib and PCV vaccination, intense malaria control, prevention of mother to child transmission of HIV infection and improvements in childhood nutrition (Roca-Feltrer et al., 2012; Chihana et al., 2012; Daza et al., 2006; Floyd et al., 2012; Jahn et al., 2010a).

Making use of this large surveillance data resource, it was determined if the number of meningitis cases and therefore incidence changed during this time period. In addition seasonality and patterns in the prevalence of different pathogens causing meningitis were examined.

4.2.1 Research questions

1. Has the number of cases of culture proven bacterial meningitis, and therefor incidence changed over 12 years?
2. Is culture proven bacterial meningitis seasonal?

Objectives

1. To examine the laboratory database of CSF culture isolates collected between 2000-2012 by pathogen and age group to estimate case burden and age-specific incidence.

2. To examine different pathogens for monthly seasonality against rainfall and temperature data.

4.2.2 Specific Methods

4.2.2.1 Database synthesis

Laboratory data were collected for each CSF isolate that was culture positive between 2000-2012, recorded in laboratory books to 2010 and via an electronic reporting system from 2010 onwards. The paper-based data were then entered into an Excel spreadsheet manually by the MLW data team, and collated with the electronic data from 2010. Data available from the

paper based system included name, age, sex, ward, date of sample, CSF WCC, gram stain and culture result. From 2007, CSF protein and glucose were also reported. From the Laboratory Information Management System (LIMS) electronic data these data were also available, with the addition of CSF WCC percentages, antibiotic sensitivities and blood film results for malaria parasites.

Isolates that appeared as duplicates were removed. A duplicate was defined as the same organism originating either on the same day or within 48 hours of the original sample from patients matched by name and age or date of birth, or where identical sample numbers had been assigned.

4.2.2.2 Patient groups by age

Cases were divided into groups based on clinically relevant age groups and bacterial isolate from the CSF: neonates (0-3 months of age), expanded programme of immunisation (EPI) eligible children (>3 months <5 years), older children (5-14 years), adolescents (15-19 years) and adults (>19 years). Over the time period studied, EPI consisted of the following vaccinations before the 1st birthday: polio, diphtheria, Hepatitis B, measles and tetanus. Hib vaccination was introduced in 2002 and 13 valent pneumococcal conjugate vaccine (PCV-13) in 2011.

Neonatal data were excluded from the analysis as this age group are epidemiologically distinct to community acquired meningitis (Swann et al., 2014).

Isolates from patients whose age was not recorded accurately were determined to be adults or children by ward of admission. Children whose age was not known were put into a separate category of ‘age unknown’ and included in the overall analysis but excluded from the age-specific analysis.

4.2.2.3 Population and rainfall data.

Data were obtained from the Ministry of Health of Malawi for population estimates and the Malawi meteorological department for two weather stations in Blantyre for temperature and rainfall data for the period studied.

4.2.2.4 **Meningitis surveillance inclusion and exclusion criteria**.

All CSF isolates that were consistent with bacterial meningitis were included (*S. pneumoniae*, *N. meningitidis*, *H. influenzae*, non-typhoidal Salmonellae species, *E. coli*, *S. aureus*, Group A, B streptococci, *Listeria monocytogenes*, *K. pneumoniae* and other gram negative pathogens). Isolates that were likely to be due to skin contamination, such as *Staphylococcus epidermidis*, Micrococcus species or Diptheroid *Spp*. were excluded.
All fungal isolates were excluded, as were all culture negative samples.

4.2.3 Statistical methods

4.2.3.1 Surveillance calculations

The Kruskal-Wallis test was used to detect global significant differences in the distribution of non-normal variables among groups of interest. These variables included CSF laboratory parameters such as white cell count and glucose by pathogen. If the results of the Kruskal – Wallis test were significant, pair-wise comparisons were done using Mann-Whitney test. Poisson regression was used to derive incident rate ratios, reported with 95% confidence intervals. Vaccine efficacy was estimated by comparing the incident rate ratios from data prior and post introduction of a vaccine. A p value <0.05 denoted statistical significance. Statistical and time series analyses were done using Stata version 11.2 (™Statacorp, College Station, Texas). These analyses were done by Dr Mavuto Mukaka (MLW) and Dr Naor Bar-Zeev in consultation with myself and Dr Dean Everett.

4.2.3.2 Incidence rates

Mid-year population estimates were calculated based upon projections from the 1998 (for 2000-2007) and 2008 Population and Housing Censuses for 2008-12 (Malawi, 1998), using a standard estimated resident population (ERP) forecasting algorithm. In Malawi, comprehensive annual data on population numbers, birth and mortality rates and net migration are lacking. A forecasting algorithm predicts population growth from census data each year, modelling on available fertility and death data available for that year. As the population of Malawi has expanded significantly over the time period studied, data from two successive censuses were used in the population estimates. Incidence was defined as number of cases in a defined period divided by population in each year observed. Changes in incidence over time were plotted.

4.2.3.3 Time-Series analysis for seasonality

Time-series decomposition was used to separate long-term trends from seasonal perturbations. This model divides data into separate predicted cyclical patterns (e.g. per-month seasonality, rainfall) and tests for associations of the cyclical data against a long term trend (in this study, meningitis incidence). To determine whether seasonal perturbation differed significantly from baseline, data were trend differenced. The distribution of residual fluctuations for each month was compared to baseline with 2-tailed t-tests. To determine the impact of rainfall and temperature, an autoregressive integrated moving average model with exogenous variables (ARMAX) model was used. This model tests the weighted monthly average of the variable tested (meningitis incidence) against changing rainfall and temperature over a 12 month cycle for each year. Models using specifications of the autoregressive and moving average parameters were compared using the absolute percentage error. These analyses were done by Dr Naor Bar-Zeev (MLW).

4.3 Results of epidemiology of bacterial meningitis

4.3.1 Surveillance trends in culture proven bacterial meningitis

Data on culture positive CSF isolates was available from the laboratory database from 2000 onwards.

4.3.1.1 All meningitis trends

Figure 4.1 shows the total numbers of culture positive cases of bacterial meningitis from 2000-2012 by year. Isolates are sub-divided into adults > 19 years, and children and adolescents <19 years compared to all CSF samples received by the laboratory for analysis (Figure 4.1, right scale).

There has been a significant decline in the total number of CSF isolates between 2000 and 2012, 12 year incident rate ratio 0.93 95% CI 0.92-0.94, p<0.001. This decline has been entirely in children and adolescents (IRR =0.87, 95% CI 0.85-0.88, p<0.001); the number of isolates from adult has remained unchanged (IRR=0.99, 95% CI 0.97-1.0, p=0.135).The number of CSF samples received by the laboratory each year remained consistent after 2002 and hospital admission rates did not change during the study period.

4.3.1.2 Surveillance trends in paediatric bacterial meningitis

Specific trends in the most commonly isolated pathogens in children and adolescents are shown in Figure 4.1. This figure includes all children from birth where the age is known, and a small number of children (<5% total) where the age was not recorded but admission under paediatrics was noted.

Hib has declined twentyfold from 80 cases/year in 2000 to less than 4 cases in 2012. The figure shows that this decline occurred rapidly after Hib vaccination was introduced in Malawi in 2002. Figure 4.1 shows the data for Expanded Programme of Immunisation (EPI) eligible

children (>3m-<5 years old). Following Hib vaccine introduction in 2002, cases declined rapidly in the vaccine eligible age group. The Hib incidence rate ratio (IRR) describes the rate of change in incidence over time. The IRR for Hib =0.73, (95% CI (0.69, 0.76) p<0.001). This figure is highly significant and gives an estimated vaccine effectiveness of 0.91 (95%CI: 0.83 to 0.99). Childhood NTS and pneumococcal meningitis cases have also significantly declined in children in this age group since 2002; the relevant incident rate rations are: NTS IRR=0.85 (95% CI 0.81, 0.89, (p<0.001), *S. pneumoniae* IRR=0.88 (95% CI 0.85, 0.91 p<0.001).

On further analysis of the pathogens causing disease in the EPI eligible children age group, Hib meningitis has dramatically declined from over 35% of all cases prior to 2002 to 3.5% in 2005, remaining between 10-15% (Figure 2a). *S. pneumoniae* accounted for 30-50% of cases 2000-2003, the pneumococcus has consistently accounted for >60% of cases since 2004. Non-typhoidal *S*almonellae (NTS) meningitis dropped from 23% of cases pre-2004, to less than 10% by 2012. Confirmed meningococcal meningitis has remained consistently at <5% (Figure 4.2)

Pathogens listed as ‘other’ have increased in proportion as the other causes have decreased. This group includes alpha and beta hemolytic streptococci, groups a and d streptococci, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Salmonella Enteritidis*, *Staphylococcus aureus* and *Haemophilus influenzae* types a,c and non-typeables.

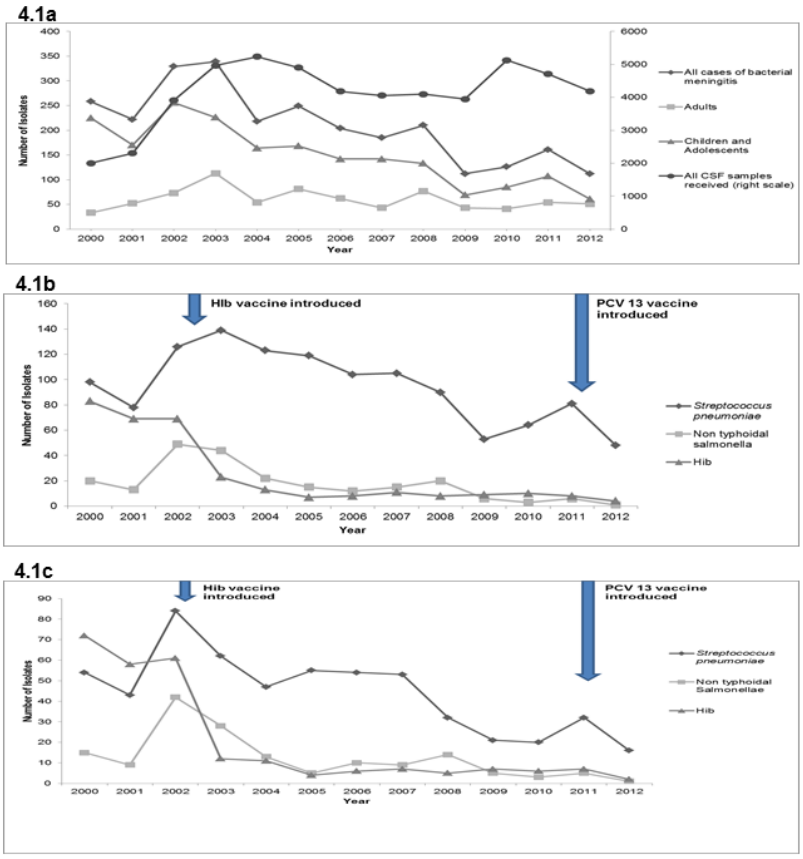


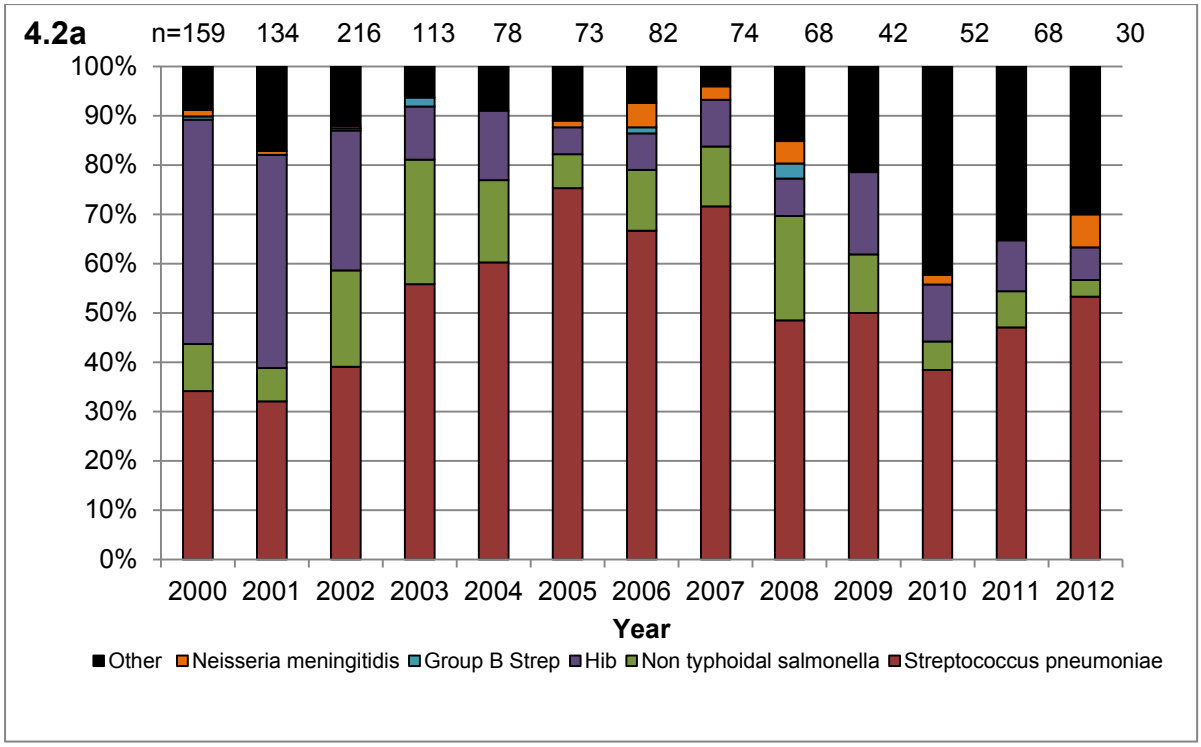
Figure 4.1 Meningitis surveillance data by age group and pathogen 2000-2012

Figure 4.1a. *Total numbers of culture positive cases of bacterial meningitis 2000-2012 by year. Isolates are sub-divided into adults > 19 years, and children and adolescents <19 years. All CSF samples received by the laboratory measured on the right scale.*

Figure 4.1b. *Total numbers of cases of meningitis caused by Hib, Streptococcus pneumoniae and non-Typhoidal Salmonellae in all children and adolescents aged <19 years.*

Figure 4.1c. Total numbers of cases of meningitis caused by Hib, Streptococcus pneumoniae and non-Typhoidal Salmonellae in EPI eligible children aged >3 months to <5years.

Bacterial meningitis in older children and adolescents is predominately caused by *S. pneumoniae*, causing 65% of infections in the 5-15 year group; Hib caused 8.6%, and NTS less than 1% (Figure 4.2). In adolescents the case numbers were few, but *S. pneumoniae* was again the predominant pathogen causing approximately 80% of disease, *N. meningitidis* caused 10% and cases defined as other caused a further 10% of infections (data not shown).



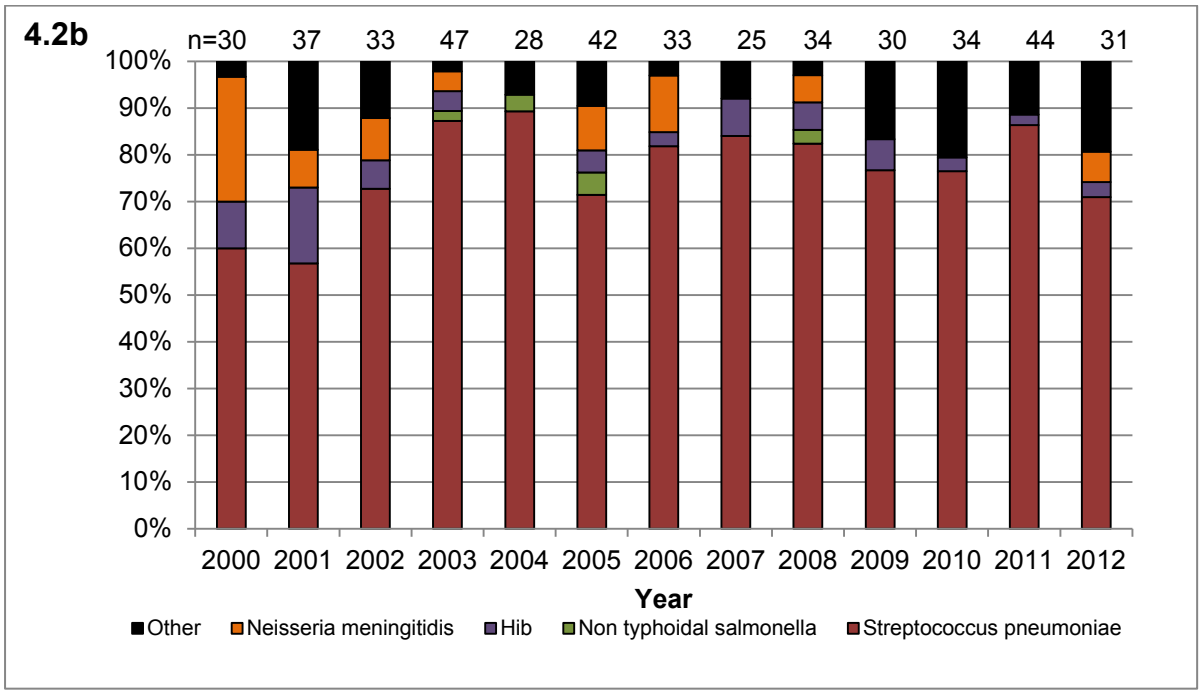


Figure 4.2 Proportions of CSF culture positive isolates by paediatric age groups per year

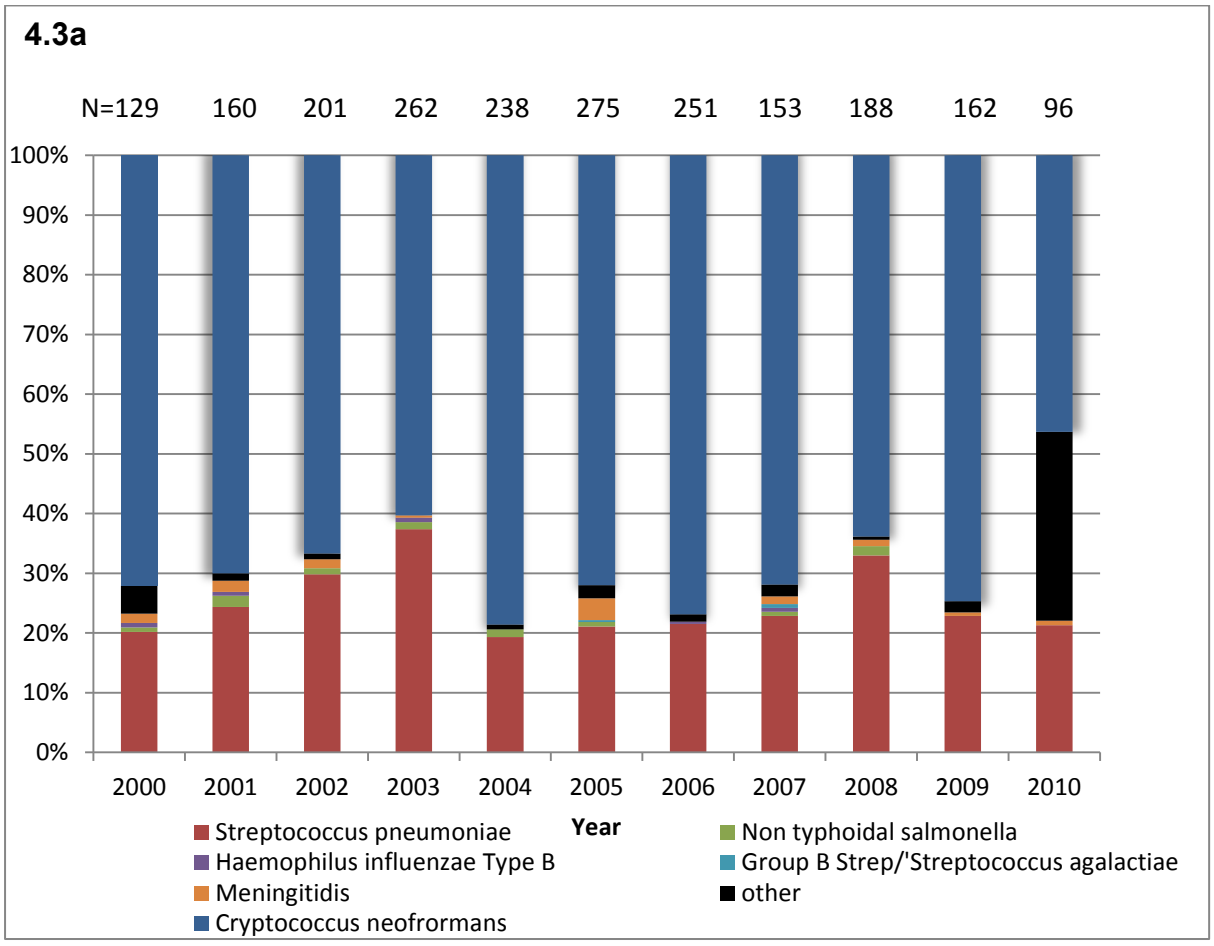
Figure 4.2a: *Proportions of culture positive CSF isolates by pathogen per year in EPI eligible children aged > 3 months to < 5 years*

Figure 4.2b: *Proportions of culture positive CSF isolates by pathogen per year in children aged 5-15 years.*

4.2.1.3 Adult bacterial meningitis and cryptococcal meningitis

Numbers of culture positive CSF isolates in adults over the age of 20 remained unchanged over the time period studied. The most common bacterial isolate in adults was *Streptococcus pneumoniae*, cases of *Neisseria meningitidis* were few (Figure 4.3). Cases defined as other are listed in section 4.2.2.

The most common culture isolate overall was *Cryptococcus neoformans*, accounting for 70% of all culture positive isolates (Figure 4.3). Case numbers of cryptococcal meningitis also did not change over the time period studied, despite the rapid roll out of ART. As cryptococcal meningitis is very different from bacterial meningitis (see Chapter 1 section 1.1), these cases were not included in the overall analysis.



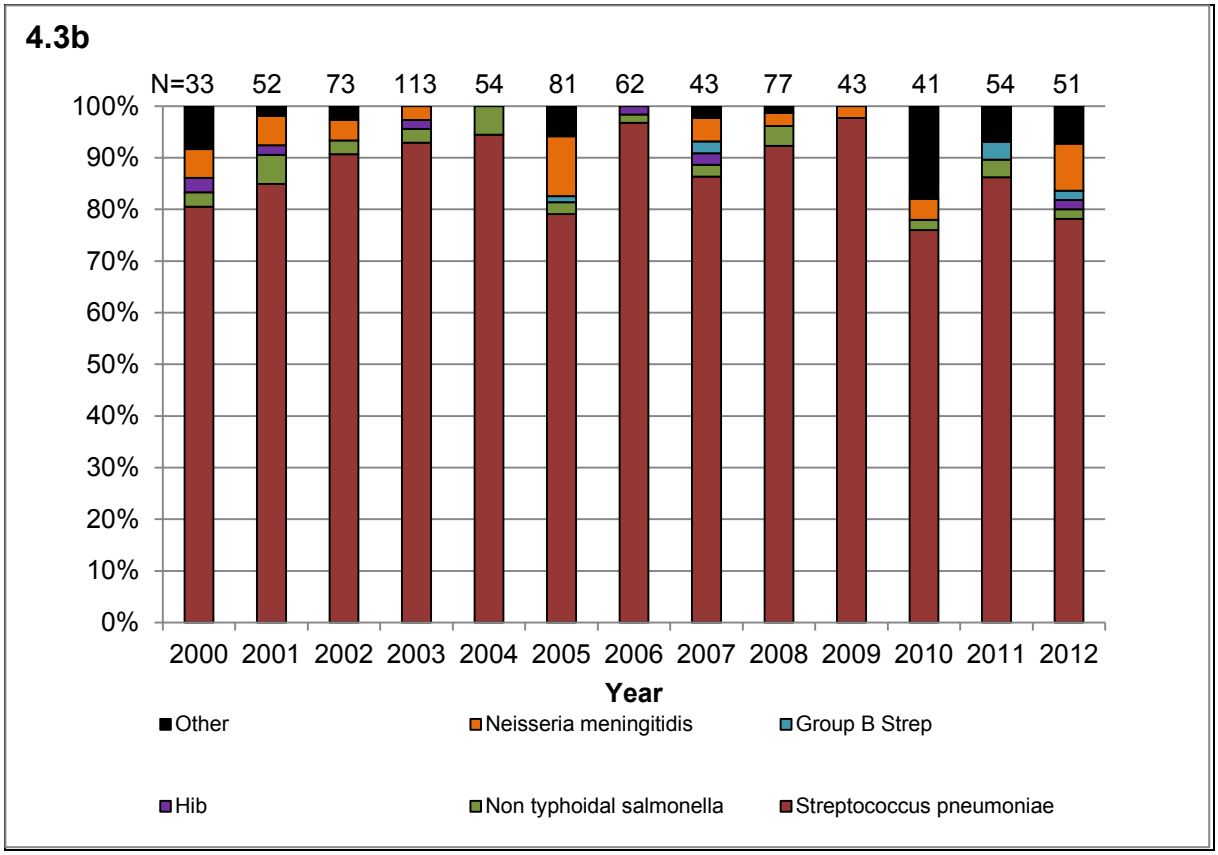


Figure 4.3 Proportions of CSF culture positive isolates in adults per year

Figure 4.3a: *Proportions of overall adult meningitis in the 20-49 years age group caused by cryptococcal infection compared to bacterial infection (2000-2010). N= number of overall cases that year*

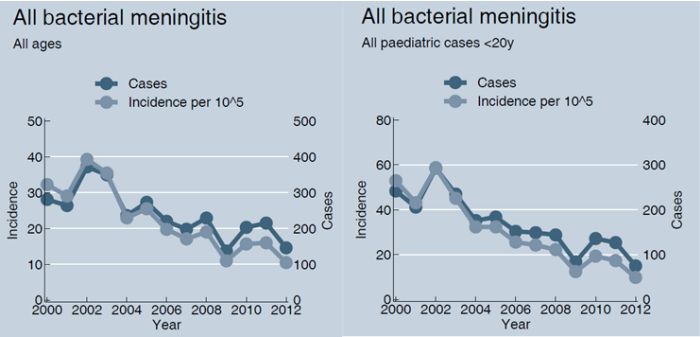
Figure 4.3b: *Cases of culture positive bacterial meningitis in adults showing the proportion caused by different pathogens for each year studied (2000-2012)*

4.3.2 Estimates of incidence of bacterial meningitis in Malawi

Estimates of overall incidence of culture positive bacterial meningitis per 100,000 population were derived from the complete culture positive data set for each year studied. Overall incidence declined from 49.6 in 2002 to 20 in 2012 (Figure 4.4).

This decline is driven entirely by the >3 months to <5 years age group (incidence 154.4 in 2002 to 20 in 2012), and remains unchanged in other age groups (5-15yrs incidence 15.7 in 2002 to 8 in 2012) (Figure 4.4). The adult incidence of bacterial meningitis remains static between 2000-2012 at 12/100000 population despite a peak in 2003-5 (Figure 4.4).

4.4a



4.4b

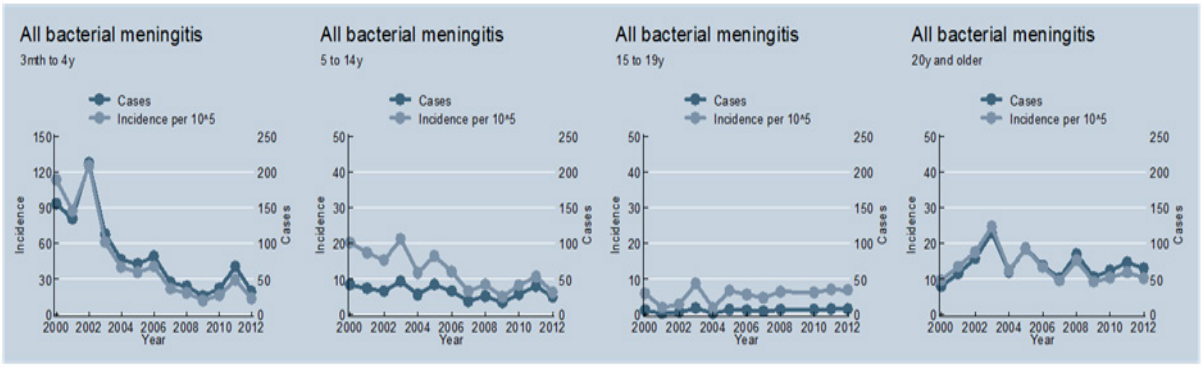


Figure 4.4 Estimates of incidence of bacterial meningitis in Malawi by age group

Figure 4.4a. *Estimates of incidence of all bacterial meningitis*

Figure 4.4b. *Estimates of incidence of culture positive bacterial meningitis by age group studied.*

4.3.3 Seasonality of bacterial meningitis by pathogen

Seasonality in pneumococcal disease has been described in Blantyre in blood stream isolates, seasonality in pneumococcal meningitis has also been observed in previous analyses from our centre (Gordon et al., 2000, Everett et al., 2011). It is not clear if other pathogens causing meningitis may also be seasonal. We tested for seasonality by examining the laboratory admission dates for all CSF culture isolates for different pathogens against rain and temperature data for the period studied. Detailed methods are found in Chapter 3, section 3.2.2.

S. pneumoniae meningitis is a seasonal disease occurring in the dry season, with an annual trough of cases during the rains (Figure 4.5). The mean numbers of cases of *S. pneumoniae* meningitis occurring each year between December to April were significantly lower than the overall mean numbers of cases occurring in the rest of the year ($p<0.05$). The seasonality pattern observed persisted despite the overall decline in disease incidence. Using the ARMAX model, rainfall was associated with 28% (95%CI: 13.7% to 41.4%, $p<0.001$) fewer cases of pneumococcal meningitis. Pneumococcal meningitis cases were increased by 42.8% (95%CI: -1.8% to 87.5%, $p=0.06$) in hot weather but the confidence interval crosses the null point. Numbers of cases of NTS meningitis were above the mean in August to December and below the mean in January, but the trend did not reach significance. There was no seasonal variation in Hib, *N. meningitidis* or Group B Streptococci case numbers.

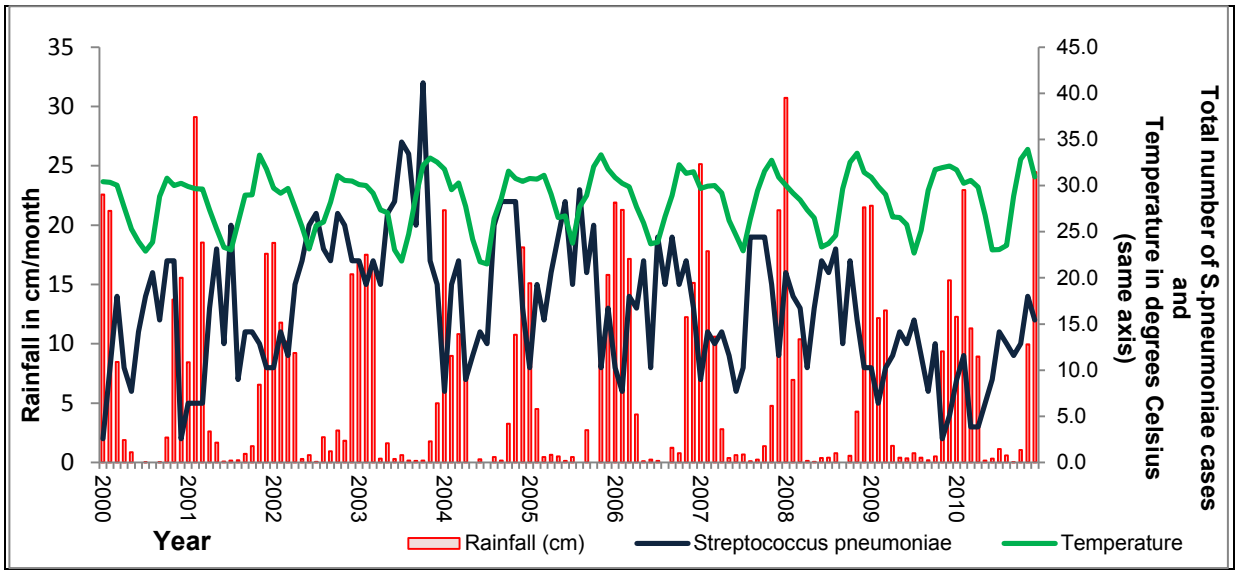


Figure 4.5 Seasonality of culture positive pneumococcal meningitis 2000-2010

4.3.4 CSF findings in bacterial meningitis

CSF biochemical parameters were recorded in the culture data from 2007. CSF white cell counts (WCC) with the addition of the CSF protein and glucose were tabulated from 2009 onwards by pathogen and parameters were compared between adults and children (Table

4.1). Data relating CSF parameters to infecting organism in bacterial meningitis from sub-Saharan Africa, are few (Jarvis et al.,2010). A significantly lower CSF WCC was seen in cases of *S. pneumoniae* meningitis compared to *N. meningitidis* meningitis in both adults and children.

The presence or absence of HIV infection did not affect the CSF WCC count due to in pneumococcal meningitis in adults (median CSF WCC in HIV positive 435 cells/mm^3 (IQR 107-1680) (n=297) and 575 cells/mm^3 (IQR 196-1740) in HIV negative (n=44), p=0.31).

Table 4.1 CSF parameters for proven bacterial meningitis in Malawi

CSF parameters in bacterial meningitis by pathogen and age group

CSF parameter	*Spn* meningitis Adults (n= 795)	*Spn* meningitis Children (n=998)	NTS meningitis Adults (n=28)	NTS meningitis Children (n=269)	*NM* meningitis Adults (n=17)	*NM* meningitis Children (n=22)
Median CSF protein (g/L) (IQR)	2.6 (0-4.6) *(n=161)*	2.4 (0.99-3.59) *(n=170)*	1.84 (0.92-3.15) *(n=3)*	1.84 (0-5.31) *(n=26)*	4.53 (3.23-5.54) *(n=4)*	1.87 (1.58-3.20) *(n=4)*
Median CSF glucose mmol/L (IQR)	0 (0-0.17) *(n=161)*	0 (0-0.33) *(n=146)*	2.75 (0-3.08) *(n=3)*	0.06 (0-0.44) *(n=25)*	0.08 (0-0.98) *(n=4)*	0.42 (0.14-0.73) *(n=4)*
Median CSF white cell count cells/mm^3 (IQR)	335 (74-1360)	407 (130-1360)	65 (5-700)	560 (150-2800)	2320 (155-5920)	1970 (250-6800)

Both NTS and cryptococcal meningitis were associated with significantly lower CSF WCC than *S. pneumoniae* and *N. meningitidis*. CSF protein was significantly higher in bacterial meningitis caused by all pathogens (with the exception of NTS) than cryptococcal meningitis (Median CSF protein SpN 2.6 g/L (0-4.6) compared to CCM 0.88 (0.51 – 1.62), p<0.001).

Similarly median CSF glucose levels, were significantly lower in *S. pneumoniae* (0 mmol/L (IQR 0-17) compared to cryptococcus (1.99 mmol/L (IQR 0.9 – 3.13) (p<0.001) (adults and children). A comparison of CSF biochemistry data by HIV status was not possible. This analysis forms part of the BAM CSF data analysis and is presented in Chapter 6 section 6.3.3.

Data were collected for culture negative CSF specimens from 2007. Of the culture negative CSF data, 643 adults had a CSF WCC of >100 cells/mm^3 and 796 children had a CSF WCC of >20 cells/mm^3 suggestive of a bacterial aetiology. These estimates were added to the culture positive totals for the time period studied. It was estimated that an additional 29% adult and 48% paediatric cases of culture negative bacterial meningitis occurred between 2007-12. Incidence calculations based on culture positive isolates probably therefore represent an under-estimate of the true incidence of bacterial meningitis in adults and children in urban Malawi.

4.4 Clinical predictors of poor outcome from bacterial meningitis

The results from section 4.3 of this chapter, demonstrate that the incidence of bacterial meningitis in adults living in Malawi is 100 times higher than in well-resourced countries, and several studies have reported a significantly higher associated mortality. Following the widespread use of penicillin in the 1940-50s, mortality rates from ABM in resource rich settings have improved from 45-50% to 11-25% in the last decade. This improvement is associated with early institution of antibiotic therapy and better supportive care (Auburtin et

al., 2006; Gjini et al., 2006a). In contrast, adult ABM mortality rates in sub-Saharan Africa have been reported to vary between 54-70% without any change over time (Gordon et al., 2000; Manga et al., 2008; Scarborough et al., 2007) and survivors experience significantly higher rates of disabling neurological sequelae compared to European patients (Edmond et al., 2010). This section details the results of a study that combined all clinical data that was available on adult patients with bacterial meningitis in Malawi to interrogate the data to determine clinical predictors of poor outcome. The results of this study were used to inform the care bundle intervention tested in a clinical trial that is the main focus of this PhD thesis (Chapter 6).

4.4.1 Research question

1. What are the clinical predictors on admission to hospital of poor outcome in bacterial meningitis in Malawi?

Objectives

1. To create a database consisting of data from clinical studies of ABM in adults and adolescents in Blantyre.
2. To test the combined data for statistical associations between individual clinical parameters and outcome at 40 days of follow up.

4.4.2 Methods details

4.4.2.1 Inclusion criteria:

- Age >14 years of age (adult ward admission age), or the age limit defined by the contributing study (e.g. age >16 years for the GLAM study).
- Enrolled in a clinical study of ABM at QECH with either
    - Proven microbiological evidence of ABM (culture or PCR positivity of an organism from the list of pathogens used in surveillance in the CSF)

- High clinical index of suspicion of ABM plus a CSF white cell count that was >50% neutrophils and >100 cells/mm^3 in HIV negative or 5 cells/mm^3 in HIV positive.

The latter was selected as the CSF inflammatory response in ABM may be attenuated in HIV (Jarvis et al., 2010).

Exclusion criteria:

- CSF microscopy or culture was positive for either Cryptococcus neoformans or Mycobacterium tuberculosis.
- CSF white cells were > 50% lymphocytes
- Study specific exclusion criteria: SAM: contraindications to study drug (dexamethasone), age < 16years, corticosteroids in the preceding 48 hrs, non-bacterial meningitis (Scarborough et al., 2007). GLAM: age < 16 years, type II diabetes mellitus, pregnancy, heart failure, CSF WCC <100 cells/mm^3 , CSF >50% lymphocytes (Ajdukiewicz et al., 2011).

4.4.2.2 **Database creation**

Details of the creation of the database are found in Chapter 3, Section 3.2.1.

4.4.2.3 Outcome measures, variable selection & data analysis

The outcome measure for included trial subjects was mortality, measured at day 40 and at day 10. Day 40 was considered the most important measure of outcome, as additional mortality from ABM was observed following hospital discharge in both SAM and GLAM, and determination of predictive factors for this variable was deemed important.

Eighteen clinical and demographic parameters were selected on the basis of previous published associations and suspected risk factors, and subjected to analysis for associations with death (van de Beek et al., 2004; Brouwer et al., 2007; Scarborough et al., 2007;

Ajdukiewicz et al., 2011). Age >40 was selected as the current life expectancy in Malawi is 53 years (Malawi, 1998) . The analysis plan was designed first to test all 18 variables against mortality using univariate analysis, followed by multivariate analysis of parameters with a significant univariate association.

Due to the heterogeneous nature of the studies included, some significant variables had missing data; where >50% of the data were missing, these variables were excluded from the multivariate analysis. The multivariate analyses were repeated using multiple imputation methods to enable subjects with missing data to be included; this did not alter the results and these analyses are not presented. These analyses were done in collaboration with Dr Brian Faragher at LSTM.

4.5 Results of clinical predictors of outcome from meningitis

4.5.1 Included clinical studies

Five studies were identified. One observational study was excluded as it was conducted prior to the introduction of ceftriaxone in 2001 under different admission conditions (Gordon et al., 2000). One vaccine trial was excluded as specific clinical data for the patients with meningitis were not available (French et al., 2010). The three included studies were two clinical trials, testing dexamethasone (Scarborough et al., 2007) or glycerol adjunct therapy (Ajdukiewicz et al., 2011) and an unpublished prospective cohort study investigating risk factors for mortality (Table 4.2). All studies recruited patients over the ward admission age (14 years, or on study specific age limits) with ABM and used ceftriaxone as the first line antibiotic.

A total of 891 participants were identified. 115 patients were excluded who had either proven fungal (n=52) or tuberculous meningitis (TBM) (n=25), or a CSF white cell count of <5 cells/mm^3 (n=38).

Table 4.2 Details of studies included in the analysis

Included studies in the Malawi meningitis database

Study name	Study dates	Type of study	Intervention tested	Number of cases (number included)	number of deaths (%)
Steroids in Adult Meningitis study (SAM)[Scarborough et al., 2007]	2001-2004	Randomised Controlled Trial	Intravenous Dexamethasone adjunct to antibiotics	465 (433)	233 (54)
Glycerol in Adult Meningitis (GLAM)[Ajdukiewicz et al., 2011]	2006-2008	Randomised Controlled Trial	Oral glycerol adjunct to antibiotics	265 (239)	129 (54)
Cohort observational study	2009	Observational cohort	No intervention	161 (43)	22 (51.1)

A further sixty one patients were excluded because no mortality data were available at day 10, yielding 715 cases for the analyses (Figure 4.6).

A relatively high proportion of participants from the cohort study (118/161) were excluded compared to 26 from the glycerol trial and 32 from the dexamethasone trial. This was due to a high number of patients in this database either not meeting the white cell count inclusion criteria for this analysis or having mortality data available.

Free access to HIV treatment become routinely available during the study period; co-trimoxazole prophylaxis in 2002 and anti-retroviral therapy (ART) from 2004 (Everett et al., 2011).

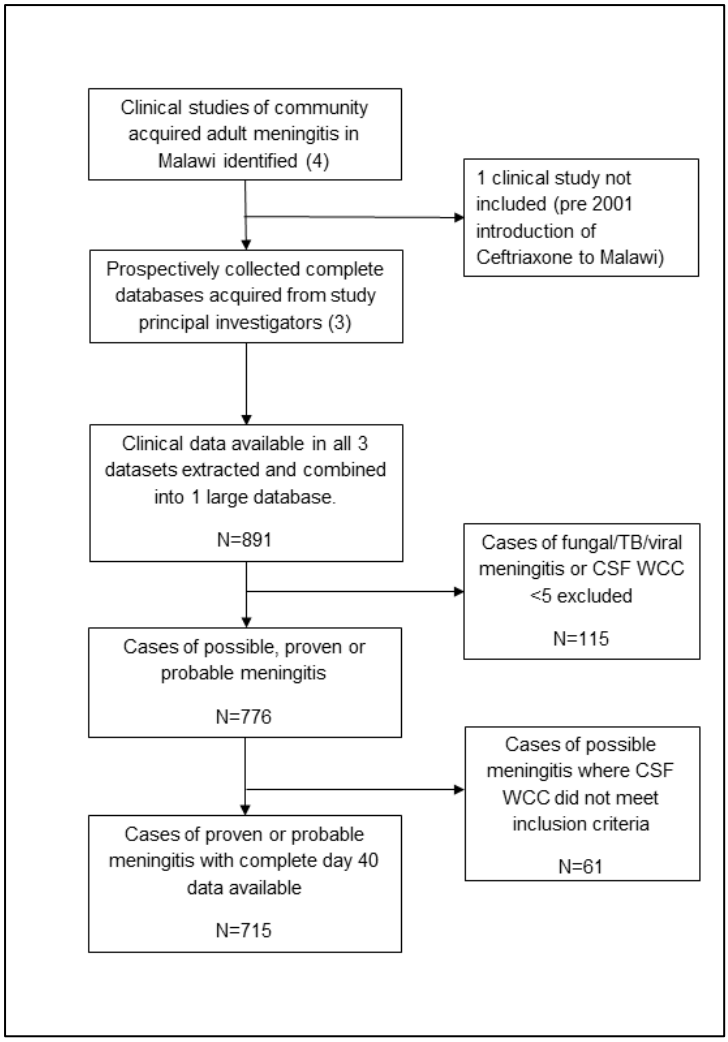


Figure 4.6 Selection of cases for the Malawi meningitis database

4.5.2 Patient characteristics and clinical and laboratory findings

The median age was 31 years (inter-quartile range IQR 25-38) and half were female (50.3%). 20 participants (2.8%) were adolescents. 608/695 (87%) of those tested were HIV antibody positive, of which 36 (5.9%) were on ART (Table 4.3). CD4 cell counts were not routinely measured during the time period studied.

The overall mortality rate was 45.3% in hospital by day 10, and 54.3% by day 40, with no significant difference across the three studies (Table 4.3). Altered mental status and coma were common. The median presenting Glasgow Coma Score (GCS) was 12 (IQR 9-14.0) and 113 (17.4%) of participants presented with a GCS of 8 or less. 319 (44.6%) participants

experienced an acute seizure and 89 participants experienced seizures after discharge. Fever >38°C (88.5%), headache or history of headache (99%) and neck stiffness (75%) were common, however photophobia was rare (15.5%). Septic shock was uncommon; the median pulse rate was 100 beats per minute (IQR 88-116) and the median mean arterial pressure (MAP) was 86 mmHg (IQR 79.9-96.7). Neurological disability (with the exception of post discharge seizures) and hearing loss were reported differently across the included studies and could not be sufficiently standardised for inclusion on the analysis.

CSF culture was positive in 419 of 712 specimens (59%) (Table 4.3). *S. pneumoniae* was the most common isolate (356/419, 85%) followed by *N. meningitidis* (17/419, 4%). Other isolates included NTS, *E. coli*, Group A streptococci and *H. influenzae.* The median CSF white cell count was 480 cells/mm^3 (IQR 170-1680). CSF opening pressure was not routinely recorded. Blood cultures were positive in 185/664 (28%) of individuals; the most commonly isolated organisms being (126/185, 68%), enteric gram negative organisms (27/185, 15%) and *N. meningitidis* (8/185, 4%). Of 352 pneumococcal CSF isolates where paired blood culture data were available, 139 (40%) were positive.

Comprehensive data on antimicrobial resistance were not available. Pneumococcal serotype was determined in 128 of 352 isolates using MLST serotyping; 56 (43.8%) were serotype 1. Other serogroups were 6 (4.7%), 7 (3.1%), 9 (4.7%), 12 (3.9%), 14 (4.7%), and 19 (3.1%). Mortality was 59% (33/56) for the participants infected with serotype 1 and 50.1% (36/71) for the other serogroups. There were too few data to analyse this trend further.

Table 4.3 Characteristics of patients included in the Malawi meningitis database

Baseline characteristics of included study participants

Characteristic on presentation	Number of observations available	Value (% or Inter Quartile Range IQR)
Clinical observations		
Deaths at day 10	715	324 (45.3)
Deaths at 40 days	668	363 (54.3)
Female sex	715	364 (50.3)
HIV positive	694	607 (87)
Antiretroviral therapy	607	35 (5.7)
Out of hours admission	655	315 (48)
Median age	715	31 (25-38)
History of acute headache	691	683(99)
Neck stiffness	715	536 (75)
Photophobia	683	106 (15.5)
Median Glasgow Coma Score	649	12.0 (9-14)
GCS >8-<11 (significant alteration of mental status)	649	169(26)
GCS <8 (coma)	649	113 (17.4)
Recorded acute seizure	714	319 (44.9)
Seizure post discharge	416	89 (21)
Median mean arterial blood pressure (mmHg) recorded	593	86 (79.9-96.7)
Median pulse (bpm)	596	100 (88-116)
Mean temperature (°C) recorded	668	38.1 (37.2-39)
Median recorded oxygen saturations	207	96% (93-97)

Microbiology	Number of observations available	Value (% or Inter Quartile Range IQR)
Positive CSF culture	712	419 (58.8) *S. pneumoniae* n= 356 (84.9) *N. meningitidis* n= 17 (4.1) *H. influenzae* n=3 (0.7) Other n=43 (10.2)
Median CSF white cell count (cells/mm^3)	707	480 (170-1680)
Positive blood culture	660	185 (27.8%) *S. pneumoniae* n= 126 (68.4) *N. meningitidis* n= 8 (4.1) *H. influenzae* n=1 (0.4) Enteric Gram negative n=27 (14.9) Other n=23 (12.4)
Blood tests		Median (IQR)
Sodium (mmol/L)	43	135 (130.9-139.1)
Haemoglobin (g/dL)	643	10.8 (9.0-12.7)
Glucose (mmol/L)	546	6.7 (6.0-98.7)
Creatinine (mg/dL)	40	1.1 (0.9-1.2)

4.5.3 Results of data analysis

Eighteen parameters measured on admission were subjected to univariate analysis for associations with survival at day 10 (n=715) and day 40 (n=668). There was no statistically significant difference in the association with outcome for all tested variables between these two time points except pneumococcal culture and therefore the day 40 follow up data are presented (Table 4.4).

The following clinical parameters were significantly associated with poor outcome on univariate analysis: age, clinical presentation with altered mental status, acute and post discharge seizures, hypoxaemia, tachycardia, hyponatraemia, anaemia and treatment with glycerol. Neither gender, out of hours admission, HIV antibody status, blood glucose, mean arterial blood pressure, oligo-anuria, respiratory rate, nor serum creatinine were associated with poor outcome (Table 4.4). Of the 339 participants with a pathogen isolated from the CSF, 181 (54%) died by day 40 - univariate OR 0.923 (0.68 : 1.25) p= 0.61. As culture of *S. pneumoniae* in the CSF and HIV status have been associated with mortality in other studies of bacterial meningitis (Nyasulu et al., 2011; Domingo et al., 2009) these data were added to the multivariate model with the other tested variables. Coma scores were grouped into three clinically relevant categories: <8, 8-11 and >11. Oxygen saturations, post discharge seizures, respiratory rate, anuria, creatinine and sodium had >50% of data missing and were excluded from multivariate analysis. Seizures were divided into acute (pre-hospital and in hospital) and seizures during follow up. Haemoglobin and pulse rate were divided into clinically significant groups to ease the clinical interpretation of the results. Hb was categorized as <5g/dL, 5-8.5g/dL, 8-11.5g/dL, 11-14.5g/dL and >14.5g/dL. Pulse was categorised into normal (<100 beats per minute), mild (100-120 bpm) and severe tachycardia (>120 bpm).

4.5.4 Results of the multivariate analysis to derive clinical prognostic markers of outcome

Presentation with coma was the strongest independent predictor of outcome after multivariate analysis; OR for death with a GCS <8/15 was 5.9 (95% CI 3.31 : 10.86) p<0.001, GCS of 8-11 was 1.665 (1.08 : 2.5) p=0.007 (Table 4.4). Acute seizures were associated with death, OR 1.55 (1.08 : 2.24) p=0.019. The severity of anaemia was associated with a corresponding increasing risk of mortality, OR death of haemoglobin (Hb) 5-8g/dL was 3.41 (1.61 : 7.23) p=0.001; at Hb <5g/dL the OR was 6.34 (1.86 : 21.62)

p=0.003. Every 1g/dL increase in haemoglobin was associated with a 12% reduction in mortality, OR 0·88 (0.82-0.94). Tachycardia >120 bpm was also associated with death, OR 1.80 (1.08 : 2.99) p=0.023. Glycerol receipt was independently associated with mortality 1.88 (1.09 : 3.25) p=0.023. Age >40 years was not significant, OR 1.33 (0.86 : 2.06) p=0.20.Neither CSF pneumococcal culture positivity nor HIV status were significantly associated with mortality at day 40. The OR of death were 0.70 (0.49 : 1.02) p=0.06 for pneumococcal infection, and 1.18 (0.70 : 2.00) p=0.52 for HIV infection. No parameter reaching non-significance at the univariate level was shown to have significant association at the multivariate level.

Mortality was not seasonal; 279 (36%) of cases presented in the cold winter season (May-September), with 145 (52%) deaths and 388 (64%) cases presented during the remaining hot months of the year with 217 (56%) deaths p=0.34 (Figure 4.7).

4.5.4.1 Delays to presentation to hospital and clinical care

Presentation out of routine working hours comprised 318 (44.5%) of admissions. For a subset of patients with culture proven ABM 'the observational cohort', (n=26) data were collected prospectively relating to the patient pathway on a standard form to investigate delays in presentation, investigation and initiation of therapy. Nine of 26 patients (35%) died before day 10. Median self-reported journey time to QECH was 0.8 hours (IQR 0.5-1.6) in non-survivors, and 1.5 hours (IQR 0.7-2.0) in survivors. Median time from presentation to medical review was 2.8 hours (IQR 0.8-5.8) in non-survivors and 1 hour (IQR 0.6-3.0) in survivors. The median time from presentation at the hospital with meningitis to receipt of intravenous antibiotics was 5.0 hours (IQR 2.5-8.9) in non-survivors and 2.7 hours (IQR 1.7-6.0) in survivors. No time difference reached statistical significance.

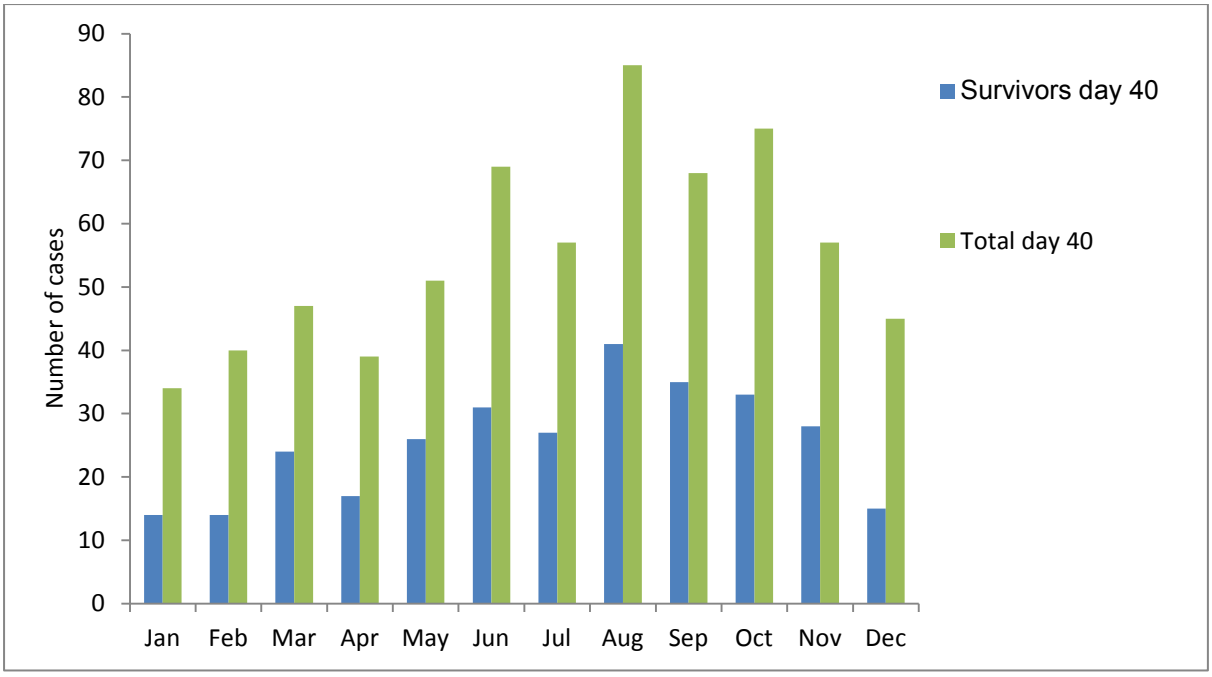


Figure 4.7 Seasonal admission and outcome data for adults with ABM 2000-2009

Table 4.4 Predictors of poor outcome from bacterial meningitis

Table 4: Univariate and multivariate predictors of poor outcome in adults with bacterial meningitis

Parameter		Day 40		Univariate (unadjusted)		Multivariate (adjusted)**	
		Alive	Dead	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sample size		304	363				
Age	**mean (sd)**	31.5 (10.7)	34.2 (11.4)	1.023 (1.009 : 1.038)‡	0.002		
	≤ 40 years	256	285 (52.7%)	---	---	---	---
	> 40 years	48	78 (61.9%)	1.460 (0.981 : 2.171)	0.062	1.327 (0.858 : 2.055)	0.204
Gender	**male**	149	177 (54.3%)	---	---	---	---
	female	155	186 (54.5%)	1.010 (0.754 : 1.370)	0.948	0.894 (0.621 : 1.287)	0.548
HIV status	**negative**	43	37 (46.2%)	---	---	---	---
	positive	254	316 (55.4%)	1.446 (0.904 : 1.370)	0.124	1.184 (0.700 : 2.001)	0.528
	not known	7	10				
Out of hours admission	**no**	138	173 (55.6%)	---	---	---	---
	yes	134	162 (54.7%)	0.964 (0.700 : 1.328)	0.824	0.949 (0.661 : 1.362)	0.777
	not known	32	28				
GCS	**mean (sd)**	12.2 (2.8)	10.2 (3.6)	0.825 (0.786 : 0.867)‡	<0.001		
	≥ 11	205	168 (45.0%)	---	---	---	---
	8 – 11	69	94 (57.7%)	1.662 (1.146 : 2.411)	0.007	1.665 (1.086 : 2.554)	0.007
	<8	20	84 (80.8%)	5.125 (3.021 : 8.695)	<0.001	5.999 (3.314 : 10.861)	<0.001
	not known	10	17				

Table 4. Univariate and multivariate predictors of poor outcome in adults with bacterial meningitis continued 2

Parameter		Day 40		Univariate (unadjusted)		Multivariate (adjusted)**	
		Alive	Dead	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
1 or more acute	no	216	207 (48.9%)	---	---	---	---
seizure episodes	yes	87	156 (64.2%)	1.871 (1.353 : 2.588)	<0.001	1.552 (1.075 : 2.241)	0.019
	not known	1	0				
Seizure post-discharge	no	271	57 (17.4%)	---	---		
	yes	23	64 (73.6%)	13.230 (7.591 : 23.057)	<0.001	†	†
	not known	10	242				
SpO_2	mean (sd)	95.2 (2.7)	93.5 (5.7)	0.888 (0.806 : 0.978)‡	0.016	†	†
	>95	52	48 (48.0%)	---	---		
	92 – 95	26	32 (55.2%)	1.333 (0.696 : 2.552)	0.385	†	†
	<92	11	29 (72.5%)	2.856 (1.287 : 6.339)	0.010		
	not known	215	254				
Pulse rate	mean (sd)	96.9 (18.6)	103.1 (19.0)	1.018 (1.008 : 1.027)‡	<0.001		
	<100	172	162 (48.5%)	---	---	---	---
	100–120	39	57 (59.4%)	1.552 (0.979 : 2.459)	0.061	1.422 (0.844 : 2.397)	0.186
	>120	44	76 (63.3%)	1.834 (1.194 : 2.816)	0.006	1.800 (1.084 : 2.987)	0.023
		49	68				
	not known						

Table 4. Univariate and multivariate predictors of poor outcome in adults with bacterial meningitis continued 3							
Parameter		**Day 40**		**Univariate (unadjusted)**		**Multivariate (adjusted)****	
		Alive	**Dead**	**Odds ratio (95% CI)**	**p**	**Odds ratio (95% CI)**	**p**
MAP (mmHg)	**mean (sd)**	89.7 (13.0)	89.0 (15.5)	0.996 (0.985 : 1.008)‡	0.530		
	<90	152	174 (53.4%)	---	---	---	---
	90-100	57	66 (53.7%)	1.011 (0.667 : 1.533)	0.957	0.972 (0.622 : 1.520)	0.902
	>100	44	56 (56.0%)	1.112 (0.708 : 1.745)	0.645	1.107 (0.636 : 1.925)	0.720
	not known	51	67				
Respiratory rate	**mean (sd)**	23.8 (9.6)	24.2 (6.3)	1.007 (0.954 : 1.064)‡	0.790	†	†
	not known	246	299				
Anuria	**no**	208	252 (54.8%)	---	---	---	---
	not known	96	111				
CSF culture positive for	**no**	145	180 (55.4%)	---	---	---	---
	yes	158	181 (53.4%)	0.923 (0.680 : 1.253)	0.606	0.704 (0.488 : 1.016)	0.061
	not known	1	2				
Plasma glucose	**mean (sd)**	7.3 (2.7)	7.4 (3.8)	1.007 (0.957 : 1.059)‡	0.793		
(mmol/L)	**≥9**	46	45 (49.5%)	---	---	---	---
	6-9	90	102 (53.1%)	1.159 (0.703 : 1.909)	0.563	1.292 (0.748 : 2.234)	0.358
	≤6	101	123 (54.9%)	1.245 (0.764 : 2.028)	0.379	1.423 (0.763 : 2.653)	0.268
	not known	67	93				

Table 4. Univariate and multivariate predictors of poor outcome in adults with bacterial meningitis continued 4

Parameter		Day 40		Univariate (unadjusted)		Multivariate (adjusted)**	
		Alive	Dead	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Haemoglobin (g/dL)	**mean (sd)**	11.4 (2.9)	10.5 (2.9)	0.893 (0.841 : 0.949)‡	<0.001		
	>14	50	32 (39/0%)	….	….		
	8-11	104	134 (56.3%)	2.013 (1.206 : 3.360)	0.007	2.598 (1.422 : 4.747)	0.002
	5-8	26	45 (63.4%)	2.704 (1.404 : 5.210)	0.003	3.404 (1.603 : 7.229)	0.001
	<5	4	11 (73.3%)	4.297 (1.259 : 14.662)	0.020	6.343 (1.861 : 21.624)	0.003
	not known	67	93				
Glycerol treatment	**no**	58	53 (47.7%)	---	---	---	---
	yes	47	76 (61.8%)	1.770 (1.051 : 2.978)	0.032	1.884 (1.092 : 3.249)	0.023
	not known	199	93				
Na (mmol/L)*	**median (range)**	137 (125:199)	132 (115:152)	0.928 (0.834 : 1.032)‡	0.166	†	†
	not known	283	341				
Creatinine (mg/dL)*	**median (range)**	1.05 (0.7:1.5)	1.15 (0.5:10.8)	4.441 (0.682 : 28.925)‡	0.119	†	†
	not known	286	341				

4.6 Summary and discussion of results findings from Chapter 4

In this chapter, a systematic analysis of all cases of bacterial meningitis that had been admitted to QECH between 2000-2012 was done. The two approaches covered in this chapter (systematic surveillance and analysis of clinical trial data) demonstrate important trends in bacterial meningitis, with clear differences between adult and paediatric disease. In addition predictors of poor outcome in adults have been identified.

Marked differences exist between adult and paediatric bacterial meningitis. The incidence of paediatric disease is very high, particularly children under the age of 5 years, but has fallen dramatically in response to vaccination campaigns, improvements in child health and potentially also the reduction of vertical HIV transmission. In contrast ABM in adults has a lower incidence, however, incidence has not fallen in response to major public health interventions including the roll-out of ART. *S. pneumoniae* is the primary cause of bacterial meningitis in both groups now Hib and GBS incidence have fallen. *N. meningitidis* remains a sporadic disease with low incidence in both groups.

Adult bacterial meningitis incidence in Malawi is 12/100 000 population in Malawi in this study, more than 100 times higher than estimates from the UK and the US of 0.1-1.4/100 000 (Gjini et al., 2006b; Thigpen et al., 2011; Okike et al., 2014). The incidence data presented here are the first formal incidence estimates from SSA based on systematic surveillance rather than meta-analysis of clinical study data. The estimate from this study is based on culture positive isolates, and therefore this figure may be a substantial under-estimate compared to the true figure (Wu et al., 2013). Further data presented in this thesis from the BAM study on the frequency of culture negative but PCR positive meningitis will assist in improving this estimate of the burden of adult disease (Chapter 6). Incidence estimates from this region of ABM in adults preceding the HIV epidemic are lacking, but many centres have reported increasing case numbers since the start of the HIV epidemic (Molyneux et al., 2003; Schutte et al., 2000). Causes for such a high burden of ABM in this region are not fully understood, but high rates of pneumococcal carriage, seasonal disease

with 20% of disease caused by *S.pneumoniae* serotype one, a high burden of HIV infection underpinned by poverty with high rates of poor housing and overcrowding may all be implicated (Glennie et al., 2011; Everett et al., 2012; Cornick et al., 2011; Roca et al., 2012; Huang et al., 2004).

From the monthly rain and temperature data, pneumococcal meningitis is clearly a seasonal disease. Peaks in cases occur in the cool, dry season each year, a pattern that has persisted despite the fall in paediatric disease. Adult mortality is static at approximately 50% throughout the year. Seasonality of pneumococcal meningitis has been reported from West and central Africa 'the meningitis belt', coinciding with the better known seasonal epidemics of meningococcal disease that occur regularly in that region in the hot dry weather preceding the onset of the rains (Molesworth et al., 2003; Gessner et al., 2010). Seasonality in meningitis during the rainy season has been reported from Uganda, but no seasonality was found in another study in Tanzania, neither centre has a systematic surveillance programme in place (Williams et al., 1986; Wiersinga et al., 2004). Very few centres beyond the meningitis belt undertake routine meningitis surveillance, so it is not known if seasonality occurs in other regions, but it is intriguing that the seasonal pattern of pneumococcal disease in Blantyre occurring in the dry season matches that of the West African centres, despite Malawi being over 3000km from the meningitis belt region.

Invasive pneumococcal disease is strongly associated with HIV infection, particularly in Africa, and in 87% of cases of probable or proven meningitis were HIV co-infected (French et al., 2010). It is surprising that the roll out of ART and better care for HIV infected adults has not resulted in a decline in pneumococcal meningitis when a decline has been seen in invasive bloodstream infections (Everett et al., 2011). The immunology group at MLW have previously demonstrated high rates of pneumococcal carriage with broad serotype diversity in HIV infected adults despite the initiation of ART (Glennie et al., 2013). Further work from this group has shown that poor T cell responses to pneumococci are present in HIV co-infected adults, irrespective of CD4 count (Glennie et al., 2011). It is therefore a reasonable

hypothesis that persistent defects in immune control in the nasopharynx and potentially the CSF compartment could account for the continuing high incidence of pneumococcal ABM in adults. In the meningitis belt, changes in weather, particularly hot weather and viral co-infection with respiratory pathogens has been associated with increased invasive meningococcal disease from the natural carriage state (Mueller et al., 2008). This has not been studied in Malawi, but it is likely that temperature changes and influenza season that coincide with the peaks of disease seen in these data influence in the incidence of pneumococcal meningitis.

ART roll out, which has led to nearly 350 000 adults nationally alive on ART at the end of 2012 has also failed to impact on the prevalence of cryptococcal meningitis as well as pneumococcal meningitis. The reasons for this are unclear, but over the time period studied more adults have started ART with a low CD4 count and no clinical evidence of CCM, rather than presenting with a first episode of CCM as a presentation of new HIV disease. The static rates of CCM in adults are therefore likely to be due to fewer patients presenting with new CCM in the absence of ART, and increasing numbers of patients presenting as part of an immune reconstitution inflammatory syndrome (IRIS) in the first 12 months of ART (Walker et al., 2012; Parkes-Ratanshi et al., 2011).

Despite the clear association between pneumococcal meningitis and HIV infection, HIV was not a predictor of poor outcome in the clinical mortality analysis. 87% of adults in the database were HIV infected; the numbers of HIV negative patients were small. Patients with HIV-infection have higher rates of pneumococcal disease and altered T-cell immunity to pneumococcal infection, so why the presence of HIV-infection did not predict mortality is intriguing (Glennie et al., 2013; Glennie et al., 2011). The results are not stratified by CD4 count as these data were unavailable for the database, however it is likely that the majority of patients were severely immunocompromised as IPD is a WHO stage 3 condition. Multiple factors are likely to be implicated in the pathological causes of poor outcome including highly virulent bacteria that produce high levels of toxins, consumption of complement within the

CSF compartment and likely overall defects in host responses to pneumococci that occur irrespective of HIV infection (Wall et al., 2012; Goonetilleke et al., 2012; Wippel et al., 2013). This will be the subject of further work after the completion of this thesis.

Poor outcome from bacterial meningitis was predicted by altered mental state and coma, seizures, anaemia, tachycardia and hypoxaemia. These variables were taken forward into the care bundle design to test if supportive clinical measures and early antibiotics may be feasible and effective in the AETC environment at QECH (Chapter 3 Section 3.3.5). It is clear that patients present very unwell to a hospital which has profound limitations on clinical resources available for treatment and supportive care. Optimisation of resuscitation with targeted restoration of normal physiological variables and early antibiotic therapy presents an opportunity to improve the clinical management of these very sick patients and potentially improve outcome. The results of goal directed therapy delivered as a care bundle are detailed in Chapter 6.

The predictor variables identified in this analysis were taken forward to synthesise a clinical predictor score for adults with bacterial meningitis, the results of which can be found in the following Chapter 5.

Summary

S.pneumoniae is the predominant cause of ABM in both adults and children in Blantyre. Paediatric ABM cases have significantly declined over the last 12 years, adult disease incidence is static. Pneumococcal meningitis is a seasonal disease in Malawi, mortality is not seasonal. Mortality is highest in adults presenting with a severe clinical phenotype including seizures, altered mental state and anaemia, HIV was not a predictor of mortality. The results from this chapter have led to the development of a severity score to predict outcome from ABM, and the design of a clinical trial to test if optimized clinical management of the clinical features associated with poor outcome is feasible in Malawi.

5 Development of a prediction tool for adult bacterial meningitis in sub-Saharan Africa: Malawian Adult Meningitis Score (MAMS)

5.1 Introduction

Individual predictors of poor outcome from severe meningitis in adults, as detailed in the previous chapter, are of interest to both clinicians and clinical scientists studying disease pathogenesis, and for use in developing and assessing clinical interventions. However individual predictors are of little practical use to the treating clinician at the patient bedside as few directly useful clinical data can be derived from each predictor in a clinical situation.

Severity scores have been developed for many illnesses to attempt to synthesise individual predictor variables into reliable bedside tools, the ascertainment of the risk of a poor outcome can assist stratifying or escalating clinical care as necessary (Adams and Leveson, 2012). For adults, a number of severity scores are in existence for infectious diseases in well-resourced settings, particularly sepsis (APACHE-II, SOFA) and pneumonia (CURB-65). These scores have been well-validated prospectively in multiple studies after publication of the original score data and are in regular clinical use (Knaus et al., 1985; Bauer et al., 2006; Richards et al., 2011). A well validated score for diagnosis of ABM in children exists, but few severity scores have been developed for children specific to meningitis (Nigrovic et al., 2012). Several scores have been developed to predict outcome from intensive care for children with meningococcal septicaemia (Castellanos-Ortega et al., 2002; Castellanos-Ortega and Delgado-Rodriguez, 2000), these patients are very different to adults with bacterial meningitis and no paediatric score has been tested on or validated on adult data.

Few severity scoring systems exist for adults with ABM. The first such score was developed in the USA (Aronin et al., 1998), using retrospectively collected data with small numbers of patient data in both the discovery and validation datasets. A predictive index or formal score was not developed by this group; patients were only divided into low/medium/high risk of negative outcome according to three predictors of outcome derived from the discovery cohort. Despite good concordance (a comparison of observed number of outcomes to the predicted number of outcomes) in the small independent validation dataset of 0.81 (95% CI 0.71 – 0.92) this score has not entered routine clinical use and has not been validated prospectively (Aronin et al., 1998). One severity score for bacterial meningitis has been developed prospectively in Europe (Weisfelt et al., 2008). This score was derived from data collected in a large study of dexamethasone for bacterial meningitis (de Gans and van de Beek, 2002) and validated on data from the European Dexamethasone cohort study (Weisfelt et al., 2006a). Patients in these studies were predominately of white European ethnicity, were middle aged and many had co-existent medical conditions such as diabetes. The study team derived a nomogram from the discovery data and validated it against the separate discovery data. The concordance index for the Dutch score was 0.84 (95% CI 0.81 - 0.86). This score was then validated externally against two datasets from clinical trials of dexamethasone for bacterial meningitis, done in Malawi and Vietnam (Schut et al., 2012). The score performed poorly when applied to both datasets, but particularly to Malawian data from the SAM trial (Scarborough et al., 2007); the concordance index of the Dutch score in Malawi was 0.68 (95% CI 0.63 : 0.73) (Schut et al., 2012). From the comparison of the clinical parameters of the patients in the two cohorts, the Malawian patients were considerably different from the Dutch patients with Malawian patients being significantly younger (median age in Europe 55 years compared to 31 years in Malawi $p<0.001$), having high rates of HIV co-infection (<1% in Europe and 90% in Malawi $p<0.001$) and much lower CSF WCC (median 3000 cells/mm^3 in Europe and 480 cells/mm^3 in Malawi $p<0.001$). It was clear that due to the failure of the European score and the marked differences between the two populations, that a score derived from patient data collected in sub-Saharan Africa

would be necessary. A locally derived score with good predictive index would have the potential to be used in further clinical research projects in the region, as well as be a useful clinical tool. In this chapter, similar methodology to the European group was used to derive a severity prediction score for adults with bacterial meningitis in Malawi.

5.1.1 Overview of severity score development including validation loop processes

To develop a severity scoring system, it is ideal to have two separate data sets, one to be used for discovery of the score, and the other for validation (Cook, 2008, Adams and Leveson, 2012). Separate datasets for discovery and validation allow for confidence in the power of the score to predict outcome in different circumstances. Validation within the same dataset, even if cases are randomly selected, is subject to the homogeneous nature of one dataset and the high probability of the randomly selected cases being very similar to those in the derivation cohort and hence excellent concordance but low transferability (Adams and Leveson, 2012).

The severity score is derived from the discovery cohort, and tested against the validation cohort for predictive performance. If performance is sub-optimal, the discovery process is refined and re-run on the discovery cohort, and then the revised score is tested again against the validation cohort. The 'validation loop' process must be repeated until optimum concordance between predicted and true outcome is reached in the validation data (Figure 5.1). External validation of the severity score is then undertaken either using independent data from an outside source, or subsequently tested in a prospective clinical trial.

Where one large study cohort dataset exists, data can be divided into separate discovery and validation cohorts by random selection (Cook, 2008). If this methodology is used then further external validation is required before the score is tested prospectively in a clinical setting (Cook, 2008).

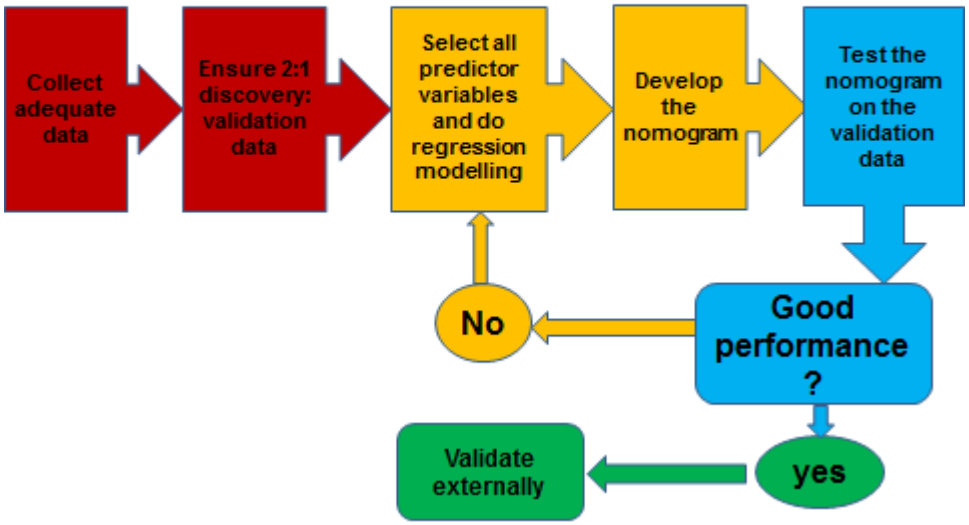


Figure 5.1 Processes for developing severity scoring systems

Based on the extensive literature for development of severity scoring systems, a ratio of 2:1 (discovery : validation) is ideally required for, either if one dataset is split for discovery and validation, or if two different datasets are used (Cook, 2008). If one dataset is split, greater than 50% of all available cases must be used for discovery to ensure the predictors derived from the discovery data are as accurate as possible and all biases and missing data minimised. As the discovery process is based on logistic regression modelling, the largest possible numbers should be entered into the modelling process to increase the power and ensure accuracy in determining the risks of each variable in predicting poor outcome. Remaining data are then used for validation (Cook, 2008). A smaller number of cases are required for validation as this process checks the accuracy of the original predictive score derived from the predictive variables only (Adams and Leveson, 2012).

Once data are correctly selected, regression modelling is then done on the data to determine the predictors of poor outcome. Two different approaches are then available for creation of the score; decision tree modelling or development of a nomogram. Decision tree modelling is

best used for scores that assist in diagnoses, or discrimination between two conditions, as the outcomes from a decision tree are based on binary decisions at all levels (Adams and Leveson, 2012). A nomogram is better used for outcome prediction as the nomogram creates a severity index that can then be applied to the validation data to determine an individual risk of poor outcome on a scale rather than as a binary variable yes/no (Cook, 2008). The nomogram approach was used in this chapter for development of the meningitis severity score.

5.2 Methods

5.2.1 Objectives and research questions

Research question

Can a prognostic risk scoring system be derived from existing meningitis clinical trial data that will reliably predict poor outcome? Will this score be useful to compare predicted outcome from admission variables in BAM across the two trial phases?

Objectives for this chapter

1. To utilise the existing data used to describe individual predictor variables developed in Chapter 4, Section 4.5.4 as a discovery cohort to develop a severity prediction score.

2. To validate this score against BAM data collected in the first observation phase to assess sensitivity and specificity of the score in predicting mortality at day 40 post admission with bacterial meningitis.

Data analysis for this chapter was done in conjunction with Dr Mavuto Mukaka and Dr Brian Faragher. Dr Mukaka performed the random selection to divide data from the meningitis database into discovery and validation data, created the nomograms performed each validation exercise, calculated the concordance index for each nomogram, drew the receiver-operated curves and estimated the sensitivity and specificity of the final nomogram.

All other analyses were performed by myself. Dr Faragher provided guidance and support to both myself and Dr Mukaka and verified the final data.

5.2.2 Inclusion and exclusion criteria for MAMS

5.2.2.1 **Discovery cohort**:

Adults and adolescents with ABM, meeting the criteria for inclusion in the historical database for discovery of predictive variables for day 40 mortality were included (Chapter 4 Section 4.5.4). These patients had been recruited to one of the SAM, GLAM and pilot studies. Patients who received glycerol in the GLAM trial were excluded due to the additional mortality attributed to glycerol receipt. The inclusion of these cases in the discovery cohort would have potentially confounded the derivation of a severity scoring system (Ajdukiewicz et al., 2011). The full inclusion and exclusion criteria for this database can be found in Chapter 4 Section 4.4.2.

5.2.2.2 **Validation cohort**:

Adults over the age of 14 years with proven or probable ABM recruited to BAM phase 1 were originally planned to be the primary validation cohort. However due to small numbers of patients recruited to BAM phase 1 (n=71), additional cases were randomly selected from the Malawi meningitis database and also included to ensure the validation cohort had adequate numbers for validation.

The latter data from the Malawi meningitis database were randomly selected and removed from the database prior to the extraction of complete case data for the discovery process. The full inclusion and exclusion criteria for the BAM study can be found in Chapter 3 Section 3.3.4 BAM inclusion criteria varied from the meningitis database inclusion criteria by including patients who met the following criteria: aged >14 years with either proven ABM on CSF culture or PCR, or probable ABM with a typical presenting phenotype, a CSF WCC >50 cells/mm^3 >50% neutrophils and with the following biochemistry: CSF:Blood glucose ratio of

<0.4, protein >0.5g/L and CSF lactate >4mmol/L. The only difference between the inclusion criteria for the two datasets were he CSF WCC inclusion criteria in the meningitis database had a CSF WCC cut off of >5 cells/mm^3 in HIV infected and >100 cells/mm^3 in HIV negative individuals. No discrimination was made using CSF biochemistry or PCR data in that database.

5.2.3 Derivation of the MAMS discovery and validation cohorts

5.2.3.1 Sample sizes

From the available data from the Malawi meningitis database and the BAM Phase 1 data, it was estimated that a total of 600 cases with complete day 40 mortality data would be available for the score discovery and validation. Using the 2:1 ratio (Cook, 2008), 400 cases would be used for discovery and 200 for validation. It is estimated that the accuracy of validation data is optimal when the number of cases available for validation is >200 (Cook, 2008). To achieve this, 71 cases were available from BAM for validation and 543 in the meningitis database for discovery and validation. The 543 cases from meningitis database were randomly split into 80 complete cases for validation and 400 complete cases for discovery, using the 'sample' command' in STATA-10 by Dr Mukaka.

Discovery cohort: From the meningitis dataset described in Chapter 4, Section 4.5, complete case data were derived for all potential variables to be tested in the severity score. The 400 complete cases discovery cohort was saved separately, and univariate analysis using logistic regression was done on this dataset to obtain odd's ratios for day 40 mortality with 95% confidence intervals for each variable tested using SPSS version 20. To ensure that there was no selection bias, these data were compared against those generated from the larger dataset (Chapter 4 Section 4.5.3-4) to ascertain if the sample was truly random and matched the larger dataset. If the 95% confidence intervals overlapped for each

variable, it was concluded that there was no major bias in the random selection of these cases.

Validation: This dataset comprised of complete case data from BAM Phase 1 merged with randomly selected data from the Malawi meningitis database that did not overlap with the discovery cohort. BAM Phase 2 data were not appropriate for MAMS development as these patients had received the care bundle intervention and therefore were not matched to either the BAM Phase 1 or the Malawi meningitis database patients. Relevant variables from BAM Phase 1 were recoded and renamed, and merged into the validation database using SPSS version 20, so the coding and names of all variables matched the discovery database.

5.2.3.2 Study endpoints

Survival or non-survival at day 40 was used as the endpoint for both databases. Only cases where these data were available were included in either the discovery or validation cohorts. Mortality was chosen as the sole endpoint as limited morbidity data were available for the day 10 or day 40 endpoint in the meningitis database. Day 40 was chosen over day 10, as additional deaths occurred in the time between day 10 and day 40 in all included studies in the meningitis database, and it was important to capture the risks of these additional deaths in the severity score.

5.2.3.3 Methods for multivariate analysis

Using exactly the same statistical methodology for Chapter 4 Section 4.4.2, the discovery cohort was subjected to multivariate logistic regression analysis. This was done by including all variables that were statistically significant on the univariate analysis at the 5% level, with the addition of variables that were determined in the analysis of the Malawi meningitis database (Chapter 4 Section 4.5.3-4) to be important confounders, such as age and HIV status. Several models were generated using this methodology and then compared.

a) The first model reported variables as continuous variables (such as GCS and age).

b) The second reported the same continuous variables categorised as clinically important groups such as coma/altered mental status/normal mental status. These groupings were first made on well-established clinical parameters, such as GCS <8 = coma, GCS >8<11 severe altered mental status, GCS >11<15 mild altered mental status (Teasdale and Jennett, 1976).
c) The third model was generated using groupings that were made by creating clinical cut-offs in the database, to match those used in the Dutch scoring system, such as CSF WCC </>1000cells/mm3 (Weisfelt et al., 2008).
d) The fourth model was generated by quartile ranges around the median. This was done to establish if the clinical cut offs (derived from other, more well-resourced settings) were relevant in Malawi, or if new cut-offs needed to be generated that were more appropriate for Malawi.

The odds ratios of the different groups and cut off points were compared to the OR generated from the continuous variable. Appropriate cut offs for the nomogram were selected on the basis of the highest OR for a particular set of groupings or clinical cut offs with associated statistical significance.

5.2.4 Synthesis of the MAMS nomogram

A severity score was derived from this analysis using a nomogram using the approach described by Cook et al (Cook, 2008). The nomogram was computed in *R* statistics package http://www.r-project.org/. Points indicating severity were applied on a sliding scale to each predictor associated with mortality, with fewer points associated with a better outcome. For example, if low GCS was associated with death, then the lower the patient's GCS the more points were be added to the total severity prediction score. The number of points for each predictor was calculated in *R*, based on the original coefficient of the logistic regression model that derived the association between the predictor and outcome. The points for each

variable were added together, and the final score was read against a predictive percentage. The maximum number of points assigned from each nomogram was associated with 95-100% prediction of negative outcome by six weeks after illness onset, and the lowest number of points associated with a less than 10% chance of predicted poor outcome. The total numbers of points available differed between nomograms, depending on the variables included.

5.2.4.1 Validation of the nomogram

Using the validation dataset, each patient had a predictive risk of death calculated by the nomogram. The predictive risk index was broken down into 10 percentiles, from 0-10 through to 90-100% risk of negative outcome. A table was synthesised that detailed the number of patients with the actual outcome in the validation data for each percentile group, compared to the number predicted by the nomogram for each percentile.

The agreement between the two columns was then calculated using Kappa to assess the degree of agreement, comparing concordance for the data between random associations and the actual agreement. Statistical significance between the two agreements was set at <0.05.

The sensitivity and specificity of the nomogram as a predictive tool was also assessed using Receiver Operated Curve (ROC) curves, an area under the curve (AUC) was determined using the same methodology in the attempt to validate the Dutch score against Malawian data (Schut et al., 2012). Chi squared testing was used to look for associations between true and predictive outcomes. In addition, sensitivity and specificity of the final nomogram were calculated, and from this, the positive and negative predictive values of the nomogram were estimated.

The preliminary nomograms and the associated validation data were presented for discussion with the MLW scientific community and the nomograms were revised following

comments. If the sensitivity and specificity of the nomogram were not adequate, the entire derivation process was repeated and the nomogram adjusted. Four validation loops were required to optimise the nomogram for acceptable performance (see section 5.5.3 in the results).

The final nomogram was selected on the basis of the performance in the validation exercise with the aim for the concordance index to improve or match the data from the Dutch score (discovery index 0.84, 95% CI 0.80-0.87, validation index 0.81, 95% CI 0.74-0.87). Multiple nomograms produced were compared against each other to produce the final optimised nomogram (summarised in Section 5.3.4). This nomogram was then put forward for further testing against BAM phase 2 data (Results Chapter 6 section 6.3.6).

5.2.5 Methods for dealing with missing data

5.2.5.1 Random imputation of all missing data

To increase the number of cases available in the discovery cohort and to account for missing data, five rounds of random imputation, as done on the Dutch meningitis score data (Weisfelt et al., 2008) were done by Dr Mukaka on the Malawi meningitis database prior to case selection for the discovery and validation cohorts. Identical univariate and multivariate regression analysis was performed on both the complete case data and on this larger imputed dataset and the results compared. Important differences, which resulted in changing of statistical significance of predictors were identified. The results of these comparisons can be found in section 5.3.2. When taken forward, these differences subsequently significantly changed the nomogram between the imputed dataset and the complete case dataset. Following review of these two datasets with Dr Mukaka and Dr Faragher, it was decided that the imputed dataset did not accurately represent the original cases as the predictors were altered by the imputation exercise, suggesting serious biases in the missing data. The table generated from the imputed dataset can be seen in Table 5.3. The complete case dataset

was used for the discovery exercise to generate the nomograms tested, and carefully selected management for individual missing data was done.

5.2.5.2 Testing for systematic biases

Following the finding that random imputation to the entire dataset altered the significance of several variables in the multivariate analysis, the database was interrogated for systematic biases in the missing data. Univariate analyses were done for each positive predictor variable using logistic regression against day 40 mortality using SPSS version 20. Data were grouped for each variable into quartiles around a median, with an additional option within that variable coded for missing data. Odds ratios for missing data and the relevant groupings within that variable were calculated and compared. Where the OR for the missing variable was significant at the 0.05 level, systematic bias was suspected. If the OR was not significant to that level, it was concluded that no systematic biases existed within that data.

5.2.5.3 Completing missing data in the final dataset

If a systematic bias in the data was found, the missing variable was then coded into the group where the nearest significant OR was seen. For example, if the OR for day 40 death for variable x to be missing was 2.5 (95% CI 1.3 : 4.6), and a variable grouping y had a matched OR within the same 95% CI, the missing data were re-coded as variable group y (Cohen, 1985).

Where significant numbers of missing data were identified within a variable, but no systematic bias in the missing data was identified, the missing data for that variable only were completed by 5 rounds of random imputation (Cohen, 1985). The random imputation was done in STATA by Dr Mukaka.

5.3 Results

5.3.1 Final univariate and multivariate analysis results on the discovery dataset

The results of the univariate and multivariate associations with day 40 mortality are presented in Table 5.1. Continuous variables with even distribution were presented as means with standard deviation, variables with skewed distribution were presented as medians with interquartile range. The following parameters were significantly association with mortality : GCS (univariate OR mortality 0.84 (95% CI 0.78 : 0.89), p<0.001), seizure episodes (1 seizure OR 1.66 (95% CI 1.01 : 2.72) p=0.043; 2 or more seizures OR 2.12 (95% CI 1.06 : 4.23) p=0.033), temperature (OR 1.2 (95% CI 1.03 : 1.42) p=0.02), pulse rate (OR 1.02 (95% CI 1.01 : 1.03) p<0.001), CSF WCC (OR 1.0 (95% CI 1.0 : 1.0) p<0.001) and Haemoglobin (OR 0.89 (95% CI 0.83 : 0.95) p=0.01). Other variables not significantly associated with mortality included age, gender, HIV status, out of hours admission, mean arterial blood pressure, blood glucose, and respiratory rate (Table 5.1). Oxygen saturations on pulse oximetry (SpO_2) were associated with mortality (OR 0.80, 95% CI 0.64 : 0.98, p=0.038) but the data were available for less than 50% of the total number of cases and could not be included in the multivariate model (Table 5.1).

The variables with significant associations with mortality (with the exception of SpO_2) were taken forward into the multivariate model, only GCS (OR 0.80 (95% CI 0.73 : 0.87) p<0.001), pulse (OR 1.01 (95% CI 1.00 : 1.03) p=0.02), CSF WCC (95% CI OR 1.0 (1.0 : 1.0) p<0.001), and Hb (OR 0.86 (95% CI 0.79 : 0.95) p=0.002) retained significance for association with death (Table 5.1). Interaction terms were run between variables that were might have been expected on clinical grounds to have been included in the multivariate model, particularly seizures. One interesting interaction found was between seizures and GCS. This led to loss of significance for seizures in the multivariate model due to the interaction with the more powerful effect of GCS

Table 5.1 Predictors of mortality in the MAMS discovery cohort

Univariate and multivariate predictors of poor outcome in the MAMS discovery cohort.

Parameter		Day 40		Univariate (unadjusted)		Multivariate (adjusted)**	
		Alive	Dead	Odds ratio (95% CI)	P	Odds ratio (95% CI)	p
Sample size		195	205				
Age	**mean (sd)**	31.8 (11.1)	33.9 (11.2)	1.01 (0.99 : 1.03)	**0.061**	1.00 (0.98 : 1.03)	0.71
	≤ 40 years	160	164	---	---		
	> 40 years	35	41	1.14 (0.69 : 1.88)	0.60	1.01 (0.56 : 1.81)	0.96
Gender	**male**	100	100	1.1 (0.74 : 1.64)	0.62	*	*
	female	95	105	…			
HIV status	**negative**	25	17	…		*	*
	positive	165	184	1.39 (0.36 : 5.27)	0.62		
	not known	5	4				
Out of hours admission	**no**	87	93	1.07 (0.70 : 1.62)	0.74	*	*
	yes	83	95	0.93 (0.62 : 1.41)	0.74		
	not known	20	22				
GCS	**mean (sd)**	11.9 (2.9)		0.84 (0.78 : 0.89)	**<0.001**	0.80 (0.73 : 0.87)	**<0.001**
	> 11	130	95	…			
	8 – 11	49	56	1.56 (0.98 : 2.49)	0.06	1.66 (0.97 : 2.82)	0.63
	<8	16	54	4.61 (2.5 : 8.5)	<0.001	5.19 (2.46 : 10.93)	<0.001
1 or more acute seizure episodes	**None**	146	128	…			
	One seizure	35	51	1.66 (1.01 : 2.72)	**0.043**	1.14 (0.64 : 2.04)	0.65
	Two seizures	14	26	2.12 (1.06 : 4.23)	**0.033**	1.0 (0.42 : 2.36)	1.0
Temperature °Celcius	**mean (sd)**	38.2 (1.2)	38.5 (1.3)	1.2 (1.03 : 1.42)	**0.02**	1.07 (0.85 : 1.36)	0.56
SpO_2 (%)	**mean (sd)**	95.6 (2.0)	93.9 (3.6)	0.80 (0.64 : 0.98)	**0.038**		

	>95	23	13	…			
	92 – 95	9	7	2.65 (0.63 : 11.1)	0.60	†	†
	<92	4	6	1.37 (0.41 : 4.56)	0.18	†	†
Pulse rate (beats/min)	**mean (sd)**	97.7 (18.4)	105.4 (19.2)	1.02 (1.01 : 1.03)	**<0.001**	1.01 (1.00 : 1.03)	**0.02**
	<100	114	91	…			
	100–120	29	36	1.55 (0.88 : 2.72)	0.123	1.51 (0.81 : 2.80)	0.19
	>120	32	57	2.23 (1.34 : 3.73)	**0.002**	1.90 (1.06 : 3.38)	**0.029**
MAP (mmHg)	**mean (sd)**	90.6 (13.1)	90.4 (16.4)	0.99 (0.98 : 1.01)	0.92	*	*
	<90	103	101	1.02 (0.53 : 1.98)	0.93		
	90-100	34	39	1.2 (0.56 : 2.55)	0.63		
	>100	36	44	1.28 (0.60 : 2.70)	0.51		
	not known	22	21	…			
Respiratory rate (breaths/min)	**mean (sd)**	23.0 (3.5)	23.1 (2.7)	1.01 (0.81 : 1.25)‡	0.91	†	†
	not known	180	184				
CSF White cell count (cells/mm^3)	**Median (IQR)**	1122 (288 : 3160)	395 (132 : 1040)	1.0 (1.0 : 1.0)	**<0.001**	1.0 (1.0 : 1.0)	**<0.001**
CSF culture positive for *S. pneumoniae*	**no**	90	93	---	---	---	---
	yes	104	111	1.03 (0.69 : 1.53)	0.87	0.475 (0.26 : 0.87)	**0.015**
	not known	0	2				
Plasma glucose (mmol/L)	**mean (sd)**	7.6 (2.8)	7.8 (4.2)	1.01 (0.96 : 1.08)	0.60	*	*
Haemoglobin (g/dL)	**mean (sd)**	11.4 (2.9)	10.4 (3.1)	0.89 (0.83 : 0.95)	**0.001**	0.86 (0.79 : 0.95)	**0.002**
	>14	35	22	..			
	11-14	69	62	1.43 (0.76 : 2.69)	0.260	1.16 (0.53 : 2.50)	0.71
	8-11	69	80	1.85 (0.98 : 3.43)	0.054	1.64 (0.77 : 3.84)	0.19
	5-8	19	33	2.76 (1.27 : 6.00)	0.010	2.35 (0.84 : 5.89)	0.067

† : proportion of missing data >50% excluded from MV analysis ‡ : odds ratio for a unit increase in predictor variable *: non-significant variable not put forward for multivariate analysis

The multivariate model was run for a second time including all variables tested by univariate analysis I, irrespective of significance levels (Table 5.2).

The multivariate predictors of mortality in this model were GCS (OR 0.80 (95% CI 0.73 : 0.88) p<0.001), CSF WCC (OR 1.0 (95% CI 1.00 : 1.00) p<0.001), and Haemoglobin (OR 0.87 (95% CI 0.79 : 0.97) p=0.008). CSF culture positivity for *S. pneumoniae* was associated with reduced chances of death compared to non-pneumococcal culture (OR 0.475 (95% CI 0.26 : 0.87) p=0.015), and pulse lost significance in this model (OR 1.01 95% CI (0.99 : 1.03) p=0.06). Individual ORs for continuous variables put into clinically relevant categories are also shown in Table 5.5.

Variables that were suspected of being important potential confounders, such as age, gender, HIV status and out of hours admission were not significantly associated with death in this model (Table 5.2), and no evidence of confounding was seen.

Table 5.2 Analysis of the discovery cohort, complete results

Multivariate analysis of the discovery data including all variables in the MV analysis, irrespective of univariate significance							
Parameter		**Day 40**		**Univariate (unadjusted)**		**Multivariate (adjusted)****	
		Alive	**Dead**	**Odds ratio (95% CI)**	**p**	**Odds ratio (95% CI)**	**p**
Sample size		195	205				
Age	**mean (sd)**	31.8 (11.1)	33.9 (11.2)	1.01 (0.99 : 1.03)	**0.061**	1.0 (0.98 : 1.03)	0.87
	≤ 40 years	160	164	---	---		
	> 40 years	35	41	1.14 (0.69 : 1.88)	0.60	0.75 (0.25 : 2.22)	0.60
Gender	**male**	100	100	1.1 (0.74 : 1.64)	0.62	0.86 (0.50 : 1.48)	0.59
	female	95	105	…			
HIV status	**negative**	25	17	…			
	positive	165	184	1.39 (0.36 : 5.27)	0.62	6.67 (0.63 : 70.3)	0.11
	not known	5	4				
Out of hours admission	**no**	87	93	1.07 (0.70 : 1.62)	0.74		
	yes	83	95	0.93 (0.62 : 1.41)	0.74	1.04 (0.61 : 1.77)	0.88
	not known	20	22				
GCS	**mean (sd)**	11.9 (2.9)	10.0 (3.5)	0.84 (0.78 : 0.89)	**<0.001**	0.80 (0.73 : 0.88)	**<0.001**
	> 11	130	95	…			
	8 – 11	49	56	1.56 (0.98 : 2.49)	0.06	1.67 (0.93 : 3.05)	0.08
	<8	16	54	4.61 (2.5 : 8.5)	<0.001	4.56 (1.97 : 10.58)	**<0.001**
	not known	0	0	…			
1 or more acute seizure episodes	**None**	146	128	…			
	One seizure	35	51	1.66 (1.01 : 2.72)	**0.043**	1.15 (0.6 : 2.19)	0.66
	Two seizures	14	26	2.12 (1.06 : 4.23)	0.033	0.92 (0.35 : 2.50)	0.88

Temperature	**mean (sd)**	**38.2 (1.2)**	**38.5 (1.3)**	**1.2 (1.03 : 1.42)**	**0.02**	**1.04 (0.78 : 1.39)**	**0.75**
SpO_2 (%)	**mean (sd)**	95.6 (2.0)	93.9 (3.6)	0.80 (0.64 : 0.98)	**0.038**	†	†
	>95	23	13	…			
	92 – 95	9	7	2.65 (0.63 : 11.1)	0.60	†	†
	<92	4	6	1.37 (0.41 : 4.56)	0.18	†	†
	not known						
Pulse rate (beats/min)	**mean (sd)**	97.7 (18.4)	105.4 (19.2)	1.02 (1.01 : 1.03)	**<0.001**	1.01 (0.99 : 1.03)	**0.06**
	<100	114	91	…			
	100–120	29	36	1.55 (0.88 : 2.72)	0.123	1.43 (0.74 : 2.80)	
	>120	32	57	2.23 (1.34 : 3.73)	**0.002**	2.43 (1.24 : 4.80)	**0.01**
	not known						
MAP (mmHg)	**mean (sd)**	90.6 (13.1)	90.4 (16.4)	0.99 (0.98 : 1.01)	0.92	1.0 (0.98 : 1.02)	0.96
	<90	103	101	1.02 (0.53 : 1.98)	0.93	0.73 (0.36 : 1.45)	0.26
	90-100	34	39	1.2 (0.56 : 2.55)	0.63	0.62 (0.28 : 1.41)	0.36
	>100	36	44	1.28 (0.60 : 2.70)	0.51		…
	not known	22	21	…			
Respiratory rate (breaths/min)	**mean (sd)**	23.0 (3.5)	23.1 (2.7)	1.01 (0.81 : 1.25)‡	0.91	†	†
	not known	180	184				
CSF White cell count ($cells/mm^3$)	**Median (IQR)**	1122 (288 : 3160)	395 (132 : 1040)	1.0 (1.0 : 1.0)	**<0.001**	1.0 (1.00 : 1.00)	**<0.001**
CSF culture positive for *S.pneumoniae*	**no**	90	93	---	---	---	---
	yes	104	111	1.03 (0.69 : 1.53)	0.87	0.475 (0.26 : 0.87)	**0.015**
	not known	0	2				
Plasma glucose (mmol/L)	**mean (sd)**	7.6 (2.8)	7.8 (4.2)	1.01 (0.96 : 1.08)	0.60	1.01 (0.93 : 1.10)	0.73

Haemoglobin (g/dL)	**mean (sd)**	**11.4 (2.9)**	**10.4 (3.1)**	**0.89 (0.83 : 0.95)**	**0.001**	**0.87 (0.79 : 0.97)**	**0.008**
	>14	35	22	..			
	11-14	69	62	1.43 (0.76 : 2.69)	0.260	1.07 0.86 : 2.49)	0.88
	8-11	69	80	1.85 (0.98 : 3.43)	0.054	1.60 (0.71 : 3.61)	0.26
	5-8	19	33	2.76 (1.27 : 6.00)	0.010	1.85 (0.68 : 5.04)	0.23
	<5	3	8	4.2 (1.01 : 17.73)	0.048	5.75 (0.88 : 37.8)	0.07
	not known	0	0				

† : proportion of missing data >50% so variable excluded from multivariate analysis

‡ : odds ratio for a unit increase in predictor variable

5.3.2 Missing data

The missing data analysis was undertaken only in variables with adequate numbers for inclusion in the multivariate analysis and significant association with day 40 mortality. Missing outcome data were found in the Malawi meningitis dataset used to derive the discovery data (n=598 cases, 543 with day 40 mortality data available), the 55 cases with missing outcome data were excluded. From this dataset, 400 complete cases (cc400) had been randomly selected for discovery, with remaining 143 cases potentially to be used for validation.

Firstly, 5 rounds of random imputation were done in STATA on the n 543 dataset to complete the missing data for the predictive variables. Only variables that were predictive of poor outcome on multivariate analysis, and where >50% of data were present were included in the imputation exercise. The univariate and multivariate analysis performed on the cc400 dataset as detailed above was repeated on the n543 imputed dataset. The results are presented in Table 5.3 and are highlighted in red font.

Important discrepancies were identified between the cc400 and the imputed n543 dataset. These included increasing significance in the imputed data for Hb between 5-8 g/dL group (OR 2.82 (95% CI 1.02 : 7.82) p=0.046 imputed compared to 1.85 (95% CI 0.68 : 5.04) p=0.21 in the cc400 data), and an increasing weight for GCS <8/15 in the imputed data (OR 7.94 (95% CI 4.02 : 15.68) p<0.001 compared to OR 4.56 (95% CI 1.97 : 10.58) p<0.001 in the cc400 data). These significant differences suggest that serious systematic biases are present in the missing data and imputation of the missing data leads to different results in the analysis of the imputed data compared to the complete case data. The imputed dataset was discarded and the missing data management strategy was revised. The concern that the imputation exercise had failed due to systematic bias in the missing data led to a further analysis of the database for systematic biases in the missing data.

Table 5.3 Analysis of the discovery data after random imputation

Multivariate analysis of discovery data showing pooled results of 5 rounds of random imputation in the n=543 dataset							
Parameter		**Day 40**		**Univariate (unadjusted)**		**Multivariate (adjusted)****	
		Alive	**Dead**	**Odds ratio (95% CI)**	**p**	**Odds ratio (95% CI)**	**p**
Sample size		187	213	…			
Age	**mean (sd)**	30.9	33.7	1.03 (1.01 : 1.04)	**0.01**	1.02 (0.99 : 1.04)	0.099
	≤ 40 years	158	168	…	…	…	…
	> 40 years	29	45	1.46 (0.87 : 2.44)	0.15	1.50 (0.80 : 2.80)	0.21
Gender	**male**	87	108	1.18 (0.80 : 1.75)	0.40	*	Δ
	female	100	105	0.84 (0.57 : 1.25)	0.40		
HIV status	**negative**	23	16	…			
	positive	164	197	1.72 (0.88 : 3.78)	0.11	0.71 (0.32 : 1.57)	0.40
	not known	0	0	…			
Out of hours admission	**no**	86	98	…			
	yes	84	96	1.00 (0.66 : 1.51)	0.98	1.07 (0.66 : 1.73)	0.78
	not known	18	18	…			
GCS	**mean (sd)**	11.9	9.8	0.80 (075 : 0.86)	**<0.001**	0.76 (0.69 : 0.83)	<0.001
	> 11	113	67	…			
	8 – 11	49	70	2.40 (1.50 : 3.87)	**<0.001**	3.17 (1.80 : 5.57)	**<0.001**
	<8	25	76	5.13 (2.98 : 8.83)	**<0.001**	**7.94 (4.02 : 15.68)**	**<0.001**
	not known	0	0				
1 or more acute seizure episodes	**None**	138	132	…			
	One seizure	34	45	1.38 (0.84 : 2.29)	0.20	**0.94 (0.50 : 1.74)**	0.83
	Two + seizures	15	36	2.5 (1.31 : 4.80)	**0.005**	1.62 (0.75 : 3.50)	0.22

		Alive	Dead	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
SpO_2 (%)	mean (sd)	95.5	92.3	0.88 (0.76 : 1.03)	0.11 (0.08 t-test)		
	>95	18	18				
	92 – 95	10	9	0.90 (0.30 : 2.74)	0.85	†	†
	<92	5	9	1.80 (0.50 : 6.43)	0.36	†	†
	not known						
Pulse rate (beats/min)	**mean (sd)**	97.9	106.0	1.03 (1.01 : 1.04)	**<0.001**	1.03 (1.01 : 1.04)	0.001
	<100	122	99	…			
	100–120	49	69	1.74 (1.10 : 2.73)	**0.017**	**1.71 (0.99 : 2.95)**	**0.051**
	>120	16	45	3.47 (1.85 : 6.50)	**<0.001**	4.14 (1.85 : 9.28)	0.001
	not known	0	0	…			
MAP (mmHg)	**mean (sd)**	89.8 (13)	89.3 (16)	0.99 (0.98 : 1.11)	0.71	*	*
	<90	99	111	0.89 (0.53 : 1.52)	0.67		
	90-100	37	37	1.12 (0.65 : 1.93)	0.66	*	*
	>100	31	39	1.16 (0.61 : 2.20)	0.65		
	not known	20	26				
Respiratory rate (breaths/min)	**mean (sd)**	24.4 (4.4)	26.3 (7.5)	1.05 (0.97 : 1.15)	0.23	†	†
	not known						
CSF culture positive for *S.pneumoniae*	**no**	72	104	…		---	---
	yes	114	108	0.65 (0.44 : 0.98)	**0.04**	**0.28 (0.16 : 0.48)**	**<0.001**
	not known	0	2				
Plasma glucose (mmol/L)	**mean (sd)**	9.5 (10.8)	10.2 (12.9)	1.00 (0.99 : 1.03)	0.56	*	*

		Alive	Dead	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Haemoglobin (g/dL)	mean (sd)	11.4 (2.7)	10.6 (2.9)	0.91 (0.85 : 0.98)	0.008	0.88 (0.80 : 0.96)	0.004
	>14	30	25				
	11-14	71	66	1.12 (0.60 : 2.09)	0.73	1.12 (0.52 (2.34)	0.79
	8-11	71	88	1.49 (0.80 : 2.75)	0.20	2.13 (1.01 : 4.49)	**0.046**
	5-8	11	27	2.95 (1.22 : 7.10)	**0.016**	**2.82 (1.02 : 7.82)**	**0.046**
	<5	4	7	2.1 (0.55 : 8.0)	0.27	4.10 (0.82 : 20.5)	0.085
	not known	0	0				

† : proportion of missing data >50% so variable excluded from multivariate analysis ‡ : odds ratio for a unit increase in predictor variable

*: non -significant variable not used in the scoring system and excluded from multivariate analysis Red font = data statistically different in these imputed data to those generated by the non-imputed data analysis

5.3.2.1 Analysis for systematic biases in the missing data

The systematic bias analysis as detailed in section 2.7.2 demonstrated very few missing data points for GCS, CSF WCC and CSF culture (<1%) and there were no systematic biases in the missing data for these variables. Due to the small number of missing data points in these variables, random imputation was not done. Missing data for pulse were noted in both the discovery data (n=400, missing data n=41) and the Malawi meningitis database (n=543, missing data n=58). Missing pulse was equally distributed between survivors and non survivors in both datasets, (OR death in the Malawi meningitis database was 1.03, 95% CI 0.59 : 1.79, p=0.91). Due to the large proportion of missing data and the lack of systematic bias present, the pulse variable only was subject to 5 rounds of random imputation to complete the missing data to make adequate numbers for both discovery and validation.

Significant missing data were also found for haemoglobin. In the larger Malawi meningitis database, a value for Hb was missing in 44 cases. The OR of missing Hb with day 40 mortality was 2.18 (95% CI 1.07 : 4.41) p=0.03. Table 5.4 shows the OR for death by Hb grouped into quartiles around the median.

Table 5.4 Haemoglobin and outcome in bacterial meningitis

Univariate associations with mortality for different groups of haemoglobin (g/dL) in the Malawi meningitis database.

N598 (543 with d40 available)

Grouping	Alive 255	Dead 288	OR (95% CI)	P value
Missing	17	27	2.18 (1.07 : 4.41)	0.031
<8.9	53	86	2.22 (1.53 : 3.66)	0.002
8.9-10.6	54	64	1.62 (0.97 : 2.71)	0.062
10.6-12.6	61	60	1.35 (0.81 : 2.24)	0.246
>12.6	70	51	Reference	

Missing Hb had almost identical OR for mortality as Hb below the 25th centile (OR 2.22, 95% CI 1.53 : 3.66) and therefore to take account of this systematic bias in the data, all missing Hb were recoded as =<8.9g/dL. Multivariate analysis was undertaken on the new dataset with imputed pulse and missing Hb recoded as <8.9, and no further biases were found. As a result of this analysis, the complete case data (n400) were derived from this dataset to be used for nomogram discovery, and the remaining complete cases were added as outlined to BAM Phase 1 patient data for validation.

Table 5.5 gives the comparison of the discovery and validation data used in the generation of the final MAMS nomogram. Univariate comparisons were made using a Student's T-test of means for normally distributed continuous variables, a Mann-Whitney U test for non-normally distributed continuous variables, and a Fisher exact test for categorical variables. Fisher exact test was used to optimise precision. Where significant differences were seen on univariate analysis between the two cohorts, a multivariate analysis model was undertaken using binary logistic regression, backwards LR mode (methods Chapter 3 section 3.2.3). Only respiratory rate retained significance after correction for the other significant variables in this model.

Table 5.5 Analysis of variables between discovery and validation cohorts

Comparison of tested variables between discovery and validation

Variable		Discovery N=400	Validation N=193	Significance
Mean Age (std)		32.9 (11.1)	31.8 (10)	0.25
Female Gender		200 (50%)	108 (52.2)	0.61
HIV co-infection		349/391 (87%)	157/193 (81%)	**0.002**
Out of hours		180/358 (50.3%)	83/190 (43%)	0.17
Day 10 mortality		164/400 (41%)	103 (49%)	**0.04**
Day 40 mortality rate		205/400 (51%)	115/193 (59%)	0.065
Mean GCS (std)		10.9 (3.3)	10.9 (3.4)	0.86
Acute seizures	One	86 (21.5%)	29/151 (19.2)	0.54
	Two or more	40 (10%)	15/151 (10%)	0.90
Mean Temp (°C) (Std)		38.3 (1.2)	38.3 (1.1)	0.91
Mean SpO_2(%) (Std)		94.4 (2.9)	94.6 (5.5)	0.71
Mean Pulse (beats/min) (Std)		101 (19.2)	102 (20.5)	0.64
Mean MAP (mmHg) (Std)		90.5 (15.3)	89.7 (17.3)	0.60
Mean RR (breaths/min) (Std)		23.9 (3.2)	26.6 (8.1)	**0.01**
Median CSF WCC (cells/mm^3) (IQR)		545 (170 – 2000)	452 (211 – 1545)	0.42
CSF culture		Negative 153 (38%) SpN 215 (53.8%) NM 13 (3.3%) Hib 3 (0.8%) Gram negative 8 (2%) Other 6 (1.5%)	Negative 70 (33%) SpN 110 (53.1%) NM 4 (1.9%) Hib 0 (0%) Gram neg 12 (5.8%) Other 11 (5.3%)	Reference 0.51 0.50 0.99 **0.013** **0.009**
Mean Plasma glucose (mmol/L) (Std)		7.7 (3.6)	7.2 (2.7)	0.12
Mean Hb (g/dL) (Std)		10.7 (2.7)	10.4 (2.7)	0.22

5.3.3 Summary of validation loop processes

5.3.1.1 Validation of all variables compared with significant variables only

Two nomograms were synthesised from the original cc400 discovery cohort of non-imputed data and tested against the complete case validation database, prior to any imputation or missing data analysis. The first (Figure 5.2a) included only variables that were significant on the multivariate analysis (including pneumococcal culture that was significant on MV but not UV analysis).

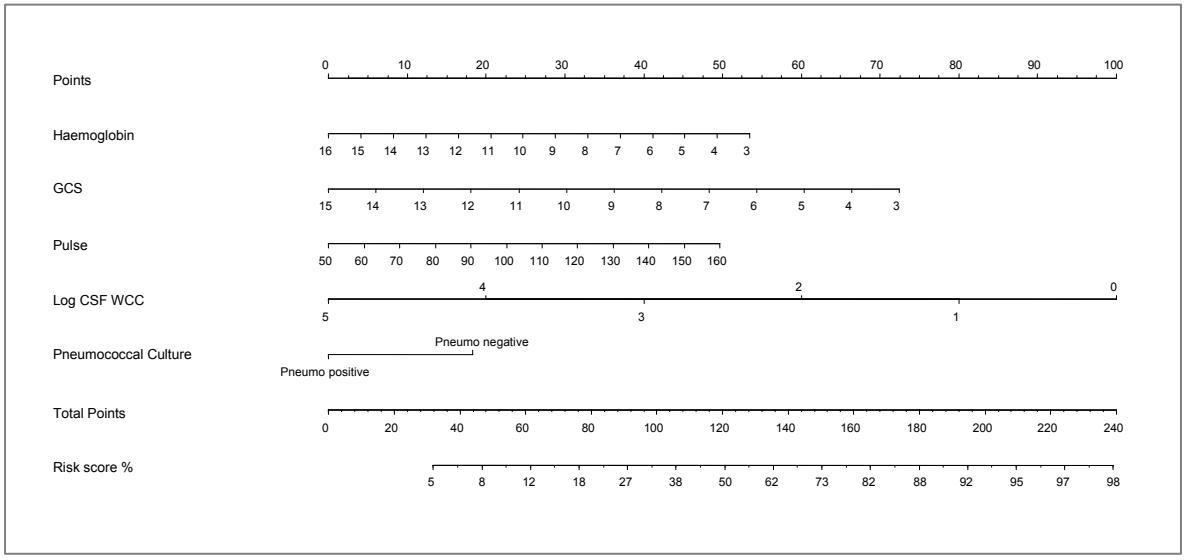


Figure 5.2.a

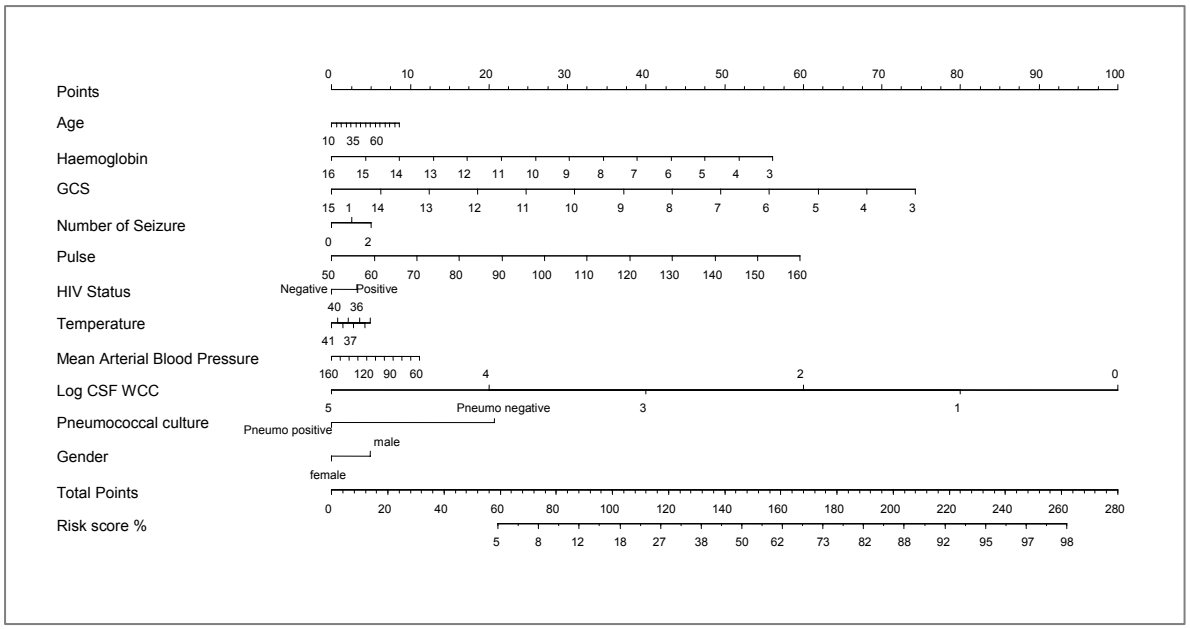


Figure 5.2 Preliminary nomograms from original complete case data

Preliminary nomograms synthesised prior to any missing data or random imputation analyses. Figure 5.2.a contains only variables with statistically significant association with negative outcome at day 40, Figure 5.2.b contains all variables tested. All units are identical to those represented in the tables

The second Figure 5.2b included all variables tested in the regression analyses irrespective of significance. For both nomograms, only 79 cases in the validation database had complete case data to validate all the variables included in the nomogram, with no imputation of missing data. Validation data for these nomograms were as follows: ROC Area under the curve (AUC) all included variables 0.77 (95%CI 0.66 - 0.88), significant variables only 0.75 (0.65 - 0.85) (Figure 5.3).

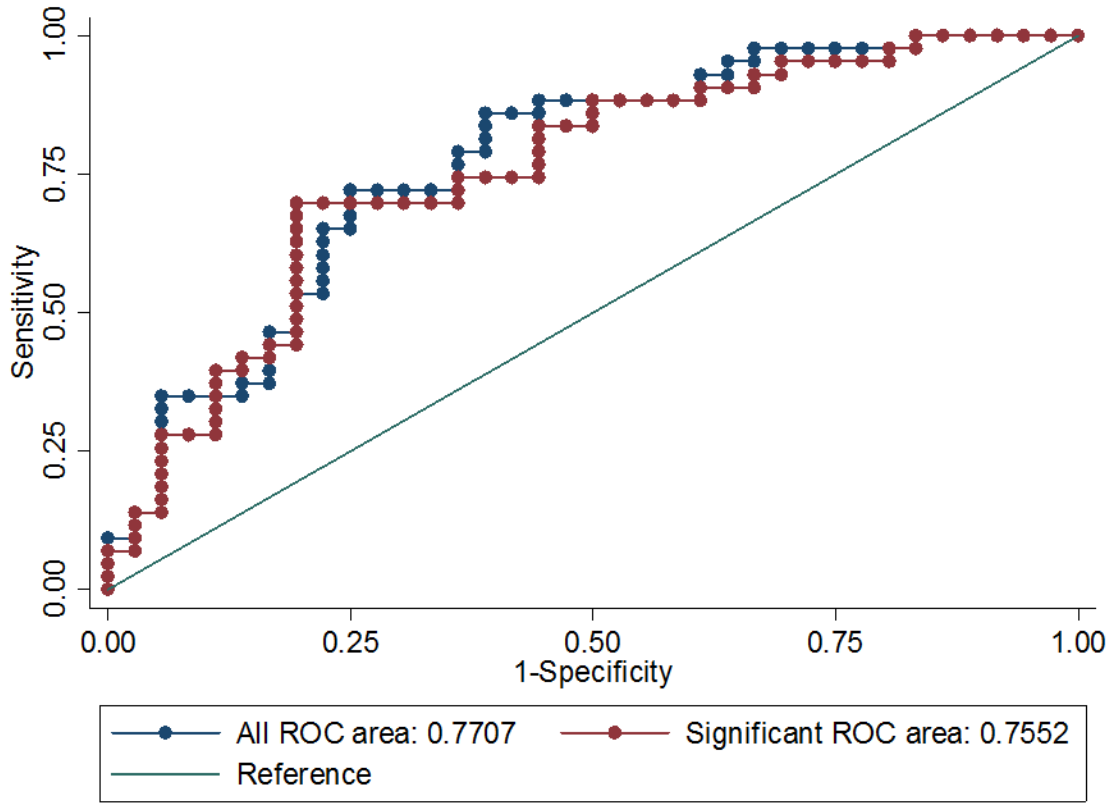


Figure 5.3 Receiver Operator Curve (ROC) comparing predictive power of both original nomograms

When the two nomograms were evaluated against the true outcome, the significant variables nomogram was more accurate, with an agreement of 37.5% (against a random agreement of 7.81% p<0.001, Kappa 0.32), (Table 5.6) compared to the all variables nomogram with an agreement of 12.5% (against a random agreement of 9.38% p=0.37, Kappa 0.034) (Table 5.7). As the all variables nomogram was clearly inferior, only the significant variables nomogram was taken forward to the validation step.

Table 5.6 MAMS agreement data for initial nomogram containing significant variables only

Summary of agreement data between observed and predicted deaths by MAMS in the original variables only with statistical significance with outcome nomogram			
% Risk of death	**Total number of participants**	**Observed Deaths (%)**	**Deaths predicted by a Nomogram**
≤10	0	-	-
11-20	6	0 (0)	1 (17)
21-30	8	2 (25)	2 (25)
31-40	0	-	-
41-50	35	12 (34)	14 (40)
51-60	15	6 (40)	8 (53)
61-70	14	9 (64)	9 (64)
71-80	10	6 (60)	7 (70)
81-90	11	9 (82)	9 (82)
90-10	5	4 (80)	5 (100)
Total	104	48 (46)	55 (53)

Table 5.7 MAMS agreement data for initial nomogram containing all tested variables

Summary of agreement data between observed and predicted deaths by MAMS in the original all included variables nomogram			
Risk score %	**Total number of participants**	**Observed Deaths (%)**	**Deaths predicted by a Nomogram**
≤10	0	-	-
11-20	6	0 (0)	1 (17%)
21-30	4	1 (25)	1 (25)
31-40	-	-	-
41-50	22	8 (36)	9 (41)
51-60	15	6 (40)	8 (53)
61-70	14	12 (86)	9 (64)
71-80	7	3 (43)	5 (71)
81-90	10	8 (80)	9 (90)
90-10	6	5 (83)	6 (100)
Total	84	43 (51)	48 (57)

However, as only complete case data without imputation had been used for the validation exercise for these nomograms, the validation data were too few to produce accurate validation and there were concerns that substantial missing data (>50% (79/193 cases) were likely to have a major influence on the power of the nomogram. The missing data analysis detailed in Section 5.3.2 was undertaken.

5.3.3.2 Significant variables nomogram after the missing data analysis

The validation exercise for the significant variables nomogram was repeated after the missing data exercise as detailed in section 5.3.2 (Figure 5.4). Using data with missing Hb coded as <8.9 g/dL and pulse imputed increased the validation dataset from 79 cases to 193 cases.

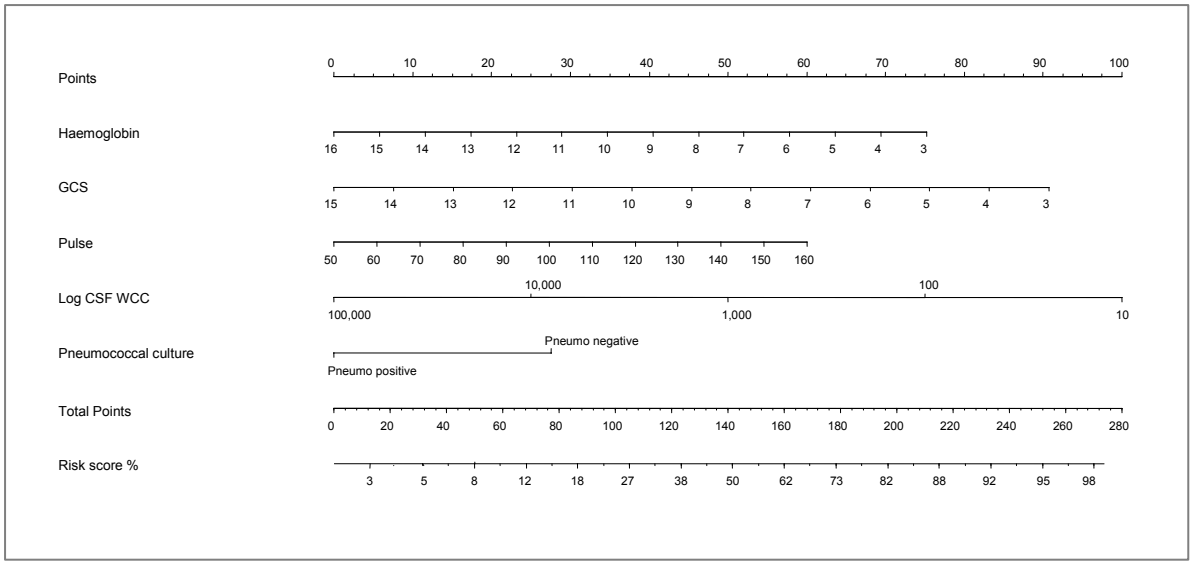


Figure 5.4 Nomogram using significant variables, post missing data completion

The significant variables nomogram validated well after completion of the missing data, with AUC of 0.74 (95% CI 0.67 : 0.82) (Figure 5.5), and agreement of 62.5% compared to random agreement of 14.1 ($p<0.001$) and Kappa of 0.6 (Table 5.8).

Sensitivity and specificity of this nomogram were calculated by Dr Mukaka. Sensitivity was74% and specificity was 55%.

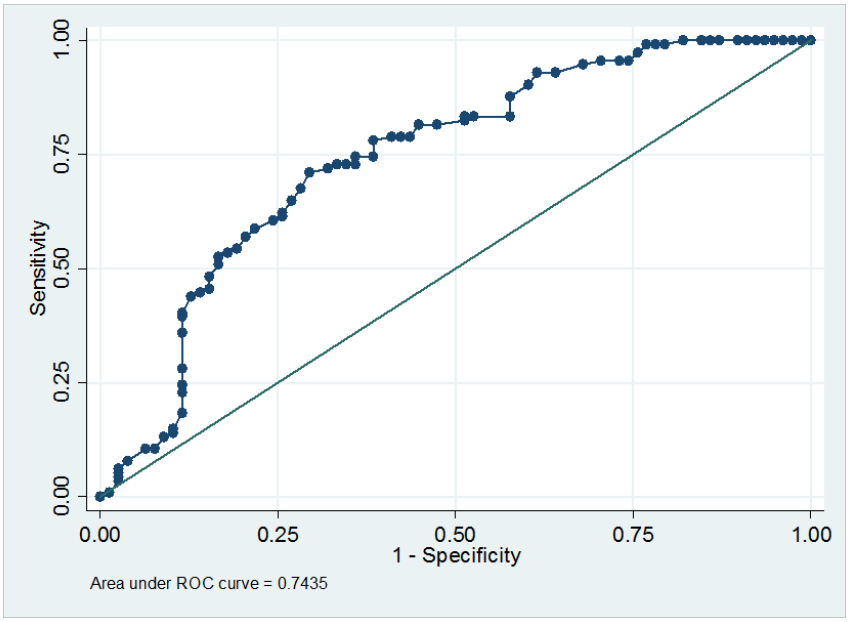


Figure 5.5 ROC for the nomogram in Figure 5.4

Table 5.8 Summary of MAMS agreement data after missing data analysis

Summary of agreement data between observed and predicted deaths by MAMS in the significant variables only nomogram after completion of the missing data			
Risk score %	**Total number of participants**	**Observed Deaths (%)**	**Deaths predicted by a Nomogram**
≤10	2	0(0)	0(0)
11-20	4	0(0)	1(25)
21-30	11	1(9)	3(27)
31-40	-	-	-
41-50	47	20(43)	20(43)
51-60	19	10(53)	10(53)
61-70	28	18(64)	18(64)
71-80	27	20(74)	20(74)
81-100	54	45(83)	48(89)
Total	192	114(59.3)	120(62.5)

5.3.3.3 Continuous compared to categorical variables

The first nomograms contained all continuous variables represented as ordinal data. We subsequently explored the effect of categorising these variables, first into clinically relevant groupings derived from standardised clinical definitions (e.g. GCS <11 = altered mental status, GCS <8 = coma, Section 5.2.3), and then into quartiles around the median. The multivariate analysis was run on the different groupings and the OR's of associations with mortality compared between the clinically relevant and quartile groupings. These data can be found in the following: Table 5.9, Table 5.10, Table 5.11, Table 5.12.

Table 5.9 Analysis of Haemoglobin with outcome by group category

Comparison of Haemoglobin association with mortality when categorised into clinical or statistical groups										
Variable		**Cc400**				**N 543 with d40 available**				
	grouping	**Alive 195**	**Dead 205**	**OR (95% CI)**	**P value**	**grouping**	**Alive 255**	**Dead 288**	**OR (95% CI)**	**P value**
Hb classical	**Missing**	0	0			**Missing**	17	27	2.56 (1.17 : 5.59)	0.018
	<5	3	8	4.24 (1.05 : 17.5)	0.048	**<5**	4	10	4.03 (1.14 : 14.2)	0.030
	5-8	19	33	2.76 (1.27 : 6.01)	0.01	**5-8**	24	42	2.82 (1.40 : 5.69)	0.004
	8-11	69	80	1.84 (0.98 : 3.43)	0.54	**8-11**	95	108	1.83 (1.04 : 3.32)	0.034
	11-14	69	62	1.43 (0.75 : 2.69)	0.27	**11-14**	73	75	1.66 (0.92 : 2.98)	0.090
	>14	35	22			**>14**	42	26		
Hb quartiles	**Missing**	0	0			**Missing**	17	27	2.18 (1.07 : 4.41)	0.031
25th centile	**<8.9**	37	68	2.49 (1.41 : 4.38)	0.002	**<8.9**	53	86	2.22 (1.53 : 3.66)	0.002
25-50th	**8.9-10.8**	49	50	1.38 (0.79 : 2.42)	0.255	**8.9-10.6**	54	64	1.62 (0.97 : 2.71)	0.062
50-75th	**10.81-12.6**	52	45	1.17 (0.66 : 2.06)	0.576	**10.6-12.6**	61	60	1.35 (0.81 : 2.24)	0.246
75th centile	**>12.6**	57	42			**>12.6**	70	51		
Hb cut off <9.0	**Missing**	0	0			**Missing**	17	27	1.68 (0.89 : 3.20)	0.10
	<9.0	38	69	2.09 (1.33 : 3.31)	0.002	**<9.0**	54	88	1.73 (1.16 : 2.57)	0.007
	>9.0	157	136			**>9.0**	184	173		

Table 5.10 Analysis of GCS and outcome by group category

Comparison of Glasgow Coma Score and association with mortality when categorised into clinical or statistical groups										
Variable		**Cc400**					**N598 (543)**			
	grouping	**Alive 195**	**Dead 205**	**OR death (95% CI)**	**p**	**grouping**	**Alive 255**	**Dead 288**	**OR death (95% CI)**	**P**
Median GCS (IQR)		13 (10-15)	10 (7-13)	0.83 (0.78 : 0.89)	<0.001		13 (10-15)	10 (7-13)	0.81 (0.77 : 0.86)	<0.001
GCS Classical	**Missing**	0	0			**Missing**	4	8	3.12 (0.91 : 10.63)	0.069
	<8	16	54	4.61 (2.49 : 8.46)	<0.001	**<8**	34	97	4.45 (2.79 : 7.08)	<0.001
	8-11	49	56	1.56 (0.98 : 2.49)	0.06	**8-11**	61	83	2.12 (1.42 : 3.21)	<0.001
	>11	130	95			**>11**	156	100		
GCS quartiles	**Missing**	0	0			**Missing**	4	8	3.89 (1.10 : 13.75)	0.034
	<9	47	90	3.76 (2.13 : 6.65)	<0.001	**<9**	55	127	4.50 (2.73 : 4.71)	<0.001
	9-11	31	37	2.34 (1.22 : 2.51)	0.011	**9-11**	40	53	2.58 (1.47 : 4.53)	0.001
	11-14	60	49	1.60 (0.89 : 2.88)	0.113	**11-14**	80	61	1.48 (0.89 : 2.47)	0.128
	>14	57	29			**>14**	76	38		
GCS cut off <9.0	**Missing**	0	0			**Missing**	4	8	2.56 (0.75 : 8.60)	0.13
	<9.0	47	90	2.46 (1.60 : 3.78)	<0.001	**<9.0**	55	127	2.95 (2.01 : 4.32)	<0.001
	>9.0	148	115			**>9.0**	196	153		
GCS cut off <11	**Missing**	0	0			**Missing**	4	8	3.12 (0.91 : 10.63)	0.069
	<11	78	127	2.44 (1.63 : 3.65)	<0.001	**<11**	95	180	2.95 (2.07 : 4.20)	
	>11	117	78			**>11**	156	100		

Table 5.11 Analysis of CSF WCC and outcome by group category

Variable		Cc400					N543			
	Grouping	Alive 195	Dead 205	OR death (95% CI)	P value	Grouping	Alive 255	Dead 288	OR death (95% CI)	P value
CSF WCC prev validated	**Missing**	1	0	0.00 (0.0 : 0.0)	1.0	**Missing**	1	0	0.00 (0.0 : 0.0)	1.0
	<1000	94	149	2.83 (1.86 : 4.29)	<0.001	**<1000**	130	205	2.35 (1.63 : 3.35)	<0.001
	>1000	100	56			**>1000**	124	83		
CSF WCC median (IQR)		1122 (288 - 3160)	395 (132 - 1040)	1.00 (1.00 : 1.00)	<0.001		960 (280 – 2915)	405 (140-1131)	1.0 (1.0 : 1.0)	0.094 LR <0.001 on M-W-U test
CSF WCC quartiles	**Missing**	1	0	0.00 (0.0 : 0.0)	1.0	**Missing**	1	0	0.00 (0.0 : 0.0)	1.0
	<170	34	66	4.61 (2.53 : 8.4)	<0.001	**<155**	44	78	3.26 (1.96 : 5.40)	<0.001
	170-545	45	55	2.91 (1.62 : 5.22)	<0.001	**155-480**	58	82	2.60 (1.60 : 4.21)	<0.001
	545-2000	46	55	2.84 (1.58 : 5.10)	<0.001	**480-1680**	60	78	2.39 (1.47 : 3.87)	<0.001
	>2000	69	29			**>1680**	92	50		
CSF WCC Log 10	**Missing**	**1**	0	0.00 (0.0 : 0.0)	1.0	**Missing**			0.00 (0.0 : 0.0)	1.0
	<10	**2**	9	9.0 (1.46 : 55.47)	0.018	**<10**	**5**	15	6.85 (1.78 : 26.3)	0.005
	10-100	**14**	32	4.57 (1.42 : 14.64)	0.010	**10-100**	**21**	44	4.78 (1.71 : 13.4)	0.003
	1e2-1e3	**78**	108	2.76 (0.99 : 7.69)	0.051	**1e2-1e3**	**104**	106	3.20 (1.27 : 8.0)	0.013
	1e3-1e4	**88**	50	1.13 (0.40 : 3.21)	0.81	**1e3-1e4**	**108**	76	1.60 (0.63 : 4.09)	0.31
	1e4-1e5	**12**	6			**1e4-1e5**	**16**	7		

Table 5.12 Analysis of pulse rate with outcome by group category

Comparison of pulse rate (beats per minute) and association with mortality when categorised into clinical or statistical groups										
		Cc400					N598 (543)			
	Grouping	Alive 195	Dead 205	OR death (95% CI)	P value	Grouping	Alive 255	Dead 288	OR death (95% CI)	P value
Pulse previously validated	**Missing**	20	21	1.12 (0.58 : 2.15)	0.72	**Missing**	28	30	1.03 (0.59 : 1.79)	0.91
	Pulse <120	155	145			**Pulse <120**	201	208		
	Pulse >120	20	39	2.08 (1.16 : 3.74)	0.014	**Pulse >120**	26	50	1.85 (1.11 : 3.10)	0.018
Pulse Median		98 (88-108)	102 (90 – 120)	1.02 (1.01 : 1.03)	0.004		98 (88-110)	100 (90-120)	1.01 (1.00 : 1.02)	<0.0001
Pulse Classical	**Missing**	20	21	0.99 (0.52 : 1.90)	0.99	**Missing**	28	30	1.19 (0.67 : 2.10)	0.53
	<100	95	74			**<100**	145	130		
	100-120	48	53	1.55 (0.88 : 2.72)	0.123	**100-120**	56	78	1.55 (1.02 : 2.35)	0.038
	>120	32	57	2.23 (1.33 : 3.72)	0.002	**>120**	26	50	2.14 (1.26 : 3.64)	0.005
Pulse quartiles	**Missing**	20	21	1.41 (0.68 : 2.93)	0.34	**Missing**	28	30	1.28 (0.69 : 2.37)	0.43
	<88	58	43			**<88**	73	61		
	88-100	56	48	1.15 (0.66 : 2.0)	0.61	**88-100**	72	69	1.14 (0.71 : 1.84)	0.57
	100-118	29	36	1.67 (0.89 : 3.13)	0.12	**100-118**	38	55	1.73 (1.01 : 2.95)	0.044
	>118	32	57	2.4 (1.33 : 4.31)	0.003	**>118**	44	73	1.98 (1.19 : 3.29)	0.008

In summary the OR for the quartile groupings were more powerful than that using the clinically relevant groups (Tables 5.9-12), and a nomogram was synthesised that included all variables categorised into these groups (Figure 5.6).

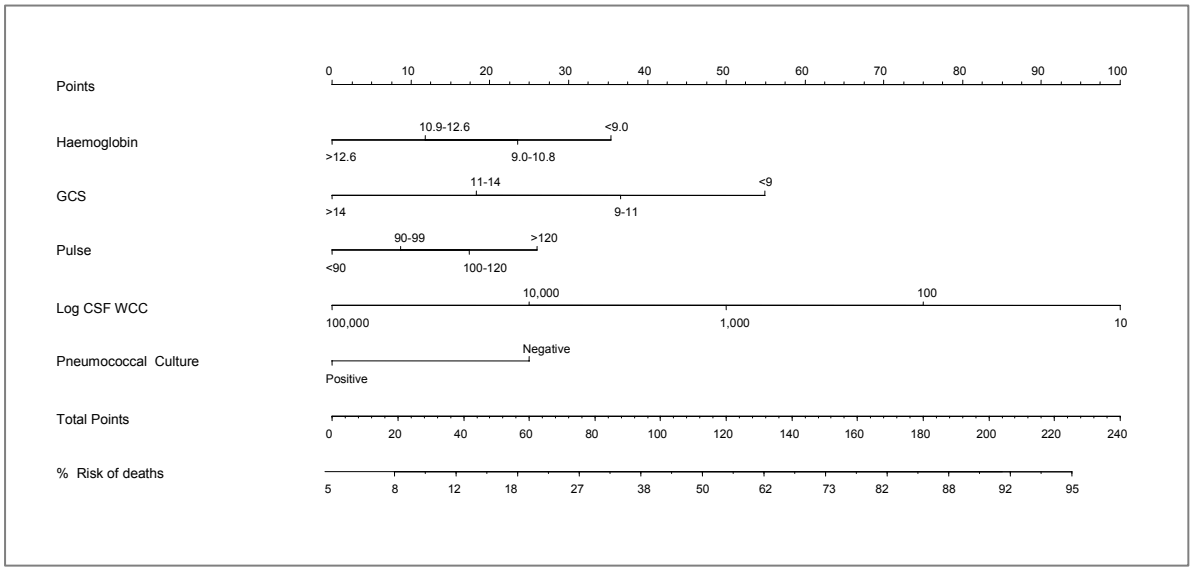


Figure 5.6 Nomogram for significant variables, categorised into quartiles

The validation data for this nomogram was as follows: AUC 0.69 (0.62 : 0.78), with poor associated agreement (Figure 5.7, Table 5.13).

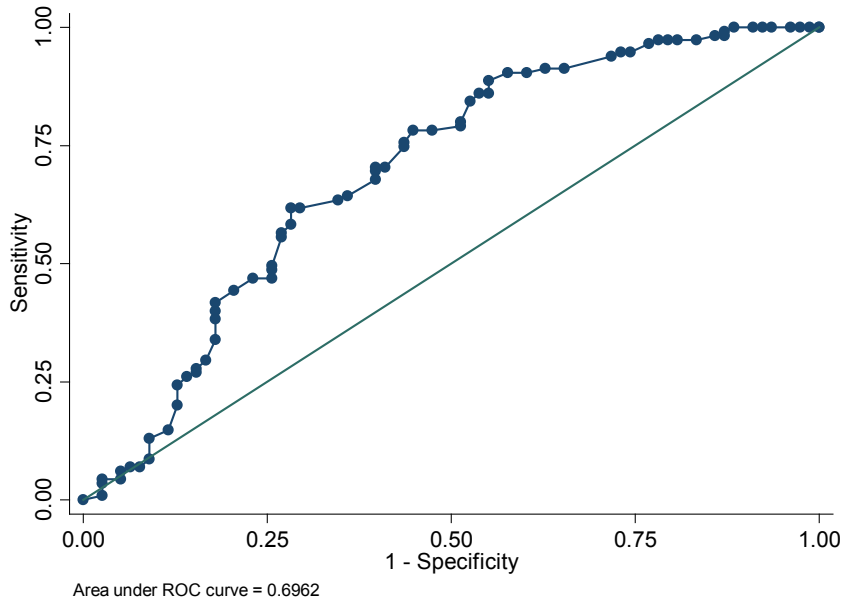


Figure 5.7 ROC for agreement of the nomogram in Figure 5.6

Table 5.13 MAMS agreement data with categorical data

Summary of agreement data between observed and predicted deaths by MAMS in the significant variables only nomogram, using categorical data instead of continuous data

Risk score %	Total number of participants	Observed Deaths (%)	Deaths predicted by the Nomogram
≤10	2	0(0.0)	0
11-20	1	0(0.0)	0
21-30	8	1(13)	2(25)
31-40	40	15(38)	17(43)
41-50	29	18(62)	16(55)
51-60	28	17(61)	18(64)
61-70	32	25(78)	24(75)
71-80	36	29(81)	31(86)
81-90	17	10(59)	16(94)
90-10	-	-	-
Total	193	115(60)	124(64)

Kappa from these data was 0.16 (Std 0.1) p=0.056, demonstrating substantially less agreement than the continuous variables nomogram. Therefore it was decided the nomogram would be more powerful with all variables other than represented on a continuous scale. The range for CSF WCC was large, so this was $\log_{10}$ transformed to accommodate the range on the scale.

5.3.3.4 Validation of the nomogram with the expansion of pneumococcal variable into pathogenic categories

In an attempt to improve the specificity of the nomogram, an analysis was done to check the accuracy of each variable when the final outcome was mis-classified by the nomogram. Although the numbers were small, it was noted that the predicted outcome in several cases was wrongly predicted by the variable 'CSF culture'. This variable was binary with either positive pneumococcal CSF culture or 'non-pneumococcal culture including culture negative'. This latter category included cases of meningococcal and Hib meningitis with zero

mortality, and gram negative (predominately *E. coli*) meningitis with 85% mortality. To test if more detailed categorisation improved predictive performance, this variable was exploded into three categories (Hib/meningococcus/other, pneumococcus or culture negative, and Gram negatives including *E. coli*) (Figure 5.8).

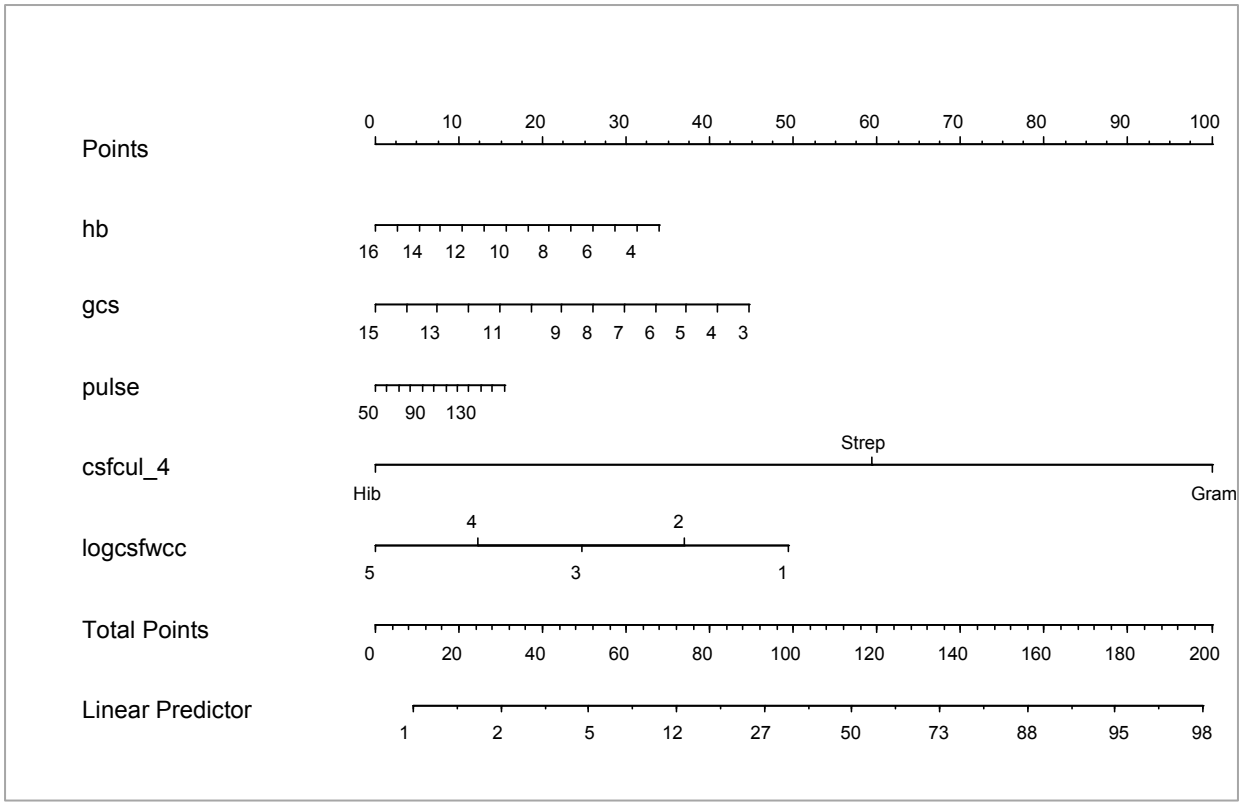


Figure 5.8 Nomogram showing continuous variables, with explosion of bacterial culture

Validation of the subsequent nomogram was substantially worse than the preceding nomogram with AUC 0.67 (95% CI 0.60 : 0.75) (Figure 5.9); agreement was only 37.5% against random agreement of 9.3% ($p<0.001$) (Table 5.14), the Kappa value was 0.31.

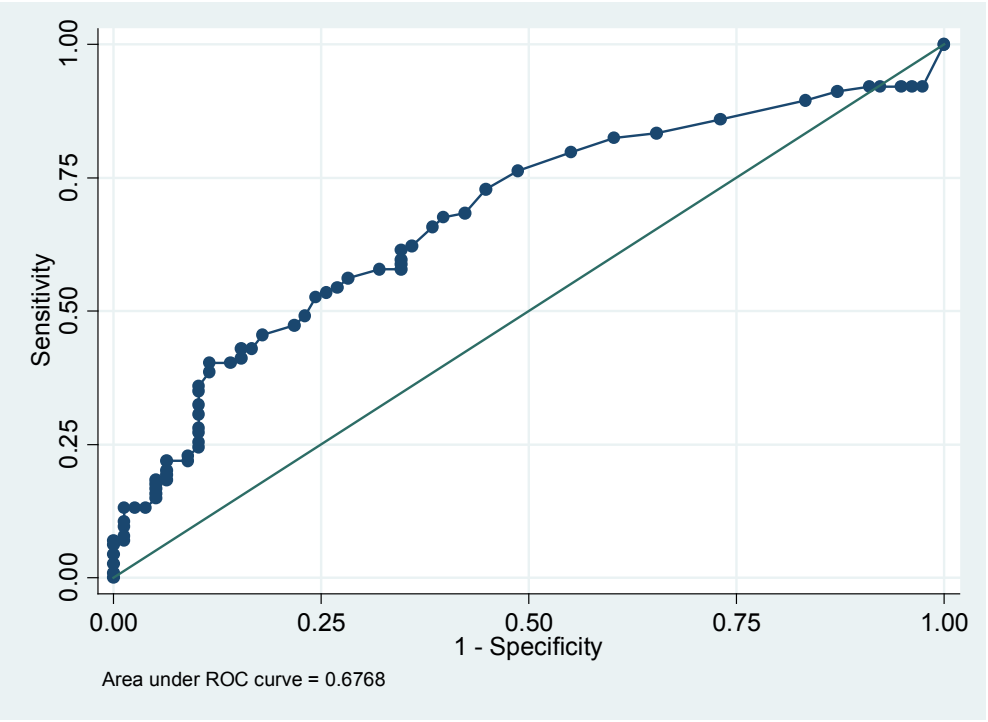


Figure 5.9 ROC curve for the nomogram in Figure 5.8

Table 5.14 MAMS agreement data with an expanded bacterial culture category

Summary of agreement data between observed and predicted deaths by MAMS in the significant variables only nomogram, using the variable 'pneumo' exploded into three categories

Risk score %	Total number of participants	Observed deaths	Predicted deaths
	n	n (%)	n (%)
≤10	51	20 (39)	3 (6)
11-20	47	27 (57)	7 (15)
21-30	32	18 (56)	8 (25)
31-40	0	0 (0)	0 (0)
41-50	34	26 (76)	14 (41)
51-60	5	4 (80)	3 (60)
61-70	6	4 (67)	4 (67)
71-80	4	3 (75)	3 (75)
81-90	13	12 (92)	12 (92)
Total	192	114 (59)	54 (28)

5.3.4 Final MAMS nomogram and validation data

As the validation exercise showed that expansion of the variable for CSF culture reduced the predictive power of the nomogram, and continuous variables were more powerful than categorical variables, the final MAMS nomogram was chosen on the basis of the optimal predictive power found during the validation cycles (Figure 5.10).

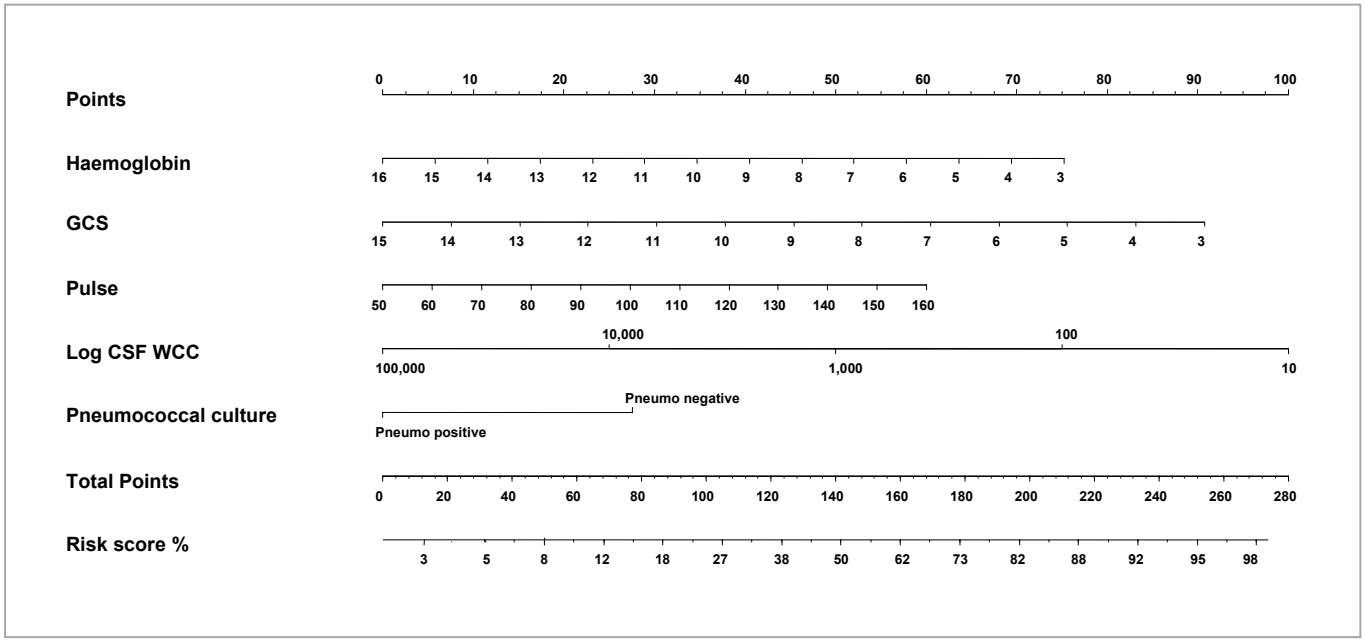


Figure 5.10 Final nomogram to predict outcome from ABM in Malawian adults

This nomogram has the four variables associated with mortality all represented as continuous variables, and CSF culture represented as a binary variable pneumococcal culture/non-pneumococcal culture. The nomogram has an AUC of 0.74 (95% CI 0.65 : 0.82), shown in the ROC curve in Figure 5.11. This AUC or concordance statistic, is within the confidence intervals of the Dutch meningitis score (validation concordance 0.81 (95% 0.74 : 0.87), and therefore is likely to perform similarly. Table 5.15 summarises the agreement of the MAMS prediction with actual outcome for each percentile of predictive risk.

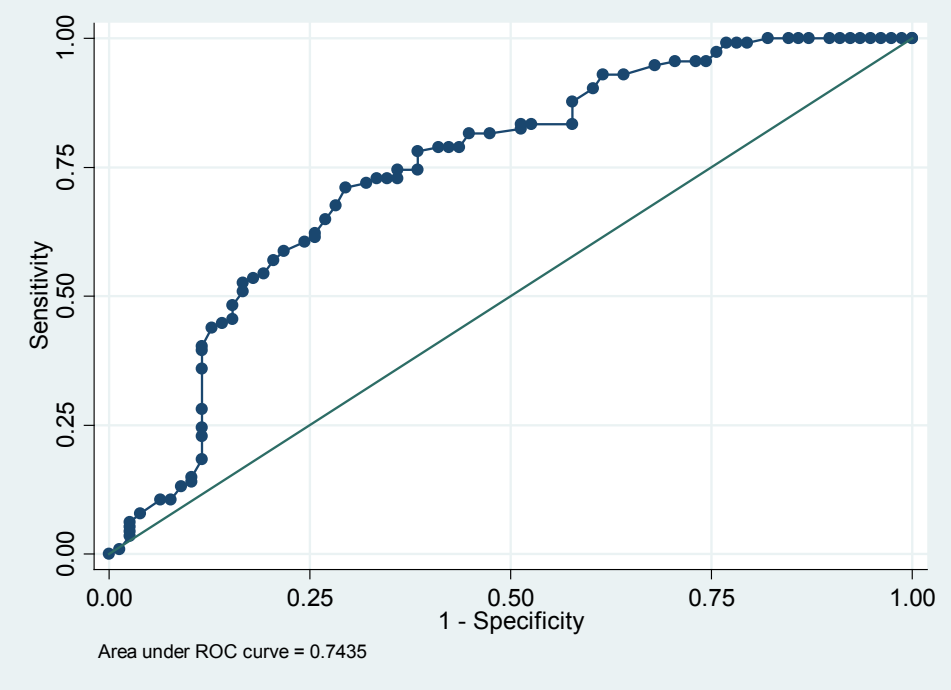


Figure 5.11 ROC showing sensitivity and specificity of the final MAMS nomogram

Table 5.15 MAMS validation data for the final nomogram

Summary of validation agreement of MAMS for different risk groups. N=192

Risk score %	Number of participants	Observed Deaths (%)	Predicted Deaths (%)
≤10	2	0(0)	0(0)
11-20	4	0(0)	1(25)
21-30	11	1(9)	3(27)
31-40	-	-	-
41-50	47	20(43)	20(43)
51-60	19	10(53)	10(53)
61-70	28	18(64)	18(64)
71-80	27	20(74)	20(74)
81-100	54	45(83)	48(89)
Total	192	114(59.3)	120(62.5)

The final validation data for the MAMS nomogram show optimal agreement, with a Kappa value of 0.6 (Std Err 0.1), and agreement of 62.5% against random agreement of 14.1% p<0.00 for all the nomograms tested in the validation exercise.

Table 5.16 shows a summary of all the nomograms tested with a comparison of the validation data by each nomogram.

Table 5.16 Summarised MAMS validation data for each nomogram

Summary of validation for each nomogram tested in the MAMS validation loops						
	AUC	**Observed mortality**	**Predicted mortality**	**Actual agreement**	**p**	**Kappa (StdErr)**
Significant variables only non-imputed	0.75 (0.65-0.85)	46%	53%	37.5%	<0.001	0.32
All variables only non-imputed	0.77 (0.66–0.88)	51%	57%	12.5%	0.37	0.03
Variables post missing data completion	0.74 (0.67-0.88)	59.3%	62.5%	62.5%	<0.001	0.6
Variables categorised into quartiles	0.69 (0.62-0.78)	60%	64%	26%	0.056	0.16
Variables with pnuemo exploded	0.67 (0.60-0.75)	59%	28%	37.5%	<0.001	0.31
Final MAMS nomogram	0.74 (0.6-0.82)	59.3%	62.5%	62.5%	<0.001	0.6

5.4 Summary and Discussion

The final MAMS nomogram compares well with the nomogram presented by the Dutch meningitis group (Table 5.17) (Weisfelt et al., 2008). The concordance statistic in MAMS in the validation dataset of 0.74 falls within the confidence intervals of the Dutch data, concordance of their score in the validation dataset was 0.81 (95% 0.74 - 0.87) (Weisfelt et al., 2008). The Dutch score did not perform well against Malawian data when tested (concordance 0.68 95% CI 0.63 - 0.73) (Schut et al., 2012). The AUC of the final nomogram for MAMS does not fall within the confidence intervals of the European score applied to Malawian data, and therefore it is clear that MAMS performs better using Malawian data than the European score when applied to the same data.

Table 5.17 Comparison of MAMS and EMS validation data

Summary of validation data from European Meningitis Scores and MAMS	
	Concordance (95% CI)
European Meningitis Score (EMS)	0.81 (0.74 - 0.87
EMS Applied to Vietnam	0.70 (0.65 - 0.75)
EMS applied to Malawi	0.68 (0.63 - 0.73)
MAMS	0.74 (0.6 - 0.82)

MAMS appears to have optimal predictive power in the mortality percentiles between 40-80%. Over prediction of poor outcome is seen in both <40% percentile and the >80% percentile, by 20% in the former and 5% in the latter. Although the numbers are small, the power of the nomogram is clearly less at these more extreme ends of the nomogram. It was thought that this was due to misclassification of poor outcome by causative pathogen (Hib/ *N. meningitidis* had 0% mortality in the discovery database and *E. coli* had 85% mortality),

but when the CSF culture variable was expanded to account for this, the power of the nomogram was significantly reduced. These unexpected deaths could be due to another predictive factor that has not been yet measured due to lacking information, such as bacterial serotype (Harboe et al., 2009; Gessner et al., 2010), lactate clearance or oxygenation (Arnold et al., 2008), or by late causes of death such as pulmonary embolism or nosocomial pneumonia that are hard to diagnose in a resource limited hospital, and data are currently lacking. No predictive scoring system can fully take account of unusual events or unpredictable deaths.

MAMS was derived using a virtually identical methodology to that used by the Dutch team, and the final validation data of MAMS compared well against the validation data of the Dutch score. There are substantial differences between the patients from whom the data for that score were derived compared to Malawian data, particularly relating to the age, HIV co-infection and CSF WCC and mortality rates. Patients in Europe were more likely to be older (52 v 31 years $p<0.001$), less likely to have had prior antibiotic treatment (9% v 26% $p<0.01$), and less likely to be HIV co-infected (<1% v 90%, $p<0.001$) (Schut et al., 2012). CSF WCCs in Europe were high compared to Malawi (mean 3000 cells/mm^3 compared to 480 cells/mm^3 $p<0.001$). The cut off used in the Dutch score of CSF WCC <1000 cells/mm^3 means that most of the Malawian patients would be assigned a high mortality score. As 90% of the patients in that study had HIV co-infection, it is possible that a low CSF WCC response to bacterial meningitis may be a marker of advanced HIV infection and possibly worse prognosis. In addition data on haemoglobin are not given in either paper from the Dutch authors, but it is likely that the European patients are less likely to be as profoundly anaemic as the Malawian patients (Lewis et al., 2005; Hedstrand and Killander, 1977), and that anaemia may also be a surrogate marker for advanced HIV co-infection and meningitis in Malawi as seen in cases of tuberculosis and non-typhoidal *S*almonella infection (Bedell et al., 2012).

The failure of the Dutch score to validate against the Malawian data is possibly due to the low age of the Malawian patients compared to the Dutch cohort, and the power of anaemia and very low CSF WCC to predict outcome in the Malawian patients. However the Dutch score also failed in a cohort of Vietnamese patients with bacterial meningitis, where the HIV co-infection rate was also low, and the mortality rate was much lower at 11% than in both Malawi (55%) and Europe (21%) (Schut et al., 2012). These patients had also a lower age (41 v 56 years p<0.001), but had high CSF WCC (2990 v 3000 cells/mm^3 p0.02). *S.pneumoniae* was the causative pathogen only in 12% of Vietnamese patients compared to 51% of Dutch patients and 55% of Malawian patients (p<0.001), the predominate organism in Vietnam was *Streptococcus suis* (26%) (Schut et al., 2012). Why that score performed differently in different populations is unclear, but suggests that ABM may have different pathophysiology in different hosts from different geographical regions.

Neither MAMS nor the Dutch meningitis score predicts outcome perfectly, and the absence of additional data on other potential risk factors that may be associated with poor outcome is likely to weaken both the scores. It is clear that inflammation associated with meningitis pathogenesis with the CSF compartment, brain and systemic inflammatory responses results in alterations of measurable physiology such as GCS and pulse, but the differences particularly in the CSF WCC response between different groups may indicate different host responses to the meningitic pathogen. Further discussion of the host responses to infection in the CNS is found in Chapter 8 Section 8.3.2.

MAMS underwent multiple changes to the nomogram during the validation loop processes to attempt to improve the predictive power. The first of these changes, optimising the missing data improved the performance of the score. However subsequent validation loops, including different ways of categorising of the variables weakened the predictive power, due to categorisation of the data, and variables represented as continuous data are optimal for this scoring system. This is likely to the loss of one degree of freedom with a continuous variable,

whereas two degrees of freedom are lost when the data are categorised. From this exercise it is clear the individual predictors of poor outcome for each patient are heterogeneous, and by creating categories for each variable, the number of cases placed in each category had significant disparities between them and were not homogeneous. The final nomogram only contains one binary variable, CSF culture, as explosion of this variable also lessened the predictive power of the score. These variations in the data were only discovered through the rigorous validation loop processes that MAMS went through in development, with full confidence that the optimal score for these data has been derived.

In the EMS in comparison, most of the variables were binary or categorical with the exception of age and GCS. It is not clear from that paper why the authors chose to use predominately binary variables compared to continuous variables when in MAMS continuous variables were more powerful (Weisfelt et al., 2008). In addition, in derivation of the EMS, the authors used 5 rounds of random imputation on all variables in the dataset and do not report if any biases were found. The validation for that score was done on European Dexamethasone Study, where patients who received Dexamethasone were included despite the receipt of dexamethasone being a factor in influencing outcome (Weisfelt et al., 2008). In the Malawian data, patients who received a treatment associated with outcome were removed, to avoid confounding in the data for the receipt of glycerol (Ajdukiewicz et al., 2011).

When evaluating a severity score, it is important that not only the discovery cohort and validation cohort are as separate as possible, but that they are also matched for demographic parameters of the patients on whom the score is tested (Cook, 2008). The MAMS discovery and validation datasets are equally matched. Although univariate significances were noted between HIV status, day 10 mortality, CSF culture results and respiratory rate, only respiratory rate retained significance between the discovery and validation datasets when tested in a multivariate model containing all univariate significant

variables. Therefore these results give us confidence that the validation data for the score are accurate and translatable can be drawn. Other variables not included in the Malawi meningitis database were collected in BAM and will be tested as predictors of poor outcome, including respiratory rate, hypoxaemia and hyper-lactataemia (Chapter 6 Section 6.3.7).

The original data from which MAMS was derived and validated against contained significant missing data. The Dutch score also had missing data in their complete case dataset (Weisfelt et al., 2008). In that study, the authors used random imputation to complete the data for score derivation, however when this approach was tried with the MAMS discovery data, significant differences were seen in the ORs of predictors of poor outcome. Systematic biases were found in the Malawian data, particularly due to the collection of Hb data, where missing Hb was significantly associated with poor outcome and this altered the results of the random imputation exercise. It is possible to speculate that Hb data were more likely to be collected on patients that survived to ward admission, either because the sample was taken on ward admission, or because the patient survived long enough on the ward for the study or clinical team to collect the data from the laboratory. Neither the SAM nor GLAM studies, from which the majority of data for this study were derived were funded for routine Hb measurements at the bedside and relied on the availability of hospital results (Scarborough et al., 2007; Ajdukiewicz et al., 2011).
Management of the missing data increased the number of cases available for validation and therefore the power of the nomogram, but possibly may have introduced separate biases through imputation of pulse and the re-coding of the Hb variable. No systematic biases were detected in further analysis of the data once imputed, and the possibility of ongoing biases influencing the completed missing data is low.

MAMS in comparison with the Dutch severity score shows that patients in Malawi with a severe clinical phenotype including severe anaemia and abnormal CSF WCC response are likely to have a poor outcome, and predictors of poor outcome are different in different

geographical settings. It is unclear at this stage if these differences can explain the failure of adjunctive treatments such as dexamethasone in Malawi, or if patients with ABM in Malawi have completely different responses to pathogens causing ABM to those in better resourced settings. The presence of important predictors such as low CSF WCC and severe anaemia indicate a severely weakened host immune system that may not be able to respond fully to invasive pathogens, correlations between these parameters and CD4 count will be explored in Chapter 7 Section 7.3.3. HIV co-infected Malawians have higher rates of pneumococcal carriage and altered mucosal and serum responses to pneumococcal carriage (Glennie et al., 2011), it remains to be determined if HIV is also a determinant of altered immunological responses within the CSF compartment.

Other potential prognostic indicators that have been demonstrated either in European or Malawian data include CSF bacterial load, persistence of pneumolysin in the CSF, hyper-lactataemia, severe immunosuppression and pre-hospital and in-hospital delays to diagnosis and treatment (Darton et al., 2009; Wall et al., 2012; Gamper et al., 2001; Wall et al., 2013c). Most pathophysiological variables such as bacterial load are not suitable for inclusion in a bedside severity scoring system, but the addition of bedside measurements including oxygen saturations and blood lactate may increase the power of the MAMS nomogram to predict outcome and therefore have optimal clinical and research utility. This will be explored in data from BAM (Chapter 6).

MAMS was developed to provide objective data on predicted severity of patients presenting to the BAM study to compare the sequential phases in order to minimise confounding biases introduced by the before/after study design. Individual MAMS will be used as an adjunct in the analysis of BAM, to compare the baseline severity and changing prognosis over 6 hours of monitoring for the care bundle intervention between Phases one and two in the following chapter. These analyses will assist in determining the relative mortality risk of patients entering that study undergoing either routine medical care or receipt of the clinical care bundle, compared to the secondary endpoints of actual mortality and morbidity.

In conclusion, the MAMS nomogram has potential to be a useful research and clinical tool. Further validation of this nomogram, in the context of a clinical trial with the addition of prospective data collection for additional variables that may be important predictors is required before MAMS can be used by the wider clinical community. In addition external validation within sub-Saharan Africa from independent data would provide important data to inform the development of the MAMS nomogram.

6 Early Goal Directed Therapy for Adult Meningitis in Malawi

6.1 Introduction

Early Goal Directed Therapy (EGDT) is a term for protocolised medical care delivered in the form of a targeted clinical care bundle over six hours (Rivers et al., 2001; Dellinger et al., 2004). Interestingly, the evidence for EGDT suggests that the overall effect of a clinical care bundle is greater than that would be predicted from the evidenced based efficacy of the individual components (Barochia et al., 2010; Dellinger et al., 2013; Chamberlain et al., 2011). All studies to date, with the exception of a limited study in Uganda (Jacob et al., 2012), have tested the concept of EGDT in resource-rich, or emerging country environments.

No trial has ever tested EGDT for acute bacterial meningitis (ABM) in any setting. However guidelines directing urgent care in the emergency department for suspected ABM in adults, particularly early antibiotic therapy do exist, based on the existing evidence base (Heyderman, 2005; Tunkel et al., 2004; Fitch and van de Beek, 2007; van de Beek et al., 2002). ABM in Africa is an appropriate illness in which to test EGDT, as shown in Chapter 4, Section 2 and Chapter five, the mortality rates are high, patients are critically unwell, and urgent care is a major priority to optimise outcome (Wall et al., 2013c).

The clinical care bundle tested in this study was derived from available literature to optimise care based on the clinical parameters associated with poor outcome in the historical ABM data (Chapter 4 section 4.4.3-4). Detail as to the choice of study design are given in Chapter 2 Section 2.6.2)

Feasibility testing in an observational design study of any complex intervention is recommended by the guidelines published by the Medical Research Council (MRC) prior to testing in a formal parallel design trial (Campbell et al., 2007; Council, 2008). EGDT requires the use of additional resources within the ED for patient care, including nursing and clinical

personnel and clinical resources such as intravenous fluids and oxygen (Rivers et al., 2001). Feasibility testing is essential in this setting where no previous data exist to either guide study design for EGDT in resource limited settings, or to design a care bundle of EGDT for ABM.

This chapter details the results of the pilot study designed to test the feasibility of delivering EGDT for acute adult meningitis in QECH in Blantyre, Malawi.

Data in this chapter are also presented exploring the causes of culture negative meningitis in Malawian adults for the first time. Culture-negative meningitis is an important phenomenon, both in our understanding of the epidemiology of bacterial meningitis, and at the patient bedside. Real-time PCR was used in this study to test for the DNA of three common pathogens causing ABM in Malawi, to determine the causes of culture negative ABM and to explore the underlying causes of why CSF may be culture negative.

6.2 Methods

Detailed trial methods can be found in Chapter 3, Section 3.4. A detailed examination of the literature supporting each individual care bundle element is found in Chapter 2 Section 2.8.

A summary of the relevant research questions, objectives, and endpoints are given here. The inclusion/exclusion criteria, ethical approvals, consent processes for acute care interventions, detailed study design, recruitment and follow up procedures and study analysis plans are detailed in Chapter 3, Section 3.4.

6.2.1 Research questions and objectives

1. Is the delivery of a clinical care bundle of resuscitation over a 6 hour time period for adults with suspected bacterial meningitis feasible and safe in a central teaching hospital in Malawi?

2. Can early goal directed therapy impact on mortality and neurological morbidity from acute bacterial meningitis in Malawian adults?

Specific objectives

1. To assess the feasibility of achieving each clinical target by using the clinical bundle, and perform a composite assessment of all targets achieved by the care bundle.
2. To assess the relative change in mortality due to ABM in adults receiving the bundle compared to standard hospital care.

6.2.2 Study endpoints

Primary

Total proportion of all clinical targets combined, achieved by the bundle at 6 hours.

Secondary (measured at day 10 and day 40)

1. Death
2. Composite poor outcome (Death or significant disability with mRS ≥2)
3. Persistent seizures requiring treatment
4. Significant neurological disability
5. Functional ability measured using the modified Rankin Score (mRS)

Death was measured as a composite secondary endpoint (days to death/last known alive) using the following methods:

Day 10 (acute) survival measured by either a date of discharge from hospital up to 10 days of inpatient antibiotic therapy.

Day 40 (convalescent) - Final outcome recorded ‘alive or dead’ from follow up or response to mobile telephone to 6 weeks.

A history of seizures and functional ability since discharge was taken at day 40, and endpoints 2-4 were measured formally using the mRS (Chapter 2 Section 3.3.9 Table 3.6). Neurological disability was considered as mRS ≥2 points.

6.2.3 Study design

This study was designed as a before/after clinical trial at single centre, with two sequential cohorts of patients receiving either standard clinical care with monitoring from the study team in year 1 (Phase 1) or EGDT delivered as a resource-appropriate clinical care bundle in year 2 (Phase 2).

The goal directed targets were:

1. Time of <1 hour to see a doctor/clinical officer and receive parenteral antibiotics
2. Treat hypoxaemia for patients with SpO_2 of <94% on admission to SpO2 of >94%
3. Treat shock and optimise perfusion with IV fluids and blood for patients with at least one or more parameter of clinical shock as defined by the 2008 surviving sepsis guidelines (Dellinger et al., 2008b)
4. Treat severe anaemia with blood
5. Insert an airway if the patient was in coma (GCS <8)
6. Raise the head to 30 degrees if the patient had significantly altered mental status (GCS <11)
7. Control seizures by the end of the 6 hour observation period
8. Correct hypoglycaemia to above 4mmol/L if present.

6.2.3.1 Diagnosis of bacterial meningitis

All CSF samples were processed by the MLW laboratory. Detailed methods are found in Chapter 3 Section 3.1. In addition, a subset of patient samples stored on admission had

multiplex real-time PCR performed for *S. pneumoniae*, *N. meningitidis* and Hib, and the results were used to determine causes of culture negative meningitis.

6.3 Results

6.3.1 Participant recruitment

Six hundred and six patients were screened by the study team in the two phases. Eleven patients did not meet the screening inclusion criteria, and a further 65 declined verbal assent and were not recruited. Five hundred and forty one patients were initially included in the study (Figure 6.1). Of these, 135 met the CSF inclusion criteria, and 132 gave informed consent for their data to be retained and were followed up.

Of the 132 participants who met the CSF inclusion criteria, 74 had culture proven ABM, and 27 participants had a negative CSF culture but subsequently had a proven pathogen on PCR. The remaining 31 participants had CSF that was culture and PCR negative (when tested) but otherwise met the inclusion criteria, and were included as probable ABM cases.

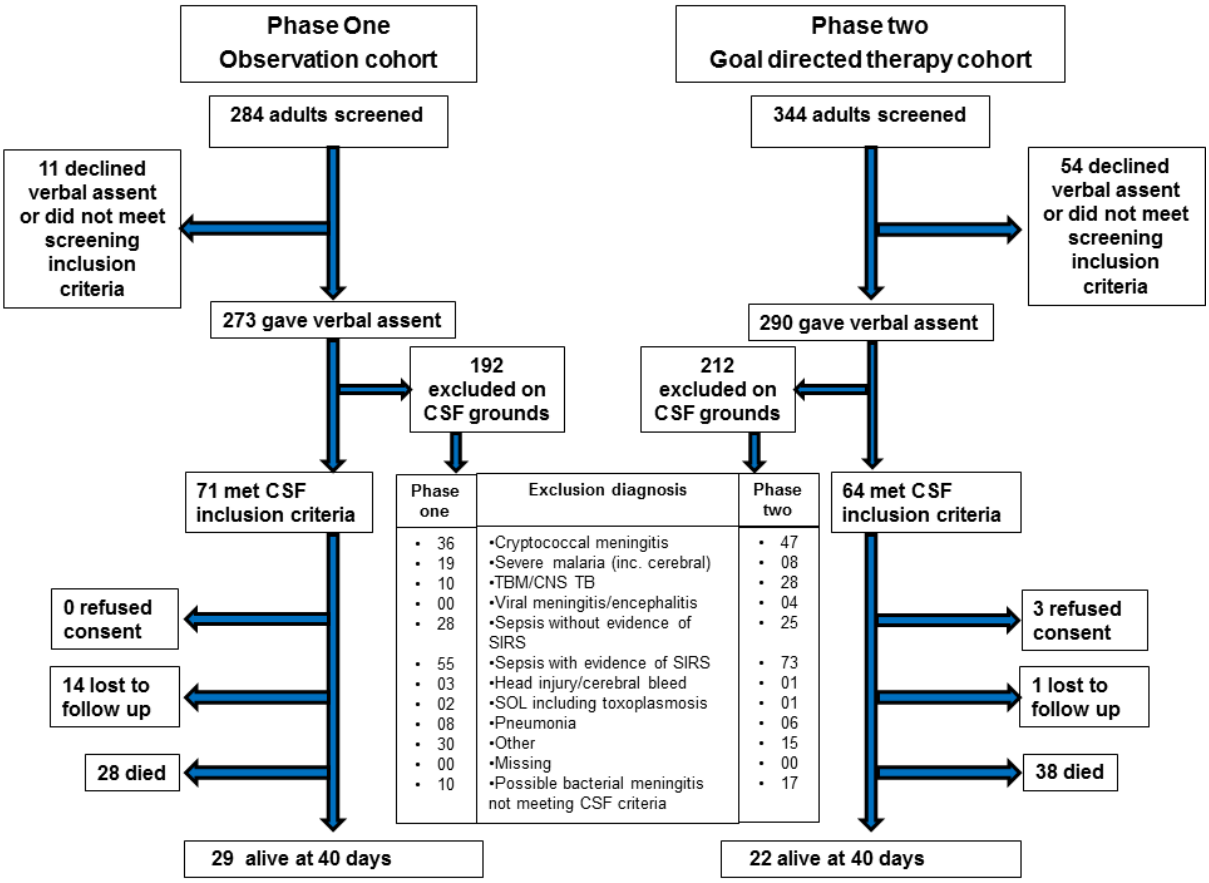


Figure 6.1 BAM trial recruitment summary

6.3.2 Causes of meningitis

Table 6.1 shows the results of CSF culture for screened patients and trial participants. *S. pneumoniae* was the commonest cause of ABM, representing 46% of culture positive cases. *N. meningitidis* and *E. coli* were rare, no cases of Hib or non-typhoidal *S*almonellae meningitis were seen.

Pathogens listed as 'other' included alpha haemolytic streptococci, group A and B streptococci, *S. aureus* and gram negative pathogens including non-typhoidal *S*almonellae.

Multiplex real-time PCR (RT-PCR) was done on 159 whole CSF samples stored at -80°C. Of these samples, seven were from excluded patients with normal CSF (acellular, culture

negative with normal biochemistry) who acted as control samples, 88 were from proven or probable ABM, and 64 were from patients with possible ABM.

Table 6.1 Results of PCR for BAM

Bacterial multiplex RT-PCR per BAM inclusion group						
Inclusion category	**CSF culture results**	**Pneumococcal DNA detected**	**Meningococcal DNA detected**	**Hib DNA detected**	**PCR negative**	**No result**
Excluded not ABM n=7	NG n= 7	0	0	0	7	0
Included proven/ probable ABM n=88	NG n=41	12	5	0	22	2
	SpN n= 40	38	0	0	0	2
	NM n= 1	0	1	0	0	0
	Hib n= 0	0	0	0	0	0
	E. coli n= 1	0	0	0	0	0
	Other GNR n= 4	0	0	0	0	0
	Other n= 1	0	0	0	0	0
Possible ABM n=64	NG n= 64	7	1	0	55	1

NG = no growth

Bacterial DNA was not found in any of the excluded samples (Table 6.1), but was found in culture negative CSF from both the included proven/probable meningitis group and the possible meningitis group.

CSF culture and PCR results were compared (Table 6.2). All CSF culture positive cases for *S. pneumoniae* and *N. meningitidis* tested were also PCR positive. DNA was detected in 18

CSF culture negative cases and from two where bacterial contamination was reported and *N. meningitidis* DNA detected in six.

Table 6.2 Comparison of culture and PCR data per pathogen

Comparison of meningitis data from bacterial culture and PCR from BAM					
Bacterial culture result	**PCR negative**	**SpN DNA positive**	**NM DNA positive**	**PCR Not done**	**Total**
No growth	12	18	4	16	50
SpN	0	36	0	25	61
NM	0	0	1	3	4
E. coli	1	0	1	0	2
Other	2	1	1	3	7
Contaminant	2	2	0	4	8
Total	17	57	7	51	132

Of the 82 cases of pneumococcal meningitis, 61 were detected by culture, and an additional 21 (25%) were detected by PCR. Of the ten cases of meningococcal meningitis, four were detected by culture and an additional six were detected by PCR (60%).

H. influenzae DNA was not detected in any samples. *S.pneumoniae* therefore accounted for 82/132 (61%) of proven ABM cases, and *N. meningitidis* accounted for 10/132 (7.5%) of cases. 18% of CSF samples were PCR and culture negative.

None of the patients in the possible group had a positive blood culture. Therefore of the total 59 cases of pneumococcal meningitis in the sample set tested with PCR, 32% were diagnosed by PCR alone. One culture positive case of meningococcal meningitis was included in the PCR study, a further 6/7 (85%) cases of ABM caused by *N. meningitidis* were detected by PCR alone. ABM caused by Hib was not detected either by culture or PCR, and

ABM with other pathogens on culture including *E. coli* and other gram negatives did not have pneumococcal DNA detected (Table 6.2).

These results were then tabulated against the receipt of antibiotics within the last 14 days for included participants to determine if antibiotic consumption altered the yield of either bacterial culture (by killing viable organisms for culture) or PCR (Table 6.3).

Table 6.3 CSF PCR and culture results with antibiotics

Comparison of CSF results by culture and PCR with recent receipt of antibiotics.

	No prior antibiotics	**Prior antibiotics**	**Unknown**
CSF culture positive n= 74	35 (47%)	32 (43%)	7 (9%)
CSF culture negative n=50	16 (32%)	29 (58%)	5 (10%)
CSF culture contaminated n= 8	0	8 (100%)	0
CSF PCR positive n= 64	27 (42%)	30 (47%)	7 (11%)
CSF PCR negative n=17	3 (17%)	14 (83%)	0
CSF PCR not done n=51	21 (41%)	25 (49%)	5 (10%)

Fewer patients whose CSF was culture negative had not received prior antibiotics (32% compared to 58% with receipt of antibiotics) p=0.02 (Chi squared test, Table 6.3). Small numbers of patients had a negative CSF PCR, more patients who had received antibiotics had a negative CSF PCR (83% compared to 17% with no antibiotics), however there was no significant relationship p=0.11 (Table 6.3).

6.3.2.1 Bacterial sub-type analysis

Serotyping was not done routinely during this study, and these data are not available. Historically, serotypes one and five have been the most common causes of IPD and ABM in Malawi (Everett et al., 2012).

All of the 4 cases of culture proven *N. meningitidis* meningitis were serogroup W135.

6.3.2.2 Bacterial resistance patterns

Antibiotic sensitivities are detailed in Table 6.4. Resistance to Co-trimoxazole was commonest, 100% of *E. coli*, 80% of and 75% of *N. meningitidis* isolates were all resistant to co-trimoxazole *in vitro*. Resistance rates to chloramphenicol and penicillin, the two most commonly prescribed parenteral antibiotics for ABM in the region were also high on disc testing, formal MIC testing was not done (in isolates:, 11% were resistant to penicillin and 24.5% resistant to chloramphenicol), but no isolates were resistant to both antibiotics. No *in vitro* resistance to ceftriaxone was noted in this study. All meningococcal isolates were resistant to penicillin on disc testing, although the numbers are small. As disc testing, not MIC testing was used, from local data these are likely to be intermediate resistance (Cornick and Bentley, 2012).

Pre-hospital antibiotics were widely prescribed (Table 6.5). Co-trimoxazole was the most frequently reported antibiotic, 20% of cases were taking this drug. Of the culture positive cases, 16 (26%) were taking Cotrimoxazole at the time of admission, and 13 (86%) of those 16 isolates were co-trimoxazole resistant. It is not clear whether patients had received cotrimoxazole for an acute indication or was long term prophylaxis for HIV co-infection, as per the Malawian guidelines for the treatment of HIV (Government, 2012).

Penicillin was the second most commonly prescribed antibiotic, with 15% of patients already taking this drug when admitted with ABM. Multiple other antibiotics were also prescribed in the community, although the overall numbers are small.

Table 6.4 Antibiotic sensitivities in CSF isolates

Antibiotic sensitivities of CSF isolates to prescribed antibiotics

Pathogen	Number of cases resistant to each antibiotic (%)									
	Ceftriaxone	Penicillin	Co-trimoxazole	Ciprofloxacin	Erythromycin	Gentamicin	Chloramphenicol	Tetracycline	Co-amoxiclav	Penicillin and Choramphenic
S.pnemoniae **N=61**	0 (0%)	7 (11%)	49 (80%)	Not tested	5 (8%)	Not tested	15 (24.5%)	33 (54%)	0 (0%)	0 (0%)
N. meningitidis **N=4**	1 (25%)	4 (100%)	3 (75%)	0 (0%)	Not tested	0 (0%)	0 (0%)	Not tested	1 (25%)	0 (0%)
E. coli **N=2**	1 (50%)	NA	2 (100%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	Not tested	0 (0%)	NA
Other n=15	0	2 (13%)	5 (33%)	0	1 (7.5%)	0	0	1 (7.5%)	0	0

Only two patients were on TB treatment at the time of admission, and no patients were on anti-malarial treatment, despite eight patients having malaria co-infection (Table 6.5). Three patients had received both parenteral penicillin and chloramphenicol from a health centre before transfer. None had received ceftriaxone.

Time from start of community prescribed antibiotic and admission with ABM was varied. Of the sixty seven patients whom were taking antibiotics, 29 (43 %) had been given the antibiotic within 24 hours of admission, 10 (15%) within 48 hours, and 8 (12%) within 48-72 hours. Only twenty (30%) of patients had been taking antibiotics for longer than 72 hours, of whom 17 had taken that drug for 7-14 days or longer.

Table 6.5 Frequencies of antibiotic consumption

Pre-hospital antibiotics	
Antibiotic **n=88**	**Frequency (%)** **n=132**
Penicillin/Amoxicillin	20 (15%)
Co-trimoxazole	26 (20%)
Ciprofloxacin	5 (3.8%)
Erythromycin	8 (6.1%)
Antimalarial including QNN*	0 (0%)
Tetracycline	0 (0%)
Ceftriaxone	3 (2.3%)
Metronidazole	3 (2.3%)
TB treatment (RHEZ)	2 (1.5%)
Fluconazole	1 (1%)
Parenteral penicillin	11 (8.3%)
Parenteral Chloramphenicol	6 (4.5%)
Parenteral Gentamicin	3 (2.2%)

***QNN = Quinine**

6.3.3 Clinical presentation with ABM

71 participants with ABM were recruited in Phase 1, and 61 in Phase 2. Table 6.6, Table 6.7, Table 6.8, and Table 6.9 detail the clinical presentation of these patients, comparing clinical, microbiological and laboratory parameters in the two phases. The patients were evenly matched for age, sex and HIV co-infection (including WHO stage and CD4 count); however some important differences were noted. These included more patients having clinically defined WHO HIV stage 3 or 4 illness in P1 (56% compared to 39% p=0.05), and longer time to presentation in P1 (Table 6.6). Markers of septic shock (MAP, pulse, lactate, capillary refill time) were equally matched between the two phases Table 6.8. The clinical history was obtained from the accompanying guardian in 108/132 (81%) of cases, with only 15 (11%) of

patients able to give a history to the study team. This did not differ by phase. Fourteen patients gave a history of involvement with a traditional healer for the acute illness prior to admission (8 in P1 and 7 in P2).

Petechial rash was observed in eleven patients in Phase 1 and none in Phase 2 (p=0.001) Table 6.7, seven with culture positive pneumococcal disease and four with negative CSF culture. CSF culture for *N.meningitidis* was negative in all of these patients, PCR was done in the four culture negative patients, all of which were negative for meningococcal DNA. Equal numbers of patients in both phases gave a history of otitis media (9 P1 and 8 P2 p=1.0). Significantly more patients in Phase 2 gave a history of vomiting (3 in P1 and 23 in P2 p<0.001). Patients in Phase 1 took longer to present to hospital (p=0.005), were more likely to have pre-hospital seizures (28% v 14.5% p=0.04), and had lower oxygen saturations (96% v 97% p=0.042) and higher respiratory rates (26 v 21 breaths per minute, p=0.007) at presentation (Table 6.7, Table 6.8, Table 6.9).

A history of a meningitic illness in the past was given by nine patients, 3 had documented ABM, one had cryptococcal meningitis (CCM) and 4 the cause was unknown. No patient gave a history of TB meningitis. Twenty patients gave a history of previous or chronic otitis media.

At history of at least one WHO HIV stage 3 or 4 defining illnesses was present in 56 (42%), equally divided between the two phases (P1 30/71 (42%), P2 27/61 (44%) p=0.86); the most common of which was significant weight loss of >10% of body weight in 26 patients. Oral or oesophageal candidiasis had been treated in 29 patients, 20 patients gave a history of TB, 13 community acquired pneumonia of which 4 were admitted, persistent fever was present in 14 (10%). Few patients had Kaposi Sarcoma (KS) (2), shingles (2) or chronic diarrhoea (7).

Table 6.6 Demographics of BAM participants

Baseline characteristics of study participants including demographics and clinical history.

Characteristic on presentation	Value or Median value (% or Inter Quartile Range IQR)		Univariate significance of differences between two phases
Clinical observations	Phase 1 N=71	Phase 2 N=61	P value
Female	30 (42%)	21 (34%)	0.37†
Median age (years)	32 (25 – 42)	34 (27.5 – 43.5)	0.14†
HIV positive	42/60 (70%)	40/52 (77%)	0.65†
Clinically defined WHO HIV stage 3/4	40 (56%)	24 (39%)	0.059†
History of WHO stage 3 or 4 condition	30 (42%)	27 (44%)	0.86
Antiretroviral therapy	20/60 (33%)	14/57 (24.5%)	0.20†
Out of hours BAM team admission	23 (32%)	1 (1.6%)	<0.001†
Median Pre-hospital symptom duration (hours) (IQR)	48 (48 – 72)	48 (24 – 72)	0.005 §
<24 hrs	13	27	Ref
24-48 hrs	25	20	0.35
48-72 hrs	21	12	0.009
72-96 hrs	5	1	0.041
>96 hrs	7	0	0.99
Pre-hospital antibiotics	35/63 (55%)	34/57 (60%)	0.58†
History of acute headache	68 (96%)	56 (93%)	0.40†
Pre-hospital seizures	20 (28%)	9 (14.8%)	0.049†

Table 6.7 Clinical features of BAM participants

Characteristic on presentation	Value or Median value (% or Inter Quartile Range IQR)		Univariate significance of differences between two phases
	Phase 1 N=71	Phase 2 N=61	P value
Neck stiffness	52 (73%)	42 (68%)	0.35†
Photophobia	9 (12%)	17 (27%)	0.024†
Cranial nerve palsy	1 (1%)	3 (4.9%) (III = 2 VIII = 1)	0.27†
Moribund on admission	11 (15.4%)	4 (6.6%)	0.08†
Subjective hearing loss	10 (14%)	5 (8.2%)	0.21†
Clinical evidence of pneumonia*	9 (12.6%)	9 (14.8%)	0.46†
Clinical evidence of anaemia*	9 (12.6%)	5 (8.2%)	0.29†
Clinical evidence of shock*	45	41	0.85†
Focal limb weakness	5 (7%)	2 (3%)	0.29†
Pre-illness modified Rankin Score >2	2 (3%)	4 (6.5%)	0.53†
Acute seizures in AETC	7/62 (11%)	7/58 (12%)	0.55†

*Clinical pneumonia defined as fast breathing with history of cough and audible chest signs consistent with pneumonia.

*Clinical anaemia defined as objective extreme pallor of the palms and conjunctivae, or bedside hemocue of Hb <6.0g/dL.

*Clinical shock defined as CRT>2 sec, BP <90 mmHg or MAP <70 mmHg, Pulse >100 bpm, lactate >4mmol/L

Table 6.8 Presenting physical parameters of BAM patients

Characteristic on presentation	Value or Median value (% or Inter Quartile Range IQR)		Univariate significance of differences between two phases
	Phase 1 N=71	Phase 2 N=61	P value
Glasgow Coma Score	13 (10 – 14)	13 (11 - 14)	0.80†
GCS >8-<11	12 (16%)	10 (16.4%)	0.63≠
GCS <8	14 (20%)	6 (9.8%)	0.09≠
Median mean arterial blood pressure (mmHg)	90 (75 – 105)	92 (82 – 104)	0.59 §
Pulse (bpm)	101 (85 – 116)	99 (86 – 119)	1.0 §
Capillary refill time (seconds)	1 (1-2)	1 (1-2)	0.66 §
Temperature (°C) recorded	38.2 (37.1 – 39.1)	38 (36 – 39)	0.01 §
Oxygen saturations	96 (94 – 98)	97 (94 – 97)	0.042§
Respiratory rate	26 (22 - 32)	21 (19 – 26)	0.007 §
Estimated body mass index (BMI)	22.4 (20.9 – 24.1)	23 (21.1 – 24.3)	0.59 §
MAMS (IQR) n=39	157 (132 - 182)	149 (110 - 190)	0.61

Table 6.9 Laboratory results in BAM patients

Microbiology		**Phase 1 N=71**	**Phase 2 N=61**	**P**
CSF culture	No growth	23 (32%)	27 (44%)	0.057 ≠
	34 (47%)		27 (44%)	0.11
	N. meningitidis	3 (4.2%)	1 (1.6%)	0.59
	E. coli	1 (1.4%)	1 (1.6%)	0.27
	Other	3 (4.2%)	4 (6.6%)	0.08
	Contaminant	7 (10%)	1 (1.6%)	Reference
CSF parameters	**Phase 1 N=71**		**Phase 2 N=61**	**P**
CSF white cell count (cells/mm^3)	68 (16 – 288)		241 (8.5 – 1116)	0.64†
CSF protein (g/L)	2.84 (1.8 – 5.4)		2.73 (1.85 – 4.16)	0.61 §
CSF : Blood glucose ratio	0.34 (0.10 – 0.46)		0.13 (0.01 – 0.16)	0.04 §
CSF lactate (mmol/L)	9.8 (7.8 – 11.1)		10.0 (9.1 – 11.2)	0.42 §
Positive blood culture	20 (28%) (18/20)		8 (13%) (3/8)	0.06 ≠
Blood parameters				
Haemoglobin (g/dL)	11.9 (10.3 – 13.3)		11.6 (9.9 – 13)	0.85 §
White cell count	9.6 (6.3 – 14.4)		10.2 (5.8 – 17.5)	0.56 §
Platelet count	228 (129 – 315)		206 (148 – 299)	0.92 §
CD4 count	97 (41 – 293)		131 (76 – 249)	0.94 §
Positive test for *P. Falciparum* Ag	5/53 (9.4%)		3/61 (4.9%)	0.46 †
Glucose (mmol/L)	7.1 (5.6 – 8.6)		7.4 (6.0 – 9.2)	0.56 §
Blood lactate (mmol/L)	2.9 (2.2 – 5.6)		3.4 (1.9 – 5.75)	0.65 §
Creatinine (mg/dL)	0.99 (0.73 – 1.07)		0.9 (0.72 – 1.30)	0.89 §
Sodium (mmol/L)	140 (138 – 144)		139 (133 – 149)	0.95 §

† Fisher exact test ≠ univariate logistic regression § Mann–Whitney U test of medians

Of the 34 patients on antiretroviral therapy (ART), 28 were taking stavudine, lamivudine and nevirapine (d4T/3TC/NVP) and 3 were taking second line tenofovir, lamivudine and efavirenz (TDF/3TC/EFV), the regime was unknown in a further 3 patients (Table 6.10).

Table 6.10 Antiretroviral treatment in BAM

Duration of ART in adults presenting with ABM n=34			
Time period on ART	All ART	d4T/3TC/NVP	TDF/3TC/EFV
<1 month	6	5	1
1-3 months	7	7	0
3-6 months	4	4	0
6-12 months	5	4	1
>12 months	8	7	1

There were no differences between the numbers of patients on ART in either phase (Table 6.6).

6.3.4 Results of the clinical targets achieved.

6.3.4.1 Patients with bacterial meningitis

The primary endpoint for this study was the total proportion of all clinical targets combined, achieved by the bundle at 6 hours.

Eight clinical targets were set in Phase 2, and first the proportion of each target achieved by the care bundle was assessed, and then the composite target achievement was calculated.

Table 6.11 shows the proportion of patients in both phases who met the criteria for treatment for each target and subsequent proportion of patients who did not meet the target set by the end of 6 hours across the phases. This was defined by either no change in the clinical parameter treated by the targeted care bundle element, or lack of availability of resources to meet that target. For example, the target for hypoxaemia (defined as SpO_2 <94%) was to

achieve oxygen saturations above that level by the end of the six hour time period. If the patient remained hypoxic by the end of 6 hours, that patient did not meet the target set.

It must be noted that nasopharyngeal airways and head tilts were not routinely available in the AETC during phase 1, but all other treatments and equipment were available. The study team monitored the care given by the AETC in Phase 1 of the study and delivered the protocolised care in Phase 2 in the AETC.

Time to see a clinician was short in both phases and no differences were observed between the two phases. This was not a specific target but identified as necessary for the prescription of antibiotics, and therefore delays at this stage could seriously influence time to receipt of parenteral antibiotics. However there was no difference observed between the phases in time to clinical review. In contrast the time to receive antibiotics, and the proportion achieving the 1 hour target was significantly improved by use of the care bundle in Phase 2 (median time 1:55 in P1 and 1:13 in P2 $p<0.001$, 14% met the 1 hour target in P1 and 44% in p2 $p<0.001$). Airway protection and head elevation were more likely to occur in Phase 2, with no patients receiving either intervention in P1 and 77% and 89% receiving at either in P2 ($p=0.04$ and 0.002 respectively).

Table 6.11 Targets achieved in BAM

Clinical targets achieved by phase for patients with ABM at the end of 6 hours				
Parameter	**Target**	**Phase 1** **N=71**	**Phase 2** **N=61**	**Univariate significance**
Timing of clinical assessment	Medical review <1 hour of arrival	Median time hh:mm (IQR) 0:26 (0:10 – 1:01)	Median time hh:mm (IQR) 0:22 (0:15 – 0:45)	 0.73
Proportion that met the target		Proportion seen in <1 hour n=44/62 (70%)	Proportion seen in <1 hour n=48/57 (84%)	 0.12
Antibiotic therapy timing	1st dose within 1 hour of arrival	Median time hh:mm (IQR) 1:55 (1:10 – 2:52)	Median time hh:mm (IQR) 1:13 (0:42 – 1:58)	 <0.001
Proportion met antibiotic target		Proportion IVABx <1 hour n=6/49 (14%)	Proportion IVABx < 1 hour 27/61 (44%)	 <0.001
Brain protection	Airway if GCS <8 given? Head tilt if GCS <11 given?	n=0/14 (0%) n=0/26 (0%)	n=7/9 (77%) n=17/19 (89%)	0.04 0.002
Oxygenation on admission	Give oxygen if Sp02 <94%	Proportion SpO_2 <94% on admission = 18/66 (27.2%)	Proportion SpO_2 <94% on admission = 15/61 (24.5%)	 0.84

Oxygenation on discharge		Proportion SpO_2 >94% on discharge to ward = 30/37 (81%)	Proportion SpO_2 >94% on discharge to ward = 48/53 (90.5%)	0.22
Perfusion status on admission	CRT<2 sec, BP >90 mmHg or MAP >70 mmHg, Pulse <100 bpm, lactate >4mmol/L	Proportion with one or more feature of shock on admission =45/69 (65%)	Proportion with one or more feature of shock on admission = 41/61 (67%)	0.85
Perfusion status on discharge		Proportion without shock on ward discharge =20/37 (54%)	Proportion without shock on ward discharge =31/53 (58%)	0.82
Blood transfusion	Haemoglobin <6g/dL Transfusion in AETC?	2/39 (5%) 0 (0%)	3/45 (6%) 2/3 (66%)	1.0 0.4
Seizures	Control of AETC seizures by ward discharge	7/7 (100%)	6/7 (85%)	1.0
Hypoglycaemia on admission	Blood glucose >4 throughout care bundle	Proportion <4mmol/L 0/53 (0%)	Proportion <4mmol/L 1/61 (1.6%)	0.71

The proportion of patients requiring oxygenation was identical on admission for both phases, the number who still required oxygen at the end of the 6 hour observation time period decreased from 18% in P1 to 9% in P2, but the number of observations was small and this difference was not statistically significant. It must be noted that oxygen therapy in P1 was not recorded, but all patients with SpO2 of <94% in P2 received oxygenation via a concentrator. A substantial number of patients had clinical evidence of shock in both phases (65% in P1 and 67% in P2 p=0.85) and although this did decrease by the end of the 6 hour observation period, there was no difference in the proportion of patients with shock across the phases after the shock protocol was given in P2 compared to P1 (46% v 41.5% p=0.82).

Very few patients either required blood transfusion or treatment of hypoglycaemia in either phase; no data were available for the one patient who required a follow up blood glucose measurements in P2 who was hypoglycaemic. Two of three patients in P2 who required blood acutely in AETC received it, compared to no patients in P1. These differences were not statistically significant. Acute seizure activity was well controlled in both phases with only one patient discharged to the ward from the entire study who continued to seize.

6.3.4.2 Target achievement for screened and excluded patients

Data from patients who were subsequently excluded from the study were then examined, as these patients had the potential to benefit from the care bundle, particularly those with pneumonia and sepsis. The overall achievement of the individual targets was reviewed across all screened participants, including those with ABM for a wider feasibility assessment.

Data were collected for the clinical targets for all screened participants. Table 6.12 details the proportion of each clinical target achieved by the different phases in all screened participants.

Table 6.12 Targets achieved in all screened participants

Clinical targets achieved by phase for all screened patients at the end of 6 hours

Parameter	Target	Phase 1 N 263	Phase 2 N 290	Univariate significance
Timing of clinical assessment	Medical review <1 hour of arrival	Median time hh:mm (IQR) 0:40 (0:15 – 1:20)	Median time hh:mm (IQR) 0:30 (0:16 – 0:54)	0.01
Proportion that met the target		Proportion seen <1 hour n= 150/253 (59%)	Proportion seen < 1 hour n= 222/279 (80%)	<0.001
Antibiotic therapy	1st dose within 1 hour of arrival	Median time hh:mm (IQR) 2:30 (1:34 – 3:30)	Median time hh:mm (IQR) 1:10 (0:50 – 2:01)	<0.001
Proportion that met the antibiotic target		IVABx <1 hour n= 16/239 (7%)	IVABx <1 hour 119/290 (41%)	<0.001
Brain protection	Airway if GCS <8? Head tilt if GCS <11?	0/26 (0%) 0/41 (0%)	14/20 (70%) 56/52 (107%)	<0.001 <0.001
Oxygenation on admission	SpO2 >94%	SpO2 <94%on admission = 50/263 (19%)	SpO2 <94%on admission = 56/290 (19%)	1.0

Oxygenation on discharge		Proportion SpO2 >94%on ward discharge = 185/207 (89%)	Proportion SpO2 >94%on ward discharge = 253/268 (94%)	0.057
Perfusion status on admission		Proportion with one or more feature of shock on admission = 165/267 (62%)	Proportion with one or more feature of shock on admission = 186/290 (64%)	0.59
Perfusion status on discharge	CRT<2 sec, BP >90 mmHg or MAP >70 mmHg, Pulse <100 bpm, lac >4mmol/L	Proportion with no features of shock on ward discharge = 131/207 (63%)	Proportion no features of shock on ward discharge = 170/269 (63%)	1.0
Blood transfusion	Haemoglobin >6g/dL	Proportion <6g/dL 8/149 (5.4%)	Proportion <6g/dL 21/289 (7.3%)	0.54
	Transfusion in AETC?	0/8 (0%)	8/19 (42%)	0.04
Seizures	Control of AETC seizures by ward discharge	29/31 (93.5%)	51/54 (94%)	1.0
Hypoglycaemia	Blood glucose >44mmol/L	Proportion <4mmol/L 11/246 (4.5%)	Proportion <4mmol/L 11/289 (4%)	0.82
Hypoglycaemia on discharge		RBS >4 mmol by end of observation period = Not done	RBS >4 mmol by end of observation period = 5/11 (40%)	NA

Amongst all screened participants, both times to see a clinician and receive parenteral antibiotics were substantially reduced in P2 (Table 6.12) with the proportion of patients meeting the 1 hour antibiotic target increased from 7% in P1 to 41% in P2 ($p<0.001$). The application of both airways and head tilt to patients meeting the criteria for those interventions were also increased from no patients in P1 to 70% and 107% respectively for both targets. Equal proportions of patients met the criteria for oxygen (19%) in P1 and P2, the number still requiring oxygen on ward transfer dropped to 11% in P1 and 5.5% in P2, $p=0.057$ suggesting a trend towards optimised oxygenation in P2 but was not significant. The number and proportions of patients with shock were the same in both phases for both admission to AETC (62% and 64% $p=0.59$) and with on-going features of shock on ward discharge 37% and 36.5% respectively $p=1.0$). Blood transfusion requirements were low, but no patients received blood in AETC in P1 and 42% received blood in P2 $p=0.04$. Equal proportions of patients had their seizures controlled across both phases, and the frequency of hypoglycaemia was rare. 40% of patients in P2 had their hypoglycaemia successfully treated by AETC discharge.

6.3.4.3 Composite target achievements for BAM included patients with bacterial meningitis

To assess the overall proportions of patients in each phase being set and meeting targeted therapy, binomial Poisson regression was used to calculate the Rate Ratio (RR) of the means of the number of targets required or met between the two phases (Table 6.13). There was no difference in the mean number of targets required by the patients across the two phases, (2.59 in P1 and 2.52 in P2) RR 1.02 (95% CI 0.85 : 1.22, $p=0.7$). However the rate of target achievement in Phase 1 was significantly less than in Phase 2, the mean number of targets achieved in P1 was 0.55 (SE 0.102), compared to 1.57 (SE 0.128) in P2, Rate Ratio 0.34 (95% CI 0.23 : 0.51) $p<0.001$.

Table 6.13 Composite target achievement in BAM

Composite achievement of clinical targets between Phase 1 and 2				
Measure	**Phase 1**	**Phase 2**	**Rate ratio (95% confidence interval)**	**p-value**
Mean Number of clinical targets per patient (s.e.)	2.59 (0.148)	2.52 (0.177)	1.027 (0.859 : 1.226)	0.773
Mean Number of required targets met per patient (s.e.)	0.55 (0.102)	1.57 (0.128)	0.348 (0.234 : 0.518)	<0.001

Following the examination of the primary endpoint (composite target achievements), the proportions of targets achieved per number of targets set were estimated across the study phases. The number of patients who met the criteria for one target alone, or two or more targets were calculated, and compared across the two phases (Figure 6.2). All patients met the criteria for at least one target (IV antibiotics within 1 hour) irrespective of clinical seriousness of their illness.

As the number of targets increased per patient, fewer targets overall were achieved across the phases, but the number of targets achieved overall was greater in P2 than in P1. No patients set more than four targets were able to achieve more than four targets in either phase. Missing data on target achievement for patients recruited out of hours has reduced the data available for the P1 analysis.

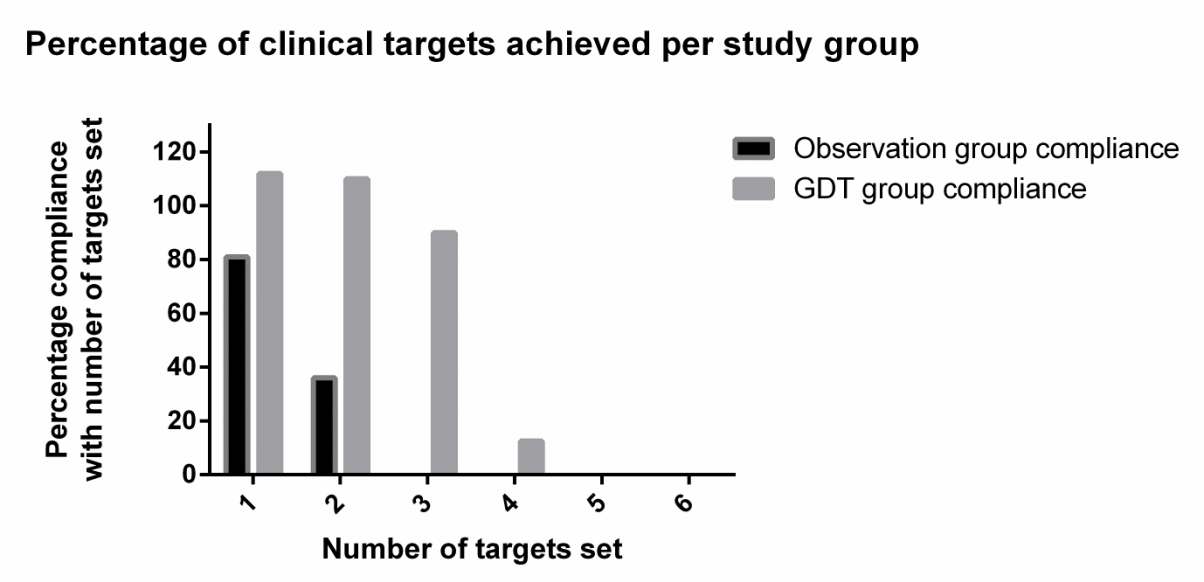


Figure 6.2 Composite proportions of targets achieved by the care bundle

6.3.4.4 Individual target achievements for BAM

Following the examination of the composite targets, individual target achievements were estimated for patients included in the study (Table 6.14). Routine clinical care was not able to meet the majority of clinical targets for these patients. However the neither the care bundle nor the routine medical care was able to achieve the targets in patients with a 5 or more target requirement.

In addition there are a small number of patients (2 in P1 and 5 in P2) who achieved more targets than they were set (Table 6.14). This was due to a clinical deterioration during the six hour observation time after admission to AETC that required the addition of further elements of the care bundle. The EGDT protocols stipulated that the patients were monitored hourly, and if a clinical deterioration was noted during the observation period that required protocolised treatment, then that treatment would be instituted. The overall study analysis was a comparison of targets set at the start of the monitoring period and those met by the end of the period, and the inclusion of these participant data who deteriorated during the care bundle led to the discrepancies seen in the Table 6.14.

Table 6.14 Cumulative target achievement in BAM

Individual cumulative target requirements across the study phases

Number of targets set	Number of patients per target set Phase 1 n=71 (%)	Number of patients meeting target no. set in Phase 1(%)		Number of patients per target set Phase 2 n=61 (%)	Number of patients meeting target no. set in Phase 2 (%)	
One target	16 (22%)	0 targets	6 (37%)	16 (26%)	0 targets	2 (12.5%)
		1 target	3 (18%)		1 target	10 (62.5%)
					2 targets	3 (18%)
		No data	7 (43%)		3 targets	1 (6%)
Two targets	22 (31%)	0 targets	2 (9%)	20 (33%)	0 targets	5 (25%)
		1 target	3 (13%)		1 target	5 (25%)
		2 targets	4 (18%)		2 targets	9 (45%)
		3 targets	2 (9%)		3 targets	1 (5%)
		No data	11 (50%)			
Three targets	14 (20%)	0 targets	4 (28%)	11 (18%)	0 targets	2 (18%)
		1 target	3 (21%)		1 target	1 (9%)
		2 targets	4 (28%)		2 targets	6 (54%)
		3 targets	2 (14%)		3 targets	2 (18%)
		No data	1 (7%)			

Four targets	13 (18%)	0 targets	4 (30%)	8 (13%)	0 targets	1 (12.5%)
		1 target	3 (23%)		1 target	2 (25%)
		2 targets	3 (23%)		2 targets	2 (25%)
		3 targets	2 (15%)		3 targets	3 (37.5%)
		4 targets	0		4 targets	0
		no data	1 (7%)			
Five targets	6 (8%)	0 targets	3 (50%)	3 (5%)	0 targets	0
		1 target	3 (50%)		1 target	0
		2 targets	0		2 targets	1 (33%)
		3 targets	0		3 targets	2 (66%)
		4 targets	0		4 targets	0
		5 targets	0		5 targets	0
Six targets	0	0 targets	0	3 (5%)	0 targets	0
		1 target	0		1 target	0
		2 targets	0		2 targets	1 (33%)
		3 targets	0		3 targets	1 (33%)
		4 targets	0		4 targets	1 (33%)
		5 targets	0		5 targets	0
		6 targets	0		6 targets	0
Seven targets	0	0		0	0	
Eight targets	0	0		0	0	

6.3.5 Mortality and morbidity comparisons between Phase 1 and Phase 2.

The BAM study was not powered to detect a difference in mortality between the two phases as the primary outcome, as no data were available on which to base an estimate of predicted mortality difference in ABM by EGDT. Estimates of mortality and morbidity were made as exploratory secondary outcome measures. Mortality was measured at discharge from AETC, daily on the ward during follow up, by discharge or day 10 whichever was earlier, and by 6 weeks post admission at day 40 (Table 6.15).

Morbidity was measured by questioning to obtain a score on the modified Rankin score (mRS), and by physical examination to determine if deafness or neurological weaknesses were present. Composite poor outcome of death or disability was made by combining patients who either died or had a mRS of >2 points at that time point. Table 6.15 details the comparison between P1 and P2 for each of these endpoints. An overall trend towards worse outcomes in P2 was seen in all measured parameters at all time points, although these only reached statistical significance at the day 10 composite endpoint of death or disability. Mortality alone at day 10 was 27/71 (38%) in P1 and 32/61 (52%) in P2, but this did not reach statistical significance (p=0.11). However, when combined with morbidity to a composite end point, the rate of poor outcome was 33/71 (46%) in P1 and 44/61 (72%) in P2 (p=0.004).

Morbidity alone differed between phases at day 10; (6/71 (8%) patients in P1 had mRS >2 but 12/61 (20%) in P2 had the same score (p=0.056).

However no patients had persistent significant disability by the 6 week follow up point.

Despite substantial loss to follow up in P1 (14 patients lost in P1 compared to 1 in P2), day 40 endpoints were again worse in P2 than P1 without reaching statistical significance. Six additional deaths were noted in P2 post discharge compared to one in P1, leading to a day 40 poor outcome rate of 29/57 (51%) in P1 and 38/60 (63%) in P2.

Table 6.15 Mortality and morbidity outcomes in BAM

Secondary outcome measures			
Outcome	**Phase 1 N=71**	**Phase 2 N=61**	**Univariate p**
Dead in AETC	4 (5%)	7 (11%)	0.34
Death day 2	20 (28%)	22 (36%)	0.33
Death day 10	27 (38%)	32 (52%)	0.11
Death + disability day 10	33/71 (46%)	44/61 (72%)	0.004
Poor functional ability mRS >2 day 10	6/71 (8%)	12/61 (20%)	0.056
Deafness day 10	15/71 (21%)	10 (16%)	1.0
Persistent seizures day 10	1 (1.4%)	0 (0%)	1.0
Death day 40	28/57 (49%)	38/60 (63%)	0.13
Death + disability day 40	29/57 (51%)	38/60 (63%)	0.19
Poor functional ability mRS >2 day 40	1/29 (3%)	0/22 (0%)	0.84
Deafness day 40	6/29 (20%)	7/22 (31%)	0.57
Persistent seizures day 40	0	0	NA

The cumulative mortality data are represented in Figure 6.3, Kaplan-Meir plot of survival comparing the two phases. The data are censored to account for the missing data.

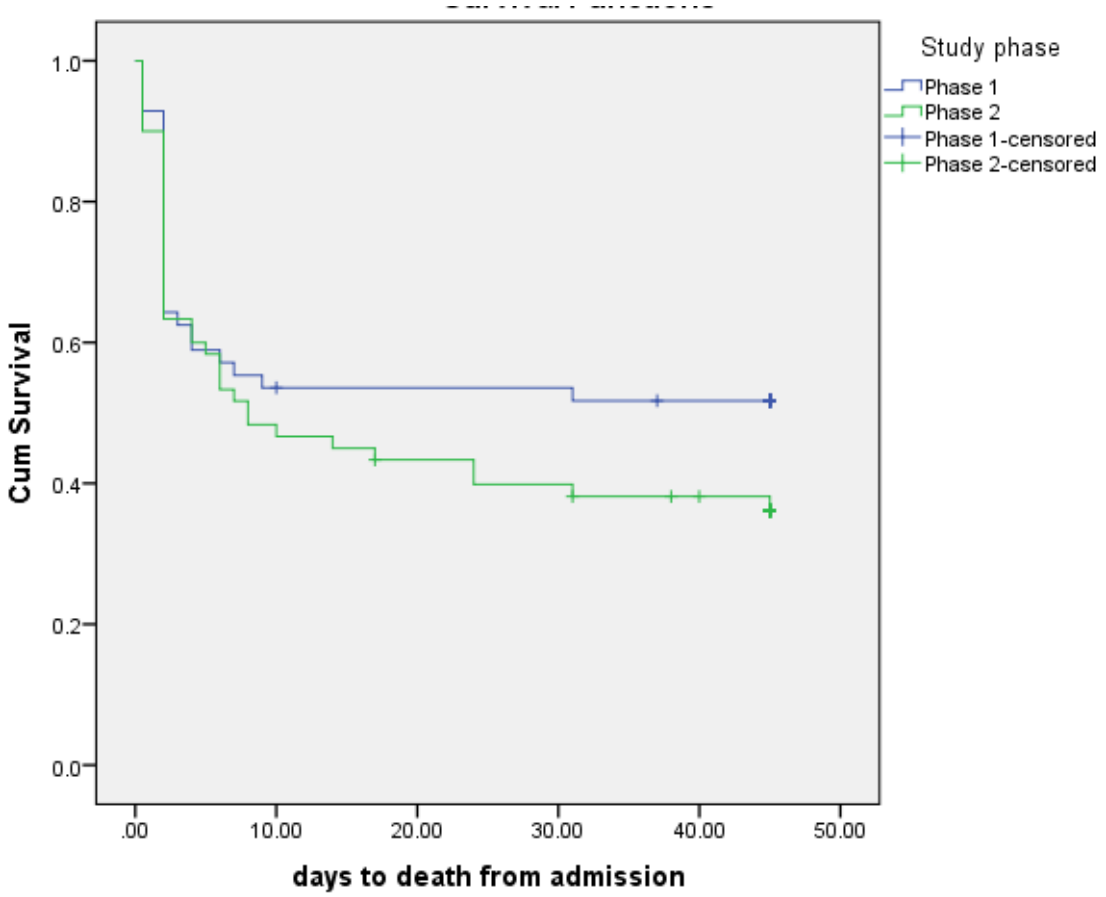


Figure 6.3 Kaplan-Meir curve of outcome from ABM by study Phase

Cumulative survival curve comparing Phase 1 and Phase 2. The difference at 40 days is not statistically significant Log rank test p=0.19

6.3.5.1 Adverse events in BAM

Details of adverse event monitoring are found in Chapter 3 Section 3.3.11.

Adverse event monitoring was undertaken throughout the study; however details on adverse events that could potentially be related to the care bundle were collected specifically in P2. Table 6.16 details the SAEs that occurred throughout the study and then specifically in P2 during the administration of the care bundle. Univariate significance was calculated using the Fisher exact test.

Table 6.16 Adverse events in BAM

Frequency of Adverse events			
Event	**Phase 1 n 71**	**Phase 2 n 61**	**Univariate significance**
Death in AETC	4	7	0.34
Death overall by day 10	27	32	0.11
Ward based events			
New or worsening focal neurology	5	1	0.56
New seizures	6	5	0.44
New or worsening deafness	3	1	0.64
Decrease in GCS >2 points from previous day	12	34	0.002
New or worsening pneumonia	2	2	0.61
Persistent fever	4	4	0.98
Oligo-anuria	1	1	1.0
Persistent hypotension requiring fluids	0	0	NA
Evidence of fluid overload	0	0	NA
Other	0	0	NA
AETC events	**P1**	**P2**	NA
Pulmonary oedema	No data	1	NA
Suspected cerebral oedema	No data	0	NA
New oxygen requirement	No data	1	NA
Worsening of coma by >2 points (survivors to AETC discharge with data)	5/37 (13.5%)	13/53 (24.5%)	0.28
New seizure	4	4	1.0
Respiratory depression (RR<12 breaths)	0	0	NA

The frequencies of adverse events in the ward during 10 days of follow up were similar, with the exception of worsening GCS of > 2 points from the previous day, which was recorded 12 times (17%) in P1 and 34 times (55%) in P2 (p=0.002). The frequency of recording a drop in GCS of >2 points during the AETC 6 hour observation period was also greater in P2 (24% v 13%) but this did not reach statistical significance (p=0.28) (Table 6.16).

6.3.5.2 Adverse events in excluded participants

Adverse events in patients who did not meet the inclusion criteria were only collected during the AETC 6 hour observation period and up to 48 hours ward follow up. SAEs for these patients were uncommon, and were only recorded in P2 due to concern about the harm that may be caused by the care bundle. The frequencies are as follows: Death 7 (3%), new oxygen requirement 3 (1.4%), GCS drop of =>2 points 2 (1%), and new seizure 1 (0.5%). Of the seven deaths none were directly attributable to the care bundle as determined by the PI and the study monitors, who reviewed the SAE forms and directed that no further action should be taken.

6.3.6 Malawi adult meningitis score (MAMS) in BAM

MAMS scores were tested prospectively in the analysis of BAM, Scores were calculated for each patient in BAM across the phases using the nomogram outlined in Chapter 5 Section 5.3.4. As the score increases, the corresponding risk of poor outcome also increases. Therefore it was expected that where a low mortality rate was observed, the MAMS would be correspondingly lower for the patients in that phase. MAMS was validated in Chapter 5 to mortality at day 40, so day 40 outcome data were used. Where day 40 data were missing, day 10 outcome data were used instead to optimise the numbers of patients in the analysis.

The score was then converted into a risk of poor outcome using the equation developed in Chapter 5, Section 5.2.4:

Predicted outcomes estimated using MAMS and true outcomes were then compared across the two phases, and the agreement calculated using Kappa (Table 6.17). A Kappa value of >0.6 was considered to represent good agreement. From the available complete case data (n=39) the calculated median MAMS were not different across the phases; median MAMS in P1 was 157 (132-182) compared to median MAMS in P2 of 149 (110 – 197) p=0.61.

Interestingly, the agreement between predicted outcome and actual outcome was very different between the phases. The predicted mortality rate for P1 was 64% and for P2 was 65%. However, the actual mortality rate was 18% in P1 and 71% in P2 (Table 6.17).

Table 6.17 Performance of MAMS in BAM

Comparison of the performance of MAMS across the BAM study phases complete case data n=39				
Probability of poor outcome group	**Phase 1 predicted deaths**	**Phase 1 actual deaths**	**Phase 2 predicted deaths**	**Phase 2 actual deaths**
0-0.1	0	0	0	0
0.1-0.2	0	0	0	0
0.2-0.3	0	0	1	3
0.3-0.4	1	0	1	1
0.4-0.5	3	0	1	1
0.5-0.6	2	1	2	1
0.6-0.7	3	0	1	1
0.7-0.8	2	1	1	1
0.8-0.9	3	2	4	4
>0.91	0	0	0	0
Total	14	4	11	12
Denominator	22	22	17	17
Mortality rate	64%	18%	65%	71%
Kappa	0.11 (p=0.42)		0.61 (p<0.001)	

Agreement of the score therefore between predicted and actual outcome was poor in Phase 1 but good in Phase 2. Kappa was 0.11 (Std 0.08) (p=0.42) in Phase 1, and Kappa was 0.69 (Std 0.18) (p<0.001) in Phase 2 (Table 6.17).

Although good agreement was seen in P2, there were limited numbers of observations available to calculate MAMS in BAM (30%). This was due predominantly to missing data in two main variables; Haemoglobin because laboratory results were not always available during the study, or CSF WCC when clumps of cells or a clot had been noted . To ensure the data were as complete as possible, a missing data analysis was undertaken as done for MAMS in Chapter 5 Section 5.2.5. The odds ratios of missing CSF WCC and missing Hb were compared with different categories for each variable (log10 CSF WCC and quartiles around the median for both CSF WCC and Hb) using logistic regression. In addition Hb was also categorised into clinically relevant groupings and these categories compared with the OR for missing values.

Neither of the missing data reported as either clumped CSF WCC or missing Hb had a statistical association with poor outcome compared to the other categories of that variable (OR 1.13 (0.35 – 3.61).and 4.1 (0.69 – 24.9) respectively. It was therefore concluded that no systematic bias of missing data with mortality was present, and random imputation could be done to increase the numbers available to test MAMS across the phases, as was done to complete missing data for the variable 'pulse' in the historical data (Chapter 5 Section 5.2.5).

Random imputation was done in SPSS version 20 and included the following variables in the imputation exercise; GCS, pulse, MAP, CSF WCC, CSF culture, age, gender, HIV status and Hb.

The overall median MAMS scores were recalculated from the mean of the pooled imputed data from five rounds of imputation and the performance of MAMS was again compared across the phases (Table 6.18).

Median MAMS in the imputed data in P1 was 151 (127-178) and in P2 was 136 (112-161) p=<0.001.

Using the imputed dataset actually worsened the relationship between predicted outcome using MAMS and actual outcome. The imputed data, although weaker than the complete case data, did demonstrate the same trend for MAMS to over predict mortality in P1 and have better agreement between predicted and actual outcomes in P2 compared with P2 agreement in the smaller non-imputed dataset (Table 6.18, Table 6.17).

Table 6.18 Performance of MAMS in BAM after imputation

Comparison of the performance of MAMS across the BAM study phases, showing pooled data from 5 rounds of random imputation

Probability of poor outcome group	Phase 1 predicted deaths n=68	Phase 1 actual deaths n=68	Phase 2 predicted deaths n=55	Phase 2 actual deaths n=55
0-0.1	0	0	0	0
0.1-0.2	0.48	0.6	0.75	2.2
0.2-0.3	0.7	1	1.6	2.8
0.3-0.4	2.9	1.6	3.9	6.4
0.4-0.5	5.6	2.6	4.3	4.8
0.5-0.6	6.5	4.4	5.3	5.4
0.6-0.7	7.5	3.2	3.2	4.2
0.7-0.8	8.5	8	4.4	4.6
0.8-1.0	5.71	4.4	3.1	3.6
Total deaths	38.2	25.8	26.6	34
Denominator	68	68	55	55
Case fatality rate	56%	38%	48.5%	61.8%
Kappa	0.1	p=0.03	0.1	p 0.003

The poor performance of the imputed data is likely to be due to subtle ascertainment biases in the original data, particularly in P1 where missing data may be associated with ward

recruitment compared to AETC recruitment. The imputed analyses are presented here for completeness, but due to the weaknesses in the data, no conclusions can be drawn from these analyses.

6.3.7 Subgroup analyses

The finding that the mortality in Phase 1 was lower than that seen in Phase 2 and lower than the baseline from the historical meningitis data presented in Chapter 4 section 4.5.3-4, was unexpected. Subgroup analyses were undertaken to explore relationships between baseline severity indices, specific interventions and physiology over the 6 hour observations in order to explore the mortality differences.

6.3.7.1 Tests for survival biases between the phases

Recruitment patterns between P1 and P2 were slightly different. In P1 funding was only available for nurses to recruit patients Mon-Fri 7am-10pm, in P2 further funding was available for an additional nurse and recruitment was 24 hours from Sunday night to Saturday morning. To detect patients that were admitted with meningitis out of study hours in P1, computerised laboratory records were searched daily. If patients had died by the point at which the results were available and therefore it was not possible for the team to obtain informed consent, data were recorded from the patient case notes. This approach to recruitment raised the possibility of survival biases in the data, that by missing early deaths, data recorded would higher rates of survival in P1 compared to P2. Outcome data were analysed using Chi squared testing and univariate logistic regression. Table 6.19 shows deaths and composite outcomes across the phases by admission in or out of hours.

Table 6.19 BAM outcomes by admission time

Study outcomes for included participants by admission in or out of hours				
Outcome measure		**Alive**	**Dead**	**OR (95% CI) p**
Death & disability day 10	Phase 1 in hours n=48	24	24 (50%)	…..
	Phase 1 out of hours n=23	14	9 (39%)	OR 0.61 (0.23 : 1.7) p= 0.39
	Phase 2 in hours n=60	29	31 (51%)	…….
	Phase 2 out of hours n=1	0	1 (100%)	OR not calculated
Death & disability day 40	Phase 1 in hours n=37	16	21 (56%)	…….
	Phase 1 out of hours n=20	12	8 (40%)	OR 0.5 (0.16 : 1.5) p=0.23
	Phase 2 in hours n=60	22	37 (61%)	
	Phase 2 out of hours n=1	0	1 (100%)	OR not calculated p=1.0

Although there were fewer cases admitted out of hours in P1 with a marginally improved composite outcome compared to those admitted in hours, these differences were not statistically significant. There was therefore no evidence that the observed reduced case fatality rate in P1 compared to P2 could be explained by unrecorded deaths occurring because of the different recruitment patterns between the two phases.

6.3.7.2 Causes of poor outcome

To explore the mortality rates seen in both phases, an analysis examining the comparison of predictors of poor outcome across the study phases was designed to test if variables suspected to be associated with poor outcome differed across the phases. Three variables were tested in the first analysis by dividing each continuous variable into quartiles around the median, and testing if an association existed between outcome and the upper quartiles of each variable, against the lowest quartile (assumed to be associated with the best outcome) using logistic regression (Table 6.20). These variables were blood lactate (BL), MAMS and time to IV antibiotics from admission. High levels of lactate (>4mmol/L) have been shown to be an independent predictor of outcome in sepsis (Rivers et al., 2001; Arnold et al., 2008), but little data are available examining the relationship between blood lactate and outcome from bacterial meningitis as opposed to sepsis. All guidelines for the treatment of ABM suggest that rapid IV antibiotic administration is important, and delay may be harmful (van de Beek et al., 2012; Koster-Rasmussen et al., 2008). MAMS is a new tool to assess risk of bad outcome developed in Chapter 5, differences in outcome by groups of each variable ranging from the >75th centile to the <25th centile were tested.

Blood lactate (BL) was measured on admission in both study Phases and again after 6 hours observation in P2. BL was grouped into quartiles around the median, with the expectation that the 75th centile would represent the highest blood lactate value and therefore be associated with increased mortality as seen in sepsis (Table 6.20). The median lactate was 3.6mmol/L (IQR 2.0 – 6.0) in survivors to day 40, and 3.4 mmol/L (IQR 1.9 – 5.6) in non survivors to day 40 (p=0.70). Mortality was evenly distributed across all quartiles of BL in both phases, the OR death in the >75th centile of BL (>5.6 mmol/L) was 1.0 (0.15 : 1.64) in P1 and 2.5 (0.58 : 10.6) in P2 p=0.21.

Median MAMS scores with the associated quartiles were evenly distributed across the two Phases with no clear association between higher scores and outcome in either Phase. OR death with different quartiles of MAMS scores in P1 were not calculated due to lack of

mortality data in the reference category. OR death in P2 with MAMS >187 = 0.37 (0.02 : 6.3) p=0.49. More deaths occurred in the lower quartiles for time to antibiotics in P2 compared to P1 stats but this trend did not reach statistical significance (Table 6.22). There was no relationship between antibiotic delay and poor outcome.

MAMS was originally validated in a dataset that consisted of BAM Phase 1 data and randomly selected data from the historical database (Chapter 5 Section 5.2.2). The failure of MAMS to predict outcome accurately in solely Phase 1 data led to testing if the predictors of mortality in the historical database detailed in Chapter 4 Section 4.5.3-4, were the same of different in BAM patients between the two Phases. In addition further analysis including important potential confounders was done (Table 6.21).

The individual predictors of poor outcome identified from the historical meningitis cohort data (Chapter 4 Section 4.5.3-42) were tested in these patients using logistic regression. Mortality at day 10 was again used as the endpoint to minimise biases from missing outcome data at day 40 in Phase 1.

Lower GCS strongly correlated with outcome across both Phases: OR GCS with death 0.84 (0.72 : 0.97) in P1 and 0.67 (0.50 : 0.91) in P2.

In P1 a higher acute seizure frequency was associated with mortality (Table 6.21) OR death in P1 of one seizure was 5.6 (95% CI 1.7 : 18.5) p=0.004, two or more seizures OR death was12.3 (1.2 : 121) p=0.03. This trend was also noted in P2. However as all seven patients who had one seizure died in P2, and 50% of those with two or more seizures died the OR for one seizure alone could not be calculated, and in the two or more seizure group, there was no significant association with mortality. All other parameters measured had no association with outcome, including those with promising trends in the historical cohort but inadequate data for full examination in that dataset such as lactate, oxygenation and respiratory rate.

Table 6.20 Association of BAM outcomes with known predictors of outcome

	All N=132	Phase 1 N=71			Phase 2 N=61		
	Deaths (%)	**Deaths (%)**	**OR**	**p**	**Deaths (%)**	**OR**	**p**
MAMS	n=39	N=22			N=17		
<130	4 (10%)	0 (0%)	…..		4 (23.5%)	……	
130-151	1 (2.5%)	0 (0%)	…..	1.0	1 (6%)	0.08 (0.004 : 1.94)	0.12
151-187	3 (7.5%)	1 (4.5%)	UC	0.99	2 (12%)	0.5 (0.19 : 12.8)	0.67
>187	5 (13%)	2 (9%)	UC	0.99	3 (17.6%)	0.37 (0.02 : 6.3)	0.49
Lactate	N=100	N=39			N=61		
<2	10 (10%)	4 (10%)	….		6 (10%)	……	
2-3.4	15 (15%)	5 (12.8%)	0.78 (0.13 : 4.3)	0.77	10 (16%)	2.7 (0.66 : 11.6)	0.16
3.4-5.6	11(11%)	4 (10%)	1.25 (0.18 : 8.4)	0.81	7 (12%)	1.6 (0.38 : 7.1)	0.49
>5.6	13 (13%)	4 (10%)	1.0 (0.15 : 6.4)	1.0	9 (15%)	2.5 (0.58 : 10.6)	0.21
Time to IV antibiotics	N=110	N=49			N=61		
<0:55	7 (6%)	0 (0%)	……		7 (11%)	……	
0:55-1:31	18 (16%)	6 (12%)	UC	0.99	12 (19.6)	3.1 (0.86 : 11.0)	0.08
1:31-2:25	13 (12%)	7 (14%)	UC	0.99	6 (10%)	1.85 (0.43 : 7.9)	0.40
>2:25	13 (12%)	6 (12%)	UC	0.99	7 (11%)	4.3 (0.84 : 22.0)	0.07

… = reference variable in logistic regression. UC = unable to calculate OR due to lack of mortality cases in the reference value.

Table 6.21 Individual predictors of outcome by study phase

		Phase one n=71				Phase 2 n=61			
		Alive 44	**Dead 27**	**OR death (univariate)**	**p**	**Alive 29**	**Dead 32**	**OR death (univariate)**	**p**
HIV infected		25	16	4.48 (0.89 : 22.3)	0.06	18	21	0.97 (0.25 : 3.72)	0.96
Mean age (Std)		32.7 (12.2)	34.1 (10.6)	1.0 (0.97 : 1.05)	0.61	35.5 (13.3)	37.6 (16.3)	1.00 (0.97 : 1.04)	0.59
Male Gender		23	18	1.8 (0.67 : 4.93)	0.23	20	20	0.75 (0.25 : 2.17)	0.59
GCS mean (Std)		12.4 (2.8)	10.4 (4.0)	0.84 (0.72 : 0.97)	**0.02**	13.3 (1.6)	11.7 (2.5)	0.67 (0.50 : 0.91)	**0.01**
Mean Pulse (bpm) (Std)		102.1 (23.5)	104.9 (25.3)	1.00 (0.98 : 1.02)	0.63	99 (0.2)	104 (22.2)	1.01 (0.98 : 1.03)	0.34
Acute seizures	**None**	37	12	…		25	21	……..	..
	One	6	11	5.6 (1.7 : 18.5)	**0.004**	0	7	Unable to compute	
	Two or more	1	4	12.3 (1.2 : 121)	**0.03**	4	4	1.1 (0.26 : 5.3)	0.82

	Phase one n=71				Phase 2 n=61			
	alive	**dead**	**OR death (univariate)**	**p**	**alive**	**dead**	**OR death (univariate)**	**p**
Oxygen saturations (%) median (IQR)	96 (95 – 98)	96 (92 –97)	0.90 (0.78 : 1.03)	0.15	97 (95-97)	97 (90-98)	0.91 (0.80 : 1.0)	0.15
O_2 saturations <94%	9	9	2.2 (0.70 : 6.6)	0.16	4	11	3.2 (0.90 : 11.8)	0.07
Respiratory rate (breaths/minute) Median (IQR)	24 (20-28)	28 (24 –39)	1.05 (0.99 : 1.12)	**0.058**	21 (20-24)	22 (19-28)	1.0 (0.96 : 1.17)	0.18
Blood lactate (mmol/L) Median (IQR)	2.8 (2.1–5.3)	3.4 (2.0–6.3)	1.0 (0.87 : 1.30)	0.54	3.4 (1.9 – 5.1)	3.5 (2.8-6.2)	1.06 (0.90 : 1.26)	0.43
Hb (g/dL) mean (Std)	11.5 (2.3)	12.1 (1.9)	1.1 (0.77 : 1.6)	0.53	11.5 (2.4)	11.0 (2.4)	0.91 (0.74 : 1.13)	0.43
CSF WCC (cells/mm^3) Median (IQR)	109 (26 – 480)	37 (8-183)	0.99 (0.99 : 1.001)	0.25	188 (0.5–1680)	241 (17.5 – 1116)	0.99 (0.99 : 1.001)	0.49
CSF culture positive	23	11	0.62 (0.23 : 1.65)	0.34	10	17	2.1 (0.76 : 6.0)	0.14

6.3.7.3 Mortality by clinical care bundle element

Mortality per clinical intervention was compared across the phases (Table 6.22). Patients not meeting the one hour antibiotic target across both phases did not have significantly increased mortality compared to those meeting the target. As expected, altered mental state and coma were associated with poor outcome across both phases, however the proportion of patients dying was higher in P2 compared to P1 in both categories (GCS <11 mortality P1 14/26 51%, P2 13/16 83%), (GCS <8 mortality P1 8/14 57%, P2 5/6 83%). Airways were inserted in patients with GCS <8 as part of the care bundle, as was a head tilt given to those with GCS <11. Both interventions were associated with poor outcome: OR death airway insertion P1 2.7 (0.82 : 9.0) p=0.09, OR P2 5.1 (0.56 : 47.3) p=0.14, OR death of head tilt P1 3.0 (1.0 : 8.3) p=0.03, in P2 OR 5.9 (1.4 : 23.0) p=0.01. However, when corrected for GCS, the insertion of an airway, neither intervention were significantly associated with poor outcome (OR death with airway insertion 0.96 (0.11 : 7.8) p=0.97, OR death with head tilt 0.48 (0.07 : 3.04) p=0.44) (Table 6.22).

Specific prescribed interventions were oxygen therapy, IV fluids and medication including antibiotics, diazepam, phenobarbitone, anti-malarials and fluconazole for suspected cryptococcal meningitis (Table 6.22). No data are available on the number of patients in P1 prescribed O_2 therapy, however receipt of O_2 in P2 was significantly associated with poor outcome OR 3.83 (1.23 : 11.9) p=0.02. The volumes of intravenous fluid prescribed in P2 were substantially greater than those in P1 (median volume P1 0.75L IQR 250-750) compared to P2 (1.5L IQR 900-2000) p=<0.001. More patients in P1 were not given any IV fluid compared to P2 but these differences were not significant.

Table 6.22 Targets in BAM and outcome by study phase at day 10

Targeted therapy		**Phase 1 n=71**				**Phase 2 n=61**			
		Alive	**Dead**	**OR (univariate)**	**p**	**Alive**	**Dead**	**OR (univariate)**	**P**
		44	**27**			**29**	**32**		
ABx <1 hr	Yes	4	2	……		15	12	…..	
	No	26	17	1.3 (0.2 : 7.9)	0.77	14	20	1.76 (0.6 : 4.9)	0.26
Airway	Yes	6	8	2.7 (0.82 : 9.0)	0.09	1	5	5.1 (0.56 : 47.3)	0.14
(GCS <8)	No	37	18	…….		28	27	…….	
Head tilt	Yes	12	14	3.0 (1.0 : 8.3)	**0.03**	3	13	5.9 (1.4 : 23.0)	**0.01**
(GCS <11)	No	31	12	…….		26	19		
Oxygen therapy	Yes	UK	UK	Unable to		6	16	3.83 (1.23 : 11.9)	**0.02**
	No			compute OR		23	16	……..	
Volume of IV	No fluid	9	2	0.55 (0.05 : 5.2)	0.60	1	1		
fluid prescribed	IV fluid	750	750	1.00 (0.99 : 1.0)	0.18	1700	1325	0.81 (0.37:1.77)	0.60
	(median	(250-	(250-			(750-	(912-1975)		
	IQR)	750)	750)			2075)			

Fluid bolus	Yes	2	3	3.7 (0.33 : 44.2)	0.28	19	19	…..	
	No	33	21	…….		3	8	2.61 (0.60 : 11.6)	0.19
Volume of blood	No blood	1	1	Unable to	1.0	0	1	Unable to	0.5
(units)	No. Units	0	0	compute OR		1	1	compute OR	
Diazepam	Yes	11	4	0.52 (0.14 : 1.8)	0.31	12	19	2.0 (0.74 : 5.75)	0.16
	No	33	23	……		17	13	……	
Phenobarbitone	Yes	3	3	1.7 (0.3 : 9.1)	0.53	2	0	Unable to	0.99
	No	41	24	…..		27	32	compute OR	
Other	Yes	1	0	Unable to	1.0	0	0	Unable to	Unable to
medication	No	43	27	compute OR		29	32	compute OR	compute
(Malaria)									
Other	Yes	2	0	Unable to	0.52	0	1	Unable to	1.0
medication	No	42	27	compute OR		29	31	compute OR	
(Fluconazole)									

IV fluid volumes were the same in survivors and non-survivors at day 10 in P1; in P2 survivors received more IV fluid compared to non-survivors but this did not reach statistical significance (median volume survivors 1.7L IQR 0.75-2.075L, non survivors 1.32L IQR 0.9-1.97) OR 0.81 (0.37:1.77) p=0.60. There was no association in either phase with the administration of a fluid bolus and death at day 10. The administration of diazepam or phenobarbitone was not associated with death overall (OR 1.38 95% CI 0.67 : 2.85), or in either phase at day 10 on univariate analysis. However, more patients received diazepam in P2 (31/61 50%) compared to P1 (15/71 21%) p<0.001. Numbers of prescriptions of other therapies including phenobarbitone, anti-malarial drugs and fluconazole were too small for meaningful analysis.

6.3.8 Physiology of patients with ABM.

All screened patients admitted to the BAM study in AETC were monitored for 6 hours post recruitment. Hourly observations were taken for pulse, respiratory rate, oxygen saturations, GCS and blood pressure. Systolic and diastolic blood pressure were converted to mean arterial blood pressure (MAP) using the following standard equation

MAP= ((2*diastolic BP)+systolic BP)/3.

Complete observations were only available for those patients in Phase 1 who were recruited by the study team in AETC, and were missing from those recruited retrospectively on the medical wards using CSF results. Further missing results were found in patients who died in AETC and did not complete the full 6 hours of observation. For completeness, only patients with complete case data to the end of 6 hours observation are presented here. By tracking the changing physiology over 6 hours it was hoped that patterns could be determined that could add further understanding to prediction and causes of poor outcome . Table 6.23 shows the mean values for each measured parameter at time zero before any treatment had been received, at time 3 hours when antibiotics and other prescribed treatments including

fluids should have been initiated , and at the last observation at 6 hours post admission. Mean values between outcomes by phase are compared using the students T-test. Complete case data were available for P1 non survivors n=13, P1 survivors n=24, P2 non survivors n=24, P2 survivors n=28. Complete case data were missing either due to death in AETC and the cessation of observations, or ward based recruitment in P1, or where data had not been collected for a particular hour due to constraints within the study team. As such the data do not fully represent the spectrum of patients recruited by BAM, but do show for the first time how physiology may change over time in the acute 6 hour period after admission with meningitis. All patients included in these analyses were alive at the end of the 6 hour observation period.

Figures Figure 6.4**,** Figure 6.5**,** Figure 6.6**,** Figure 6.7 and Figure 6.8 shows the trend lines for each observation by phase and outcome at day 10. Day 10 was selected in all cases for analysis as missing data in P1 to day 40 may potentially bias outcome results. After review of the graphs with Dr Faragher, formal statistical comparisons of the gradients of the graphs were not done, as no important differences in graph slope by outcome were seen in any of the physiological sub-sets sets presented. In the following figures, each physiological parameter is shown in patients with complete case data only measured over 6 hours from admission (T0) to AETC discharge (T6). The data are summarised in Table 6.23.

All pulse measurements on admission were very similar (Figure 6.4), however a divergence occurred between T2 and T3 in P2, where the pulse rate declined over time, the pulse rate in P1 remains static over time with no separation by outcome in either phase. The differences in pulse at any of the time points are not statistically significant.

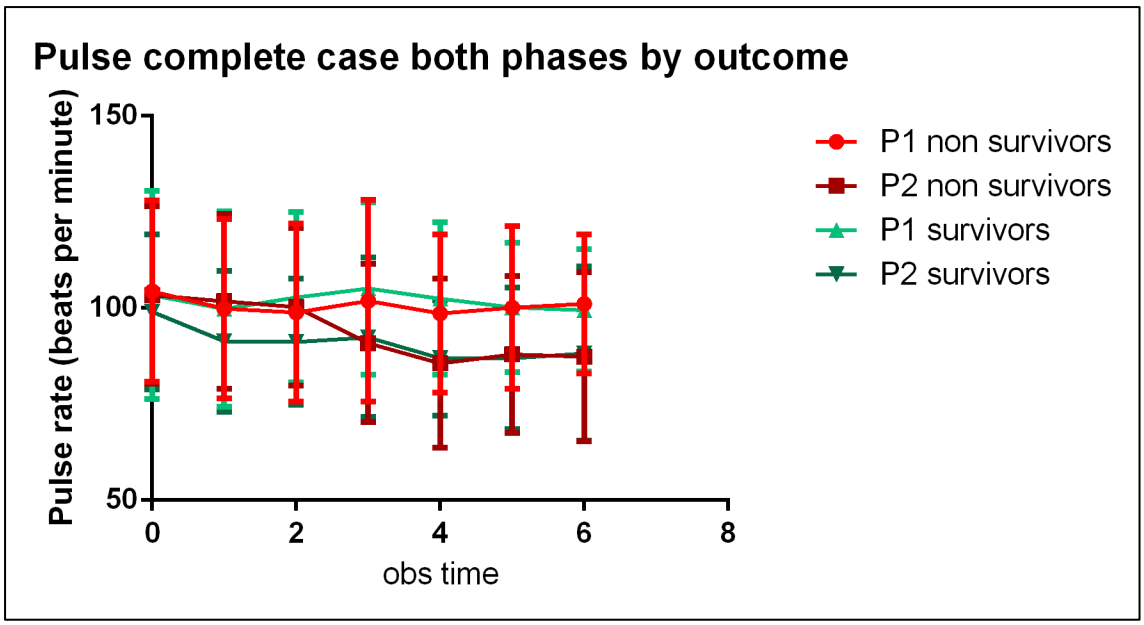


Figure 6.4 Pulse rate over time in patients with ABM

Mean Pulse rate with standard deviation over 6 hours by outcome and phase, Data from non survivors are depicted in red, and survivors in green. Points represent means, and bars errors around the mean.

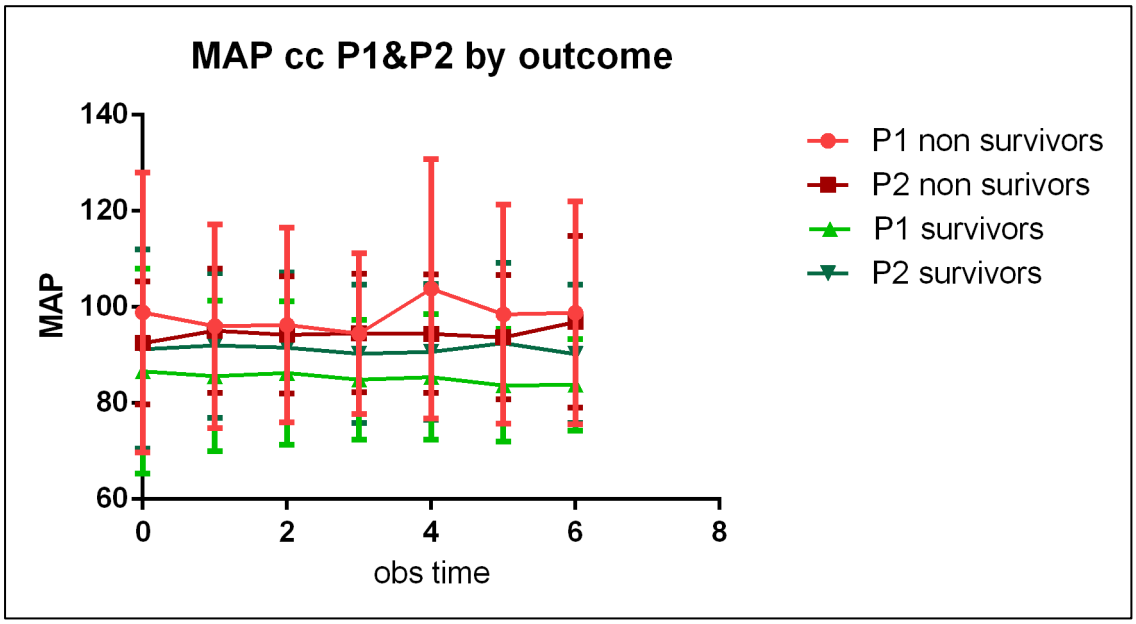


Figure 6.5 Mean MAP over time in patients with ABM

Mean MAP with standard deviation over 6 hours by outcome and phase, non survivors are depicted in red, and survivors in green. Points represent means, and bars errors around the mean.

Mean arterial blood pressure (MAP) was higher in non-survivors in both phases than survivors (Figure 6.5). This difference was more marked in P1 and reached statistical

significance by T3 in P1 (Table 6.23), (86.3 mmHg in survivors v 94.4 mmHg in non survivors p=0.05), with increasing disparity at T6, (83.8 mmHg in survivors v 98mm Hg in non survivors p=0.004) and borderline statistical significance in P2 at T6 (90.2 mmHg in survivors v 96.9 mmHg in non survivors p=0.07) (Table 6.23).

The divergence of MAP and particularly the trend towards increasing MAP in non-survivors over time is a novel finding.

As expected, there were marked differences in GCS by outcome in both phases (Table 6.23), although the overall mean GCS at all-time points in P1 non survivors were lower than in P2 non survivors (Figure 6.6)

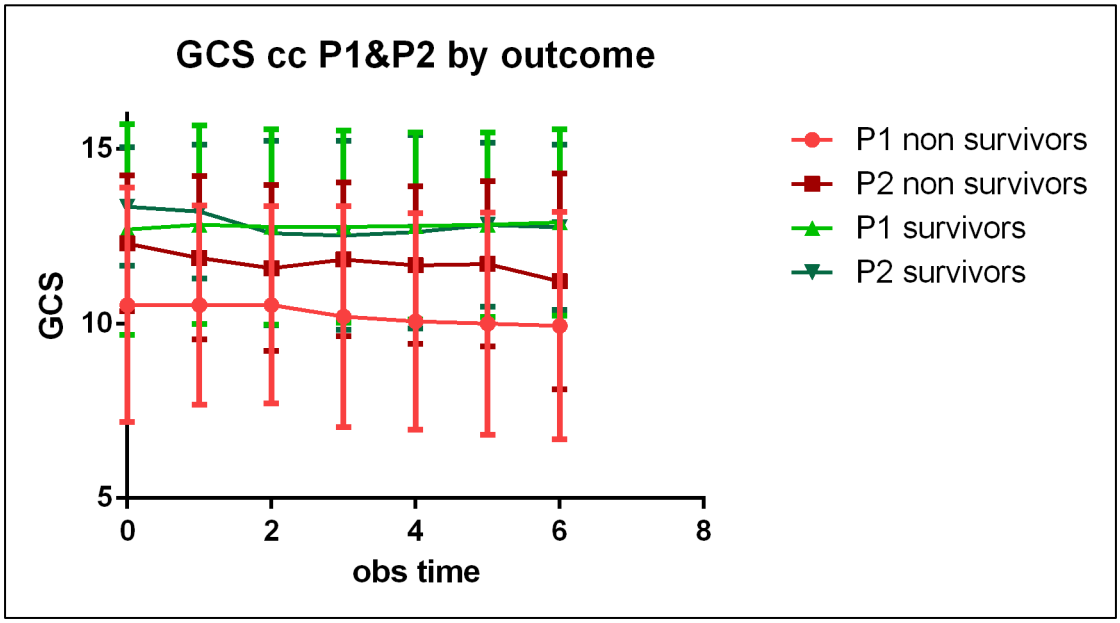


Figure 6.6 Mean GCS over time in patients with ABM

Mean GCS with standard deviation over time by outcome and study phase, non survivors are depicted in red, and survivors in green. Points represent means, and bars errors around the mean.

Mean GCS in survivors did not differ by phase in survivors, non survivors in P1 had lower mean GCS than non survivors in P2. There were no novel trends in these data, no significant declines over time in GCS were noted in any of the groups (Figure 6.6).

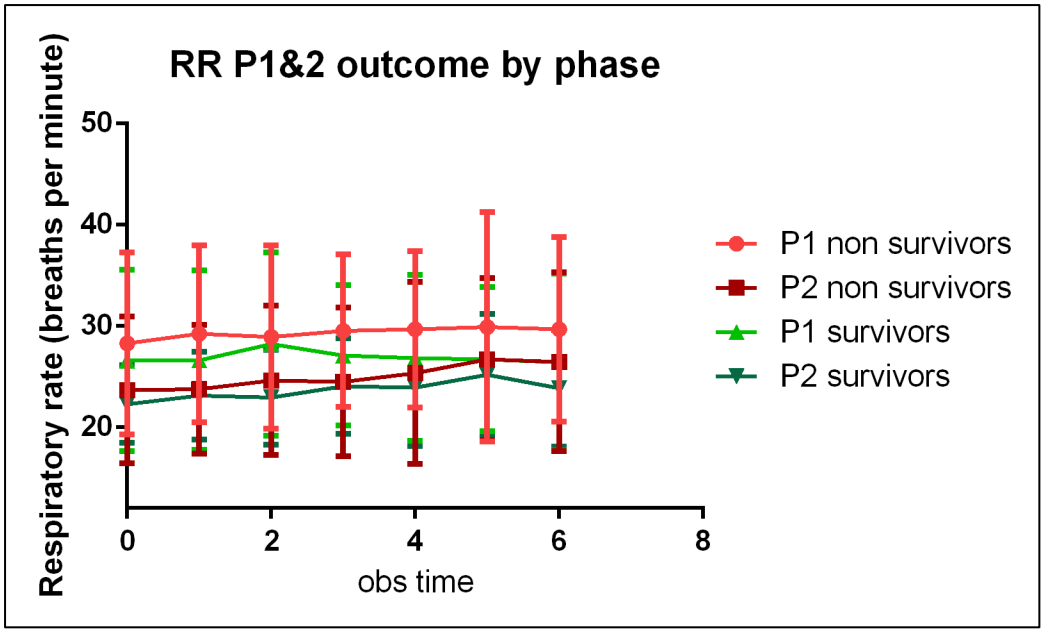


Figure 6.7 Mean respiratory rate over time in patients with ABM

Figure 6.4.4 Mean respiratory rate with standard deviation over 6 hours by outcome and study phase, non survivors are depicted in red, and survivors in green. Points represent means, and bars errors around the mean.

Respiratory rates are shown in Figure 6.7. Respiratory rates were overall higher in non survivors than survivors, increasing over time in P2 non survivors and static in P1 non survivors. RR fell in survivors, leading to a significant difference in RR at T3 (RR 26.5 v 29.5 p=0.099) and T6 (RR 25.5 v 29.6 p=0.05) in P1, this trend did not reach significance in P2 (Table 6.23).

In comparison, mean oxygen saturation rates across the phases were also similar; however a dip in SpO_2 was noted in both phases at T3 in non survivors (Figure 6.8). The differences between SpO_2 by outcome at this time point were statistically significant (Table 6.23). Mean SpO_2 in P1 non survivors at T3 was 93.3% compared to 96.7% p=0.03, and in P2 mean SpO2 in non survivors at T3 was 95.7% compared to 97.2% in survivors p=0.05. However these differences resolved by T6 in both phases and mean oxygenation across all groups was above 95% at ward discharge for all patients (Figure 6.8).

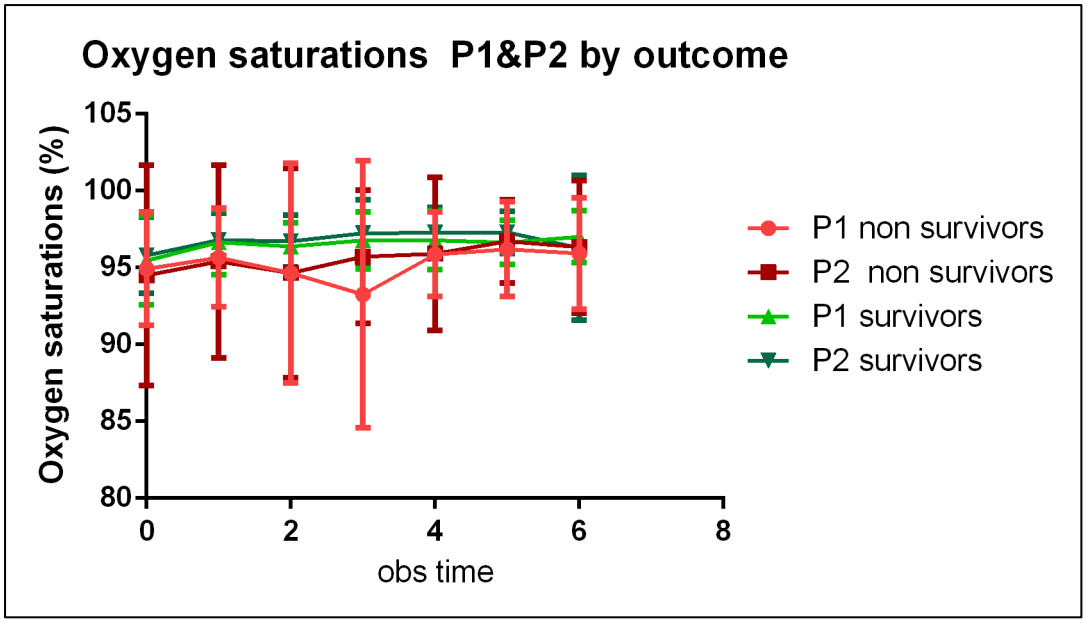


Figure 6.8 Mean oxygen saturations over time in patients with ABM

Mean oxygen saturation with standard deviation over time by outcome and study phase, non survivors are depicted in red, and survivors in green. Points represent means, and bars errors around the mean.

Table 6.23 Physiology of meningitis over time in BAM

Comparison of physiological parameters over time by phase and outcome (complete case data).

	Phase 1 n=37			Phase 2 n=52		
Mean (STD)	**Alive 24**	**Dead 13**	**P**	**Alive 28**	**Dead 24**	**p**
Pulse T0	103.3 (26)	104.4 (22)	0.45	99 (19.8)	103.3 (22.6)	0.23
Pulse T3	105 (21.5)	101.9 (25)	0.35	92.3 (20)	90.8 (20.1)	0.24
Pulse T6	99.4 (15)	101.0 (17)	0.39	88.2 (22)	87.4 (21.5)	0.44
MAP T0	89.6 (20.9)	98.8 (28)	0.06	91.2 (20)	92.2 (12.5)	0.40
MAP T3	86.3 (12.2)	94.4 (16)	**0.05**	90.3 (14.1)	94.5 (12.5)	0.13
MAP T6	83.8 (9.3)	98.8 (22)	**0.004**	90.2 (14.1)	96.9 (17.9)	0.07
GCS T0	12.7 (2.9)	10.5 (3.2)	**0.01**	13.3 (1.6)	12.2 (1.9)	**0.02**
GCS T3	12.7 (2.7)	10.2 (3.0)	**0.003**	12.5 (2.6)	11.8 (2.1)	0.16
GCS T6	12.9 (2.6)	9.9 (3.1)	**0.001**	12.7 (2.3)	11.2 (3.0)	**0.02**
RR T0	27.2 (9.2)	28.3 (8.6)	0.28	22.2 (3.7)	23.6 (7.1)	0.19
RR T3	26.5 (5.8)	29.5 (7.2)	**0.09**	24.0 (4.6)	24.5 (7.2)	0.39
RR T6	25.5 (5.8)	29.6 (8.7)	**0.05**	23.8 (5.6)	26.4 (8.6)	0.10
SpO_2 T0	95.4 (2.8)	95.0 (4.1)	0.31	95.7 (2.4)	94.5 (7.0)	0.18
SpO_2 T3	96.7 (1.8)	93.2 (8.4)	**0.03**	97.2 (2.1)	95.7 (4.2)	**0.05**
SpO_2 T6	97.9 (1.6)	96.2 (3.9)	0.11	96.2 (4.6)	96.3 (4.2)	0.48

A further analysis of the physiological data was undertaken by examining trends in a sub-set of the complete case data that were noted to have clinical shock on admission as defined in Table 3.4. The complete case numbers available for these analyses were small, P1 survivors n=14, P1 non survivors n=8, P2 survivors n=18, P2 non survivors n=15 (Figure

6.5.1-5). Due to the small numbers and very similar trends to the complete cases data, no formal statistical analyses were done on these data.

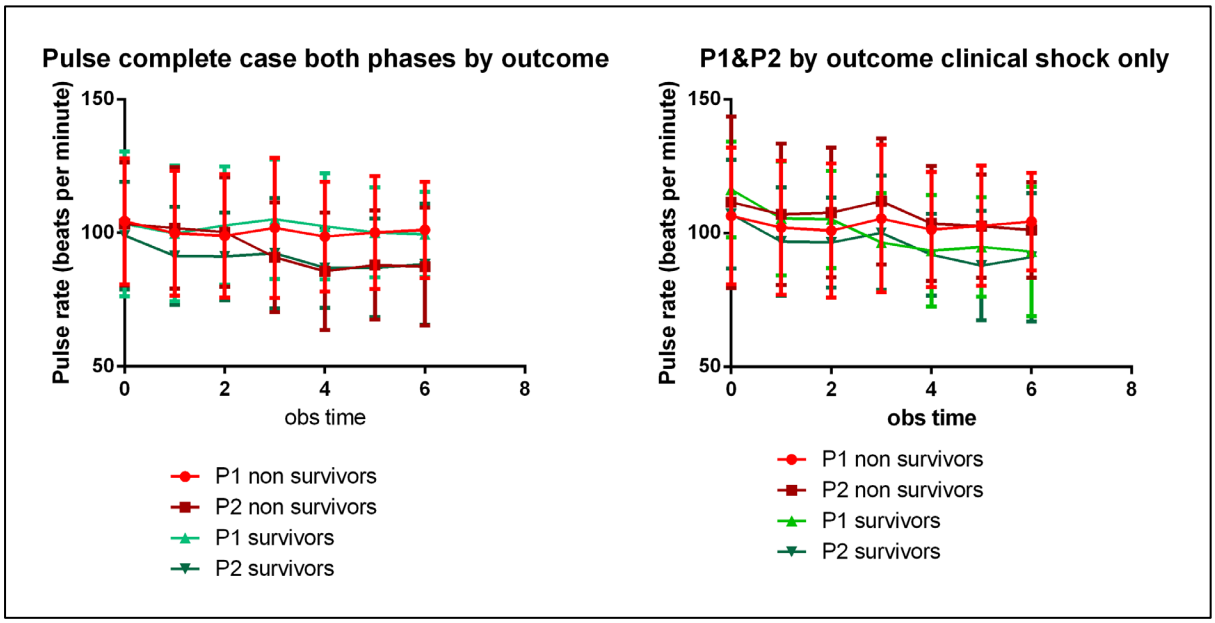


Figure 6.9 Mean pulse over time, comparing all ABM to those with clinical shock only

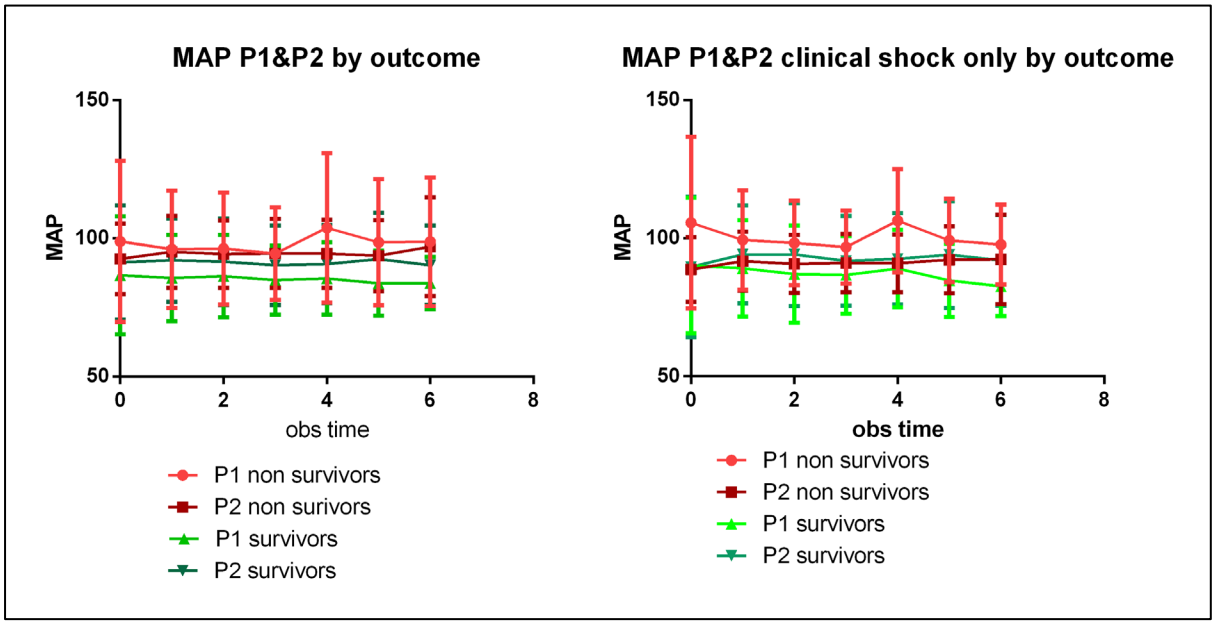


Figure 6.10 Mean MAP over time, comparing all ABM with those with clinical shock only

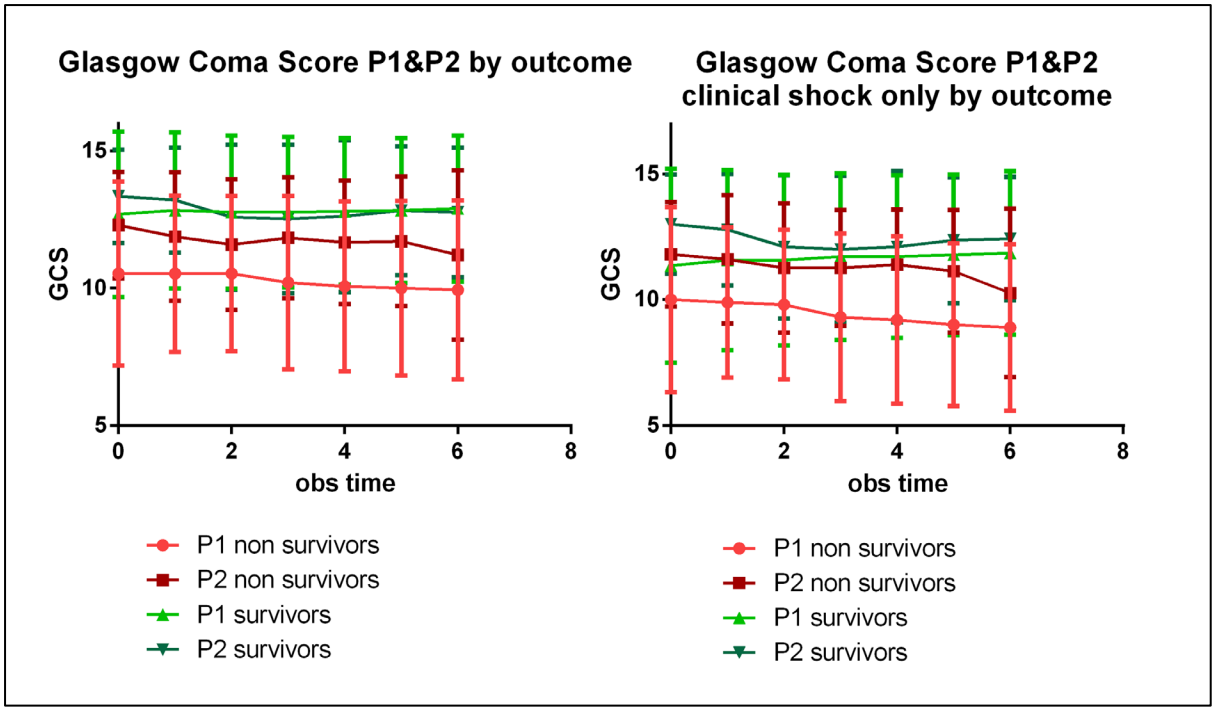


Figure 6.11 Mean CGS over time comparing all ABM with those with clinical shock only

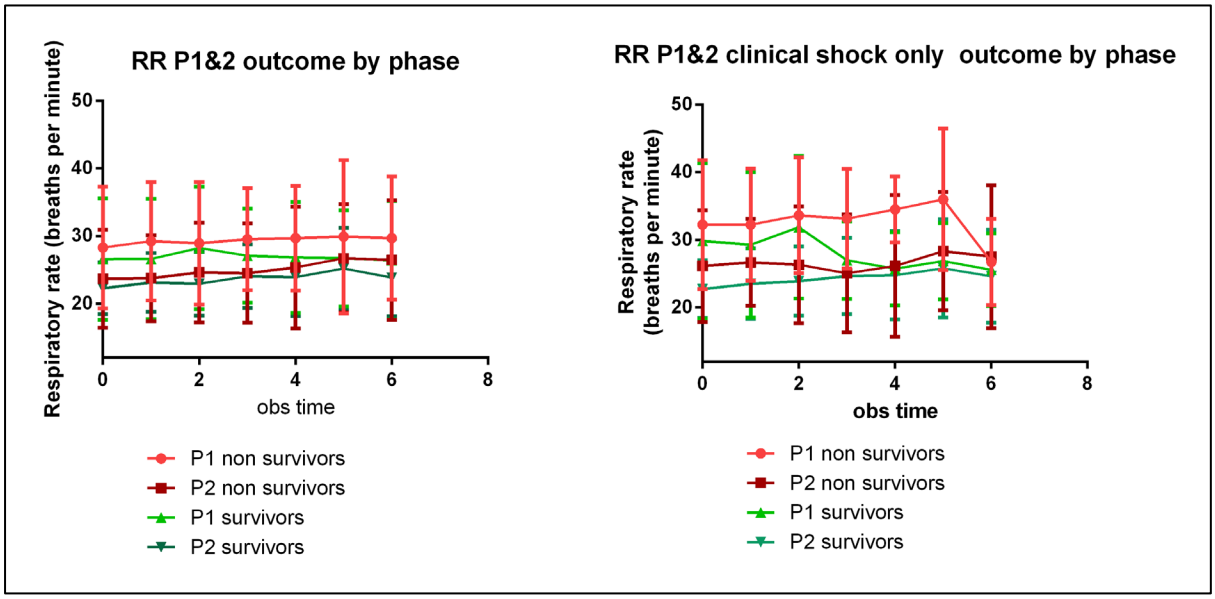


Figure 6.12 Respiratory rate over time comparing all ABM with those with clinical shock only

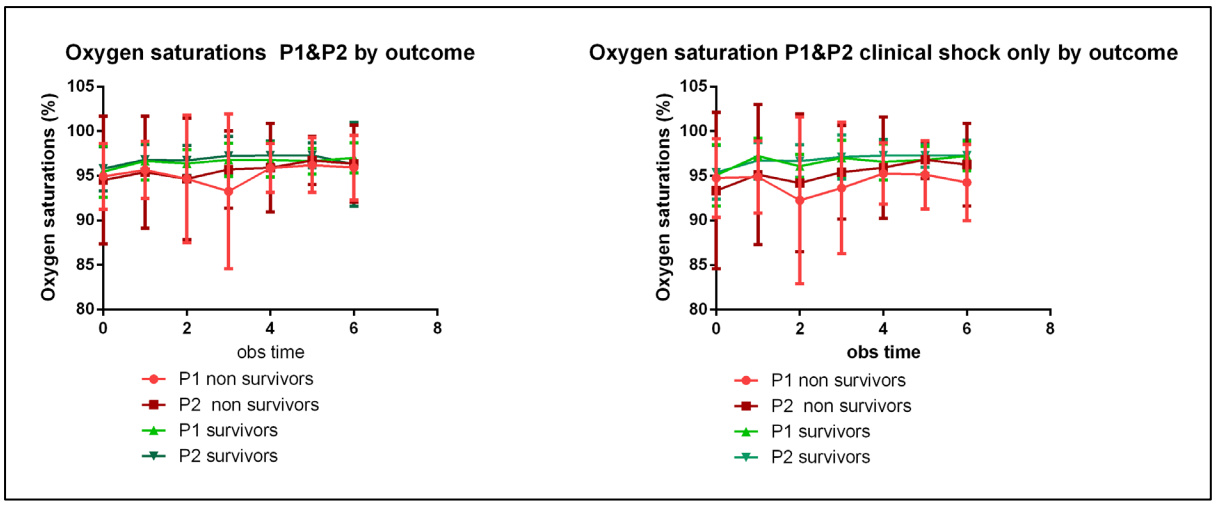


Figure 6.13 Oxygen saturations over time comparing all ABM with those with clinical shock only

In a comparison of shocked patients to all patients, non survivors with clinical shock appear to have a marginally more severe clinical phenotype developing over time, with non-declining pulse rate (Figure 6.9), higher MAPs at T6 (Figure 6.10) slightly greater declines in GCS over time (Figure 6.11), increasing or greater respiratory rates (Figure 6.12) and overall lower oxygen saturations (Figure 6.13). The small numbers preclude further analyses of these trends; however they provide important data to take forward into larger prospective data collection.

6.3.9 Excluded patients

6.3.9.1 Sepsis

Detailed analysis of the feasibility of targeted therapy for sepsis is beyond the scope of this study for several reasons. Firstly the BAM study was designed primarily to recruit patients with ABM and not sepsis, so other patients with sepsis will have been missed and selection bias is a major issue. Secondly with limited follow up data on these patients, no assessments of the safety of EGDT can fully be made from these data, particularly as no SAE data were collected after patients were excluded from the main trial.

6.4 Discussion

This study was designed to test if EGDT for ABM in Malawi was feasible as the primary endpoint, and determine if the care bundle impacted on mortality as an exploratory endpoint. This section will divide into three parts, the first discussing the results of the meningitis diagnostic testing, the second the feasibility assessment of the intervention, and the third discussing the outcomes observed.

6.4.1 Causes of culture negative meningitis

DNA of three common causes of bacterial meningitis were tested in culture negative samples in this study, *S. pneumoniae*, *N. meningitidis* and *H. influenzae* type b. Both bacterial culture and PCR detected cases of the former two pathogens, no cases of Hib were detected. The specificity of PCR appeared to be high, all culture positive cases were also PCR positive. In addition, PCR detected more cases of pneumococcal and meningococcal meningitis than culture alone (32% more cases of *S. pneumoniae* detected with PCR and 85% more cases of *N. meningitidis* detected).

Hib not detected by culture or PCR in this study. In the historical data in Chapter 4 Section 4.5.2, only 3/715 (0.4%) cases of ABM were due to Hib, the lack of positive culture in a sample size of 132 is not unexpected. It is possible that the lack of Hib meningitis in this study may be due to indirect effects of the paediatric vaccination programme, although there are no data testing for indirect effects of Hib in adults, and adult Hib meningitis is a rare disease (Cohen et al., 2010d). Indirect effects of Hib vaccine have been shown in siblings of vaccinated children, determination of indirect effects in adults would be very difficult as it is a rare adult disease (Adegbola et al., 2005; Muhlemann et al., 1996). As shown in Chapter 4 Section 4.3.1, Hib meningitis is declining dramatically in vaccine age-eligible Malawian children following the roll-out of vaccination in 2010 (Wall et al., 2014a).

In this study, a history of antibiotic prescription within 2 weeks of meningitis onset was associated with lower rates of bacterial culture in this study, 58% of culture negative cases had received prior antibiotics, compared to 32% who had no antibiotic exposure. This association is similar to that reported from a surveillance programme in Brazil, where the presence of detectable antibiotic in the CSF substantially increased the odds of the sample being culture negative but PCR positive (OR 12.2 (95% CI 5.9 – 25.2) (Sacchi et al., 2011). Given the high specificity of PCR, and potential very high sensitivity of PCR (Wu et al., 2013), molecular diagnostics are potentially better than culture in detecting ABM caused by common pathogens, particularly when antibiotics have been administered (Wu et al., 2013; Sacchi et al., 2011). PCR in whole blood was better at detecting meningococcal sepsis compared to blood culture (Carrol et al., 2000), unfortunately blood PCR was not performed in this study, particularly to detect if cases presenting with a petechial rash had meningococcaemia.

However PCR should not replace culture completely, as the range of pathogens detected by culture as causative pathogens in BAM was greater than that covered by the PCR tool alone, and sole reliance on PCR would mean these cases being missed. In addition PCR does not yet give useful information such as antibiotic sensitivity, which is crucial data for the clinician selecting the correct treatment, and for surveillance to monitor emerging antibiotic resistance. PCR therefore has the most utility in culture negative meningitis, where important additional information can be derived.

Despite data in this study showing that PCR apparently exceeded culture in detecting more cases of both pneumococcal and meningococcal meningitis, formal estimates of sensitivity and specificity of PCR to diagnose bacterial meningitis cannot be made from these data. Calculation of these estimates uses the classical 2x2 validation analysis, where the accuracy of the new test is tested against the current best standard test, with the assumption that the latter test is the best. When the new test has estimated higher sensitivity or specificity the calculation fails (Hadgu et al., 2005). Latent class analysis (LCA) has been used to model

diagnostic accuracy and estimate sensitivity of PCR over culture, but this method also has limitations, particularly the model may assign disease free state to a patient with only PCR positive meningitis where other factors support the diagnosis such as CSF WCC and clinical history are not included in the LCA model and therefore sensitivity is under-estimated (Wu et al., 2013; Hadgu et al., 2005; Sacchi et al., 2011). As more detailed diagnostics evolve that are not reliant on bacterial viability, newer statistical tools need to be developed to measure sensitivity accurately of tests that are potentially better than the measurement standard. Given the high sensitivity of PCR, is it possible to justify including PCR negative cases of ABM in this study? All cases included in the study met clinical and CSF inclusion criteria for the diagnosis of ABM.

The PCR only measured three pathogens, other causes of PCR negative ABM are likely to be *S. aureus*, *E. coli*, Group A streptococcus and NTS. In addition of the meningococcal culture positive patients in this study, all were sero-group W135. Conventional multiplex PCR detects meningococcal sero-groups A, B and C, but not all other sero-groups of *N. meningitidis* which contain the citrA gene, including W135 (Wang et al., 2012). More specific meningococcal PCR assays have been developed for other sero-groups of *N. meningitidis*, and the application of these may yield further cases of currently PCR negative meningococcal meningitis (Wang et al., 2012). No studies have evaluated PCR for the rarer causes of bacterial meningitis in adults, and therefore given the limitations of both bacterial culture and PCR in this setting, cases of probable ABM that are culture and PCR negative should remain included in this study, when the clinical and CSF criteria are met.

It is possible that cases of acute TBM or CCM were erroneously included in the culture and PCR negative group. CSF TB culture was not available to this study, PCR using the GeneXpert® technology was available in Blantyre, but has little data to support use for TBM diagnostics; large volumes of CSF are required and the test has relatively low estimated sensitivity of 62% (Patel et al., 2013). CSF bacterial culture, when positive for *C.*

neoformans, led to the exclusion of the patient from the study. CSF culture for this pathogen is very sensitive in SSA and it is unlikely that many cases of culture negative CCM cases were included in this study (Kabanda et al., 2014). In the latter half of the BAM study, Cryptococcal Antigen testing (CrAg) by lateral flow assay was introduced as a screening tool to the laboratory to all adult CSF samples, CrAg is at least as sensitive as culture in diagnosing CCM, and highly specific (Jarvis et al., 2009). Four patients who were CrAg positive and fungal culture negative were included in this study; all had concurrent proven bacterial infection in the CSF.

Antibiotic sensitivities in this study were commensurate with those seen in previous data from MLW (Everett et al., 2011; Cornick et al., 2011; Cornick et al., 2014). Penicillin resistant pneumococci are prevalent in this study and in Malawi, but very few isolates were resistant to both penicillin and chloramphenicol (Klugman et al., 2008; Cornick and Bentley, 2012). Nearly all cases were treated with ceftriaxone, and no ceftriaxone failures were identified in the study. Penicillin resistance is predicted to evolve into ceftriaxone resistance, however ceftriaxone resistant pneumococci are yet to be identified in Malawi (Cornick et al., 2011; Cornick and Bentley, 2012).

6.4.2 Is Early Goal Directed Therapy feasible for bacterial meningitis in settings such as Malawi?

This study tested if the delivery of eight clinical targets or goals, set on an individual patient basis according to clinical need for patients presenting with a clinical suspicion of bacterial meningitis was feasible in the AETC of Queen Elizabeth Central Hospital in Malawi. Although the care bundle included eight targets, the maximum required by any patient was six, the mean target number was 2.59 in P1 and 2.57 in P2. Substantially more targets were met by the clinical care bundle of EGDT than ordinary clinical care in the composite analysis 0.55 in P1 compared to 1.57 in P2 $p<0.001$.

The clinical care bundle was designed to improve oxygenation and tissue perfusion, and the targets set to achieve these, fluid resuscitation, nasal oxygen at 24% concentration and blood transfusion did not lead to significantly increased improvements in the proportions of patients with shock and hypoxia compared to routine care in P1 after 6 hours post recruitment to the study. The patients screened and recruited in P1 and P2 were equally matched, the failure to improve parameters of shock or hypoxia beyond routine care is therefore surprising. Data on the use of oxygen in P1 are lacking, however no patients that required a blood transfusion in P1 received one, and all shocked patients received minimal IV fluids in P1 compared to P2. This pattern was seen not only in the patients with ABM, but in the all screened patient analysis as well. It is possible that a proportion of patients with refractory shock or hypoxaemia at the end of the 6 hour observation period required more intervention than EGDT alone could provide, such as intensive care support, but it is intriguing that despite greater volumes of IV fluid, blood and potentially more use of oxygen, the proportion of these patients without resolution of shock or hypoxaemia were the same in P1 and P2 at the end of 6 hours observation.

A study of sepsis bundles from Singapore measuring compliance with a target of MAP>70mmHg at the end of six hours only achieved this target with aggressive resuscitation in 40% of cases with septic shock enrolled into the study (Kuan et al., 2013). Causes of hypotension in meningitis and sepsis are multifactorial, in ABM, the central effects of inflammation on blood pressure control to maintain intracranial pressure may lead to hypo or hypertension, which is not amenable with intravenous fluids (Tureen, 1995; Park and Chang, 2000; Park et al., 2003). In all sepsis bundle studies where the bundle was shown to have a good outcome, mortality was reported as the primary outcome. All centres publishing these studies had access to intensive care support with inotropes for the patients (Barochia et al., 2010), very few actual data on the achievability of the blood pressure targets are available (Kuan et al., 2013). It is likely that the resources of crystalloid fluid and low flow oxygen were inadequate for the physiological needs of these patients with shock.

Times to receipt of parenteral antibiotics were significantly improved by the care bundle, however the proportion of patients who met the 1 hour target was below 50% in P2. Given the degree of complexity in managing these patients and recruiting them with verbal assent to the trial, it is un-surprising that this target was not met in so many patients. The improvement from a median 2h 20 mins in P1 to 1hr 10 mins in P2 is a substantial achievement in such a resource limited environment such as Malawi.

6.4.2.1 What were the challenges in delivery of the care bundle?

The study team reported that all the care bundle elements were easy to deliver, but the most difficult target to achieve was administration of IV antibiotics within one hour. This was due to the time taken particularly when dealing with a combative patient with altered mental status, added to the time involved in performing the pre-antibiotic LP and obtaining verbal assent, while collecting data for study samples and completing the study case record form (CRF). In P2 one patient was reported to experience pulmonary oedema as a SAE, another a new oxygen requirement, but beyond these small data, it is difficult to conclude that the other targets such as oxygenation or improvement in patients with parameters of shock were no different across the phases due to difficulties in administering, or harm from the care bundle. Lack of access to quality high dependency facilities for patients with on-going medical needs after the 6 hour observation period may be associated with high mortality, however all patients in P2 who required ongoing oxygen did receive it, but due to resource constraints, patients admitted to the HDU or wards did not receive ongoing monitoring.

Study resources were not available to provide on-going medical and nursing care for these patients throughout the inpatient admission period. The literature from sepsis EGDT suggests that the six hour acute resuscitation window is the important time point in which to attempt to correct physiological abnormalities (Barochia et al., 2010), which was used for

BAM, the longest time period of any care bundle intervention is 24 hours (Chamberlain et al., 2011).

The addition of a new study nurse in P2 enabled 24 hour recruitment between Sunday-Friday. The change in shift patterns for the BAM study team to take account of time off after long night shifts meant that less communication between the study team was possible except at handover, and regular meetings were less frequent. The nurse working at night was alone and un-supported by the study team, although she did have access to the PI by telephone 24 hours per day. Each CRF was reviewed the following morning, and no difficulties in either administering the care bundle, or achieving the targets at night were noted, and issues were discussed with the study manager before that nurse went off shift. The nurses found working independently at night challenging, particularly as little support was available from colleagues in the AETC.

The team had to work within the AETC, becoming integrated particularly in the triage area. Constant surveillance by the team manager was required to ensure that the study team were able to perform both AETC and study duties without their study work being compromised.

In addition to internal challenges of recruitment and care bundle delivery, external challenges included intermittent supplies of resources including medication and laboratory results. As a result the study team had to purchase an independent supply of antibiotics, diazepam and fluids to continue administering the care bundle, as hospital supplies were erratic. A political crisis led to currency devaluation, which led to marked inflation in prices, particularly in fuel, limiting affordability of public transport to many patients.

6.4.2.2 Were the wrong targets set?

The clinical targets were not derived from other studies of bacterial meningitis, as this approach has not been tested in ABM in any setting world-wide. Instead the targets were derived from, and based on assumptions that treatment for sepsis with EGDT improved

outcomes, and as a serious bacterial infection, ABM may benefit from the same approach. Other targets were derived from data on patients with sub-arachnoid haemorrhage and from the critical care literature (Chapter 2 section 2.8). Important differences exist between the settings and patients in those studies, particularly in the clinical resources available, and the high rates of HIV co-infection in patients in Malawi and throughout the region. Further discussion of these differences can be found in Chapter 8 section 8.3.

Despite the significant achievements of the care bundle to meet more targets than routine care alone, the failure of the EGDT to improve markers of shock or hypoxaemia beyond routine care, suggests these patients may have different physiological responses to invasive infection within the CSF compartment. A sepsis-based approach may not be the optimal way to improve clinical care and outcomes from bacterial meningitis. This is discussed further in Chapter 8 section 8.5.4. These data may be used to derive new targets for an ABM-specific care bundle in the future, focusing on potentially treatable abnormalities found in the ABM patients, such as rising blood pressure and seizures.

6.4.3 Does EGDT have an impact on outcome from ABM in Malawi?

This study was constrained by the before/after design into recruiting equal numbers of patients over two set time periods, and as such no formal sample size was set. Sequential cohort before/after studies are open to the effects of multiple confounders, the most important of which is time, but others include changing hospital and patient circumstances, varying availability of medications and resources, and changes in staff and available expertise. These confounders limit the interpretation of differences found between the sequential phases.

The numbers of patients recruited were smaller than planned based on eligibility from historical data (100 per 10 month period), and comparison of the two patient populations in P1 and P2 is therefore confounded by time and limited by these small numbers.

Important differences were noted between the patients in the two phases. P1 and P2 patients were equally matched for age, gender, HIV co-infection status and all other demographic parameters measured. Physiological variables, CSF culture results and MAMS severity scores were also equally matched on admission. The overall numbers are small, however marginally better composite outcomes were noted in P1 compared to P2, with a case fatality rate (CFR) at day 10 of 38% in P1 with a 52% mortality rate in P2 p=0.11, the composite death/disability rate was 46% in P1 compared to a composite day 10 outcome rate of 72% p=0.004. This trend continued to day 40, with an increased but non-significant CFR observed at day 40 in P2 compared to P1 49% compared to 63% p=0.13. With 14 missing data points for day 40 outcome in P1 compared to 1 in P2, complete conclusions cannot be drawn from the mortality data at day 40.

Why patients in P1 had lower mortality than P2 is intriguing. P1 mortality was lower than CFRs in the historical meningitis data (Chapter 4 section 4.5.2, n=715), which were 45% at day 10 and 54% at day 40, compared to P2 patients whose CFR was 49% at day 10 and 63% at day 40. In studies from elsewhere in sub-Saharan Africa, mortality rates from ABM range from 50-70% (Gessner et al., 2010; Hakim et al., 2000; Yaro et al., 2006), commensurate with both the historical database and the case fatality rate (CFR) observed in P2. In comparison, the CFR in the P1 patients was 38% at day 10 and 49% at day 40. The MAMS was designed as a tool to test if predicted mortality differed across the study phases. It was hypothesised that patients in P2 would have worse physiological parameters and hence worse predicted mortality, compared to P1, explaining the difference in CFR between the two phases by P2 patients being substantially sicker. Surprisingly MAMS scores were identical across the phases, showing that P1 and P2 patients were equally matched for their critical illness severity on admission. The agreement between predicted and actual outcome was poor in P1 and good in P2, suggesting MAMS has good utility in predicting outcome in P2 but not P1.

Why, therefore, P1 patients survived despite having equal predictors of poor outcome and less intensive medical care compared to P2 is unclear. The P1 CFR of 38% at day 10 and 49% at day 40 is unusually low for the region and matches more closely data from European and American settings, where CFR from pneumococcal meningitis ranges between 20-35% (van de Beek et al., 2004; Thigpen et al., 2011; Durand et al., 1993). The high rate of survival in P1 is therefore the unusual aspect of the outcome of this study, and warrants further investigation. Patients in P1 received marginally later antibiotics and lumbar puncture, less parenteral fluids and less diazepam. It is possible that the clinical care received by patients in P1 more closely matched that given in more well-resourced settings, and therefore resulted in better outcomes. However the mortality differences are small, and the study is observational, the hypothesis of more simplified quality medical care leading to better outcomes would need to be tested in a parallel randomised study.

From the MAMS scores and the physiological trends presented, it is clear that the P1 non survivors had very poor clinical and physiological parameters on admission compared even to P2 non survivors. P2 non survivors were matched physiologically more equally with P1 and P2 survivors than the P1 non survivors, suggesting that P1 non survivors may have had a particularly severe physiological phenotype. It is possible to hypothesise that the care bundle may have altered the outcome in P2, so that patients who may have been physiologically primed to survive at day 10 if they had received care in P1, did not do so. However there is not enough evidence from the current study to support this hypothesis, and testing of the care bundle concept, revised on the basis of this pilot study in a randomised controlled trial with parallel recruitment is required.

Is it then possible that another factor was implicated in the low mortality rates seen in P1, such as the quality of routine medical care delivered in the AETC compared to the older system of admission (Chapter 3 Section 3.3.5) This system was chaotic and resulted in major delays for several hours before clinical review and antibiotics were administered. Or

could the mortality differences represent harm from one or more bundle elements in P2, or an outbreak particularly aggressive pneumococcal serotype in P2? Serological data are awaited for patients with culture proven pneumococcal meningitis in both Phases, and these data will be reported in due course. The only differences seen in clinical care bundle administration in P2 compared to routine care in P1 were not statistically harmful; larger volumes of IV fluid resuscitation were given, airways inserted and head tilts applied, however the numbers for each intervention were small and a larger sample would give definitive safety information. All the current data support the safety of the care bundle.
New data are presented monitoring the physiology of patients in both phases over time, the first time data of this type have been reported for ABM. In this chapter it was shown that rising MAP and falling GCS over time are associated with a high poor outcome. These trends suggest that inflammation within the CSF compartment and the CNS tissue are likely to be driving the abnormal physiological phenotype and high mortality rates, and not traditional peripheral sepsis responses with depressed myocardial function and poor tissue perfusion, as were assumed in the design of the care bundle.

To explain the differences in mortality, using the physiological trends, it is clear that either more non survivors in P2 could have survived but didn't, either due to an as yet unascertained harmful element of the clinical care bundle, or patients in P1 received better care than historically recruited patients in the AETC but were not given part of the care bundle that was harmful. The possibilities for a harmful clinical care bundle element that was either not given or given in smaller volumes in P1 compared to P2 are larger volumes of IV fluids, airway and head-tilt, or administration of diazepam. However one limitation of examination of care bundles is that extracting individual data on harm of one care bundle element when given with others is statistically impossible, and data are highly confounded (Barochia et al., 2010). For example, the more unwell a patient is, the more targets are likely to be set, and the more care bundle elements are likely to be received. From her original illness severity she has a higher predicted chance of death, and due to the her illness

severity will receive more care bundle elements, confounding the analysis of causes of poor outcome.

For a true examination of the benefits and harms of a care bundle, a trial with a parallel randomised design is required. The BAM study shows that EGDT for meningitis is feasible, and the care bundle needs refinement before further testing in a larger trial. This will be discussed further in Chapter 8 Section 8.5.3-4.
An alternative possibility to explain higher rates of non-survival in P2, is the hypothesis that P2 non survivors had an additional factor present that influenced mortality within the CSF compartment and this will be the focus of the following Chapter 7.

In conclusion, limited early goal directed therapy for bacterial meningitis in Malawi is feasible, but the care bundle requires careful re-design encompassing elements of care delivered in routine hospital care before testing in a randomised controlled trial to be more specific to ABM.

7 The relationship between bacterial load, viral co-infection and outcome from bacterial meningitis in Malawian adults

7.1 Introduction

Bacterial meningitis in Malawian adults has an unacceptably high mortality rate of 54% in adults (Wall et al., 2013c). Full estimates of morbidity are lacking, but in the SAM trial, 'any disability' occurred in 47/202 (23%) of the 46% of participants that survived (Scarborough et al., 2007). While mortality is obviously the most serious outcome measure for bacterial meningitis, survivors that experience morbidity face extreme challenges in a profoundly resource-limited environment such as Malawi. Loss of the ability to work and consequent reduced income, without any access to governmental or insurance related support, plus degrees of dependence on friends or family for activities of daily living puts a substantial financial and emotional burden on the family of the patient (Desmond et al., 2013). Poor outcome can therefore be defined as either death or survival with poor function, and understanding the causes of poor outcome is the first step towards designing improved preventative and treatment strategies to reduce incidence, death and morbidity from bacterial meningitis in Africa.

In this study, 59 of 132 (44.6%) adults with proven or probable ABM had died by day ten post admission, and a further 18 (14%) were disabled (mRS >2 points). By day 40, 66/117 (56%) had died, and one person (0.8%) was alive with disability, the other survivors were healthy. The burden of poor survival was therefore considerable, in the immediate post discharge period, particularly as these patients had predominantly died post discharge by six weeks.

Clinical bedside predictors of poor outcome have been explored extensively in the previous chapters of this thesis. In this chapter, potential laboratory based biomarkers in the blood

and CSF were explored to determine if either bacterial load, viral co-infection (as discussed in Chapter 2 Section 2.3-4) or the host inflammatory response were associated with a poor outcome from ABM in the BAM cohort.

The host inflammatory response is a key determinate of outcome from sepsis and meningitis, discussed in Chapter 2 Section 2.2 (van der Poll and Opal, 2008; Kasten et al., 2010; Mook-Kanamori et al., 2011). Blood lactate is a well-recognised marker of outcome from sepsis (Arnold et al., 2008), but the role of either CSF or blood lactate as a biomarker of outcome in ABM is less clear. Both blood and CSF biomarkers of inflammation were therefore tested against outcome.

To investigate whether there is a relationship between CSF bacterial or viral load and outcome, these microbial parameters were analysed with respect to day ten composite outcomes, day 40 outcomes were not analysed separately as missing data limited data available. The association between CSF cellular and biochemical findings, and outcome was also analysed.

7.2 Methods

7.2.1 Objectives and research question

Research questions

a) Is bacterial load associated with outcome from bacterial meningitis?
b) What is the relationship between bacterial meningitis and viral co-infection?
c) What is the influence on outcome of markers of inflammation outcome from bacterial meningitis?

Objectives

i) To use multiplex real-time PCR to determine the causes of culture negative meningitis

ii) To estimate the quantity in bacterial and viral copy number of each pathogen identified in the CSF using RT-PCR.

iii) To determine if any of the biomarkers of inflammation including bacterial load and viral co-infection are associated with outcome from ABM

7.2.1 Inclusion and exclusion criteria and outcome measures

The BAM study cohort has been described in detail in Chapter 6. All patients with ABM retained in BAM with available samples for PCR testing were used in this study, including those with a strong clinical suspicion of bacterial meningitis with abnormal CSF biochemistry not meeting the other CSF inclusion criteria above were included in this study (full inclusion criteria Chapter 3 Section 3.3.4). All culture negative but PCR positive patients from the BAM study were included in the formal BAM trial analysis.

Outcome measures

The outcome in this study was a composite outcome of healthy survival compared to death or disability at ten days post admission. Data were collected to six weeks (day 40) post admission, but due to a substantial proportion of missing data at day 40 in the first phase of BAM, analysis was confined to the earlier time-point. Disability was measured by the modified Rankin score (mRS), and was defined as a score of ≥ 2 points compared to healthy survival (mRS <2 points).

7.2.2 Laboratory methods DNA extraction and PCR amplification

Bacterial and viral DNA was extracted from whole CSF using standard manual DNeasy kits (Qiagen, Germany), with a pneumococcal cell wall extraction step prior to the extraction. Real-Time PCR was performed using primers and probes supplied by Fast Track

Diagnostics (FTD Holland). Full methods are supplied in Chapter 3 Section 3.1. Bacterial loads were estimated using calculations supplied by FTD based on the Ct values. Viral loads were calculated using standard curves generated from samples with known DNA quantity per sample supplied by FTD.

7.2.3 Data analysis and statistics

For all tables, binary variables such as gender, HIV status, the number and the proportions (as percentages) of the category of interest were summarized. Normally distributed continuous variables were summarised using means and standard deviations (SDs) while continuous variables with skewed distribution were summarised using median and range. The appropriate measures for specific variables have been indicated in the table.

Univariate logistic regression was used to assess which variables were associated with either viral co-infection or poor outcome compared to healthy survival. The odds ratios were reported together with the 95% confidence intervals. Normally distributed variables were compared with parametric tests (Students T-test), variables with a skewed distribution were $\log_{10}$ transformed and compared with non-parametric tests (Mann-Whitney test and univariate logistic regression). All statistical tests were performed at 5% significance level.

7.3 Results

7.3.1 Is bacterial load associated with outcome from bacterial meningitis?

One hundred and sixty five CSF samples were stored from BAM for this study, of which 159 had adequate CSF available and were tested for SpN/NM/Hib DNA using RT-PCR. The bacterial loads were calculated for all samples where DNA was detected (n= 56).

Pneumococcal bacterial loads were not different to meningococcal loads ($6.5x10^6$ copies/ml pneumococcal DNA (IQR $6.7x10^5$-$4.8x10^7$) compared to $6.7x10^5$ copies/ml meningococcal DNA (IQR $1.06x10^5$ – $1,4x10^6$) p=0.49.

7.3.1.1 Pneumococcal meningitis

Pneumococcal loads were not significantly associated with worse outcome, median bacterial load in survivors $2.45x10^6$ ($6.7x10^3$-$8.4x10^6$) copies/ml compared to $1.5x10^7$ ($1.8x10^6$-$6.49x10^7$) in non survivors p=0.33 (Figure 7.1, Table 7.1). Due to significant skewing in the data, bacterial loads were then $\log_{10}$ transformed and the differences compared. The data were grouped into quartiles around the median and univariate regression analysis was done to test if higher quartiles of bacterial load were associated with poor outcome. Table 7.1 shows $\log_{10}$ transformed pneumococcal loads represented as medians by day 10 composite outcome group, and categorised into quartiles around the median. The quartile analysis was to look for trends at either end of the skewed data to see if an effect of high or low quartiles was associated with outcome, when no difference between the overall medians were seen.

The median $\log_{10}$ bacterial load in survivors with good outcome was 6.38 copies/ml CSF (IQR (3.82 – 6.90), compared to 7.19 copies/ml (IQR 6.27 – 7.81), p=0.06 (Table 7.1). When corrected for HIV co-infection, the difference between the log transformed loads was borderline significant p=0.056. The quartile analysis demonstrated a slight trend towards higher quartiles of bacterial load and poor outcome with marginally significant association with poor outcome seen in the 50-75th quartile, (OR of poor outcome with a bacterial load in this quartile was 8.4 (95% CI 0.94 : 75.0), p=0.057). These data are shown in Figure 7.2.

Table 7.1 Bacterial load and outcome

Univariate associations between bacterial load and outcome				
	Healthy survival n= 30	**Death/poor survival n= 51**	**Univariate OR (95% CI)**	**Significance p**
Median log_{10} SpN bacterial load Copies/ml (IQR) n=57	6.38 (3.82 – 6.90)	7.19 (6.27 – 7.81)	1.35 (0.98 : 1.84)	0.06
SpN PCR negative	9	15	….	
<25th centile	8	6	0.45 (0.17 : 1.72)	0.24
25-50th centile	8	6	0.45 (0.17 : 1.72)	0.24
50-75th centile	1	14	8.4 (0.94 : 75.0)	0.057
>75th centile	4	10	1.5 (0.36 : 6.23)	0.57

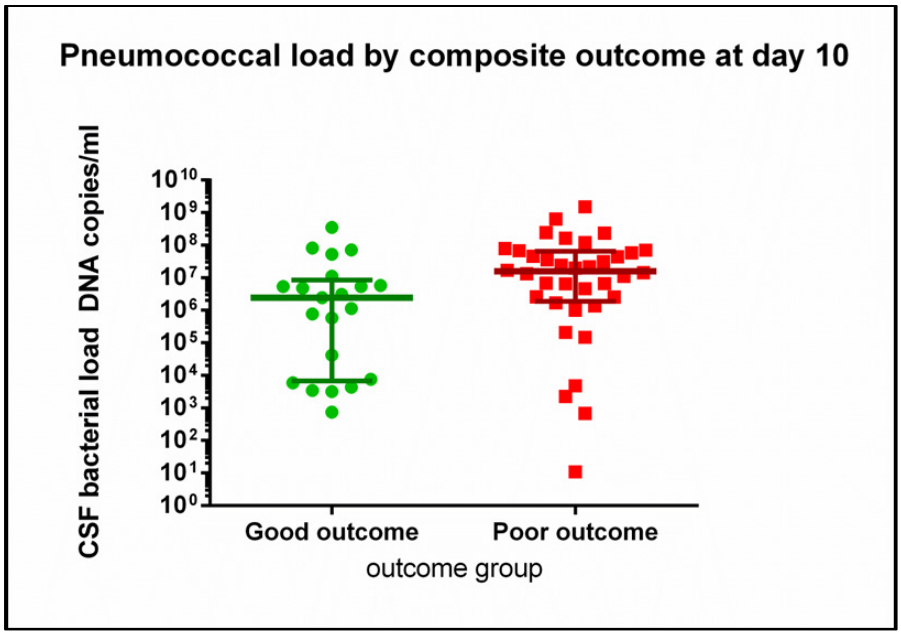


Figure 7.1 CSF bacterial load by outcome

Median CSF S. pneumoniae pathogenic load by outcome group at day 10. Error bars represent inter-quartile range.

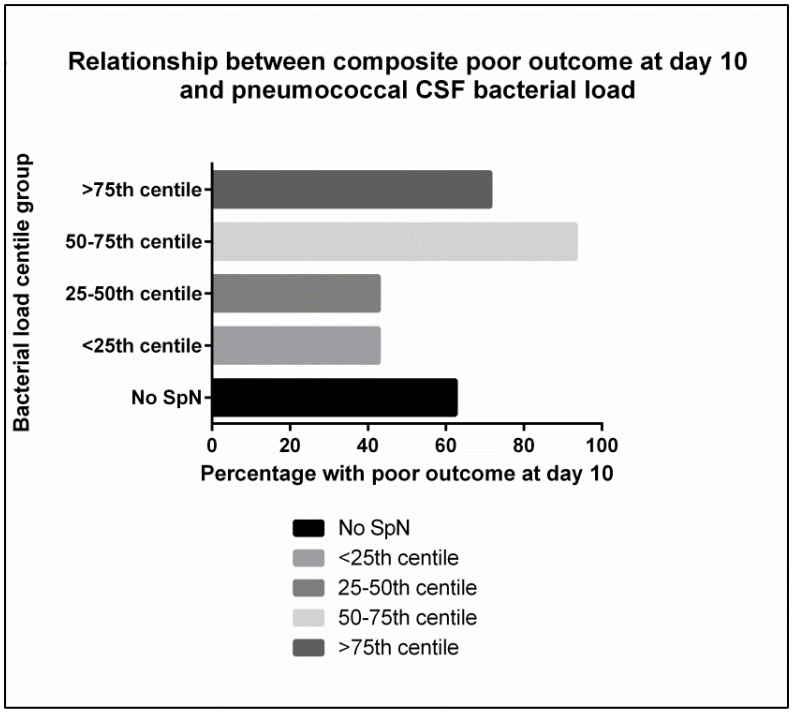


Figure 7.2 Bacterial load with outcome by quartile

7.3.1.2 Meningococcal meningitis

Despite the small numbers of *N. meningitidis* meningitis, the median bacterial load in survivors with good outcome (n=4) was 5.3_{10} (IQR 3.86 – 5.76) compared to 6.16_{10} (IQR 5.88 – 6.16) in those with poor outcome (n=3) p=0.02. The small numbers available determined that these data could not be included in any more detailed analysis including by quartile.

7.3.2 Is viral co-infection in the CSF of adults with bacterial meningitis associated with morbidity?

EBV was detected in the CSF of 34/81(41%) patients included in BAM who were tested for EBV, and in 30/50 (60%) of patients who were included in the PCR study, but subsequently excluded from BAM on the basis of CSF and bacterial PCR results. EBV was commonly detected in the CSF of patients with pneumococcal meningitis (20/57, 35%), and not detected in those with meningococcal meningitis (0/7, 0%).

CMV was detected in only three patients, all were EBV infected. One further case of CMV was detected, in the excluded patient group (Table 7.2).

Table 7.2 Viral co-infection in meningitis

Viral copy number per pathogen detected on PCR		
Bacterial PCR result	**Median EBV viral load (IQR) copies/ml CSF**	**Median CMV viral load (IQR) copies/ml CSF**
SpN DNA positive N=57	1268 (977-5185) n=20	1125 (No IQR) n=1
NM DNA positive N=7	0 n=0	0 n=0
Hib DNA positive N=0	NA	NA
PCR negative included in BAM N=39	2422 (1459-6924) n=14	16909 (1766-16909) n=2
PCR negative and excluded from BAM N=50	2149 (1125-5670) n=30	5028 n=1

NA = not applicable

No relationship between EBV and outcome at day 10 was determined on univariate analysis. EBV DNA was positive in 22/51 patients with poor outcome at day 10, p=0.48. In addition both the median viral load and individual quartiles around the median were also unrelated to outcome (Figure 7.3 and Figure 7.4, Table 7.3). No relationship exists between EBV loads and composite poor outcome at day 10. When corrected for HIV, higher loads of EBV infection

were not associated with outcome at day 10 (OR >75th centile of EBV load and poor outcome was 0.59 (95% CI 0.13 : 2.16) p=0.49). EBV co-infection was not associated with outcome in pneumococcal meningitis (OR 0.66 (95% CI 0.18 : 2.83 p=0.53).

Table 7.3 Viral load and outcome in meningitis

Relationship between pathogenic loads and composite outcome at day 10 in adults included in BAM n=81

	Healthy survival n 30	**Death/poor survival n 51**	**Univariate OR (95% CI)**	**Significance p**
Median EBV viral load Copies/ml (IQR)	1944 (1138-6771)	1913 (1068-3824)	1.0 (1.0 : 1.0)	0.35
No EBV	18	29	…….	…..
<25th centile	2	6	1.8 (0.33 : 10.2)	0.47
25-50th centile	4	5	0.77 (0.18 : 3.2)	0.73
50-75th centile	2	7	2.1 (0.40 : 11.6)	0.36
>75th centile	4	4	0.62 (0.13 : 2.7)	0.53

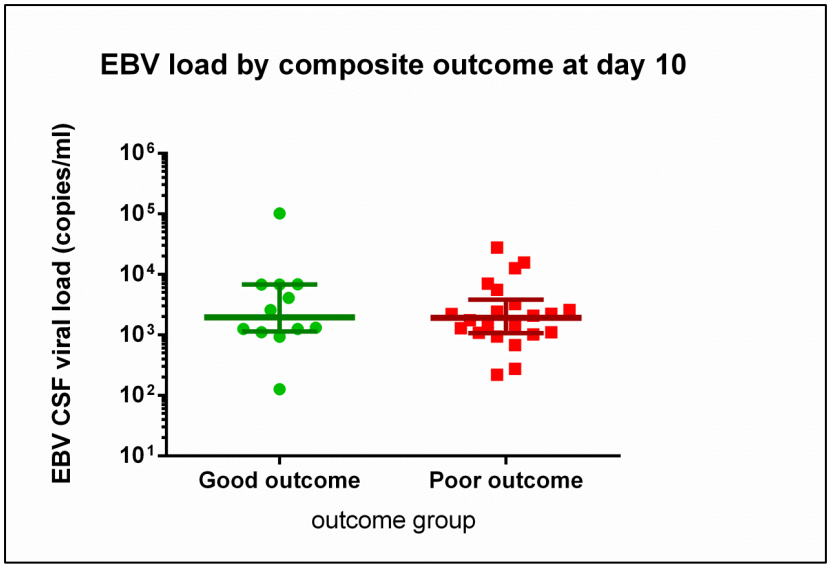


Figure 7.3 Median viral CSF load of EBV by outcome

Error bars represent inter-quartile range.

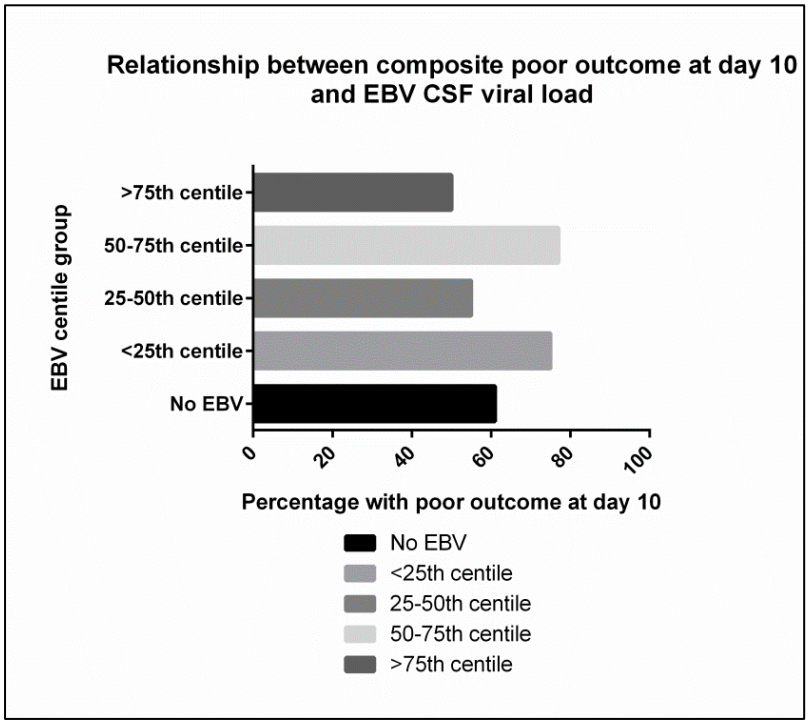


Figure 7.4 EBV viral load with outcome by quartile group

Equal numbers of patients with a poor outcome at day 10 had EBV co-infection compared to healthy survivors.

The relationship between EBV and quality of survival were then explored in a sub-set of patients who survived at day 10 with PCR data available, either healthy survival (mRS <2) n=26 or had poor survival (mRS =>2) n=16 (Table 7.4). No relationship between EBV viral load and the quality of survival at day 10 was observed.

Table 7.4 Viral load and quality of survival

EBV viral load and quality of survival				
	Healthy survival n = 26	**Poor survival n = 16**	**Univariate OR (95% CI)**	**Significance P**
Median EBV viral load Copies/ml (IQR) n=17	1316 (1102-6774)	4667 (1586-1.6x10^5)	1.0 (1.0 : 1.0)	0.81
No EBV	15	10	….	

7.3.3 CSF and blood inflammatory biomarkers of poor outcome from bacterial meningitis

In Chapter 4 Section 4.5, an analysis was presented showing clinical predictors of mortality at day 40 post bacterial meningitis from 715 adults enrolled in clinical trials or other studies of ABM in Malawi. In this section, CSF and blood biomarkers from patients recruited to BAM with ABM were examined for potential associations between these biomarkers and outcome. These analyses were repeated with correction for HIV co-infection, to determine if HIV had impact on blood and CSF inflammatory parameters.

7.3.3.1 CSF results (Table 7.5)

Eleven CSF variables were recorded for patients in BAM. Data were lacking in some variables such as CSF biochemistry when laboratory results were not available, or in bedside data such as CSF lactate when the strips were not available. Therefore some

variables in this group did not have adequate data to take forward to multivariate analysis. The following CSF variables had no significant association on univariate analysis: CSF protein, CSF lactate, the predominant CSF WCC type, culture results, or pneumococcal infection on either culture or PCR. The only variable showing significance at the <0.05 level was CSF WCC (OR 0.99 (0.99 : 1.0) p=0.04), but this was not significant when corrected for other CSF parameters or when the analysis was repeated to correct for HIV co-infection.

7.3.3.2 Blood results (Table 7.6)

Five markers of inflammation in the blood were measured in BAM, plus the presence of bacteraemia. On univariate analysis blood culture positivity for *S.pneumoniae* was associated with good outcome (OR poor outcome 0.29 (95% CI 0.03 : 1.71) p=0.01, but when corrected for other variables on multivariate analysis, significance was not retained. CD4 count had too few data available to be included in the multivariate analysis. No measured blood variables were independently associated with outcome on multivariate analysis. Correction for HIV co-infection did not alter the results and these are not presented.

Table 7.5 Associations between CSF parameters and outcome in meningitis

Parameter		Good survival	Dead or poor survival	Univariate (unadjusted)		Multivariate (adjusted)**	
		N=55	N=77	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
CSF protein (g/dL)	Median (IQR)	2.76 (1.71 – 3.72)	2.83 (1.91 – 5.79)	1.18 (0.93 : 1.49)	0.16	1.41 (0.83 : 2.40)	0.98
CSF: Blood glucose ratio		0.35 (0.15 -0.43)	0.1 (0.02-0.18)	0.004 (0.00 : 1.43)	0.06	*	
CSF lactate (mmol/L)	Median (IQR)	10.6 (7.4-11.2)	9.6 (9.0-10.8)	0.96 (0.80 : 1.15)		1.08 (0.76 : 1.53)	0.64
CSF White cell count (cells/mm^3)	Median (IQR)	169 (32 – 1760)	56 (10-300)	0.99 (0.99 : 1.0)	0.047	0.99 (0.99 : 1.0)	0.31
CSF WCC reported as clumps	No	31	43	….			
	Yes	24	34	1.02 (0.50 : 2.0)	0.95	*	
CSF culture results	No growth	23	27	1.17 (0.26 : 5.2)	0.83		
	SpN	25	36	1.44 (0.32 : 6.30)	0.62	1.12 (0.08 : 15.5)	0.93
	Other^	7	14	0.33 (0.23 : 4.7)	0.41	1.23 (0.01 : 1.07)	0.92
CSF culture or PCR positive for *S. pneumoniae*	no	30	41	….			
	yes	25	36	1.05 (0.52 : 2.11)	0.88	1.00 (0.19 : 5.12)	0.99

Table 7.6 Association with haematological parameters and outcome in meningitis

Parameter		Good survival	Dead or poor survival	Univariate (unadjusted)		Multivariate (adjusted)** N=63	
		N=55	N=77	Odds ratio (95% CI)	P	Odds ratio (95% CI)	p
Blood culture results	No growth	31	53	0.85 (0.14 : 4.9)	0.86	….	
	SpN	14	7	0.29 (0.03 : 1.71)	0.01	0.21 (0.03 : 1.30)	**0.09**
	Other ^	4	9	1.31 (0.37 : 4.6)	0.66	1.43 (0.22 : 9.20)	0.70
Haemoglobin (g/dL)	Median (IQR)	11.9 (10.1 – 13.4)	11.4 (9.9-13.0)	0.94 (0.79 : 1.13)	0.55	0.94 (0.76 : 1.18)	0.63
Peripheral blood white cell count (cells/mm^3)	Median (IQR)	9.8 (5.8 – 14.7)	10.7 (6.9 – 16.1)	1.01 (0.96 : 1.07)	0.58	1.03 (0.95 : 1.11)	0.41
Peripheral platelet count (cells/mm^3)	Median (IQR)	211 (131-285)	206 (139-331)	1.00 (0.99 : 1.005)	0.25	1.0 (0.99 : 1.0)	0.54
CD4 count (cells/mm^3)	Median (IQR)	238 (41-296)	97 (72–186)	0.99 (0.98 : 1.002)	0.14	*	
Blood lactate (mmol/L)	Median (IQR)	2.9 (1.9 – 5.5)	3.4 (2.1-5.6)	1.05 (0.91 : 1.20)	0.48	0.97 (0.79 : 1.18)	0.77

UC= unable to compute with logistic regression * = numbers too small for multivariate analysis ^ other culture includes *N. meningitidis*, *E. coli, S. aureus*, Group A streptococu

7.4 Discussion

In this chapter, CSF bacterial loads, CSF viral co-infection and markers of inflammation in the CSF and blood of patients recruited to BAM were explored in relation to a composite outcome of death/poor survival compared to good survival at ten days post admission. Data has been published from Malawi examining some of these variables with relation to binary mortality outcome at day 40 (Wall et al., 2014b; Scarborough et al., 2007; Kelly et al., 2012), but not using a day ten composite outcome point. This discussion is divided into three sections, each relating to the relevance of the findings of the three analyses done in context of the previously published data.

7.4.1 Pneumococcal load in CSF of adults with ABM

Data from the SAM and GLAM trials showed clearly that pneumococcal loads were not associated with outcome at day 40 (Wall et al., 2014b). The bacterial loads measured in this study did not exhibit a trend towards higher loads and poor composite outcome at day 10. The median pneumococcal loads in this study were higher than previously observed; $6.5x10^6$ (IQR $6.7x10^5$ – $4.88x10^7$) compared to 6.5_x10^5 copies/ml (IQR $1.08_{\ x}10^5$ – 2.96_x10^6) in the data from the SAM and GLAM study (Wall et al., 2014b). The numbers of cases of meningococcal meningitis were too few to analyse in detail, so the focus was on primarily pneumococcal meningitis. In this study the overall pneumococcal loads were skewed, log transformation to normalise the data distribution were required, a trend towards higher loads and poor outcome was seen in the log transformed data when corrected for HIV co-infection.

Previous studies of CSF pneumococcal loads in Malawian children also reported log transformed data, and found CSF and blood pneumococcal loads were higher in HIV co-infected children than non-HIV infected children: higher CSF loads were also associated with mortality (Carrol et al., 2007a). That study did not look at trends by quartile bacterial load,

only continuous data, and utilised data from 77 children, compared to the 57 in this study. The HIV co-infection rate in that study was comparable to this study, 62% of children compared to 68% of BAM included participants in this study were HIV co-infected (Carrol et al., 2007a). The methodology used to measure the bacterial load in both previous Malawian studies relied on a standard curve of sufficient quality to calculate the results (Wall et al., 2014b; Carrol et al., 2007a). In this study, bacterial loads were estimated based on a calculation provided by the company supplying the multiplex kits, and were not independently verified. Therefore the estimated data presented may not represent completely accurate data, and comparisons with the data from the previous adult and paediatric studies are subject to this limitation.

Although a marginal trend towards poor outcome and higher bacterial loads was seen in this study, no differences were seen in the previous adult data, with a larger sample size and more robust methodology (Wall et al., 2014b). In that study, significantly longer times to hospital from symptom onset were recorded compared to other studies where a clear relationship between bacterial load and outcome have been shown (Scarborough et al., 2007; Ajdukiewicz et al., 2011; Darton et al., 2011; Darton et al., 2009; Hackett et al., 2002; Rello et al., 2009; Roine et al., 2009), the paediatric Malawian study reported median 3 days symptom onset before hospital admission (Carrol et al., 2007a). It is possible that by the time the lumbar puncture was done in the Malawian adults in the SAM and GLAM studies, bacterial growth in both outcome groups had reached a static rather than exponential growth phase, and as such no differences were seen between the groups. It is also possible that with impaired CSF WCC responses in these patients, bacterial growth is relatively unhindered by immunological control, and as such is less important in determining outcome than host factors (Wall et al., 2014b). In the data presented, the CSF WCC were low, but the median time to admission was 48 hours (Chapter 6 Section 6.3.3-4), lower than in the previous study. It is therefore possible that these samples were taken at an earlier time point during the growth phase and not the static phase.

As such, with small numbers and estimated data, further studies to explore the relationship between composite outcome and pneumococcal load in adults with bacterial meningitis are needed from SSA and other settings.

7.4.2 Viral CSF co-infection of adults with bacterial meningitis

EBV was frequently identified in the CSF of patients included in the BAM study (33%). However CMV was only identified in 2.9%, all of whom were co-infected with EBV, and all were dead or disabled by day 10. EBV was also detected in 46% of cases that were subsequently excluded from BAM and assigned an alternative diagnosis, a rate similar to previous studies (Kelly et al., 2012). There were too few cases of CMV infection to analyse in detail; EBV was only detected in pneumococcal or culture negative meningitis, and not meningococcal meningitis. However, EBV was not statistically associated with HIV co-infection in patients with meningitis, equal numbers of patients with and without HIV co-infection had EBV detected, and the measurements of association with outcome did not change when corrected for HIV. This is in contrast to that observed in a study of viral co-infection in Malawian adults with meningitis, where HIV co-infection was strongly associated with the presence of EBV, and the association with mortality became significant when corrected for HIV (section 7.3.2) (Kelly et al., 2012). In that study 53% of adults with bacterial meningitis were co-infected with EBV and a strong association with HIV co-infection was seen. In another study from Zambia, EBV was found in the CSF of 27.5% of HIV co-infected adults presenting with CNS opportunistic infections (Siddiqi et al., 2014), a study from Angola found EBV in 40% of children with ABM, but no association with outcome was seen (Pelkonen et al., 2013).

EBV has rarely been detected in the CSF of adults with bacterial meningitis outside Africa (Brouwer et al., 2013, Kleines et al., 2011)and none of these studies detecting reported

associations between with other parameters and EBV infection (Kelly et al., 2012, Kleines et al., 2011). Concomitant viral infection was not looked for in European studies of patients with HIV co-infection and bacterial meningitis. Given the high prevalence of HIV and EBV in SSA, it may be that this association is only found within this region (Schaftenaar et al., 2014). Further data are required to explore the relationship between EBV, HIV and meningitis in other settings.

In contrast with the Kelly study, EBV was not associated with outcome in BAM, either when tested as a continuous variable, when categorised into quartiles around the median, or when adjusted for HIV co-infection. EBV was also not associated with the quality of survival. An association with EBV viral load mortality was reported in the previous Malawian study between the highest quartile of EBV load and mortality, when corrected for HIV co-infection p=0.02 (Kelly et al., 2012). The lack of association in this study may have been due to smaller numbers (149 patients with a 54% prevalence of EBV in Kelly et al compared to 81 patients with 41% prevalence in this study), as the only association with outcome in that study was in the highest quartile of viral CSF load, so the association was small but significant. Viral loads per patient (median in BAM 1913 copies/ml CSF (IQR 1095 – 5852) compared to 1012 copies/ml (IQR 365 – 7269) in HIV negative and 6849 copies/ml (IQR 511 – 223323) in HIV co-infected) did not significantly differ between this study and the previous study. The Kelly study did not explore EBV co-infection by ABM causative pathogen. In this current study, EBV co-infection was equally distributed between pneumococcal, PCR negative meningitis, and excluded cases, with similar viral loads in each group. The role of EBV in the CSF of African adults with a meningitic illness is not clear, there are limited data to determine if it is an active component of CSF inflammation or a by-stander activated by inflammatory white cells in the CSF during ABM. Viraemia from latently infected B cells in the blood entering the CSF with blood-brain-barrier breakdown is a possible source of CSF EBV, however low level circulating CSF virus released from lymphoid tissue within the ependymal cells lining the blood brain barrier is also a possible source of virus (Kleines et

al., 2011, Salvetti et al., 2009). In the prior Malawian study, active CNS EBV replication rather than the detection of latent infection was hypothesised, triggered by bacterial infection in the CSF (Kelly et al., 2012). Studies specifically designed to explore this problem are required to determine the source of EBV in the CNS of patients with ABM, and explore the role of the virus in pathogenesis.

7.4.3 Laboratory predictors of poor outcome

This analysis was designed to detect biomarkers in the blood and CSF of adults with meningitis that were associated with poor outcome. The most striking finding was the relative normality of the measured parameters of inflammation and tissue perfusion in the blood, including peripheral white cell counts and blood lactate. The CSF data did represent an inflammatory picture with very high protein and lactate levels, although CSF WCC were overall markedly lower than in other settings (de Gans and van de Beek, 2002; Durand et al., 1993; Dzupova et al., 2009).

Only CSF WCC was associated with poor outcome on univariate analysis in this study; other predictors demonstrated to be predictive in other studies showed no association including platelet count, peripheral white cell count, blood lactate, haemoglobin (van de Beek et al., 2004; Weisfelt et al., 2008; Weisfelt et al., 2006e; Wall et al., 2013c). However, analysis was significantly limited by the quantity of data available. Pneumococcal culture and anaemia were not significantly associated with poor outcome in BAM, which compared to their positive correlation in the historical data is interesting (Wall et al., 2013c). The differences in the data may partly be explained by the relatively small case numbers in BAM compared to the historical cohort (n=132 compared to n=715), with missing data limiting the analysis.

HIV co-infection was not associated with poor outcome, as shown previously. However the proportion of HIV uninfected adults was low (20% of patients were HIV uninfected, a further 18% did not have a test done so have unknown status. The relatively small numbers of HIV uninfected may mean that any statistical association is minimised, leading to a type two statistical error. No data have been published from other settings in adult meningitis with sufficient numbers to determine if a true association with HIV infection and outcome exists. This is the subject of further discussion in Chapter 8, Section 8.3.4.

Very high CSF protein and low CSF: Blood glucose ratios were shown to be potential biomarkers for pathogenic severity, as have been shown in a study in the Czech Republic (Dzupova et al., 2009), however the analysis of these variables in this study was limited by little data available. High CSF lactates were ubiquitous, but lactate production in the CSF did not predict outcome, unlike in older studies of ABM, and in TB and cryptococcal meningitis (Lu et al., 1999; Vengerov et al., 2008; Lu et al., 2001a) .

Recruitment to Phase 2 was the strongest predictor of poor outcome in the day 10 analysis (Chapter 6 Section 6.3.5, 6.3.7). Due to the weight given to this variable in the multivariate modelling, it is possible that biomarker data may have had more weight if no differences had been seen in outcome across the study phases. The possible causes for the poor outcome seen in Phase 2 have been explored extensively in Chapter 6, and because of the strength of the association between P2 admission and outcome, full interpretation of the biomarkers analysed in this study is not possible.

A larger sample size in a future study would be necessary to determine if any biomarkers tested with univariate associations with outcome are true predictors of poor outcomes. All patients had abnormally low CSF WCC, and high CSF protein and lactates indicative of CSF inflammation, but in contrast measures of inflammation in the blood were limited. No

measures of inflammation in either compartment were significantly associated with outcome on multivariate analysis.

7.4.4 Limitations, biases and contrasts

The analyses presented in this chapter have several limitations. Complete data and sample collection for the PCR study altered between P1 and P2 (Chapter 3 Section 3.3.6) effectively limiting the numbers of samples collected in P1 compared to P2. This change increased the data and numbers of samples available for the study, but meant that data collection for physiological detail and the PCR study were predominately from patients in Phase 2 where mortality was higher. In all other important characteristics these patients were the same on admission as those in Phase 1, and the P2 mortality rates were the same as seen in the historical database. The extent to which the outcome data were altered by this mortality difference is unclear, but this is likely to be minimal.

Bacterial loads presented in this study are estimated based on calculations provided by the company that supplied the PCR kits. These calculations had been extensively validated by the company, but were not cross-checked in Malawi against a standard curve of known copy numbers, the conventional way to calculate copy number accurately. Viral copy numbers were calculated using a standard curve provided by the manufacturer, and therefore the results may be more accurate. Definitive conclusions as the relationships between bacterial loads and outcome therefore cannot be definitively made.

Data were missing from this study to allow full inclusion in the analysis of markers of inflammation and poor outcome. These related either to missing laboratory data due to reliance on hospital laboratory facilities, or periods where test strips for measuring lactate and glucose were not available. At QECH during the time of the study these facilities were severely limited, and results for HIV-1 antibodies, FBC, CD4 counts and biochemistry were commonly lacking. Many CSF samples processed in the MLW laboratory had very abnormal

results produced that were off the scale measured by the laboratory, and were reported as '#' rather than as a numerical value and as such were not included in these analyses. In addition, the lack of numerical CSF WCC data, when many patient samples were reported as 'clumped' cells limited the data available to determine full trends with CSF WCC and outcome as seen in the historical data.

The presence of entrance to study Phase 2 as an independent predictor of poor outcome suggests that admission to that Phase may have made the interpretation of the analysis more complex for the other variables included. The differences in data collection between P1 and P2 may have led to the discrepancies observed between the variables associated with poor outcome at day 10 and day 40. These data are potentially confounded by the study design. Further data from a prospective study with parallel, randomised design not subject to these confounding would provide data from patients randomised to the intervention, and would give more robust data comparing biomarkers and outcome.

In conclusion, pneumococcal loads in patients with bacterial meningitis are high, and are not associated significantly with outcome. EBV co-infection in the CSF of patients with ABM is common, and does not appear to be linked to either mortality or quality of survival, in contrast to a previous study. Predictors of poor outcome from ABM in this study appear to be associated with poor immune-responses to invasive pathogens within the CSF. Further, prospective data collection in a randomised study is required to test these trends and laboratory biomarkers further.

8 Main Discussion

8.1 Introduction

The work in this thesis addresses two important questions about mortality from and the optimum treatment of acute bacterial meningitis in African adults:

1) Why is mortality from ABM so much higher in sub-Saharan Africa (SSA) compared to other regions and why did have evidence-based adjunctive interventions from other regions failed to improve outcome in SSA?
2) Can the application of the principles of Early Goal Directed Therapy improve quality of acute clinical care for adults with suspected ABM in SSA?

Three main themes arising from this work will be addressed and discussed using a framework of the patient journey from symptom onset to outcome:

a) The causes of high mortality and the evolving picture of ABM in SSA in the last 12 years.

b) The feasibility of implementing Early Goal Directed Therapy as a protocolised treatment approach for ABM and assessment of the practicality and utility of the care bundle in the context of the trial and the setting.

c) Future directions for the study of ABM in SSA, particularly with a focus on reduction in case fatality rates in adults.

The experience of a patient with ABM from symptom onset to final outcome is influenced by many factors, some of which are dependent on the socioeconomic environment of the patient (e.g. travelling costs, societal pressures) and some of which are universal (e.g. disease recognition in the community and hospital, access to adequate health resources). Many interventions have been tested in the hope of improving survival (for example early recognition in the community; early antibiotics; adjunctive therapy, management of complications) at different points on this pathway. The possible causes of poor outcomes

from ABM, particularly in SSA, are complex and multi-factorial. This discussion section will first summarise the stages of the patient journey, then discuss themes from this thesis with regards to the potential pathogenic causes of poor outcome and the intervention tested in this thesis. Finally, future potential adjunctive interventions for ABM in adults in SSA will be discussed.

8.2 The patient journey from symptom onset to hospital outcome: where can things go wrong for adult patients with bacterial meningitis in SAA?

An adult with bacterial meningitis will start to experience symptoms in the community, most commonly headache and fever (Scarborough and Njalale, 2004). Symptoms of bacterial meningitis may progress rapidly, and need to be recognised as life-threatening. Commonly the patient will require assistance of a relative, friend or guardian to seek help at a local health-centre, or traditional healer (Desmond et al., 2013). Substantial social and community barriers can lead to delay in accessing care locally (Desmond et al., 2013), including failure of appropriate disease recognition and lack of access to qualified health professionals. Once assessed at a health centre, the patient may be referred to a central hospital with inpatient facilities, or the diagnosis may be missed and the patient given inappropriate treatment or discharged, leading to delay in referral and admission or even death in the community (Cullinan and Pieterick, 1998; Okubadejo and Danesi, 2004; Reyburn et al., 2004). Even in resource-rich settings, a diagnosis of meningitis may be missed (Brennan et al., 2003) and clinicians at the primary care level in Malawi are less well trained and have a far greater workload. In the primary care centre, although symptoms may be mild initially, the rapid progression and potential severity of the disease needs to be recognised, a referral made and access to transport and associated travel costs need to be available to ensure adequate treatment (Desmond et al., 2013). Most transport only operates in the day-time, fuel shortages common and access to ambulances for transport is severely

limited or not available (Cullinan and Pieterick, 1998). A patient referred in the evening may have to wait until the morning, often without treatment, before the referral can be completed.

By the time the patient arrives in the central hospital, he or she may have deteriorated substantially en-route (Harnden et al., 2006; Gjini et al., 2006a; Scarborough and Thwaites, 2008). Systems and facilities to prioritise very unwell cases are rare in most hospitals in SSA (Scarborough and Njalale, 2004). Triage, or early triage and treatment practices are recommended by the WHO for adults, but often not in place due to lack of trained staff (Robertson and Molyneux, 2001; WHO, 2004). Clinical care is dependent on the quality of the facilities available, including timely access to qualified medical and nursing staff, and treatment including parenteral antibiotics, seizure control medications and intravenous fluids, and diagnostic facilities.

Once patients have been admitted, ongoing quality ward based care is important, including continuation of antibiotic treatment, airway and fluid management, HIV antibody counselling and testing if appropriate , Discharge follow up arrangements are needed to ensure that post-meningitis sequelae can be treated. Many limitations occur in both the ward based care and follow up arrangements: lack of test kits precludes a diagnosis of HIV co-infection, lack of drugs on the ward limit the treatment options available, and lack of access to the correct follow up clinics commonly limits patients' access to HIV or on-going medical care. These limitations are caused predominately by the profound resource constraints that QECH and other hospitals in Malawi operate under, combined with weak procurement systems, low staffing levels and poor management and organisational structures. A significant number of the patients who survive to hospital discharge die in the community of unknown causes before their six week follow up visit.

These stages between symptom onset and eventual outcome are summarised in Figure 8.1.

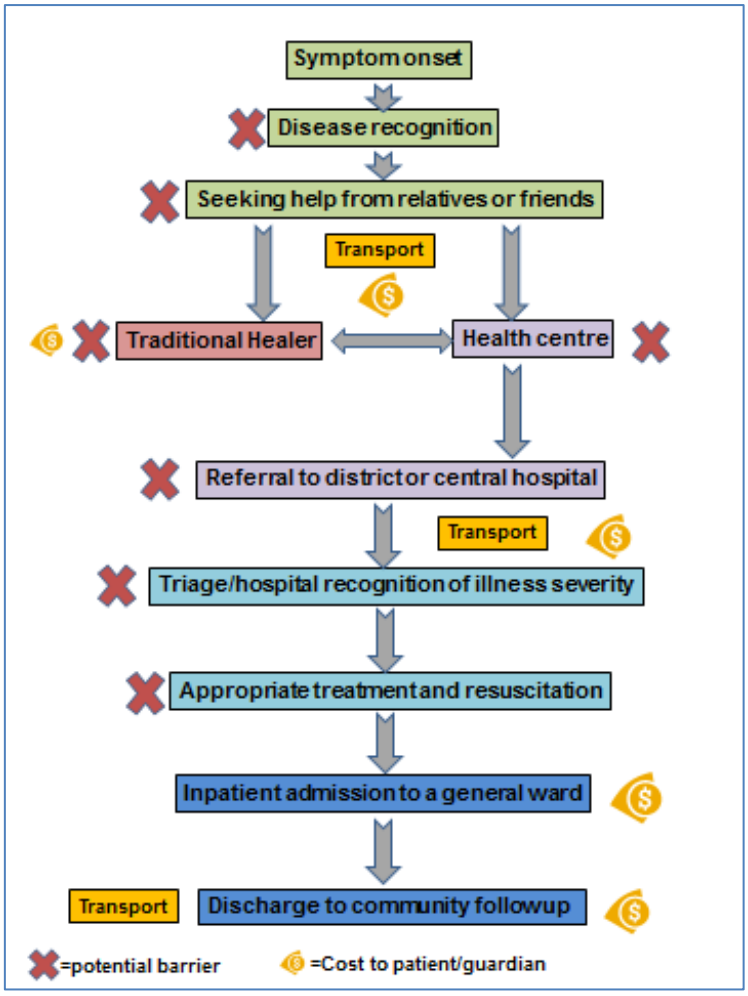


Figure 8.1 Patient journey with ABM in Malawi

Summary of the medical journey of a patient with bacterial meningitis in Malawi indicating opportunities for barriers to care and patient costs.

It is clear that barriers and bottle-necks exist at each stage of the patient journey between symptom onset and community recovery (Figure 8.1). Any single intervention is not going to be able to solve all the problems with healthcare delivery for these patients in a profoundly resource-limited environment such as Malawi. Key points on the patient journey have been identified in Figure 8.1 where barriers exist and interventions have either been tested or are planned. A joined-up approach to meningitis recognition and treatment at both community and hospital level is required to improve the overall care of these patients, as has been done, particularly for children in more resource-rich settings (Nadel et al., 1998; Thompson et al., 2006; Vaina et al., 2013; Harnden et al., 2006). EGDT for bacterial meningitis in

Malawi is part of this approach, with the aim to improve early hospital recognition and treatment of ABM.

8.3 Persistently high mortality from ABM in African adults

Survival rates in adult ABM in clinical trials are commonly measured at 48 hours, ten days or discharge from hospital and four-twelve weeks post symptom onset, (Scarborough et al., 2007; Ajdukiewicz et al., 2011; de Gans and van de Beek, 2002; Nguyen et al., 2007; Glimaker et al., 2014; Mourvillier et al., 2013). Mortality in all of these studies shows the same trend, with high frequency of initial deaths by 48 hours, with additional deaths in-hospital and further community deaths post discharge. This same pattern was seen in this study, with the most deaths occurring early in the illness, but with significant in-hospital and out of hospital mortality.

The case fatality rates (CFR) from ABM at day 10 (45%) and day 40 (57%) in BAM were the same as those in the SAM (45% and 56% respectively) and GLAM trials (45% and 61% respectively) (Scarborough et al., 2007; Ajdukiewicz et al., 2011), and an observational study (60%) (Gordon et al., 2000). These high CFRs are typical for the region (Gessner et al., 2010; Traore et al., 2009; Trachtenberg et al., 2007), and are substantially higher than those recorded in other settings (Mourvillier et al., 2013; de Gans and van de Beek, 2002; Nguyen et al., 2007; Glimaker et al., 2014; Durand et al., 1993). The CFR for comparison for pneumococcal meningitis in more well-resource settings remains at 20-35%, remaining at this level despite the introduction of dexamethasone therapy and high quality intensive care support (Almirante et al., 1995; Brouwer et al., 2010a; Lepur and Barsic, 2007).

It is disappointing that shorter times from presentation to ART receipt for HIV co-infected individuals, and substantial overall improvements in adult mortality in HIV co-infected individuals associated with improvements in health-centre level care in Malawi do not appear

to have affected mortality in adults with ABM (Sloan et al., 2013; Floyd et al., 2010). This may partly be due to the considerable social barriers that many patients meet before arriving at the treating hospital, in addition patients with poor outcome present with a features of severe disease that are difficult to manage within the resource limitations of healthcare in Malawi (Wall et al., 2013c; Desmond et al., 2013).

8.3.1 Prediction of poor outcome from ABM

A severity score (MAMS), derived from five predictors of poor mortality from previous studies was shown to be a good predictor of outcome in BAM Phase 2 and was superior to a previous score developed in Europe when analysing Malawian data (Weisfelt et al., 2008; Schut et al., 2012). This observation suggests that severity scores for meningitis may be population specific, leading to the inference that significant differences exist in ABM in different regions, due to either differences in invasive disease in the host, pathogens or the environment. Detailed comparative studies are required to explore this further.

Altered mental state remained the strongest predictor of poor outcome in BAM and historical studies (Wall et al., 2013c), in BAM it was shown that lower GCS combined with rising mean arterial blood pressure (MAP) over six hours was predictive of poor outcome. This pattern is consistent with rising intracranial pressure (Koenig et al., 2008; Glimaker et al., 2014). An association between altered mental state and poor outcome has been observed in many studies of ABM in other settings (Flores-Cordero et al., 2003; Ishihara et al., 2009; Lepur and Barsic, 2007; van de Beek et al., 2004); no other study has reported outcome with serial GCS and MAP measurements. The underlying causes of altered mental state due to CNS inflammation and raised intracranial pressure in ABM are complex, work from developing severity scores in Europe and in Chapter 5 shows that as GCS falls, the risk of poor outcome increases proportionally (Weisfelt et al., 2008).

The measurement of clinical parameters associated with outcome is useful for the development of prediction tools, which in turn may have useful roles in future clinical trials. These measurements alone do not increase our understanding of the specific causes of poor outcome, but, together with data from meningitis pathophysiology from animal models, have led to attempts to ameliorate clinical findings associated with poor outcome, such as reducing intracranial pressure (Glimaker et al., 2014; Ajdukiewicz et al., 2011), CNS inflammation or brain cooling (Scarborough et al., 2007; de Gans and van de Beek, 2002; Mourvillier et al., 2013). However, these treatments to date have been targeted at global reductions in brain inflammation and swelling, and have met with limited success.

Human post-mortem studies show that patients who do not survive ABM have a very severe inflammatory findings such as reduced synaptic density and cortical necrosis (Wippel et al., 2013), but these represent only the terminal disease state and therefore provide at best a partial insight into the physiology of the disease. Imaging studies with CT scanning or MRI would be a good way to see if macroscopic changes such as space occupying lesions, hydrocephalus or cerebrovascular accidents exist within the CNS of adults who survive or do not survive. The few data available are exclusively from resource-rich settings. In a study of patients with suspected ABM on admission to hospital, CT scans were abnormal in approximately 24%, with a wide variety of findings. Focal lesions were most common, particularly stroke, mass lesion and disease of the peri-ventricular white matter; non-focal lesions including sub-arachnoid haemorrhage, meningeal enhancement and hydrocephalus were also seen (Hasbun et al., 2001). Abnormal CT findings on admission in this cohort were associated with poor outcome, increased age (>60 years) and an immunocompromised state, but details of specific CT changes in the sub-set of patients with proven ABM are not included (Hasbun et al., 2001). This study shows that macroscopic brain changes are present in a subset of adults with meningitis, however to date no study has related imaging findings to outcome.

Cerebral thrombosis on MRI has been shown to be a rare cause of late mortality and morbidity from ABM after initial recovery, with infarctions of the brainstem or thalamus in all patients. The risk of post-meningitis stroke may be able to be predicted from raised cerebral blood flow velocity during the acute illness; further prospective clinical data are needed (Klein et al., 2010; Schut et al., 2009a; Klein et al., 2011). Imaging data from proven ABM in children shows that infants with late presentation, abnormal mental status and seizures were more likely to have abnormal MRI findings, commonly meningeal enhancement, infarcts and subdural empyema (Oliveira et al., 2014). MRI is available in Blantyre but was not financially or logistically possible during BAM. Nonetheless, future studies may provide evidence for risk stratification, based on the degree of cerebral inflammation or presence of stroke, leading to additional treatments such as prolonged antibiotics for patients with imaging abnormalities or targeted management of stroke-like phenomena.

8.3.2 Host biomarkers of outcome

Trends towards an association between lower CSF white cell counts and mortality were observed in SAM, GLAM and BAM. Low CSF WCC have been associated with poor outcome in other more well-resourced settings, although the median counts recorded in those studies are 10 to 100 fold higher than those observed in Malawi (van de Beek et al., 2004; Weisfelt et al., 2006d). Malawian patients with ABM appear to have substantially lower CSF WCC responses to ABM compared to those in other regions, with a univariate association between CSF WCC and poor outcome (Wall et al., 2014b). CSF white cells in ABM are made up of predominately neutrophils, which phagocytose and destroy invasive bacteria through autolysis (Mook-Kanamori et al., 2011; Nazifi et al., 1997). It is possible that inadequate numbers of CSF white cells lead to inability to phagocytose and autolyse sufficient bacteria, even in the presence of adequate bactericidal antibiotics leading to delayed sterilisation. However in an animal model of meningitis where infiltration of neutrophils into the CSF compartment was blocked using monoclonal antibodies, lack of

CSF WCC enhanced animal survival (Tuomanen et al., 1989). No human data exist. CSF WCC differed in both adults and children according to specific meningitis pathogens, with the meningococcus having a 10 fold higher median cell count than the pneumococcus or NTS (Wall et al., 2014a). HIV status had no effect upon the median cell count in adult meningitis (Wall et al., 2014a).

Whether the overall lower CSF WCC observed in SSA is associated with pathogenesis, or is a function of ethnic neutropaenia is not clear. People of African origin have been noted to have lower peripheral neutrophil counts compared to normal ranges derived from Caucasian populations (Wells et al., 2006; Kourtis et al., 2005; Bain et al., 1984); no lack of functionality has been attributed to the lower peripheral cell counts (Haddy et al., 1999). Ethic neutropenia may be associated with increased risk of HIV acquisition, possibly due to Duffy-null associated low neutrophil counts (Ramsuran et al., 2011).

The wide range of CSF neutrophil counts observed in BAM, compared to less variation in the peripheral white cell count suggests that that the issue lies with reactivity of the host system rather than ethnic neutropaenia and the association with negative outcome supports this. Testing which hypothesis is be correct would be possible but complicated, and require fresh CSF and peripheral blood samples from patients with meningitis, and peripheral blood from matched control patients. Flow cytometry on fresh CSF samples may be able to determine expression of different receptors on CSF neutrophils in patients with different counts, cell reactivity to pneumococci in the periphery between controls and patients, and gene expression in the peripheral blood neutrophils and CSF neutrophils may also provide useful data to explore this area.

Interestingly a similar pattern has not been highlighted in studies of children in SSA. Children in this region have an overall high CSF WCC in ABM. In a study from South Africa, children with ABM had a median CSF cell count of 848 x 10^6 cells/mm^3 (IQR 13 – 10 027x10^6 cells/mm^3), n=67, 23% were HIV co-infected, 18 patients had cell counts of <300x10^6

cells/mm^3 (Michelow et al., 2000). No association between low cell count and outcome was tested in that study. A review of prognostic indicators for paediatric ABM in Malawi did not look at CSF WCC or peripheral white cell count (McCormick et al., 2012), neither did a study examining the relationship between bacterial load and cytokines in CSF with outcome in Malawian children with ABM (Carrol et al., 2007a). Other studies of causative factors for poor outcome from SSA also did not examine the relationship between CSF or peripheral white cell count and outcome (Akpede et al., 1999; Johnson et al., 2007; Pelkonen et al., 2009). Although details are not given, it is likely that these laboratory data were not available for inclusion from systemic surveillance.

A systematic review of poor prognostic indicators in paediatric bacterial meningitis world-wide showed that overall, low CSF WCC and low peripheral CSF WCC were associated with poor neurological outcomes from ABM including coma and seizures (de Jonge et al., 2010). Substantial differences existed in the quality of the included studies, those which looked for and found CSF WCC to be associated with poor outcome were mostly retrospective studies that have been done in both the pre and post Hib vaccine eras, around the start of the HIV epidemic (Koomen et al., 2004; Chao et al., 2008; Lovera and Arbo, 2005; Pagliano et al., 2007; Kaaresen and Flaegstad, 1995; Herson and Todd, 1977). The ranges of CSF WCC reported in these studies varied enormously, the numbers available in most studies were small. A meta-analysis only showed an association between poor neurological outcomes and low CSF WCC; relationships between CSF WCC and other outcomes were not significant (de Jonge et al., 2010).

Peripheral neutrophil counts were commonly not available in the BAM study, however total peripheral white cell counts were within the normal ranges (Chapter 6 section Table 6.9, **Table 7.6**), despite the presence of invasive pneumococcal infection where a reactive neutrophil pleocytosis might have been expected (Nazifi et al., 1997). Lower peripheral blood white cell counts were observed in BAM non-survivors, however the trend did not reach

statistical significance and there are no historical data for comparison. Further understanding/ insights into this issue will be generated from RNA expression analysis of whole blood and CSF peripheral blood mononuclear cells (PBMCs) in these patients, to determine if patterns of RNA expression differ in either compartment in relation to outcome.

Other markers of inflammation in blood and CSF were not associated with poor outcome in BAM, including blood lactate and haemoglobin. The lack of association between blood lactate and outcome was surprising, given the extensive evidence in sepsis and septic shock that higher lactates directly correlate with poor outcome, particularly failure to clear or reduce blood lactate levels with resuscitation (Arnold et al., 2008; Kortgen et al., 2006; Rivers et al., 2001; Nguyen et al., 2011). The relatively low blood lactate values seen in BAM suggest that few patients had septic shock causing poor tissue perfusion, supporting the hypothesis that poor outcome was driven by CNS inflammation, not sepsis and shock.

Few studies are available exploring if CSF lactate is associated with poor outcome in meningitis, a relationship has been shown in children with cerebral malaria (White et al., 1985). A single study from Nigeria in children showed that there was a trend towards higher CSF lactates and outcome, although this did not reach statistical significance (Imuekehme et al., 1997). An animal study showed that high CSF lactate was associated with development of deafness after ABM in an animal model of pneumococcal meningitis (Bhatt et al., 1993). CSF lactate has been developed as a test to discriminate between viral/aseptic meningitis and ABM, not as a marker of outcome (Berg et al., 1982; Brook et al., 1978; Controni et al., 1977). CSF lactate is produced by anaerobic respiration in active cells in the CSF including pneumococci and neutrophils as they autolyse, and is a marker of intense inflammation (Mustafa et al., 1989a; Bhatt et al., 1993; Mustafa et al., 1989b; Ammendolia et al., 2009). All BAM patients had high CSF lactates (>8mmol/L), commensurate with the high bacterial loads seen. No previous studies have attempted to correlate CSF lactate with bacterial load; the BAM data showed no significant correlation.

A clear relationship was demonstrated between severe anaemia and poor outcome in the historical meningitis data presented in Chapter 4 Section 4.5.4 (Wall et al., 2013c). This association was not seen in BAM, overall mean haemoglobin measurements were higher than the in the historical dataset with only a very small proportion of severely anaemic patients (Hb <8.0g/dL). It is not clear why these patients were less anaemic, but the introduction of ART, and generalised improved nutritional status of Malawians over the last 10 years may be contributory (Chihana et al., 2012; Floyd et al., 2012; Jahn et al., 2010b). Anaemia in SSA is commonly a marker of severe chronic disease, and possibly a marker of the degree of immunosuppression caused by HIV (Lewis et al., 2005; Phiri et al., 2008). Future monitoring of trends in anaemia in patients with ABM from other centres in SSA would be helpful to explore this further.

8.3.3 The roles of pathogenic bacteria in determining outcome

Pneumococcal meningitis, particularly amongst adults in Malawi is predominately caused by serotype 1 (Everett et al., 2012), a pneumococcal serotype that is rarely carried but causes highly lethal epidemics of meningitis in West and Central Africa (Mueller et al., 2012; Yaro et al., 2006; Leimkugel et al., 2005). In contrast, serotype 1 is not commonly associated with meningitis in Western countries, and is associated with a relatively lower CFR compared to other serotypes; important differences in virulence are likely to exist between serotype 1 in different continents (Burgos et al., 2012a).

High levels of pneumococcal toxins and high bacterial loads have been observed in African patients with pneumococcal meningitis, and CNS infection is aggressive and rapidly progressive (Wall et al., 2012; Mueller et al., 2012; Roine et al., 2014b; Wall et al., 2014b; Carrol et al., 2007a). The two sole few human studies relating bacterial toxins in the CSF to outcome from ABM (Wall et al., 2012; Ovstebo et al., 2004) both show a relationship between bacterial product and outcome. In both human post mortem and animal work, the pneumococcal toxin pneumolysin causes severe structural neuronal and synaptic damage

which may be irreversible and fatal (Wippel et al., 2013). Tests of the host ability to clear toxin from the CSF of the animals were not done in this study. The degree to which outcome is determined by the pathogen or the host is yet un-determined.

8.3.4 The role of HIV co-infection in outcome

HIV co-infection was not associated with outcome in either the historical dataset or in the BAM trial (Wall et al., 2013c). This finding was unexpected in the context of the important role HIV co-infection plays in the acquisition and increased risk of invasive pneumococcal disease world-wide and particularly in sub-Saharan Africa. HIV co-infection is associated with increasing pneumococcal carriage frequency, and increased rates of pneumonia, sepsis and meningitis, despite increasing access to ART (Anthony et al., 2012; Burgos et al., 2012b; Domingo et al., 2009; French et al., 2010; Grau et al., 2005; Molyneux et al., 2003; Nunes et al., 2011; Glennie et al., 2013; Wolter et al., 2014b).

HIV is clearly a significant risk factor for pneumococcal meningitis (Feikin et al., 2010; Bekondi et al., 2006; Burgos et al., 2012b; Domingo et al., 2009; French et al., 2010; Gordon et al., 2000; Molyneux et al., 2003; Hakim et al., 2000; Wall et al., 2013c), however the role of the HIV virus in pathogenesis of ABM is less clear.

HIV co-infection has been associated with poor outcome in children with meningitis caused by both *S. pneumoniae* and *N. meningitidis* in South Africa and *S.pneumoniae* in Malawi (Cohen et al., 2010b; Nyasulu et al., 2011; McCormick et al., 2012). In these vertically infected children, failure to thrive and age <1 year were also predictors of poor outcome (Nyasulu et al., 2011). No differences were noted in mortality for all IPD in adults between HIV co-infected and HIV negative individuals in Spain; meningitis mortality was not compared (Burgos et al., 2012b). Also in Spain, an earlier study showed HIV co-infected adults with bacterial meningitis had a higher case fatality rate than HIV uninfected, but the

numbers were very small (n=34) (Domingo et al., 2009). . No causative data were given in these descriptive epidemiological studies, but the authors of all the specific meningitis data showing an association with HIV and mortality, associate weaker humoral immunity caused by HIV co-infection with the increased CFR observed.

In the study of historical Malawian data, concerns were raised that the lack of association of outcome with HIV co-infection was due to the small proportion of HIV negative individuals in the data analysed (13% of the total) (Wall et al., 2013c). BAM again did not show an association between HIV status and outcome again, with 30% HIV un-infected making an association between HIV and outcome across a total of 827 patients with meningitis very unlikely. Data are lacking from studies of ABM pathogenesis in HIV co-infected humans, but it is known that the presence of rapidly dividing bacteria within the CSF triggers a series of innate immunological responses from both within the CNS and the periphery to control the infection (Mook-Kanamori et al., 2011; Ernst et al., 1983; Fishman et al., 1975; Grandgirard et al., 2007; Mertsola, 1991; Tauber et al., 1992; Tauber and Sande, 1984). It is possible that these responses are only minimally affected by HIV and that other determinates of the outcome of this intense inflammation are likely to be more important in the final outcome than the presence of HIV infection.

It is well established that the CNS is a particular reservoir for HIV, and HIV causes specific brain related pathology in a small proportion of HIV co-infected individuals (Geffin and McCarthy, 2013; Ances and Ellis, 2007). BBB endothelial cells can be activated to suppress HIV replication and possibly entry of HIV into the CNS from the circulation; they may be important in regulating the interaction between HIV and the CNS (Li et al., 2013). The impact of HIV on innate CNS immunology has not been studied in detail.

Although comparisons are limited, studies of cryptococcal meningitis (CCM) suggests differences when activities of paired Natural Killer (NK) cells and monocytes between the blood and CSF are compared during acute infection (Naranbhai et al., 2014) and that chemokine responses in the CNS to CCM are different to the blood (Chang et al., 2013). These data support the hypothesis of a specific compartmentalised response arising from within the CNS in response to the presence of a pathogen; it is unknown if this is instead of, or complementary to the peripheral circulation in response to infection in the meninges.

However, it is not clear if any of these mechanisms described in CCM are relevant to the immune attack within the CNS against pneumococci multiplying in the CSF. It is likely that failures of immunological mechanisms recruiting inflammatory cells to the CSF, leads to abnormal CSF WCC responses and uncontrolled bacterial growth and inflammation and poor outcome. The extent to which that this process is affected by HIV is unknown, but as HIV co-infection was not associated with outcome, it is unlikely the effects of HIV on pathogenesis were significant. This hypothesis will be explored in further work examining RNA expression patterns of PBMCs in the blood and CSF of patients from BAM.

8.3.5 The importance of Herpes viruses co-infection in ABM pathogenesis

In contrast to HIV co-infection, historically EBV has been shown to be positively associated with HIV and poor outcome in patients from the GLAM study (Kelly et al., 2012; Ajdukiewicz et al., 2011). This analysis was repeated in BAM, there was no association between EBV and a composite poor outcome. In a paediatric study from Angola, EBV was isolated in the CSF of 13% of children with ABM, with no association with outcome observed (Pelkonen et al., 2013). Possible causes for the discrepancy between the Malawian studies may be due to smaller numbers available for the BAM analysis, or the increased frequency of ART receipt in BAM compared to the SAM and GLAM trials. EBV infection is ubiquitous in SSA and it is unclear whether the presence of EBV in the CSF is important in the pathogenesis of ABM, or

if the virus is a bystander, produced by actively replicating B-memory cells. (Schaftenaar et al., 2014; Kleines et al., 2011; Petrara et al., 2013).

The presence of CMV infection in the CSF appeared to be associated only with the presence of EBV, and strongly associated with mortality in GLAM study (Kelly et al., 2012); the numbers were too few to test in BAM. It is likely that CMV is a marker of profound immunosuppression and again, it is unclear if this virus has an active role in pathogenesis of meningitis (Siddiqi et al., 2014) (Griffiths, 2004).

Other herpes viruses including HSV-1, HSV-2 and VZV are not found in adults in SSA with ABM, they are found in the CSF of adults presenting with aseptic meningitis (Kelly et al., 2012; Benjamin et al., 2013). Children with ABM in SSA appear to have these viruses present as co-infection, however again the role in pathogenesis of these viruses is unclear (Pelkonen et al., 2013). Enterovirus co-infection was also present in a small number of patients with ABM in this paediatric study, conclusions are limited by the small sample size available (Pelkonen et al., 2013; Pelkonen et al., 2012).

In summary, the pathogenic causes of poor outcome in Malawian adults with ABM are likely to be multifactorial, and do not appear to be influenced by horizontally acquired HIV co-infection. Both host and pathogenic factors are likely to be important, and investigation of this area represents an important future step in the understanding of meningitis pathogenesis in SSA, and in the development of future adjunctive treatments. The effects of pathological features on poor outcome are likely to be compounded by barriers met by the patient and guardians between symptom onset and hospital treatment.

8.4 The stages of the patient journey with ABM, opportunities for intervention

8.4.1 Community onset of symptoms, understanding of disease and urgency of seeking medical help

Bacterial meningitis may have either an abrupt acute clinical onset with fulminant disease course, or start with mild, non-specific symptoms which then progress over hours to days to more fulminant symptoms (Radetsky, 1992). In countries with a high prevalence of malaria, the meningitic symptoms of fever, headache, confusion and convulsions, are commonly mis-diagnosed as malaria (Molyneux, 1996; Walker et al., 1992; Reyburn et al., 2004; Okubadejo and Danesi, 2004).

Perceptions within the community of the high burden of malaria, may lead to inappropriate home based malaria treatment in the early stages of a meningitic illness in SSA, where free or heavily subsidised combination anti-malaria treatments are easily available (Desmond et al., 2013; Aubouy, 2011). A qualitative study from Malawi examining the treatment seeking behaviour of patient carers or guardians of both adults and children with subsequently proven meningitis showed socially complex healthcare seeking behaviour (Desmond et al., 2013). In this study male guardians could make decisions and had access to funds to take a patient to the health provider of choice, but female guardians could only do so with social validation of their social superiors, from whom they had also to access funds for all costs incurred (Desmond et al., 2013). Guardians in this situation were commonly directed towards a traditional health care provider and a health-centre, in some cases permission to access to health centre or hospital was only granted only after deterioration of symptoms or perceived lack of effect of traditional remedies. Community perceptions about the poor quality of health care available at the health-centre were also important factors influencing delays to hospital care (Desmond et al., 2013). A study from Ghana examining attitudes to meningitis in the community revealed lack of knowledge of the risks of meningitis and early symptoms amongst healthy adults surveyed, an awareness of the high costs involved with a relative sick with meningitis and a lack of understanding of the protective effects of vaccination.

These areas were identified by the authors as barriers to accessing healthcare in that region (Hayden et al., 2013; Akweongo et al., 2013).
These factors were not studied directly in the BAM study, but were undoubtedly important in influencing delays for patients with ABM recruited in all studies of meningitis in Malawi.

The study by Desmond et al led to a novel initiative in Malawi that was conducted during the BAM study, where local language radio programmes dealing with important healthcare issues including meningitis were broadcast. The programme involved interviews with patients and guardians, and a text message/phone in question and answer service. Full reports of the response to the programmes are awaited, but preliminary data shows that they substantially increased social awareness of important diseases, and the facilities available for treatment, counteracting poor community perceptions which may limit health-seeking behaviour (Nyirenda 2013 unpublished data). This type of intervention may be very powerful in improving community disease recognition and health-seeking behaviours, with subsequent reduction in pre-hospital delays and associated mortality and morbidity (Bamani et al., 2013; Ndlovu and Sihlangu, 1992).

8.4.2 Health centre management and community treatment/referrals for ABM

In cases of suspected meningitis and meningococcal septicaemia, early antibiotics are advised (Theilen et al., 2008). In well-resourced settings, pre-hospital intramuscular antibiotics have been associated with improvements in outcome from meningitis in observational studies (Harnden et al., 2006), and primary healthcare providers are commonly equipped with antibiotics for this purpose. It is recommended to give a community dose of antibiotic rapidly pre-referral to a hospital (Aronin et al., 1998; Fitch and van de Beek, 2007), although no randomised controlled trials have been done (Sudarsanam et al., 2013). Trials from the meningitis belt region showed that single dose, long acting oily chloramphenicol is adequate treatment for sensitive meningococcal meningitis in Africa (Wali

et al., 1979). However, single dose long acting penicillin is not associated with bacterial sterility in the CSF at 48 hours, and is not recommended as monotherapy for meningococcal meningitis, without follow up regular parenteral penicillin (Macfarlane et al., 1979). Single dose ceftriaxone is as effective as chloramphenicol for epidemic meningococcal meningitis (Nathan et al., 2005). No data on long acting antibiotic therapies are available for the treatment of pneumococcal meningitis. Single dose ceftriaxone would be appropriate pre-hospital therapy for suspected cases of ABM in Malawi, as no ceftriaxone resistance has been identified.

The sensitivity of CSF microbial culture is reduced by pre-hospital antibiotic administration (Sacchi et al., 2011), but this risk is outweighed by the potential mortality benefits. In Malawi, mis-diagnosis of meningitis and delays at crowded and poorly resourced healthcare centres potentially contribute to the severe clinical presentation and associated poor outcome from ABM in adults and children (Desmond et al., 2013; Scarborough and Thwaites, 2008; Cullinan and Pieterick, 1998). No studies of pre-hospital parenteral antibiotics have been done in adults in this setting for suspected meningitis. The SAM trial showed intramuscular ceftriaxone to be non-inferior to the intravenous route in obtaining adequate CSF levels to obtain the minimum inhibitory concentration (MIC) for *S. pneumoniae* (Scarborough et al., 2007).

A study of a paediatric package of care delivered in rural health centres in Southern Malawi showed that for unwell children presenting to a health centre with a presumptive diagnosis of either meningitis or malaria (with no access to diagnostic tests for either), delivery of a simple package of care including intramuscular quinine and chloramphenicol substantially improved mortality in patients who remained in the centre for complete care, and may have reduced mortality for those referred to a central hospital, follow up data are lacking in the study for those patients (Cullinan and Pieterick, 1998). This study was observational and subject to several biases, but provides interesting pilot data for studies of pre-hospital presumptive treatment. A qualitative assessment showed that the users rated the package

easy to use (Cullinan and Pieterick, 1998). Subsequently, a much larger study to introduce the concept of prioritising clinical care for children on the basis of universal clinical predictors of severe illness, the 'Chipitala Robot' study (translation: Hospital traffic light study) has been funded. No results are yet available from this study, which is currently in progress, however the mobile phone based triage-tool appears to be acceptable and facilitate referrals to the central hospital (Byrne 2013 unpublished data).

A study of health centre administered pre-hospital antibiotics for adults with suspected ABM has not been done, but from the evidence currently available, the strategy may have significant benefits in reducing treatment delays and improving outcome.

8.4.3 Delay/travel to central hospitals for diagnosis and treatment

The time from symptom onset to triage in-hospital was shorter in BAM than in both the SAM and GLAM trials. Pre-hospital delays in ABM in Blantyre are complex, and only partially relate to travelling times and mis-diagnosis in health-centres, other social, financial and cultural factors are also important (Desmond et al., 2013; Hayden et al., 2013). Increased availability of ART and clinical care for HIV co-infected people in Blantyre (Sloan et al., 2013), may have facilitated community understanding of meningitis and hospital referrals in the BAM study.

However obtaining accurate data for illness duration in Malawi is difficult. Patient guardians, who frequently provide the history, may not live with the patient, and may fear anger and poor clinical care if they admit to delays. Accurate time keeping in such an environment may also be challenging. Although a median time of 48 hours from (IQR 24-72 hours) was observed in both BAM phases, times of onset were recorded in days (24/48/72 hours) by the study team as more accurate were difficult to obtain from patients or guardians.

Scarborough and Thwaites have suggested that community delays may be due to misdiagnosis of ABM as pneumonia, malaria or other cause of headache (Scarborough and Thwaites, 2008). Nothing in BAM suggested that this was a significant cause of pre-hospital delay in BAM, although errors in diagnosis in the AETC leading to referrals to psychiatry and ophthalmology were observed in a small number of patients in P1.

8.4.4 Role of in-hospital delays and pre-treatment with antibiotics on outcome.

Patients in BAM experienced much shorter in-hospital delays than those in the historical database who were admitted through the old Room 6-Ward 4B system (Wall et al., 2013c). We expected to find that shorter times to clinical review and parenteral antibiotics would be associated with better outcomes, similarly to the sepsis care bundle studies where shorter times to parenteral antibiotics were associated with improved survival (Barochia et al., 2010). However this association was not observed in either phase of the study.

Pre-hospital treatment with antibiotics did not improve outcome or cause apparent delays in referral, although the numbers of patients who received parenteral antibiotics were small. Pre-hospital treatment with parenteral antibiotics in other settings has improved outcome from severe meningococcal disease, with the exception of those have a fulminant disease when presenting to primary care (Harnden et al., 2006; Hahne et al., 2006; Perea-Milla et al., 2009). No data are available for pneumococcal meningitis in any setting. Although the presence of pre-hospital antibiotics was recorded, it the indication was not always clear in the BAM study: patients who received co-trimoxazole may have received this for prophylaxis as part of the national HIV treatment programme and not for their meningitic symptoms (van Oosterhout et al., 2005; Government, 2010). A specific study designed to examine the utility of pre-hospital treatment of meningitis is urgently required.

8.4.5 Comparisons of admission characteristics for adults with bacterial meningitis

Early recognition and diagnosis of ABM on admission to hospital is essential to initiate appropriate care (Brouwer et al., 2012; Attia et al., 1999). Important public health interventions with substantial benefits have been introduced into Malawi since the SAM trial started recruitment, including free access to ART, intensive malaria control, and Hib and pneumococcal vaccination (Chihana et al., 2012; Harries et al., 2009; Sloan et al., 2013; van Oosterhout et al., 2005; Roca-Feltrer et al., 2012; Daza et al., 2006; Wall et al., 2014a). These interventions have resulted in decreased rates of admission with bacterial meningitis for children but not adults (Wall et al., 2014a; Bar-Zeev N, 2014). The BAM data were compared against the historical meningitis clinical trial data to see if the clinical characteristics of patients presenting to BAM had changed, and whether admissions with ABM had declined since 2001. There was little difference in the patient profile between patients from the historical database presented in Chapter 4, and in the BAM trial presented in Chapter 6; the patients have similar median ages, rates of HIV co-infection, frequency of pneumococcal disease and disease severity on admission. However, the rate of severe anaemia was higher historically, and substantially more patients who were HIV co-infected in BAM were receiving ART (42%) compared to those in SAM (0%) or GLAM (5%) (Scarborough et al., 2007; Ajdukiewicz et al., 2011).

The surveillance data presented in Chapter 4 suggested that the incidence of adult culture positive meningitis has not changed between 2000-2012 (Wall et al., 2014a). It was therefore expected that the planned sample size (>100 patients with ABM per year), based on estimates from recruitment data from the SAM and GLAM trials would be met easily. Instead, recruitment rates were substantially lower than the previous trials. Why this was remains uncertain. The BAM trial ran for 20 months, with a mean recruitment rate of 6.6 cases/month, half the rate of recruitment to the SAM trial (mean 15.1 cases/month) (Scarborough et al., 2007), and lower than that in the GLAM trial (mean 10.6 cases/month) (Ajdukiewicz et al., 2011). No new facilities treating adults with ABM opened in Blantyre

during the course of any of the studies, and therefore reduced case load due to admission elsewhere is unlikely. The median symptom history in BAM was also shorter than in GLAM and SAM (48 hours compared to >48-72 hours), arguing against increased early deaths in the community causing lower recruitment.

All three studies included culture negative meningitis but it is unlikely that decreasing rates of culture negative meningitis would account for the decrease in recruitment rate. Differences in inclusion criteria could obviously possibly account for the different recruitment rates, however the only difference in the CSF inclusion criteria between the three studies were a CSF WCC of >100 cells/mm^3 in SAM and GLAM, and >50 cells/mm^3 in BAM. By using this lower threshold, higher recruitment rates for BAM were expected. All trials recruited daily, including out of hours. It is possible that the reduced number of cases seen in BAM could be due to an indirect result of increasing ART roll out, and possibly through herd immunity from the introduction of pneumococcal vaccination in Malawian children in 2012.

More patients in BAM were on ART (34%) compared to SAM (0%) and GLAM (5%). ART has been postulated to reduce the frequency of invasive pneumococcal disease (IPD) in HIV co-infected adults, (Almirante et al., 1998; Burgos et al., 2012b) .
Further surveillance data reporting meningitis trends in Malawi will be very important. It remains unclear why the recruitment rate for BAM was so much lower than SAM and GLAM despite static pneumococcal disease incidence rates in 2012,

8.4.6 Meningitis Diagnostics and the role of culture negative meningitis

In the BAM study, CSF culture detected a pathogen in 63% of included participants, compared to 68-72% of participants in the SAM trial, 77% in the Dutch dexamethasone trial and 50% in the Vietnam dexamethasone trial (Scarborough et al., 2007; de Gans and van de Beek, 2002; Nguyen et al., 2007). The sensitivity of CSF culture in well-resourced routine

laboratories is estimated to be 81%, decreasing to <75% when antibiotic activity was detected in the CSF (Wu et al., 2013), higher than that seen in clinical trials. This may be due to early antibiotic or delay influencing culture viability in the trials, implying molecular identification of the causative organism in culture negative patients may be appropriate for surveillance (Michael et al., 2010; Sacchi et al., 2011).

Unsurprisingly the utility of PCR was greatest when prior antibiotics had been administered (Sacchi et al., 2011). Prescription of antibiotics in the community was common in BAM. With increasing access to treatment in health centres for adults and children, particularly enhanced HIV care, the proportion of culture negative cases of ABM may increase, limiting surveillance of ABM based on culture positive cases only. Enhanced surveillance using PCR, particularly for pneumococcal and meningococcal meningitis would give more accurate data, but the substantial cost may be prohibitive in this setting (Sacchi et al., 2011).

8.5 Acute in-hospital treatment: Early Goal Directed Therapy in sub-Saharan Africa

8.5.1 The feasibility of EGDT for ABM in a resource-poor setting

The BAM study is the first study of goal directed therapy for bacterial meningitis, and the first formal study of EGDT in SSA. The study was primarily designed as a pilot study to test the feasibility of introducing EGDT in QECH. The data presented in Chapter 6 show that the use of EGDT for ABM was feasible; significantly more clinical targets were met using the care bundle compared to standard hospital management. Times to medical review and parenteral antibiotics were faster, airways were used more frequently, greater volumes of IV fluids and more sedative drugs were given to patients receiving the care bundle.

Despite the widespread introduction of EGDT for sepsis and other uses in critical care in resource-rich countries (Rivers et al., 2001; Dellinger et al., 2004; Dellinger et al., 2013; Levy et al., 2012; Robb et al., 2010), primary feasibility assessment data for care bundles are rarely published. Very few studies have piloted goal directed interventions and undertaken feasibility assessments.

Feasibility testing is important to ensure that the targets set in the care bundle are achievable within the time frame set, and that the intervention is appropriate for the disease studied (Campbell et al., 2000; Campbell et al., 2007). Feasibility testing is also recommended by the MRC guidelines on testing complex interventions, to ensure that problems with the study are resolved before the start of a large and expensive clinical trial (Campbell et al., 2007; Council, 2008).

In BAM, delivery of targets that were feasibly achieved were: target times for assessment and antibiotics, insertion of airway and head tilt, and increased administration of blood and fluid for patients who were anaemic or shocked. In the composite assessment, the mean targets achieved by the bundle were greater than those achieved by normal, non-targeted care, and greater proportions of targets could be met in sicker patients with the bundle compared to routine care (Chapter Six, section 6.3.4). These data are encouraging, suggesting that despite resource constraints, targeted care is possible. It must be noted that the care bundle was delivered by trained research nurses, not those with a large patient work-load. This was so changes in physiology with targeted therapy could be monitored closely, to determine efficacy of the care bundle. It does not replicate real-life situations in most African hospitals.

The first large trial testing EGDT for sepsis reported the relative proportions of each target achieved by six hours across the groups, but the authors did not publish prior feasibility data

for their sepsis bundle (Rivers et al., 2001). Subsequently most centres adopted the evidence based surviving sepsis guidelines (SSG) published after the Rivers study, drawing on data from Rivers *et al* and other studies (Dellinger et al., 2004; Dellinger et al., 2008b; Dellinger et al., 2013) then reported problems with implementing care bundles and associated required quality improvement processes (Levy et al., 2004).

Feasibility testing for care bundle studies is difficult. One large study did test for feasibility of achievement of the individual care bundle elements. This observational study in Singapore, in before/after design introduced a sepsis bundle to the emergency department (ED) of their hospital, and measured bundle compliance against the increasing number of targets set for patients with correspondingly greater clinical needs (Kuan et al., 2013). Intensive quality improvement (QI) initiatives were put in place to facilitate bundle compliance. This study showed that as bundle compliance increased with the QI initiatives, mortality correspondingly decreased (Kuan et al., 2013). The easiest care bundle elements to achieve in that study were measurement of lactate, fluid bolus administration, early antibiotics and blood culture draw before antibiotics (Figure 8.2). Achieving the MAP and CVP targets were much more challenging, with only 40% completing bundle compliance by the end of 6 hours after the fourth round of QI initiatives (Figure 8.2) (Kuan et al., 2013).

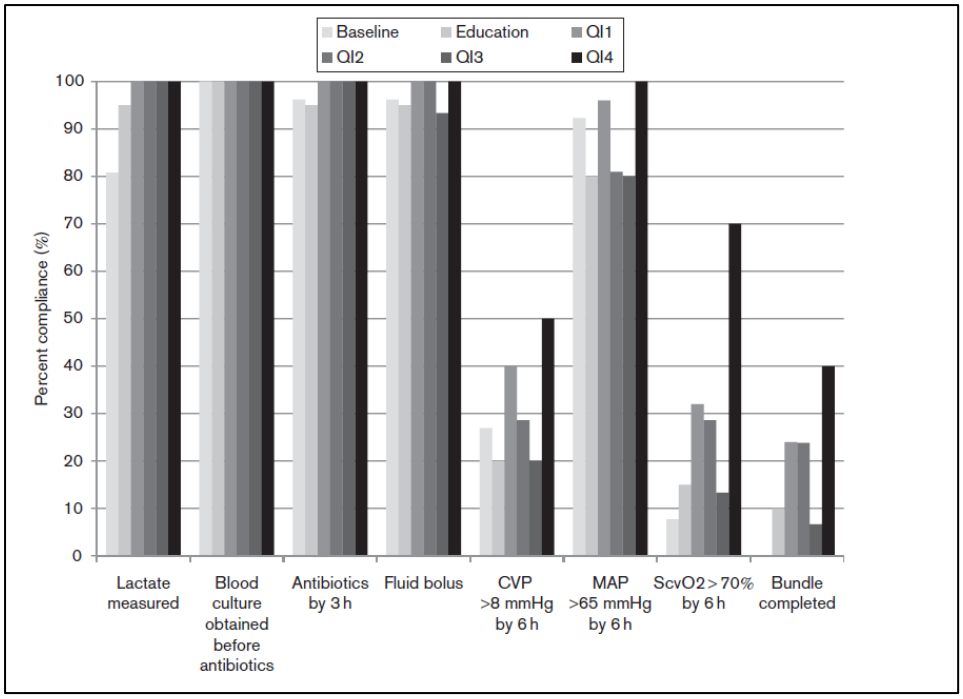


Figure 8.2 Published care bundle feasibility

Compliance with bundle targets during four quality improvement initiatives in a hospital in Singapore. Care bundle feasibility data published from (Kuan et al., 2013)

A small number of other studies have also tested sepsis bundles for feasibility. One study tested a sepsis bundle of EGDT for feasibility in the emergency departments of four centres, reporting that the goal directed algorithms were acceptable to 97% of physicians (McIntyre et al., 2008), objective measurements of feasibility were not reported.

Another observational before/after study across four emergency departments and intensive care units, did examine feasibility of use of their sepsis bundle , reporting that time to antibiotics was significantly reduced by the care bundle for adults with suspected septic shock (99 minutes v 142 minutes p=0.02), and those patients also received significantly greater intravenous fluid volumes (Jones et al., 2007b). Absolute mortality differed by 9%, with lowered mortality in the bundle group (Jones et al., 2007b).

Other studies of care bundles with feasibility as the primary objective have examined post cardiac arrest cooling protocols, improvement of ICU sedation protocols and acceptability of sepsis protocols (Shehabi et al., 2013; Gaieski et al., 2009; Casserly et al., 2011). However,

only one study reported the proportion of targets achieved by the care bundle compared to previous care (Gaieski et al., 2009).

Only one of the feasibility studies available give composite proportions of targets achieved and overall assessments of care bundle delivery achievability (Kuan et al., 2013). Given the paucity of data on care bundles and feasibility, the BAM study shows that targeted care is feasible in Malawi, and provides important data to inform other centres in SSA considering designing EGDT protocols for use in resource limited settings, and has shown comparable compliance data to other more well-resourced settings.

8.5.2 The impact of EGDT on outcome for ABM in Malawi

The main results of the study were disappointing with higher case fatality rates in the patients receiving the care bundle compared to standard care at day 10 (72% compared to 46% p=0.04), although this difference was not statistically significant by day 40 (63% compared to 51% p=0.19). These results are in contrast to all the studies published on EGDT and sepsis, with the exception of the PROCESS trial where outcome was equal amongst the three groups (Yealy et al., 2014). However, the estimates of effect from these BAM data are not as precise as would be seen in a trial with parallel design and random selection, due to the observational nature of the study. The explanation for the apparent lack of improvement in outcomes with the bundle is not clear. The possibility that the care bundle may have been associated with harm cannot be excluded

Patients in both phases had an equal risk of a poor outcome, the median severity scores (MAMS) on admission were equal across the study phases. Post admission care on the wards or in the outpatient setting did not differ across the study phases.

Patients presenting in phase 1 received a good standard of care according to the resources available, in line with guidelines from the UK and Holland (Heyderman, 2005; Fitch and van de Beek, 2007). Rapid assessment was carried out including LP, appropriate antibiotics were given in an acceptable time period and seizures were controlled. These patients received conservative parenteral fluids and oxygen if this was required and available. Patients in phase 2 who were given EGDT received the same overall baseline of care; in addition these patients received antibiotics more rapidly, greater volumes of parenteral fluid, more diazepam and more blood. It is impossible to derive a single harmful element from the bundle, it is possible that one or more of these elements led to increased harm from care bundle delivery associated with the higher mortality observed. Overtreatment with parenteral fluids in ABM could lead to increased intracranial pressure and cerebral oedema (Pfister et al., 1990), although clinical data are lacking. Excess sedation for patient management beyond seizure control also could have a harmful effect (Ume and Gelb, 1988), although an independent relationship between either fluid administration or sedative prescription and poor outcome was not seen.

The aggressive resuscitation protocol in BAM was based on the guidelines for treatment of severe sepsis (Dellinger et al., 2008b), with the underlying assumption that patients with ABM would have either meningitis and sepsis, or similar inflammatory responses to meningitis that would respond to the sepsis approach (Heyderman, 2005). One component of the definition of shock in the SSG that was used in BAM was a pulse rate >100 beats/minute (Dellinger et al., 2008b). Patients were therefore defined as shocked if they had a tachycardia, even if they did not have hypotension or hyper-lactataemia, and were resuscitated with the shock protocol. The physiology documented in the observation period suggests very few patients had multiple features of shock (as defined by the SSG) and instead showed physiological responses to rising intracranial pressure rather than systemic inflammatory responses to sepsis (SIRS). It is possible that fluid resuscitation in adults with a centrally mediated tachycardia and no other features of shock was harmful, although the

numbers are too few to look for trends. Fluid resuscitation is harmful for children in SSA presenting with or without clinical evidence of shock, the causes of this harm have yet to be fully established, but may be due to lack of available facilities to manage pulmonary oedema appropriately (Maitland et al., 2011a).

Patients with ABM in Malawi appear to have inflammation predominately confined to the CNS compartment, and therefore the assumption that treatment based on systemic sepsis outcomes for ABM was not necessarily correct.

The relatively low CFR observed in Phase 1 compared to Phase 2 also raises the question did patients with ABM recruited to BAM need the full EGDT protocol tested, or did they just need good clinical care with rapid diagnostics and appropriate support? Further studies are required to answer this question definitively, but it does appear that good quality rapid clinical care is appropriate for adults with suspected ABM.

Definitive conclusions as to the harm or clinical benefit of elements of the BAM EGDT protocol cannot be fully drawn from the data.

8.5.3 What is the right approach for the acute resuscitation of ABM in adults in sub-Saharan Africa?

The BAM study does not provide definitive data to support guidance for the clinical care of adults with ABM. However, results from the study can be extrapolated to support the development of a further strategy for acute management of these patients.

The principle of goal-directed therapy was feasible, but the current bundle, with eight individual potential targets is in its current format was too complex for routine use outside of a research study. Revising the concept of goal-directed therapy, to the design of a simple bundle with a focus on the best possible basic clinical management as observed in P1 is likely to be safe, effective and reproducible beyond a research setting.

Based on the data from BAM P1, a care bundle that would optimise the resources available to give the best clinical management for ABM would consist of rapid recognition of ABM, parenteral antibiotics within three hours of admission; and management of seizures, oxygenation and anaemia if required using available resources. No conclusions can currently be drawn from BAM about the most appropriate fluid strategy for bacterial meningitis; a study randomising conservative compared to aggressive resuscitation strategies is required.

Based predominately on these, and other observational studies, the surviving sepsis guidelines recommend a six hour period for intensive resuscitation to achieve the clinical targets set, which can be in the emergency department or after transfer to the intensive care unit (Dellinger et al., 2004; Dellinger et al., 2008b; Dellinger et al., 2013). The randomised controlled trials of EGDT for sepsis in well-resourced settings tested the relevant resuscitation protocol over a six hour window, compared to high quality standard care over the same time period, after which time all patients were transferred to high dependency or critical care wards for ongoing management (Rivers et al., 2001; Yealy et al., 2014; 2010). In two studies, EGDT was superior to routine care (Rivers et al., 2001; 2010), however the PROCESS study showed that EGDT was equivalent to good quality routine in-hospital care (Yealy et al., 2014).

The full care bundle in the SSG was not appropriate for adults in a resource-limited setting, where many resources required are not available, such as central venous monitoring. In addition, the SSG bundle is designed primarily for sepsis, not meningitis. Therefore, the BAM targets set were appropriate for meningitis in a resource-limited setting, and were substantially simpler than those outlined in the SSG. Resuscitation was given over the same six hour observation period as per the SSG, testing if targets set were achieved by the end of the time; all observation was done in the AETC.

Due to limited facilities available, no patients were transferred to the intensive care facilities available, most received basic ward based care, with oxygen if required. Beyond attempts to obtain blood transfusion and on-going fluid resuscitation in BAM, the only further intervention that patients received during the six hour observation period was on-going seizure management in a small number of patients. The presence of persistent seizures was associated with poor outcome, monitoring and treatment over time is likely to appropriate for this small number of critically unwell patients. However, if the fluid resuscitation protocols were removed from the care bundle, very few patients would require further treatment during the six hour observation window, with considerable resource savings.

None of the large sepsis bundle trials have tested the most appropriate resuscitation time period, six hours appears to have been adopted with little evidence to support this time over other time-frames. The BAM study does not provide evidence that a six hour monitoring and target-driven resuscitation period is required for all patients with suspected ABM in QECH. To conserve resources, particularly nursing time and effort, this may not be a useful part of a future care bundle for a resource limited setting. Important parts of the clinical care bundle with the strongest evidence base, such as early recognition and delivery of appropriate antibiotics need to be tested, but are likely to continue to be important in the management of ABM in this setting. Other elements such as the fluid resuscitation protocol and full six hour monitoring period may not be required. Any studies testing appropriate in-hospital treatment ideally should be combined with a strategy of addressing quality issues in the hospital community relating to disease recognition and management of delay, including the importance of prompt recognition and treatment to give the best outcomes.

8.5.4 Future directions for EGDT for ABM in sub-Saharan Africa

The BAM study was observational in design, with sequential phases for observation and intervention. The estimates of effect size are subject to observational biases and

confounding by time, and as such, cannot provide a definitive answer about the efficacy of EGDT for ABM in Malawi. A randomised controlled trial, with parallel recruitment would produce data that was not subject to the limitations of observational studies which include observer and allocation biases, and would provide a more accurate comparison between routine hospital care and modified EGDT.

Ideal studies testing EGDT are cluster randomised, with randomisation done by hospital and by patient (Campbell et al., 2007). This is for two reasons; firstly to minimise ethical issues in delivering both routine hospital care and EGDT within an individual centre, where a study team who are aware of the clinical evidence behind the care bundle may feel that delivering routine care is un-ethical, and are therefore at risk of biasing the study by improving routine care (Huang et al., 2013). Secondly, this design minimises contamination between study teams in a single centre. Blinding in a traditional RCT is impossible in studies of EGDT, and therefore if EGDT is being tested in an emergency department, with all the educational resources required, ensuring that the team delivering routine care were not exposed to, and therefore delivered clinical care biased by the presence of the study is difficult (Council, 2008; Campbell et al., 2000). Cluster randomised design would provide the most reliable and robust results by separating the randomisation from a patient to a hospital level.

It is likely that the clinical care delivered by the AETC team in P1 and by the BAM study team in P2 is likely to be substantially better than the quality of clinical care in most central and district hospitals in Malawi. Expanding the study of a new meningitis care bundle in a cluster randomised design beyond the AETC at QECH to multiple centres would determine the baseline medical management and CFR for ABM in other hospitals in the region, and give a true result as to efficacy of EGDT for ABM. Based on the data from BAM, the care bundle would need to be revised before the design of such a trial. Such a care bundle would include rapid recognition of suspected ABM and prompt administration of antibiotics, seizure

control, correction of hypoxia and hypoglycaemia, with possible randomisation on an individual level to conservative or resuscitation fluid strategies.

8.5.5 Ward based care

Once a preliminary diagnosis of ABM has been made in hospital, the patient is transferred to either the medical ward, or medical intensive care unit/high dependency unit, dependent on the on-going clinical requirements of the patient (Heyderman et al., 2003; Fitch and van de Beek, 2007; Tunkel et al., 2004). In Malawi, the only options are either general medical ward care, or care in the high dependency unit for those with persistent seizures or an on-going oxygen requirement.

Strategies to improve outcome from ABM which have been tested in both adults and children in SSA are dexamethasone, glycerol, high dose paracetamol, short course compared to traditional long course antibiotics and bolus compared to infusion doses of ceftriaxone (Ajdukiewicz et al., 2011; Scarborough et al., 2007; Molyneux et al., 2011; Molyneux et al., 2014; Molyneux et al., 2002; Pelkonen et al., 2011). None of these interventions resulted in improvements in outcome, although short course antibiotics were equivalent to long course in children. Interventions tested in either ward or intensive care settings in other populations include, management of raised intracranial pressure (ICP), hypothermia, and medical adjuncts including dexamethasone and glycerol (Glimaker et al., 2014; Mourvillier et al., 2013; Kilpi et al., 1995; Peltola et al., 2007; de Gans and van de Beek, 2002; van de Beek et al., 2010). These interventions have either met with limited success (dexamethasone, glycerol, ICP management) in improving outcome, or substantial harm (hypothermia) (Mourvillier et al., 2013). Full discussion of the evidence behind these interventions can be found in Chapter 2, Section 2.8. No ward based treatment for ABM has yet to substantially improve outcome in adults with bacterial meningitis to the effect sizes seen in sepsis with EGDT in well-resource settings.

The Malawi Adult Meningitis Severity score (MAMS) was shown in Chapter 5 to predict risk of outcome from ABM with good accuracy. This score requires further, prospective validation, but may have several roles in the ward based management of patients with ABM. However without further data exploring the underlying causes of high mortality in African adults with meningitis to inform future trials of acute or ward based therapeutic treatments, these interventions may not be the most appropriate next step for the treatment of ABM in SSA.

8.5.6 Post hospital care

A significant number of deaths (5-10% of the total mortality) occur in patients with ABM post discharge from hospital in SSA. In BAM, a significant proportion of patients were discharged at day 10 with substantial morbidity, 8% in P1 and 20% in P2, although missing data in P1 do not give an accurate morbidity data by day 40, in P2 66% of those discharged with morbidity had died by this time. Very little data are available from BAM to explore the possible causes of this. These patients are poorly studied as community follow up is very difficult in an environment where transport is expensive, no formal road map or house numbering systems exist and patients may not have access to a reliable telephone number.

Patients that die in the community do so without notification, access to cause of death data are difficult, as most deaths are not certified and no autopsies are carried out. In the BAM study we were able to obtain data on patient outcomes after taking a map of the physical address on discharge, and driving to that address and the surrounding areas asking for information, and we were able to establish for most patients for whom we had an address the eventual six week outcome. We had no access to any data that would support a cause of death, beyond those patients who were readmitted and whose notes we could access. Studies into post-discharge mortality are urgently required, but obtaining high quality data is likely to be complex.

In Europe, cerebral thrombosis has been well described as a late complication after pneumococcal meningitis, no studies have examined whether this occurs in SSA (Klein et al., 2010; Lucas et al., 2013; Schut et al., 2009a). In the analysis presented in Chapter 4, seizures post discharge were strongly correlated with risk of death by six weeks, this may be due to persistent structural lesions within the CNS due to ABM, as seen on an older imaging study in the USA, or new-onset stroke. No imaging studies are available to explore this hypothesis further (Hasbun et al., 2001; Klein et al., 2011; Schut et al., 2009a). There were too few patients in BAM who died post-discharge to be able to analyse associations with risk factors for late death, however these patients were more likely to have morbidity (mRS ≥2) on discharge to those who had healthy survival at six weeks.

Although an increased risk of death from ABM has been observed in the first year on ART in SSA (Walker et al., 2012), the time period between discharge from hospital and the six week follow up appointment in all meningitis studies in Malawi is likely to be too short for ART to be initiated and an IRIS phenomenon to manifest in death (Chang et al., 2014).

An interesting observation from all trials of ABM in Malawi, is the relatively low frequency of deafness in survivors at six weeks which was <10% in all of the studies across the studies (Scarborough et al., 2007; Ajdukiewicz et al., 2011), although this was higher in BAM, approximately 20-25% of survivors reported subjective hearing loss, the numbers were small and, audiometry data were not sufficient to analyse for trends. Deafness rates of up to 14-21% occurred in patients with pneumococcal meningitis in the adult dexamethasone trial (de Gans and van de Beek, 2002). These discrepancies could be due to ascertainment biases, as formal audiometry was commonly not available historically; audiometry was variably available in BAM.

Overall the causes of late mortality from ABM in SSA are unclear. Future studies should focus more on examining this potentially treatable area, to complement pre-hospital and in-hospital strategies for improving poor outcome from ABM.

8.6 Future directions for bacterial meningitis studies in sub-Saharan Africa beyond EGDT

8.6.1 Utility of severity scoring systems in resource limited settings for ABM

The Malawi Adult Meningitis Score (MAMS) was shown to have good power in generating a risk score for patients with ABM. This score needs further prospective evaluation in the context of a clinical trial, but could be used to help answer the following research questions:

a) What is the optimal length of antibiotic therapy?

A paediatric study in Malawi determined that 5 days of ceftriaxone was non-inferior to 10 days for bacterial meningitis, it reduced length of stay and was cost-efficient for the hospital, the patient and guardians (Molyneux et al., 2011). Currently the Malawian guidelines for adult meningitis advise a ten day course of antibiotics. In adult patients at high risk of in-hospital mortality and complications, such as observed in BAM, SAM and GLAM, it is unlikely 5 day courses would be safe for all patients. However for patients stratified by MAMS as low risk, for example those with meningococcal meningitis, shorter course antibiotics may be a suitable compared to the 10 day standard (Heckenberg et al., 2008; Sudarsanam et al., 2013; Girgis et al., 1988).

b) Are adjunctive treatments effective in different risk groups?

Dexamethasone had differing effectiveness in the three big trials done in adults in the Netherlands, Vietnam and Malawi, being predominantly effective in the Netherlands on pneumococcal meningitis (de Gans and van de Beek, 2002),effective in Vietnam on

meningitis caused by *Streptococcus suis* (Nguyen et al., 2007) and having no effect in Malawi (Scarborough et al., 2007). In a study of TB meningitis in Vietnam, dexamethasone was most effective in the groups with the worst predicted outcome (Thwaites et al., 2004). It is possible that by using MAMS to stratify risk of outcome from ABM, sub-group analyses of patients divided by risk group could test if new adjunctive interventions were effective in some, but not all patients, and determine if high or low risk patients would be most likely to benefit from the intervention. For example, although dexamethasone was ineffective in adults with ABM in Malawi, it may have effects in sub-groups of patients with high or low risk of poor outcome, MAMS may be useful in permitting such evaluations.

8.6.2 Prospects for future therapies

No therapeutic adjuncts beyond dexamethasone and glycerol have been tested in clinical trials of ABM in SSA (van de Beek et al., 2012). Other interventions such as brain cooling have been shown to be ineffective and are not appropriate for use in SSA (Mourvillier et al., 2013), as is reduction of intracranial pressure (Glimaker et al., 2014). High dose paracetamol is ineffective in reducing mortality in children (Molyneux et al., 2014), and is unlikely to be effective in adults. Novel treatments including inhibition of specific cytokines using monoclonal antibodies are unlikely to be effective or affordable in Malawi (Park et al., 1999; Saez-Llorens et al., 1991). Enhancement of macrophage opsonisation of pneumococci using P4 presents a potential novel adjunct, however this compound is not yet in phase one clinical trials, and may not penetrate the CSF (Bangert et al., 2012), large clinical trials testing this compound in adults are many years away.

For adjuncts to be effective in reducing mortality in SSA, a much greater understanding of the differences in pathophysiology between bacterial meningitis in sub-Saharan Africa and more well-resourced settings is required. Whole blood, CSF, serum and nasal swabs and pneumococcal isolates were collected during the course of the BAM study, and these

samples will be used to initially to describe and explore host RNA expression, the pneumococcal proteome in the CSF and the common serotypes carried in pneumococcal meningitis. In addition, optimal clinical care for patients with ABM in resource limited settings must be evaluated formally, either using EGDT or consensus guidelines, as the effects of new adjuncts are only likely to be observed when optimal antibiotic timing and cerebral perfusion and fluid management strategies have been determined by a trial of modified EGDT. Data generated from these studies will identify further areas of study and potentially new areas for treatment of bacterial meningitis.

8.6.3 Prevention of ABM

Data presented in Chapter 4 Section 4.3.1 showed that Hib meningitis has decreased dramatically following the introduction of vaccination against this pathogen in 2002 (Wall et al., 2014a). Early data suggests that paediatric invasive pneumococcal disease may be decreasing following improvements in neonatal and maternal mortality rates, prevention of mother to child transmission of HIV, and introduction of PCV-13 to the routine infant vaccination schedule in 2011 (Jahn et al., 2010a; Wall et al., 2014a; Bar-Zeev N, 2014). Pneumococcal vaccination prevents recurrent IPD in HIV co-infected adults in Malawi (French et al., 2010), but vaccination of this group as a meningitis preventative strategy is unlikely to be cost-effective or realistic in Malawi, (Egere et al., 2012; Ota et al., 2012; Roca et al., 2012; Cohen et al., 2010a; French et al., 2010). Clinical trials in this population are required. No trials of primary pneumococcal vaccination with PCV-7 or PCV-13 in HIV co-infected adults in Africa have been reported (Nunes and Madhi, 2012), the older 23-valent vaccine in HIV co-infected adults in Uganda did not prevent primary IPD in this population (French et al., 2000; Watera et al., 2004).

Substantial protection through herd immunity from childhood vaccination may well be the best preventative strategy in current practice. Indirect effects of PCV-13 vaccination in

children may lead to reduction in the burden of disease in both HIV negative and co-infected individuals, as has been seen in other settings; trends towards lower rates of IPD are being observed in children in Malawi (Burgos et al., 2012b; Muhammad et al., 2013; Hsu et al., 2009b; Bar-Zeev N, 2014). However, the degree to which childhood vaccination may reduce IPD incidence in adults is not yet known, and may be limited. HIV co-infected adults in Africa have high rates of pneumococcal carriage compared to adults in other settings, and substantially altered humoral responses to carriage (Glennie et al., 2013; Glennie et al., 2011; Ferreira et al., 2013; Palmu et al., 2012), antibody responses to both vaccination and carriage are limited compared to healthy adults (Nielsen et al., 1998; Nunes and Madhi, 2012; Opravil et al., 1991; Glennie et al., 2013). The widespread use of PCV-13 may also result in serotype-replacement disease as has been seen in other settings after the introduction of PCV-7, where the overall incidence of IPD has not decreased in many places, despite substantial decreases in vaccine-type disease (Byington et al., 2010; Burgos et al., 2012a; Elston et al., 2012).

Based on current evidence, the best preventative strategy for pneumococcal meningitis in Malawi is to ensure adults and children receive appropriate vaccinations, ART and good HIV care. Development of improved vaccines that are immunogenic in HIV co-infected individuals and that do not cause serotype replacement disease is an urgent public health priority.

8.7 Concluding remarks

Over the course of the pathway from symptoms to management for patients with ABM in SSA, it is clear that there are several places where interventions may improve outcome (Figure 8.3). The work presented in this thesis started with the remit to try to understand why mortality was so high in patients with ABM in SSA, and to test if using a sepsis-orientated

approach to resuscitation, adjusted for meningitis and resource limitation was feasible in this setting.

The thesis has helped to better understand the epidemiology of ABM in adults and children, the clinical predictors of poor outcome in adults with actual prediction of outcome using MAMS; detailed physiological data has shown that compartmentalised CNS responses are the cause of poor outcome and not septic shock. Goal-directed therapy is feasible, but it is unclear if the care bundle tested in BAM in its current format is appropriate or not for bacterial meningitis.

Several important points on the patient journey present opportunities for further intervention to improve mortality (Figure 8.3), in pre-hospital recognition and early treatment, and in optimising in-hospital care and post-hospital outcome. The following research questions have arisen from this work, which should be the direction that research into ABM in SSA should take.

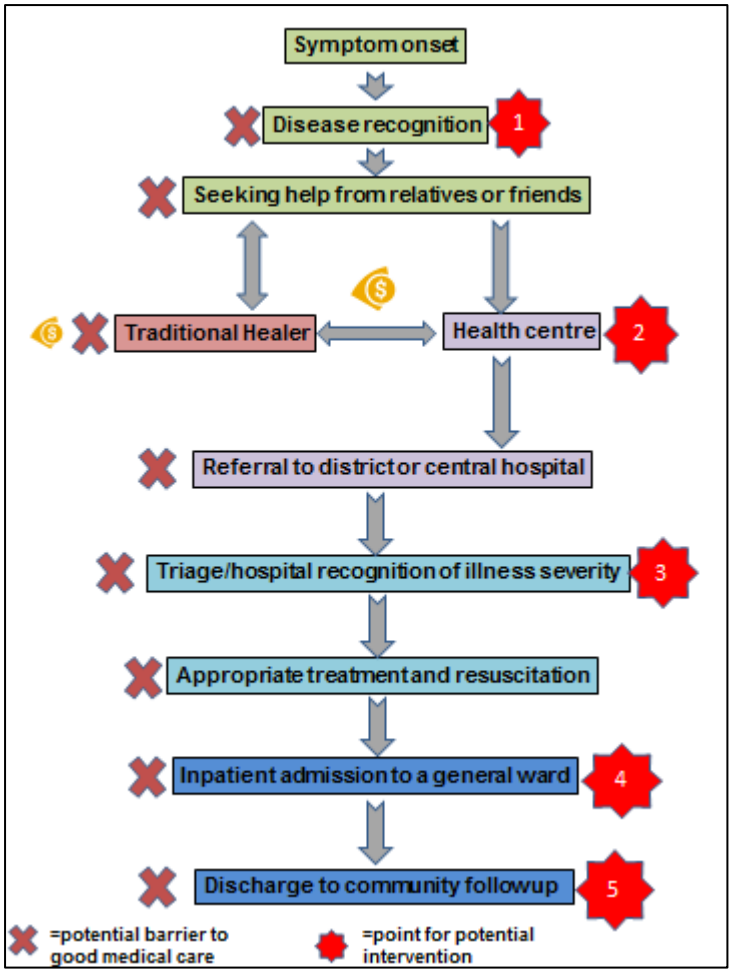


Figure 8.3 Points for intervention on the patient journey with ABM in Malawi

The patient journey with ABM, with points highlighted for intervention. 1 = community awareness campaigns. 2 = randomised controlled trial of early antibiotics at health centre level. 3 = formal randomised trial of EGDT 4 = new adjunctive ward based therapies. 5 = causes of post discharge mortality, to identify avenues for improvements in outcome

i) What are the relative contributions of host and pathogen to early and late mortality from ABM in Malawian adults?

A greater in-depth understanding of the differences in those patients that die and those that survive is required to develop future treatments and strategies that will increase the proportion of survivors from ABM. Further understanding of the differences between ABM in

different populations is also required, the work on severity scoring systems shows that different hosts in different countries have different risk factors for death from meningitis.

Studies of the host need to include: blood and CSF gene expression, deeper exploration of the host proteome, CSF neutrophil phenotype and function, CNS imaging studies and genetic sequencing data to determine if there is a particular host genotype or phenotype that is associated with mortality. Equal attention needs to be given to exploring the pathogen, including the relative contribution of different serotypes to outcome, pneumococcal gene sequencing and expression, and the pneumococcal secretome (Tjalsma et al., 2000), including the proteome produced in active infection. Some of these studies will be done on samples collected during BAM, but future prospective studies designed to examine these questions in detail are required.

ii) Would community strategies including public information and pre-hospital antibiotics improve outcome from ABM in Malawi in both adults and children?

Very little data are currently available to inform community-based strategies for early recognition and treatment of ABM in SSA. Early parenteral community antibiotics are likely to be helpful, and unlikely to be harmful, but are the effects of community based treatments are likely to be enhanced if recognition of meningitis and health-seeking behaviours are also addressed to reduce time from symptom onset to attendance in a health-centre or hospital. A large surveillance study of many health centres in a large town or district would be required to test if a public information campaign resulted in more frequent attendance or diagnosis and referral of ABM in the community. This would then need to be followed by a trial testing pre-referral parenteral antibiotics given to adults with suspected ABM.

iii) What is the optimal resuscitation strategy for adults with ABM in SSA?

The data presented show that EGDT is feasible in Malawi, with similar care bundle compliance to sepsis data from Singapore. However, the differences in mortality across the

study phases, although limited in analysis by the observational nature of the data, are intriguing and require further understanding before future trials of EGDT can be planned. Measurable markers of severity of disease caused by ABM have been described in the data presented, and are reproducible across studies. The Malawi Adult Meningitis Score shows good predictive power with a promise of future utility in clinical research and possibly as a bedside tool.

Early Goal Directed Therapy as a concept is feasible in SSA, a cluster-randomised trial of a care bundle, modified from the original BAM bundle (Chapter 8 Section 8.5.3) would give definitive information to design optimal clinical care guidelines for the management of ABM in SSA, which are urgently required to improve in-hospital care and outcome.

iv) What are the causes of late mortality and morbidity from ABM?

It is clear that poor outcomes are partly due to late in-hospital mortality and out of hospital mortality. A study designed to examine the causes of this late mortality, using imaging studies and close community follow-up would help to determine if structural CNS abnormalities, such as empyema and stroke, or if other factors such as TB or CCM co-infection, early initiation of ART, or seizures are associated with late poor outcome.

Substantial advances in the understanding of poor outcome from ABM in adults in SSA have been made since Scarborough and colleagues published the dexamethasone trial (Scarborough et al., 2007). Key points in our improved understanding include the determination of clinical phenotype of patients at high risk of poor outcome, including the role of persistence of pneumolysin in the CSF, poor white cell responses in the CSF to very high bacterial loads, the role of HIV as a risk factor for acquiring ABM, but not as a causative factor in mortality, the causes of culture negative meningitis and the role of PCR, and the possible role of pneumococcal serotypes and viral co-infections in poor outcome (Wall et al., 2013c; Wall et al., 2012; Wall et al., 2014b; Wall et al., 2014a; Kelly et al., 2012; Ajdukiewicz

et al., 2011). The studies presented in this thesis shows that outcome from meningitis can be predicted with a simple bedside tool, and the strategy of EGDT is feasible in adult medicine in Malawi, and may be effective for reduction in meningitis mortality; a larger trial is required to provide definitive data on the acute management of ABM in the region.

The definitive aim of any meningitis study must be towards curative strategies that will lead to lower case fatality rates. Appropriate studies that should be done subsequently to BAM should test enhanced community awareness information campaigns, early community based treatment and optimised in-hospital care.

References

Abbott, N. J. 2002. Astrocyte-endothelial interactions and blood-brain barrier permeability. *J Anat,* 200(6)**,** pp 629-38.

Abulhasan, Y. B., Al-Jehani, H., Valiquette, M. A., McManus, A., Dolan-Cake, M., Ayoub, O., Angle, M. & Teitelbaum, J. 2013. Lumbar drainage for the treatment of severe bacterial meningitis. *Neurocrit Care,* 19(2)**,** pp 199-205.

Adam, H. J., Richardson, S. E., Jamieson, F. B., Rawte, P., Low, D. E. & Fisman, D. N. 2010. Changing epidemiology of invasive Haemophilus influenzae in Ontario, Canada: evidence for herd effects and strain replacement due to Hib vaccination. *Vaccine,* 28(24)**,** pp 4073-8.

Adams, S. T. & Leveson, S. H. 2012. Clinical prediction rules. *BMJ,* 344(d8312.

Adegbola, R. A., Secka, O., Lahai, G., Lloyd-Evans, N., Njie, A., Usen, S., Oluwalana, C., Obaro, S., Weber, M., Corrah, T., Mulholland, K., McAdam, K., Greenwood, B. & Milligan, P. J. 2005. Elimination of Haemophilus influenzae type b (Hib) disease from The Gambia after the introduction of routine immunisation with a Hib conjugate vaccine: a prospective study. *Lancet,* 366(9480)**,** pp 144-50.

Ajdukiewicz, K. M., Cartwright, K. E., Scarborough, M., Mwambene, J. B., Goodson, P., Molyneux, M. E., Zijlstra, E. E., French, N., Whitty, C. J. & Lalloo, D. G. 2011.

Glycerol adjuvant therapy in adults with bacterial meningitis in a high HIV seroprevalence setting in Malawi: a double-blind, randomised controlled trial. *Lancet Infect Dis,* 11(4)**,** pp 293-300.

Akpede, G. O., Akuhwa, R. T., Ogiji, E. O. & Ambe, J. P. 1999. Risk factors for an adverse outcome in bacterial meningitis in the tropics: a reappraisal with focus on the significance and risk of seizures. *Ann Trop Paediatr,* 19(2)**,** pp 151-9.

Akweongo, P., Dalaba, M. A., Hayden, M. H., Awine, T., Nyaaba, G. N., Anaseba, D., Hodgson, A., Forgor, A. A. & Pandya, R. 2013. The economic burden of meningitis to households in Kassena-Nankana district of Northern Ghana. *PLoS One,* 8(11)**,** pp e79880.

Almirante, B., Cortes, E., Pigrau, C., Gasser, I., del Valle, O., Campos, L. & Pahissa, A. 1995. [Treatment and outcome of pneumococcal meningitis in adults. Study of a recent series of 70 episodes].[see comment]. *Medicina Clinica,* 105(18)**,** pp 681-6.

Almirante, B., Saballs, M., Ribera, E., Pigrau, C., Gavalda, J., Gasser, I. & Pahissa, A. 1998. Favorable prognosis of purulent meningitis in patients infected with human immunodeficiency virus. *Clin Infect Dis,* 27(1)**,** pp 176-80.

Ammendolia, M. G., Superti, F., Bertuccini, L., Seganti, L., Cipriani, D. & Longhi, C. 2009. Necrotic cell death in human amniotic cells infected by Listeria monocytogenes. *International Journal of Immunopathology & Pharmacology,* 22(1)**,** pp 153-62.

Ances, B. M. & Ellis, R. J. 2007. Dementia and neurocognitive disorders due to HIV-1 infection. *Semin Neurol,* 27(1)**,** pp 86-92.

Angus, D. C. & van der Poll, T. 2013. Severe sepsis and septic shock. *N Engl J Med,* 369(21)**,** pp 2063.

Anthony, L., Meehan, A., Amos, B., Mtove, G., Mjema, J., Malahiyo, R., Yin, J. K., Oftadeh, S., Gilbert, G. L., Shingadia, D., Reyburn, H., Deen, J., Richmond, P. C. & Booy, R. 2012. Nasopharyngeal carriage of Streptococcus pneumoniae: prevalence and risk factors in HIV-positive children in Tanzania. *Int J Infect Dis,* 16(10)**,** pp e753-7.

Arditi, M., Manogue, K. R., Caplan, M. & Yogev, R. 1990. Cerebrospinal fluid cachectin/tumor necrosis factor-alpha and platelet-activating factor concentrations and severity of bacterial meningitis in children. *J Infect Dis,* 162(1)**,** pp 139-47.

Arnold, R. C., Shapiro, N. I., Jones, A. E., Schorr, C., Pope, J., Casner, E., Parrillo, J. E. & Dellinger, R. P. 2008. Multi-Center Study of Early Lactate Clearance as a Determinant of Survival in Patients with Presumed Sepsis. *Shock*.

Aronin, S. I., Peduzzi, P. & Quagliarello, V. J. 1998. Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing. *Ann Intern Med,* 129(11)**,** pp 862-9.

Artenstein, A. W. & LaForce, F. M. 2012. Critical episodes in the understanding and control of epidemic meningococcal meningitis. *Vaccine,* 30(31)**,** pp 4701-7.

Arulkumaran, N., Corredor, C., Hamilton, M. A., Ball, J., Grounds, R. M., Rhodes, A. & Cecconi, M. 2014. Cardiac complications associated with goal-directed therapy in high-risk surgical patients: a meta-analysis. *Br J Anaesth,* 112(4)**,** pp 648-59.

Attia, J., Hatala, R., Cook, D. J. & Wong, J. G. 1999. The rational clinical examination. Does this adult patient have acute meningitis? *JAMA,* 282(2)**,** pp 175-81.

Aubouy, A. 2011. Promotion of malaria home-based treatment in Africa: The dangers of creating a second health system. *Int Health,* 3(4)**,** pp 219-20.

Auburtin, M., Wolff, M., Charpentier, J., Varon, E., Le Tulzo, Y., Girault, C., Mohammedi, I., Renard, B., Mourvillier, B., Bruneel, F., Ricard, J. D. & Timsit, J. F. 2006. Detrimental role of delayed antibiotic administration and penicillin-nonsusceptible strains in adult intensive care unit patients with pneumococcal meningitis: the PNEUMOREA prospective multicenter study. *Crit Care Med,* 34(11)**,** pp 2758-65.

Azevedo, L. C., Toscano, C. M. & Bierrenbach, A. L. 2013. Bacterial Meningitis in Brazil: Baseline Epidemiologic Assessment of the Decade Prior to the Introduction of Pneumococcal and Meningococcal Vaccines. *PLoS One,* 8(6)**,** pp e64524.

Bain, B., Seed, M. & Godsland, I. 1984. Normal values for peripheral blood white cell counts in women of four different ethnic origins. *J Clin Pathol,* 37(2)**,** pp 188-93.

Bamani, S., Toubali, E., Diarra, S., Goita, S., Berte, Z., Coulibaly, F., Sangare, H., Tuinsma, M., Zhang, Y., Dembele, B., Melvin, P. & MacArthur, C. 2013. Enhancing community knowledge and health behaviors to eliminate blinding trachoma in Mali using radio messaging as a strategy. *Health Educ Res,* 28(2)**,** pp 360-70.

Bangert, M., Bricio-Moreno, L., Gore, S., Rajam, G., Ades, E. W., Gordon, S. B. & Kadioglu, A. 2012. P4-mediated antibody therapy in an acute model of invasive pneumococcal disease. *J Infect Dis,* 205(9)**,** pp 1399-407.

Bangert, M., Wright, A. K., Rylance, J., Kelly, M. J., Wright, A. D., Carlone, G. M., Sampson, J. S., Rajam, G., Ades, E. W., Kadioglu, A. & Gordon, S. B. 2013. Immunoactivating peptide P4 augments alveolar macrophage phagocytosis in two diverse human populations. *Antimicrob Agents Chemother*.

Bar-Zeev N, E. D., Alaerts M. Katsulukuta C, Mwansambo C, Costello A, Verani JR, Whitney CG, Cunliffe NA, Heyderman RS, French N. 2014. IS REDUCTION IN PNEUMOCOCCAL MENINGITIS IN NON-VACCINATED AGE GROUPS IN MALAWI ATTRIBUTABLE TO THE INTRODUCTION OF 13-VALENT PNEUMOCOCCAL CONJUGATE VACCINE? *International Symposium on Pneumococci and Pneumococcal Disease.* Hyderabad, India.

Barichello, T., Santos, A. L., Silvestre, C., Generoso, J. S., Cipriano, A. L., Petronilho, F., Dal-Pizzol, F., Comim, C. M. & Quevedo, J. 2011. Dexamethasone treatment reverses cognitive impairment but increases brain oxidative stress in rats submitted to pneumococcal meningitis. *Oxid Med Cell Longev,* 2011(173035.

Barochia, A. V., Cui, X. & Eichacker, P. Q. 2013. The Surviving Sepsis Campaign's Revised Sepsis Bundles. *Curr Infect Dis Rep,* 15(5)**,** pp 385-93.

Barochia, A. V., Cui, X., Vitberg, D., Suffredini, A. F., O'Grady, N. P., Banks, S. M., Minneci, P., Kern, S. J., Danner, R. L., Natanson, C. & Eichacker, P. Q. 2010. Bundled care for septic shock: an analysis of clinical trials. *Crit Care Med,* 38(2)**,** pp 668-78.

Bauer, T. T., Ewig, S., Marre, R., Suttorp, N. & Welte, T. 2006. CRB-65 predicts death from community-acquired pneumonia. *J Intern Med,* 260(1)**,** pp 93-101.

Bedell, R. A., Anderson, S. T., van Lettow, M., Akesson, A., Corbett, E. L., Kumwenda, M., Chan, A. K., Heyderman, R. S., Zachariah, R., Harries, A. D. & Ramsay, A. R. 2012. High prevalence of tuberculosis and serious bloodstream infections in ambulatory individuals presenting for antiretroviral therapy in Malawi. *PLoS One,* 7(6)**,** pp e39347.

Bekondi, C., Bernede, C., Passone, N., Minssart, P., Kamalo, C., Mbolidi, D. & Germani, Y. 2006. Primary and opportunistic pathogens associated with meningitis in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. *Int J Infect Dis,* 10(5)**,** pp 387-95.

Benjamin, L. A., Kelly, M., Cohen, D., Neuhann, F., Galbraith, S., Mallewa, M., Hopkins, M., Hart, I. J., Guiver, M., Lalloo, D. G., Heyderman, R. S. & Solomon, T. 2013. Detection of herpes viruses in the cerebrospinal fluid of adults with suspected viral meningitis in Malawi. *Infection,* 41(1)**,** pp 27-31.

Berg, B., Gardsell, P. & Skansberg, P. 1982. Cerebrospinal fluid lactate in the diagnosis of meningitis. Diagnostic value compared to standard biochemical methods. *Scandinavian Journal of Infectious Diseases,* 14(2)**,** pp 111-5.

Bhatt, S. M., Lauretano, A., Cabellos, C., Halpin, C., Levine, R. A., Xu, W. Z., Nadol, J. B., Jr. & Tuomanen, E. 1993. Progression of hearing loss in experimental pneumococcal meningitis: correlation with cerebrospinal fluid cytochemistry. *Journal of Infectious Diseases,* 167(3)**,** pp 675-83.

Bicanic, T., Brouwer, A. E., Meintjes, G., Rebe, K., Limmathurotsakul, D., Chierakul, W., Teparrakkul, P., Loyse, A., White, N. J., Wood, R., Jaffar, S. & Harrison, T. 2009. Relationship of cerebrospinal fluid pressure, fungal burden and outcome in patients with cryptococcal meningitis undergoing serial lumbar punctures. *AIDS,* 23(6)**,** pp 701-6.

Bogaert, D., van Belkum, A., Sluijter, M., Luijendijk, A., de Groot, R., Rumke, H. C., Verbrugh, H. A. & Hermans, P. W. 2004. Colonisation by Streptococcus pneumoniae and Staphylococcus aureus in healthy children. *Lancet,* 363(9424)**,** pp 1871-2.

Bonita, R. & Beaglehole, R. 1988. Recovery of motor function after stroke. *Stroke,* 19(12)**,** pp 1497-500.

Borel, T., Rose, A. M., Guillerm, M., Sidikou, F., Gerstl, S., Djibo, A., Nathan, N., Chanteau, S. & Guerin, P. J. 2006. High sensitivity and specificity of the Pastorex latex agglutination test for Neisseria meningitidis serogroup A during a clinical trial in Niger. *Trans R Soc Trop Med Hyg,* 100(10)**,** pp 964-9.

Borish, L. C. & Steinke, J. W. 2003. 2. Cytokines and chemokines. *J Allergy Clin Immunol,* 111(2 Suppl)**,** pp S460-75.

Bottcher, T., Ren, H., Goiny, M., Gerber, J., Lykkesfeldt, J., Kuhnt, U., Lotz, M., Bunkowski, S., Werner, C., Schau, I., Spreer, A., Christen, S. & Nau, R. 2004. Clindamycin is neuroprotective in experimental Streptococcus pneumoniae meningitis compared with ceftriaxone. *Journal of Neurochemistry,* 91(6)**,** pp 1450-60.

Boutron, I., Moher, D., Altman, D. G., Schulz, K. F. & Ravaud, P. 2008. Extending the CONSORT statement to randomized trials of nonpharmacologic treatment: explanation and elaboration. *Ann Intern Med,* 148(4)**,** pp 295-309.

Branson, R. D., Gomaa, D. & Rodriquez, D., Jr. 2014. Management of the artificial airway. *Respir Care,* 59(6)**,** pp 974-90.

Brennan, C. A., Somerset, M., Granier, S. K., Fahey, T. P. & Heyderman, R. S. 2003. Management of diagnostic uncertainty in children with possible meningitis: a qualitative study. *Br J Gen Pract,* 53(493)**,** pp 626-31.

Brissac, T., Mikaty, G., Dumenil, G., Coureuil, M. & Nassif, X. 2012. The meningococcal minor pilin PilX is responsible for type IV pilus conformational changes associated with signaling to endothelial cells. *Infect Immun,* 80(9)**,** pp 3297-306.

Brook, I., Bricknell, K. S., Overturf, G. D. & Finegold, S. M. 1978. Measurement of lactic acid in cerebrospinal fluid of patients with infections of the central nervous system. *Journal of Infectious Diseases,* 137(4)**,** pp 384-90.

Brouwer, M. C., Heckenberg, S. G., de Gans, J., Spanjaard, L., Reitsma, J. B. & van de Beek, D. 2010a. Nationwide implementation of adjunctive dexamethasone therapy for pneumococcal meningitis. *Neurology,* 75(17)**,** pp 1533-9.

Brouwer, M. C., Jim, K. K., Benschop, K. S., Wolthers, K. C., van der Ende, A., de Jong, M. D. & van de Beek, D. 2013. No evidence of viral coinfection in cerebrospinal fluid from patients with community-acquired bacterial meningitis. *J Infect Dis,* 208(1)**,** pp 182-4.

Brouwer, M. C., Thwaites, G. E., Tunkel, A. R. & van de Beek, D. 2012. Dilemmas in the diagnosis of acute community-acquired bacterial meningitis. *Lancet,* 380(9854)**,** pp 1684-92.

Brouwer, M. C., Tunkel, A. R. & van de Beek, D. 2010b. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. *Clin Microbiol Rev,* 23(3)**,** pp 467-92.

Brouwer, M. C. & van de Beek, D. 2011. Glycerol in bacterial meningitis: one strike and out? *Lancet Infect Dis,* 11(4)**,** pp 257-8.

Brouwer, M. C., van de Beek, D., Heckenberg, S. G., Spanjaard, L. & de Gans, J. 2007. Hyponatraemia in adults with community-acquired bacterial meningitis. *QJM,* 100(1)**,** pp 37-40.

Bruyn, G. A., van der Meer, J. W., Hermans, J. & Knoppert, W. 1988. Pneumococcal bacteremia in adults over a 10-year period at University Hospital, Leiden. *Reviews of Infectious Diseases,* 10(2)**,** pp 446-50.

Burgos, J., Falco, V., Borrego, A., Sorde, R., Larrosa, M. N., Martinez, X., Planes, A. M., Sanchez, A., Palomar, M., Rello, J. & Pahissa, A. 2012a. Impact of the emergence of non-vaccine pneumococcal serotypes on the clinical presentation and outcome of adults with invasive pneumococcal pneumonia. *Clin Microbiol Infect*.

Burgos, J., Penaranda, M., Payeras, A., Villoslada, A., Curran, A., Garau, M., Riera, M., Crespo, M., Navarro, J., Van den Eynde, E., Planes, A. M., Ribera, E., Pahissa, A. & Falco, V. 2012b. Invasive pneumococcal disease in HIV-infected adults: clinical changes after the introduction of the pneumococcal conjugate vaccine in children. *J Acquir Immune Defic Syndr,* 59(1)**,** pp 31-8.

Bustamante, A., Giralt, D., Garcia-Bonilla, L., Campos, M., Rosell, A. & Montaner, J. 2012. Citicoline in pre-clinical animal models of stroke: a meta-analysis shows the optimal

neuroprotective profile and the missing steps for jumping into a stroke clinical trial. *J Neurochem,* 123(2)**,** pp 217-25.

Byington, C. L., Hulten, K. G., Ampofo, K., Sheng, X., Pavia, A. T., Blaschke, A. J., Pettigrew, M., Korgenski, K., Daly, J. & Mason, E. O. 2010. Molecular epidemiology of pediatric pneumococcal empyema from 2001 to 2007 in Utah. *J Clin Microbiol,* 48(2)**,** pp 520-5.

Campbell, M., Fitzpatrick, R., Haines, A., Kinmonth, A. L., Sandercock, P., Spiegelhalter, D. & Tyrer, P. 2000. Framework for design and evaluation of complex interventions to improve health. *BMJ,* 321(7262)**,** pp 694-6.

Campbell, N. C., Murray, E., Darbyshire, J., Emery, J., Farmer, A., Griffiths, F., Guthrie, B., Lester, H., Wilson, P. & Kinmonth, A. L. 2007. Designing and evaluating complex interventions to improve health care. *BMJ,* 334(7591)**,** pp 455-9.

Carrol, E. D., Guiver, M., Nkhoma, S., Mankhambo, L. A., Marsh, J., Balmer, P., Banda, D. L., Jeffers, G., White, S. A., Molyneux, E. M., Molyneux, M. E., Smyth, R. L. & Hart, C. A. 2007a. High pneumococcal DNA loads are associated with mortality in Malawian children with invasive pneumococcal disease. *Pediatr Infect Dis J,* 26(5)**,** pp 416-22.

Carrol, E. D., Mankhambo, L. A., Balmer, P., Nkhoma, S., Banda, D. L., Guiver, M., Jeffers, G., Makwana, N., Molyneux, E. M., Molyneux, M. E., Smyth, R. L. & Hart, C. A. 2007b. Chemokine responses are increased in HIV-infected Malawian children with invasive pneumococcal disease. *J Acquir Immune Defic Syndr,* 44(4)**,** pp 443-50.

Carrol, E. D., Thomson, A. P., Shears, P., Gray, S. J., Kaczmarski, E. B. & Hart, C. A. 2000. Performance characteristics of the polymerase chain reaction assay to confirm clinical meningococcal disease. *Arch Dis Child,* 83(3)**,** pp 271-3.

Casserly, B., Baram, M., Walsh, P., Sucov, A., Ward, N. S. & Levy, M. M. 2011. Implementing a collaborative protocol in a sepsis intervention program: lessons learned. *Lung,* 189(1)**,** pp 11-9.

Castellanos-Ortega, A. & Delgado-Rodriguez, M. 2000. Comparison of the performance of two general and three specific scoring systems for meningococcal septic shock in children. *Crit Care Med,* 28(8)**,** pp 2967-73.

Castellanos-Ortega, A., Delgado-Rodriguez, M., Llorca, J., Sanchez Buron, P., Mencia Bartolome, S., Soult Rubio, A., Milano Manso, G., Dominguez Sampedro, P., Blanco Montero, R., Rodriguez Nunez, A., Zambrano Perez, E., Rey Galan, C., Lopez Negueruela, N. & Reig Saenz, R. 2002. A new prognostic scoring system for meningococcal septic shock in children. Comparison with three other scoring systems. *Intensive Care Med,* 28(3)**,** pp 341-51.

Cecconi, M., Corredor, C., Arulkumaran, N., Abuella, G., Ball, J., Grounds, R. M., Hamilton, M. & Rhodes, A. 2013. Clinical review: Goal-directed therapy-what is the evidence in surgical patients? The effect on different risk groups. *Crit Care,* 17(2)**,** pp 209.

Chamberlain, D. J., Willis, E. M. & Bersten, A. B. 2011. The severe sepsis bundles as processes of care: a meta-analysis. *Aust Crit Care,* 24(4)**,** pp 229-43.

Chandran, A., Watt, J. P. & Santosham, M. 2005. Prevention of Haemophilus influenzae type b disease: past success and future challenges. *Expert Rev Vaccines,* 4(6)**,** pp 819-27.

Chang, C. C., Omarjee, S., Lim, A., Spelman, T., Gosnell, B. I., Carr, W. H., Elliott, J. H., Moosa, M. Y., Ndung'u, T., French, M. A. & Lewin, S. R. 2013. Chemokine levels and chemokine receptor expression in the blood and the cerebrospinal fluid of HIV-infected patients with cryptococcal meningitis and cryptococcosis-associated immune reconstitution inflammatory syndrome. *J Infect Dis,* 208(10)**,** pp 1604-12.

Chang, C. C., Sheikh, V., Sereti, I. & French, M. A. 2014. Immune Reconstitution Disorders in Patients With HIV Infection: From Pathogenesis to Prevention and Treatment. *Curr HIV/AIDS Rep*.

Chang, W. N., Lu, C. H., Huang, C. R., Tsai, N. W., Chuang, Y. C., Chang, C. C., Chen, S. F. & Chien, C. C. 2008. Changing epidemiology of adult bacterial meningitis in southern taiwan: a hospital-based study. *Infection,* 36(1)**,** pp 15-22.

Chao, Y. N., Chiu, N. C. & Huang, F. Y. 2008. Clinical features and prognostic factors in childhood pneumococcal meningitis. *J Microbiol Immunol Infect,* 41(1)**,** pp 48-53.

Chen, Q. H., Zheng, R. Q., Lin, H., Wang, H. L., Lu, N. F., Shao, J. & Yu, J. Q. 2008. [The impact of sepsis bundles on mortality in patients with sepsis shock: a prospective clinical study]. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue,* 20(9)**,** pp 534-7.

Chihana, M., Floyd, S., Molesworth, A., Crampin, A. C., Kayuni, N., Price, A., Zaba, B., Jahn, A., Mvula, H., Dube, A., Ngwira, B., Glynn, J. R. & French, N. 2012. Adult mortality and probable cause of death in rural northern Malawi in the era of HIV treatment. *Trop Med Int Health,* 17(8)**,** pp e74-83.

Chihara, T., Hashimoto, M., Osman, A., Hiyoshi-Yoshidomi, Y., Suzu, I., Chutiwitoonchai, N., Hiyoshi, M., Okada, S. & Suzu, S. 2012. HIV-1 proteins preferentially activate anti-inflammatory M2-type macrophages. *J Immunol,* 188(8)**,** pp 3620-7.

Choi, C. W., Hwang, J. H., Chang, Y. S., Park, W. S., Kim, B. I., Choi, J.-H. & Lee, M. 2005. Effects of hypertonic (7%) saline on brain injury in experimental Escherichia coli meningitis. *Journal of Korean Medical Science,* 20(5)**,** pp 870-6.

Chowdhury, M. H. & Tunkel, A. R. 2000. Antibacterial agents in infections of the central nervous system. *Infect Dis Clin North Am,* 14(2)**,** pp 391-408, ix.

Chung, C. S., Venet, F., Chen, Y., Jones, L. N., Wilson, D. C., Ayala, C. A. & Ayala, A. 2009. Deficiency of Bid Protein Reduces Sepsis-Induced Apoptosis, Inflammation and Improves Septic Survival. *Shock*.

Cisse, M. F., Breugelmans, J. G., Ba, M., Diop, M. B., Faye, P. C., Mhlanga, B., Mueller, J. E., Koffi, D. & Gessner, B. D. 2010. The Elimination of Haemophilus influenzae type b meningitis following conjugate vaccine introduction in Senegal. *Pediatr Infect Dis J,* 29(6)**,** pp 499-503.

Clark, T., Duffell, E., Stuart, J. M. & Heyderman, R. S. 2006. Lumbar puncture in the management of adults with suspected bacterial meningitis--a survey of practice. *J Infect,* 52(5)**,** pp 315-9.

Cobos-Jimenez, V., Booiman, T., Hamann, J. & Kootstra, N. A. 2011. Macrophages and HIV-1. *Curr Opin HIV AIDS,* 6(5)**,** pp 385-90.

Cohen, A. L., Harrison, L. H., Farley, M. M., Reingold, A. L., Hadler, J., Schaffner, W., Lynfield, R., Thomas, A. R., Campsmith, M., Li, J., Schuchat, A. & Moore, M. R. 2010a. Prevention of invasive pneumococcal disease among HIV-infected adults in the era of childhood pneumococcal immunization. *AIDS,* 24(14)**,** pp 2253-62.

Cohen, C., Singh, E., Wu, H. M., Martin, S., de Gouveia, L., Klugman, K. P., Meiring, S., Govender, N. & von Gottberg, A. 2010b. Increased incidence of meningococcal disease in HIV-infected individuals associated with higher case-fatality ratios in South Africa. *AIDS,* 24(9)**,** pp 1351-60.

Cohen, D. B., Zijlstra, E. E., Mukaka, M., Reiss, M., Kamphambale, S., Scholing, M., Waitt, P. I. & Neuhann, F. 2010c. Diagnosis of cryptococcal and tuberculous meningitis in a resource-limited African setting. *Trop Med Int Health,* 15(8)**,** pp 910-7.

Cohen, J. C., P. (ed.) 1985. *Applied Multiple Regression and Correlation Analysis for the Behavioral Sciences*: Lawrence Erlbaum Associates.

Cohen, S. A., Ahmed, S., Klassen, A. C., Agree, E. M., Louis, T. A. & Naumova, E. N. 2010d. Childhood Hib vaccination and pneumonia and influenza burden in US seniors. *Vaccine,* 28(28)**,** pp 4462-9.

Coimbra, R. S., Loquet, G. & Leib, S. L. 2007. Limited efficacy of adjuvant therapy with dexamethasone in preventing hearing loss due to experimental pneumococcal meningitis in the infant rat. *Pediatr Res,* 62(3)**,** pp 291-4.

Commins, S. P., Borish, L. & Steinke, J. W. 2010. Immunologic messenger molecules: cytokines, interferons, and chemokines. *J Allergy Clin Immunol,* 125(2 Suppl 2)**,** pp S53-72.

consent, P. o. h. s. i. 1996. Final rule. *Federal Register,* 61(192)**,** pp 51498-533.

Controni, G., Rodriguez, W. J., Hicks, J. M., Ficke, M., Ross, S., Friedman, G. & Khan, W. 1977. Cerebrospinal fluid lactic acid levels in meningitis. *Journal of Pediatrics,* 91(3)**,** pp 379-84.

Cook, C. E. 2008. Potential pitfalls of clinical prediction rules. *J Man Manip Ther,* 16(2), pp 69-71.

Corless, C. E., Guiver, M., Borrow, R., Edwards-Jones, V., Fox, A. J. & Kaczmarski, E. B. 2001. Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR. *J Clin Microbiol,* 39(4), pp 1553-8.

Cornick, J. E. & Bentley, S. D. 2012. Streptococcus pneumoniae: the evolution of antimicrobial resistance to beta-lactams, fluoroquinolones and macrolides. *Microbes Infect,* 14(7-8), pp 573-83.

Cornick, J. E., Everett, D. B., Broughton, C., Denis, B. B., Banda, D. L., Carrol, E. D. & Parry, C. M. 2011. Invasive Streptococcus pneumoniae in Children, Malawi, 2004-2006. *Emerg Infect Dis,* 17(6), pp 1107-9.

Cornick, J. E., Harris, S. R., Parry, C. M., Moore, M. J., Jassi, C., Kamng'ona, A., Kulohoma, B., Heyderman, R. S., Bentley, S. D. & Everett, D. B. 2014. Genomic identification of a novel co-trimoxazole resistance genotype and its prevalence amongst Streptococcus pneumoniae in Malawi. *J Antimicrob Chemother,* 69(2), pp 368-74.

Corry, J. J. 2012. Use of hypothermia in the intensive care unit. *World J Crit Care Med,* 1(4), pp 106-122.

Council, M. R., 2008. Developing and evaluating complex interventions, Council, M. R. (London).

Council, U. R. 2010. *Advanced Life Support guidelines 2010* [Online]. Available: http://www.resus.org.uk/pages/Guide.htm 2014].

Coutinho, L. G., Grandgirard, D., Leib, S. L. & Agnez-Lima, L. F. 2013. Cerebrospinal-fluid cytokine and chemokine profile in patients with pneumococcal and meningococcal meningitis. *BMC Infect Dis,* 13(1), pp 326.

Cullinan, T. R. & Pieterick, C. 1998. Packaged treatment for first-line care in cerebral malaria and meningitis. *Bull World Health Organ,* 76(3), pp 257-64.

Dangor, Z., Izu, A., Moore, D. P., Nunes, M. C., Solomon, F., Beylis, N., von Gottberg, A., McAnerney, J. M. & Madhi, S. A. 2014. Temporal association in hospitalizations for tuberculosis, invasive pneumococcal disease and influenza virus illness in South African children. *PLoS One,* 9(3)**,** pp e91464.

Darton, T., Guiver, M., Naylor, S., Jack, D. L., Kaczmarski, E. B., Borrow, R. & Read, R. C. 2009. Severity of meningococcal disease associated with genomic bacterial load. *Clin Infect Dis,* 48(5)**,** pp 587-94.

Darton, T. C., Guiver, M., Naylor, S., Kaczmarski, E. B., Borrow, R. & Read, R. C. 2011. Bacterial genomic detection within cerebrospinal fluid of patients with meningococcal disease is influenced by microbial and host characteristics. *Clin Infect Dis,* 53(5)**,** pp 463-7.

Daugla, D. M., Gami, J. P., Gamougam, K., Naibei, N., Mbainadji, L., Narbe, M., Toralta, J., Kodbesse, B., Ngadoua, C., Coldiron, M. E., Fermon, F., Page, A. L., Djingarey, M. H., Hugonnet, S., Harrison, O. B., Rebbetts, L. S., Tekletsion, Y., Watkins, E. R., Hill, D., Caugant, D. A., Chandramohan, D., Hassan-King, M., Manigart, O., Nascimento, M., Woukeu, A., Trotter, C., Stuart, J. M., Maiden, M. C. & Greenwood, B. M. 2014. Effect of a serogroup A meningococcal conjugate vaccine (PsA-TT) on serogroup A meningococcal meningitis and carriage in Chad: a community study [corrected]. *Lancet,* 383(9911)**,** pp 40-7.

Daza, P., Banda, R., Misoya, K., Katsulukuta, A., Gessner, B. D., Katsande, R., Mhlanga, B. R., Mueller, J. E., Nelson, C. B., Phiri, A., Molyneux, E. M. & Molyneux, M. E. 2006. The impact of routine infant immunization with Haemophilus influenzae type b conjugate vaccine in Malawi, a country with high human immunodeficiency virus prevalence. *Vaccine,* 24(37-39)**,** pp 6232-9.

de Gans, J. & van de Beek, D. 2002. Dexamethasone in adults with bacterial meningitis. *N Engl J Med,* 347(20)**,** pp 1549-56.

de Jonge, R. C., van Furth, A. M., Wassenaar, M., Gemke, R. J. & Terwee, C. B. 2010. Predicting sequelae and death after bacterial meningitis in childhood: a systematic review of prognostic studies. *BMC Infect Dis,* 10(232.

de Louvois, J., Halket, S. & Harvey, D. 2007. Effect of meningitis in infancy on school-leaving examination results. *Arch Dis Child,* 92(11)**,** pp 959-62.

Deisenhammer, F., Bartos, A., Egg, R., Gilhus, N. E., Giovannoni, G., Rauer, S., Sellebjerg, F. & Force, E. T. 2006. Guidelines on routine cerebrospinal fluid analysis. Report from an EFNS task force.[see comment]. *European Journal of Neurology,* 13(9)**,** pp 913-22.

Dellinger, R. P., Carlet, J. M., Masur, H., Gerlach, H., Calandra, T., Cohen, J., Gea-Banacloche, J., Keh, D., Marshall, J. C., Parker, M. M., Ramsay, G., Zimmerman, J. L., Vincent, J. L. & Levy, M. M. 2004. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med,* 32(3)**,** pp 858-73.

Dellinger, R. P., Levy, M. M., Carlet, J. M., Bion, J., Parker, M. M., Jaeschke, R., Reinhart, K., Angus, D. C., Brun-Buisson, C., Beale, R., Calandra, T., Dhainaut, J. F., Gerlach, H., Harvey, M., Marini, J. J., Marshall, J., Ranieri, M., Ramsay, G., Sevransky, J., Thompson, B. T., Townsend, S., Vender, J. S., Zimmerman, J. L. & Vincent, J. L. 2008a. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Intensive Care Med,* 34(1)**,** pp 17-60.

Dellinger, R. P., Levy, M. M., Carlet, J. M., Bion, J., Parker, M. M., Jaeschke, R., Reinhart, K., Angus, D. C., Brun-Buisson, C., Beale, R., Calandra, T., Dhainaut, J. F., Gerlach, H., Harvey, M., Marini, J. J., Marshall, J., Ranieri, M., Ramsay, G., Sevransky, J., Thompson, B. T., Townsend, S., Vender, J. S., Zimmerman, J. L. & Vincent, J. L. 2008b. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med,* 36(1)**,** pp 296-327.

Dellinger, R. P., Levy, M. M., Rhodes, A., Annane, D., Gerlach, H., Opal, S. M., Sevransky, J. E., Sprung, C. L., Douglas, I. S., Jaeschke, R., Osborn, T. M., Nunnally, M. E., Townsend, S. R., Reinhart, K., Kleinpell, R. M., Angus, D. C., Deutschman, C. S., Machado, F. R., Rubenfeld, G. D., Webb, S., Beale, R. J., Vincent, J. L. & Moreno, R. 2013. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med,* 39(2)**,** pp 165-228.

Desmond, N. A., Nyirenda, D., Dube, Q., Mallewa, M., Molyneux, E., Lalloo, D. G. & Heyderman, R. S. 2013. Recognising and treatment seeking for acute bacterial

meningitis in adults and children in resource-poor settings: a qualitative study. *PLoS One,* 8(7), pp e68163.

Djibo, S., Njanpop Lafourcade, B. M., Boisier, P., Moussa, A., Kobo, G., Sidikou, F., Hien, A., Bieboure, G., Aguilera, J. F., Parent du Chatelet, I., Gessner, B. D. & Chanteau, S. 2006. Evaluation of the Pastorex meningitis kit for the rapid identification of Neisseria meningitidis serogroups A and W135. *Trans R Soc Trop Med Hyg,* 100(6), pp 573-8.

Domingo, P., Suarez-Lozano, I., Torres, F., Pomar, V., Ribera, E., Galindo, M. J., Cosin, J., Garcia-Alcalde, M. L., Vidal, F., Lopez-Aldeguer, J., Roca, B., Gonzalez, J., Lozano, F. & Garrido, M. 2009. Bacterial meningitis in HIV-1-infected patients in the era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr,* 51(5), pp 582-7.

Dougherty, J. M. & Jones, J. 1986. Cerebrospinal fluid cultures and analysis. *Ann Emerg Med,* 15(3), pp 317-23.

Duke, T., Mokela, D., Frank, D., Michael, A., Paulo, T., Mgone, J. & Kurubi, J. 2002. Management of meningitis in children with oral fluid restriction or intravenous fluid at maintenance volumes: a randomised trial. *Annals of Tropical Paediatrics,* 22(2), pp 145-57.

Durand, M. L., Calderwood, S. B., Weber, D. J., Miller, S. I., Southwick, F. S., Caviness, V. S., Jr. & Swartz, M. N. 1993. Acute bacterial meningitis in adults. A review of 493 episodes. *N Engl J Med,* 328(1), pp 21-8.

Dzupova, O., Rozsypal, H., Prochazka, B. & Benes, J. 2009. Acute bacterial meningitis in adults: predictors of outcome. *Scand J Infect Dis,* 41(5), pp 348-54.

Ebm, C., Cecconi, M., Sutton, L. & Rhodes, A. 2014. A cost-effectiveness analysis of postoperative goal-directed therapy for high-risk surgical patients. *Crit Care Med,* 42(5), pp 1194-203.

Edmond, K., Clark, A., Korczak, V. S., Sanderson, C., Griffiths, U. K. & Rudan, I. 2010. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. *Lancet Infect Dis,* 10(5), pp 317-28.

Egere, U., Townend, J., Roca, A., Akinsanya, A., Bojang, A., Nsekpong, D., Greenwood, B., Adegbola, R. A. & Hill, P. C. 2012. Indirect effect of 7-valent pneumococcal conjugate vaccine on pneumococcal carriage in newborns in rural Gambia: a randomised controlled trial. *PLoS One,* 7(11)**,** pp e49143.

Egermann, U., Stanga, Z., Ramin, A., Acosta, F., Stucki, A., Gerber, P., Cottagnoud, M. & Cottagnoud, P. 2009. Combination of daptomycin plus ceftriaxone is more active than vancomycin plus ceftriaxone in experimental meningitis after addition of dexamethasone. *Antimicrobial Agents & Chemotherapy,* 53(7)**,** pp 3030-3.

El Solh, A. A., Akinnusi, M. E., Alsawalha, L. N. & Pineda, L. A. 2008. Outcome of septic shock in older adults after implementation of the sepsis "bundle". *J Am Geriatr Soc,* 56(2)**,** pp 272-8.

Eldridge, S., Spencer, A., Cryer, C., Parsons, S., Underwood, M. & Feder, G. 2005. Why modelling a complex intervention is an important precursor to trial design: lessons from studying an intervention to reduce falls-related injuries in older people. *J Health Serv Res Policy,* 10(3)**,** pp 133-42.

Elena Santolaya, M., O'Ryan, M., Teresa Valenzuela, M., Prado, V., Vergara, R. F., Munoz, A., Toneatto, D., Grana, G., Wang, H. & Dull, P. M. 2013. Persistence of antibodies in adolescents 18-24 mo after immunization with one, two or three doses of 4CMenB meningococcal serogroup B vaccine. *Hum Vaccin Immunother,* 9(11)**,** pp.

Elston, J. W., Santaniello-Newton, A., Meigh, J. A., Harmer, D., Allgar, V., Allison, T., Richardson, G., Meigh, R., Palmer, S. R. & Barlow, G. 2012. Increasing incidence of invasive pneumococcal disease and pneumonia despite improved vaccination uptake: surveillance in Hull and East Yorkshire, UK, 2002-2009. *Epidemiol Infect,* 140(7)**,** pp 1252-66.

Emonts, M., Sweep, F. C. G. J., Grebenchtchikov, N., Geurts-Moespot, A., Knaup, M., Chanson, A. L., Erard, V., Renner, P., Hermans, P. W. M., Hazelzet, J. A. & Calandra, T. 2007. Association between high levels of blood macrophage migration inhibitory factor, inappropriate adrenal response, and early death in patients with severe sepsis. *Clinical Infectious Diseases,* 44(10)**,** pp 1321-8.

Ernst, J. D., Decazes, J. M. & Sande, M. A. 1983. Experimental pneumococcal meningitis: role of leukocytes in pathogenesis. *Infection & Immunity,* 41(1)**,** pp 275-9.

Everett, D. B., Cornick, J., Denis, B., Chewapreecha, C., Croucher, N., Harris, S., Parkhill, J., Gordon, S., Carrol, E. D., French, N., Heyderman, R. S. & Bentley, S. D. 2012. Genetic characterisation of Malawian pneumococci prior to the roll-out of the PCV13 vaccine using a high-throughput whole genome sequencing approach. *PLoS One,* 7(9)**,** pp e44250.

Everett, D. B., Mukaka, M., Denis, B., Gordon, S. B., Carrol, E. D., van Oosterhout, J. J., Molyneux, E. M., Molyneux, M., French, N. & Heyderman, R. S. 2011. Ten years of surveillance for invasive Streptococcus pneumoniae during the era of antiretroviral scale-up and cotrimoxazole prophylaxis in Malawi. *PLoS One,* 6(3)**,** pp e17765.

Feikin, D. R., Jagero, G., Aura, B., Bigogo, G. M., Oundo, J., Beall, B. W., Karani, A., Morpeth, S., Njenga, M. K. & Breiman, R. F. 2010. High rate of pneumococcal bacteremia in a prospective cohort of older children and adults in an area of high HIV prevalence in rural western Kenya. *BMC Infect Dis,* 10(186.

Feldman, C. & Anderson, R. 2014. Review: Current and new generation pneumococcal vaccines. *J Infect*.

Ferreira, D. M., Neill, D. R., Bangert, M., Gritzfeld, J. F., Green, N., Wright, A. K., Pennington, S. H., Bricio-Moreno, L., Moreno, A. T., Miyaji, E. N., Wright, A. D., Collins, A. M., Goldblatt, D., Kadioglu, A. & Gordon, S. B. 2013. Controlled human infection and rechallenge with Streptococcus pneumoniae reveals the protective efficacy of carriage in healthy adults. *Am J Respir Crit Care Med,* 187(8)**,** pp 855-64.

Finberg, R. W., Moellering, R. C., Tally, F. P., Craig, W. A., Pankey, G. A., Dellinger, E. P., West, M. A., Joshi, M., Linden, P. K., Rolston, K. V., Rotschafer, J. C. & Rybak, M. J. 2004. The importance of bactericidal drugs: future directions in infectious disease. *Clin Infect Dis,* 39(9)**,** pp 1314-20.

Fishman, R. A., Sligar, K. P., Spigel, J. H. & Hake, R. B. 1975. The pathogenesis of the encephalopathy of purulent meningitis: effects of leukocytes on brain metabolism. *Transactions of the American Neurological Association,* 100(80-4.

Fitch, M. T. & van de Beek, D. 2007. Emergency diagnosis and treatment of adult meningitis. *Lancet Infect Dis,* 7(3)**,** pp 191-200.

Flannery, B., Heffernan, R. T., Harrison, L. H., Ray, S. M., Reingold, A. L., Hadler, J., Schaffner, W., Lynfield, R., Thomas, A. R., Li, J., Campsmith, M., Whitney, C. G. & Schuchat, A. 2006. Changes in invasive Pneumococcal disease among HIV-infected adults living in the era of childhood pneumococcal immunization. *Ann Intern Med,* 144(1)**,** pp 1-9.

Flexner, S. 1907. Experimental Cerebro-Spinal Meningitis in Monkeys. *J Exp Med,* 9(2)**,** pp 142-67.

Flexner, S. 1913. The Results of the Serum Treatment in Thirteen Hundred Cases of Epidemic Meningitis. *J Exp Med,* 17(5)**,** pp 553-76.

Flores-Cordero, J. M., Amaya-Villar, R., Rincon-Ferrari, M. D., Leal-Noval, S. R., Garnacho-Montero, J., Llanos-Rodriguez, A. C. & Murillo-Cabezas, F. 2003. Acute community-acquired bacterial meningitis in adults admitted to the intensive care unit: clinical manifestations, management and prognostic factors.[see comment]. *Intensive Care Medicine,* 29(11)**,** pp 1967-73.

Floyd, S., Marston, M., Baisley, K., Wringe, A., Herbst, K., Chihana, M., Kasamba, I., Barnighausen, T., Urassa, M., French, N., Todd, J. & Zaba, B. 2012. The effect of antiretroviral therapy provision on all-cause, AIDS and non-AIDS mortality at the population level--a comparative analysis of data from four settings in Southern and East Africa. *Trop Med Int Health,* 17(8)**,** pp e84-93.

Floyd, S., Molesworth, A., Dube, A., Banda, E., Jahn, A., Mwafulirwa, C., Ngwira, B., Branson, K., Crampin, A. C., Zaba, B., Glynn, J. R. & French, N. 2010. Population-level reduction in adult mortality after extension of free anti-retroviral therapy provision into rural areas in northern Malawi. *PLoS One,* 5(10)**,** pp e13499.

Foundation, B. T. 2000. The American Association of Neurological Surgeons. The Joint Section on Neurotrauma and Critical Care. Initial management. *J Neurotrauma,* 17(6-7)**,** pp 463-9.

Frank, M. S., Nahata, M. C. & Hilty, M. D. 1981. Glycerol: a review of its pharmacology, pharmacokinetics, adverse reactions, and clinical use. *Pharmacotherapy,* 1(2)**,** pp 147-60.

French, N., Gordon, S. B., Mwalukomo, T., White, S. A., Mwafulirwa, G., Longwe, H., Mwaiponya, M., Zijlstra, E. E., Molyneux, M. E. & Gilks, C. F. 2010. A trial of a 7-valent pneumococcal conjugate vaccine in HIV-infected adults. *N Engl J Med,* 362(9)**,** pp 812-22.

French, N., Nakiyingi, J., Carpenter, L. M., Lugada, E., Watera, C., Moi, K., Moore, M., Antvelink, D., Mulder, D., Janoff, E. N., Whitworth, J. & Gilks, C. F. 2000. 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomised and placebo controlled trial. *Lancet,* 355(9221)**,** pp 2106-11.

Friedland, I. R., Jafari, H., Ehrett, S., Rinderknecht, S., Paris, M., Coulthard, M., Saxen, H., Olsen, K. & McCracken, G. H., Jr. 1993. Comparison of endotoxin release by different antimicrobial agents and the effect on inflammation in experimental Escherichia coli meningitis.[erratum appears in J Infect Dis 1993 Nov;168(5):1342]. *Journal of Infectious Diseases,* 168(3)**,** pp 657-62.

Friedland, I. R. & Klugman, K. P. 1992. Failure of chloramphenicol therapy in penicillin-resistant pneumococcal meningitis. *Lancet,* 339(8790)**,** pp 405-8.

Fullerton, D. G., Jere, K., Jambo, K., Kulkarni, N. S., Zijlstra, E. E., Grigg, J., French, N., Molyneux, M. E. & Gordon, S. B. 2009. Domestic smoke exposure is associated with alveolar macrophage particulate load. *Trop Med Int Health,* 14(3)**,** pp 349-54.

Futier, E., Constantin, J. M., Petit, A., Chanques, G., Kwiatkowski, F., Flamein, R., Slim, K., Sapin, V., Jaber, S. & Bazin, J. E. 2010. Conservative vs restrictive individualized goal-directed fluid replacement strategy in major abdominal surgery: A prospective randomized trial. *Arch Surg,* 145(12)**,** pp 1193-200.

Gaieski, D. F., Band, R. A., Abella, B. S., Neumar, R. W., Fuchs, B. D., Kolansky, D. M., Merchant, R. M., Carr, B. G., Becker, L. B., Maguire, C., Klair, A., Hylton, J. & Goyal, M. 2009. Early goal-directed hemodynamic optimization combined with therapeutic hypothermia in comatose survivors of out-of-hospital cardiac arrest. *Resuscitation,* 80(4)**,** pp 418-24.

Gamper, G., Oschatz, E., Herkner, H., Paul, G., Burgmann, H., Janata, K., Roggla, M. & Laggner, A. N. 2001. Sepsis-associated purpura fulminans in adults. *Wiener Klinische Wochenschrift,* 113(3-4)**,** pp 107-12.

Garvey, L. J., Pavese, N., Politis, M., Ramlackhansingh, A., Brooks, D. J., Taylor-Robinson, S. D. & Winston, A. 2014. Increased microglia activation in neurologically asymptomatic HIV-infected patients receiving effective ART. *AIDS,* 28(1)**,** pp 67-72.

Geffin, R. & McCarthy, M. 2013. Innate immune responses to HIV infection in the central nervous system. *Immunol Res,* 57(1-3)**,** pp 292-302.

Geldhoff, M., Mook-Kanamori, B. B., Brouwer, M. C., Troost, D., Leemans, J. C., Flavell, R. A., Van der Ende, A., Van der Poll, T. & Van de Beek, D. 2013. Inflammasome activation mediates inflammation and outcome in humans and mice with pneumococcal meningitis. *BMC Infect Dis,* 13(358.

Georges, H., Chiche, A., Alfandari, S., Devos, P., Boussekey, N. & Leroy, O. 2009. Adult community-acquired bacterial meningitis requiring ICU admission: epidemiological data, prognosis factors and adherence to IDSA guidelines. *Eur J Clin Microbiol Infect Dis,* 28(11)**,** pp 1317-25.

Gerber, J. & Nau, R. 2010. Mechanisms of injury in bacterial meningitis. *Curr Opin Neurol,* 23(3)**,** pp 312-8.

Gessner, B. D., Mueller, J. E. & Yaro, S. 2010. African meningitis belt pneumococcal disease epidemiology indicates a need for an effective serotype 1 containing vaccine, including for older children and adults. *BMC Infect Dis,* 10(22.

Gilks, C. F., Ojoo, S. A., Ojoo, J. C., Brindle, R. J., Paul, J., Batchelor, B. I., Kimari, J. N., Newnham, R., Bwayo, J., Plummer, F. A. & et al. 1996. Invasive pneumococcal disease in a cohort of predominantly HIV-1 infected female sex-workers in Nairobi, Kenya. *Lancet,* 347(9003)**,** pp 718-23.

Girgis, N. I., Abu el-Ella, A. H., Farid, Z., Haberberger, R. L. & Woody, J. N. 1988. Ceftriaxone alone compared to ampicillin and chloramphenicol in the treatment of bacterial meningitis. *Chemotherapy,* 34 Suppl 1(16-20.

Giufre, M., Cardines, R., Caporali, M. G., Accogli, M., D'Ancona, F. & Cerquetti, M. 2011. Ten years of Hib vaccination in Italy: prevalence of non-encapsulated Haemophilus influenzae among invasive isolates and the possible impact on antibiotic resistance. *Vaccine,* 29(22)**,** pp 3857-62.

Gjini, A. B., Stuart, J. M., Cartwright, K., Cohen, J., Jacobs, M., Nichols, T., Ninis, N., Prempeh, H., Whitehouse, A. & Heyderman, R. S. 2006a. Quality of in-hospital care for adults with acute bacterial meningitis: a national retrospective survey. *QJM,* 99(11)**,** pp 761-9.

Gjini, A. B., Stuart, J. M., Lawlor, D. A., Cartwright, K. A., Christensen, H., Ramsay, M. & Heyderman, R. S. 2006b. Changing epidemiology of bacterial meningitis among adults in England and Wales 1991-2002. *Epidemiol Infect,* 134(3)**,** pp 567-9.

Glennie, S. J., Banda, D., Gould, K., Hinds, J., Kamngona, A., Everett, D. D., Williams, N. A. & Heyderman, R. S. 2013. Defective pneumococcal-specific Th1 responses in HIV-infected adults precedes a loss of control of pneumococcal colonization. *Clin Infect Dis,* 56(2)**,** pp 291-9.

Glennie, S. J., Sepako, E., Mzinza, D., Harawa, V., Miles, D. J., Jambo, K. C., Gordon, S. B., Williams, N. A. & Heyderman, R. S. 2011. Impaired CD4 T cell memory response to Streptococcus pneumoniae precedes CD4 T cell depletion in HIV-infected Malawian adults. *PLoS One,* 6(9)**,** pp e25610.

Glimaker, M., Johansson, B., Halldorsdottir, H., Wanecek, M., Elmi-Terander, A., Ghatan, P. H., Lindquist, L. & Bellander, B. M. 2014. Neuro-intensive treatment targeting intracranial hypertension improves outcome in severe bacterial meningitis: an intervention-control study. *PLoS One,* 9(3)**,** pp e91976.

Goh, D. & Minns, R. A. 1993. Cerebral blood flow velocity monitoring in pyogenic meningitis. *Archives of Disease in Childhood,* 68(1)**,** pp 111-9.

Goonetilleke, U. R., Scarborough, M., Ward, S. A. & Gordon, S. B. 2010. Proteomic analysis of cerebrospinal fluid in pneumococcal meningitis reveals potential biomarkers associated with survival. *J Infect Dis,* 202(4)**,** pp 542-50.

Goonetilleke, U. R., Scarborough, M., Ward, S. A., Hussain, S., Kadioglu, A. & Gordon, S. B. 2012. Death is associated with complement C3 depletion in cerebrospinal fluid of patients with pneumococcal meningitis. *MBio,* 3(2)**,** pp.

Gordon, M. A., Graham, S. M., Walsh, A. L., Wilson, L., Phiri, A., Molyneux, E., Zijlstra, E. E., Heyderman, R. S., Hart, C. A. & Molyneux, M. E. 2008. Epidemics of invasive Salmonella enterica serovar enteritidis and S. enterica Serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis,* 46(7)**,** pp 963-9.

Gordon, M. A., Walsh, A. L., Chaponda, M., Soko, D., Mbvwinji, M., Molyneux, M. E. & Gordon, S. B. 2001. Bacteraemia and mortality among adult medical admissions in Malawi--predominance of non-typhi salmonellae and Streptococcus pneumoniae. *J Infect,* 42(1)**,** pp 44-9.

Gordon, S. B., Walsh, A. L., Chaponda, M., Gordon, M. A., Soko, D., Mbwvinji, M., Molyneux, M. E. & Read, R. C. 2000. Bacterial meningitis in Malawian adults: pneumococcal disease is common, severe, and seasonal. *Clin Infect Dis,* 31(1)**,** pp 53-7.

Gossger, N., Snape, M. D., Yu, L. M., Finn, A., Bona, G., Esposito, S., Principi, N., Diez-Domingo, J., Sokal, E., Becker, B., Kieninger, D., Prymula, R., Dull, P., Ypma, E., Toneatto, D., Kimura, A. & Pollard, A. J. 2012. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. *JAMA,* 307(6)**,** pp 573-82.

Government, M., 2010. Quarterly HIV Programme Report, Government, M. (Lilongwe).

Government, M., 2012. HIV Treatment Guidelines 2012, Government, M. (Lilongwe, Malawi).

Grandgirard, D., Gaumann, R., Coulibaly, B., Dangy, J. P., Sie, A., Junghanss, T., Schudel, H., Pluschke, G. & Leib, S. L. 2013. The causative pathogen determines the inflammatory profile in cerebrospinal fluid and outcome in patients with bacterial meningitis. *Mediators Inflamm,* 2013(312476.

Grandgirard, D., Steiner, O., Tauber, M. G. & Leib, S. L. 2007. An infant mouse model of brain damage in pneumococcal meningitis. *Acta Neuropathol,* 114(6)**,** pp 609-17.

Grau, I., Pallares, R., Tubau, F., Schulze, M. H., Llopis, F., Podzamczer, D., Linares, J. & Gudiol, F. 2005. Epidemiologic changes in bacteremic pneumococcal disease in patients with human immunodeficiency virus in the era of highly active antiretroviral therapy. *Arch Intern Med,* 165(13)**,** pp 1533-40.

Green, J. A., Tran, C. T., Farrar, J. J., Nguyen, M. T., Nguyen, P. H., Dinh, S. X., Ho, N. D., Ly, C. V., Tran, H. T., Friedland, J. S. & Thwaites, G. E. 2009. Dexamethasone, cerebrospinal fluid matrix metalloproteinase concentrations and clinical outcomes in tuberculous meningitis. *PLoS One,* 4(9)**,** pp e7277.

Greiner, O., Day, P. J., Bosshard, P. P., Imeri, F., Altwegg, M. & Nadal, D. 2001. Quantitative detection of Streptococcus pneumoniae in nasopharyngeal secretions by real-time PCR. *J Clin Microbiol,* 39(9)**,** pp 3129-34.

Griffin, M. R., Zhu, Y., Moore, M. R., Whitney, C. G. & Grijalva, C. G. 2013. U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. *N Engl J Med,* 369(2)**,** pp 155-63.

Griffiths, P. 2004. Cytomegalovirus infection of the central nervous system. *Herpes,* 11 Suppl 2(95A-104A.

Gutierrez, F., Masia, M., Rodriguez, J. C., Ayelo, A., Soldan, B., Cebrian, L., Mirete, C., Royo, G. & Hidalgo, A. M. 2003. Evaluation of the immunochromatographic Binax NOW assay for detection of Streptococcus pneumoniae urinary antigen in a prospective study of community-acquired pneumonia in Spain. *Clin Infect Dis,* 36(3)**,** pp 286-92.

Guyatt, G. H., Oxman, A. D., Schunemann, H. J., Tugwell, P. & Knottnerus, A. 2011. GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology. *J Clin Epidemiol,* 64(4)**,** pp 380-2.

Haarman, E. G., Vermeulen, R. J., van Furth, A. M., Verbeke, J. I. & Plotz, F. B. 2008. Cushing's triad in pneumococcal meningitis due to brainstem ischemia: early detection by diffusion-weighted MRI. *Pediatric Neurology,* 38(4)**,** pp 276-8.

Hackett, S. J., Guiver, M., Marsh, J., Sills, J. A., Thomson, A. P., Kaczmarski, E. B. & Hart, C. A. 2002. Meningococcal bacterial DNA load at presentation correlates with disease severity. *Arch Dis Child,* 86(1)**,** pp 44-6.

Haddy, T. B., Rana, S. R. & Castro, O. 1999. Benign ethnic neutropenia: what is a normal absolute neutrophil count? *J Lab Clin Med,* 133(1)**,** pp 15-22.

Hadgu, A., Dendukuri, N. & Hilden, J. 2005. Evaluation of nucleic acid amplification tests in the absence of a perfect gold-standard test: a review of the statistical and epidemiologic issues. *Epidemiology,* 16(5)**,** pp 604-12.

Hahne, S. J., Charlett, A., Purcell, B., Samuelsson, S., Camaroni, I., Ehrhard, I., Heuberger, S., Santamaria, M. & Stuart, J. M. 2006. Effectiveness of antibiotics given before admission in reducing mortality from meningococcal disease: systematic review. *BMJ,* 332(7553)**,** pp 1299-303.

Hajishengallis, G., Darveau, R. P. & Curtis, M. A. 2012. The keystone-pathogen hypothesis. *Nat Rev Microbiol,* 10(10)**,** pp 717-25.

Hakim, J. G., Gangaidzo, I. T., Heyderman, R. S., Mielke, J., Mushangi, E., Taziwa, A., Robertson, V. J., Musvaire, P. & Mason, P. R. 2000. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. *AIDS,* 14(10)**,** pp 1401-7.

Harboe, Z. B., Thomsen, R. W., Riis, A., Valentiner-Branth, P., Christensen, J. J., Lambertsen, L., Krogfelt, K. A., Konradsen, H. B. & Benfield, T. L. 2009. Pneumococcal serotypes and mortality following invasive pneumococcal disease: a population-based cohort study. *PLoS Med,* 6(5)**,** pp e1000081.

Harnden, A., Ninis, N., Thompson, M., Perera, R., Levin, M., Mant, D. & Mayon-White, R. 2006. Parenteral penicillin for children with meningococcal disease before hospital admission: case-control study. *BMJ,* 332(7553)**,** pp 1295-8.

Harries, A. D., Zachariah, R., Jahn, A., Schouten, E. J. & Kamoto, K. 2009. Scaling up antiretroviral therapy in Malawi-implications for managing other chronic diseases in resource-limited countries. *J Acquir Immune Defic Syndr,* 52 Suppl 1(S14-6.

Hasbun, R., Abrahams, J., Jekel, J. & Quagliarello, V. J. 2001. Computed tomography of the head before lumbar puncture in adults with suspected meningitis. *N Engl J Med,* 345(24)**,** pp 1727-33.

Hayden, M. H., Dalaba, M., Awine, T., Akweongo, P., Nyaaba, G., Anaseba, D., Pelzman, J., Hodgson, A. & Pandya, R. 2013. Knowledge, attitudes, and practices related to meningitis in northern Ghana. *Am J Trop Med Hyg,* 89(2)**,** pp 265-70.

Heckenberg, S. G., de Gans, J., Brouwer, M. C., Weisfelt, M., Piet, J. R., Spanjaard, L., van der Ende, A. & van de Beek, D. 2008. Clinical features, outcome, and meningococcal genotype in 258 adults with meningococcal meningitis: a prospective cohort study. *Medicine (Baltimore),* 87(4)**,** pp 185-92.

Hedstrand, H. & Killander, A. 1977. Anaemia in middle-aged men. Prevalence and aetiology in a population study. *Scand J Haematol,* 19(5)**,** pp 417-23.

Heldrich, F. J., Walker, S. H. & Crosby, R. M. 1986. Risk of diagnostic lumbar puncture in acute bacterial meningitis. *Pediatr Emerg Care,* 2(3)**,** pp 180-2.

Herson, V. C. & Todd, J. K. 1977. Prediction of morbidity in Hemophilus influenzae meningitis. *Pediatrics,* 59(1)**,** pp 35-9.

Heyderman, R. S. 2005. Early management of suspected bacterial meningitis and meningococcal septicaemia in immunocompetent adults--second edition. *J Infect,* 50(5)**,** pp 373-4.

Heyderman, R. S., Lambert, H. P., O'Sullivan, I., Stuart, J. M., Taylor, B. L. & Wall, R. A. 2003. Early management of suspected bacterial meningitis and meningococcal septicaemia in adults. *J Infect,* 46(2)**,** pp 75-7.

Higgins JPT, G. S. (ed.) 2009. *Cochrane Handbook for Systematic Reviews of Invertentions Version 5.0.2*: The Cochrane Collaboration.

Hirst, R. A., Kadioglu, A., O'Callaghan, C. & Andrew, P. W. 2004. The role of pneumolysin in pneumococcal pneumonia and meningitis. *Clin Exp Immunol,* 138(2)**,** pp 195-201.

Hoffman, M., Mofolo, I., Salima, C., Hoffman, I., Zadrozny, S., Martinson, F. & Van Der Horst, C. 2012. Utilization of family members to provide hospital care in Malawi: the role of Hospital Guardians. *Malawi Med J,* 24(4)**,** pp 74-8.

Hoffman, O. & Weber, R. J. 2009. Pathophysiology and treatment of bacterial meningitis. *Ther Adv Neurol Disord,* 2(6)**,** pp 1-7.

Hsu, C. L., Chang, C. H., Wong, K. N., Chen, K. Y., Yu, C. J. & Yang, P. C. 2009a. Management of severe community-acquired septic meningitis in adults: from emergency department to intensive care unit. *J Formos Med Assoc,* 108(2)**,** pp 112-8.

Hsu, H. E., Shutt, K. A., Moore, M. R., Beall, B. W., Bennett, N. M., Craig, A. S., Farley, M. M., Jorgensen, J. H., Lexau, C. A., Petit, S., Reingold, A., Schaffner, W., Thomas, A., Whitney, C. G. & Harrison, L. H. 2009b. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med,* 360(3)**,** pp 244-56.

Huang, D. T., Angus, D. C., Barnato, A., Gunn, S. R., Kellum, J. A., Stapleton, D. K., Weissfeld, L. A., Yealy, D. M., Peake, S. L., Delaney, A., Bellomo, R., Cameron, P., Higgins, A., Holdgate, A., Howe, B., Webb, S. A., Williams, P., Osborn, T. M., Mouncey, P. R., Harrison, D. A., Harvey, S. E. & Rowan, K. M. 2013. Harmonizing international trials of early goal-directed resuscitation for severe sepsis and septic shock: methodology of ProCESS, ARISE, and ProMISe. *Intensive Care Med,* 39(10)**,** pp 1760-75.

Huang, S. S., Finkelstein, J. A., Rifas-Shiman, S. L., Kleinman, K. & Platt, R. 2004. Community-level predictors of pneumococcal carriage and resistance in young children. *Am J Epidemiol,* 159(7)**,** pp 645-54.

Hung, K. L., Liao, H. T. & Tsai, M. L. 2000. Epstein-Barr virus encephalitis in children. *Acta Paediatr Taiwan,* 41(3)**,** pp 140-6.

Ichai, C., Armando, G., Orban, J. C., Berthier, F., Rami, L., Samat-Long, C., Grimaud, D. & Leverve, X. 2009. Sodium lactate versus mannitol in the treatment of intracranial hypertensive episodes in severe traumatic brain-injured patients. *Intensive Care Med,* 35(3)**,** pp 471-9.

Ichiyama, T., Hayashi, T., Nishikawa, M. & Furukawa, S. 1997. Levels of transforming growth factor beta 1, tumor necrosis factor alpha, and interleukin 6 in cerebrospinal fluid: association with clinical outcome for children with bacterial meningitis. *Clin Infect Dis,* 25(2)**,** pp 328-9.

Imuekehme, S., Obi, J. & Alakija, W. 1997. Cerebro-spinal lactate status in childhood pyogenic meningitis in Nigeria. *Journal of Tropical Pediatrics,* 43(6)**,** pp 361-3.

Irving, T. J., Blyuss, K. B., Colijn, C. & Trotter, C. L. 2012. Modelling meningococcal meningitis in the African meningitis belt. *Epidemiol Infect,* 140(5)**,** pp 897-905.

Ishihara, M., Kamei, S., Taira, N., Morita, A., Miki, K., Naganuma, T., Minami, M., Shiota, H., Hara, M. & Mizutani, T. 2009. Hospital-based study of the prognostic factors in adult patients with acute community-acquired bacterial meningitis in Tokyo, Japan. *Intern Med,* 48(5)**,** pp 295-300.

Jacob, S. T., Banura, P., Baeten, J. M., Moore, C. C., Meya, D., Nakiyingi, L., Burke, R., Horton, C. L., Iga, B., Wald, A., Reynolds, S. J., Mayanja-Kizza, H. & Scheld, W. M. 2012. The impact of early monitored management on survival in hospitalized adult Ugandan patients with severe sepsis: a prospective intervention study*. *Crit Care Med,* 40(7)**,** pp 2050-8.

Jahn, A., Floyd, S., Crampin, A. C., Mvula, H., Mwinuka, V., Mwaiyeghele, E., McGrath, N., Zaba, B., Fine, P. E. & Glynn, J. R. 2010a. Declining child mortality in northern Malawi despite high rates of infection with HIV. *Bull World Health Organ,* 88(10)**,** pp 746-53.

Jahn, A., Floyd, S., McGrath, N., Crampin, A. C., Kachiwanda, L., Mwinuka, V., Zaba, B., Fine, P. E. & Glynn, J. R. 2010b. Child mortality in rural Malawi: HIV closes the survival gap between the socio-economic strata. *PLoS One,* 5(6)**,** pp e11320.

Jansen, A. G., Sanders, E. A., A, V. D. E., AM, V. A. N. L., Hoes, A. W. & Hak, E. 2008. Invasive pneumococcal and meningococcal disease: association with influenza virus and respiratory syncytial virus activity? *Epidemiol Infect,* 136(11)**,** pp 1448-54.

Jarvis, J. N., Lawn, S. D., Vogt, M., Bangani, N., Wood, R. & Harrison, T. S. 2009. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis,* 48(7)**,** pp 856-62.

Jarvis, J. N., Meintjes, G., Rebe, K., Williams, G. N., Bicanic, T., Williams, A., Schutz, C., Bekker, L. G., Wood, R. & Harrison, T. S. 2012. Adjunctive interferon-gamma immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. *AIDS,* 26(9)**,** pp 1105-13.

Jarvis, J. N., Meintjes, G., Williams, A., Brown, Y., Crede, T. & Harrison, T. S. 2010. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis,* 10(67.

Joffe, A. R. 2007. Lumbar puncture and brain herniation in acute bacterial meningitis: a review. *Journal of Intensive Care Medicine,* 22(4)**,** pp 194-207.

Johnson, A. W., Adedoyin, O. T., Abdul-Karim, A. A. & Olanrewaju, A. W. 2007. Childhood pyogenic meningitis: clinical and investigative indicators of etiology and outcome. *J Natl Med Assoc,* 99(8)**,** pp 937-47.

Jones, A. E., Focht, A., Horton, J. M. & Kline, J. A. 2007a. Prospective external validation of the clinical effectiveness of an emergency department-based early goal-directed therapy protocol for severe sepsis and septic shock. *Chest,* 132(2)**,** pp 425-32.

Jones, A. E., Shapiro, N. I. & Roshon, M. 2007b. Implementing early goal-directed therapy in the emergency setting: the challenges and experiences of translating research innovations into clinical reality in academic and community settings. *Acad Emerg Med,* 14(11)**,** pp 1072-8.

Kaaresen, P. I. & Flaegstad, T. 1995. Prognostic factors in childhood bacterial meningitis. *Acta Paediatr,* 84(8)**,** pp 873-8.

Kabanda, T., Siedner, M. J., Klausner, J. D., Muzoora, C. & Boulware, D. R. 2014. Point-of-care diagnosis and prognostication of cryptococcal meningitis with the cryptococcal antigen lateral flow assay on cerebrospinal fluid. *Clin Infect Dis,* 58(1)**,** pp 113-6.

Kabyemera, R., Masalu, N., Rambau, P., Kamugisha, E., Kidenya, B., De Rossi, A., Petrara, M. R. & Mwizamuholya, D. 2013. Relationship between non-Hodgkin's lymphoma and blood levels of Epstein-Barr virus in children in north-western Tanzania: a case control study. *BMC Pediatr,* 13(4.

Kadurugamuwa, J. L., Hengstler, B. & Zak, O. 1989. Cerebrospinal fluid protein profile in experimental pneumococcal meningitis and its alteration by ampicillin and anti-inflammatory agents. *J Infect Dis,* 159(1)**,** pp 26-34.

Karon, B. S., Scott, R., Burritt, M. F. & Santrach, P. J. 2007. Comparison of lactate values between point-of-care and central laboratory analyzers. *Am J Clin Pathol,* 128(1)**,** pp 168-71.

Kasten, K. R., Tschop, J., Adediran, S. G., Hildeman, D. A. & Caldwell, C. C. 2010. T cells are potent early mediators of the host response to sepsis. *Shock,* 34(4)**,** pp 327-36.

Kelly, M. J., Benjamin, L. A., Cartwright, K., Ajdukiewicz, K. M., Cohen, D. B., Menyere, M., Galbraith, S., Guiver, M., Neuhann, F., Solomon, T., Lalloo, D. G. & Heyderman, R. S. 2012. Epstein-barr virus coinfection in cerebrospinal fluid is associated with increased mortality in Malawian adults with bacterial meningitis. *J Infect Dis,* 205(1)**,** pp 106-10.

Kilpi, T., Peltola, H., Jauhiainen, T. & Kallio, M. J. 1995. Oral glycerol and intravenous dexamethasone in preventing neurologic and audiologic sequelae of childhood bacterial meningitis. The Finnish Study Group. *Pediatric Infectious Disease Journal,* 14(4)**,** pp 270-8.

Kim, P. E., Musher, D. M., Glezen, W. P., Rodriguez-Barradas, M. C., Nahm, W. K. & Wright, C. E. 1996. Association of invasive pneumococcal disease with season, atmospheric conditions, air pollution, and the isolation of respiratory viruses. *Clin Infect Dis,* 22(1)**,** pp 100-6.

Kinlin, L. M., Spain, C. V., Ng, V., Johnson, C. C., White, A. N. & Fisman, D. N. 2009. Environmental exposures and invasive meningococcal disease: an evaluation of effects on varying time scales. *Am J Epidemiol,* 169(5)**,** pp 588-95.

Kjaergaard, S., Goldinger, M. P. & Hojberg, A. S. 2006. [Pediatric septic shock--timing, volume resuscitation and anticoagulation therapy]. *Ugeskr Laeger,* 168(3)**,** pp 289-90.

Klein, M., Koedel, U., Kastenbauer, S., Pfister, H. W., van de Beek, D., Schut, E. S., Brouwer, M. C. & Steiner, I. 2010. Delayed cerebral thrombosis after initial good recovery from pneumococcal meningitis: past as prologue: delayed stroke as a parainfectious process of bacterial meningitis? *Neurology,* 75(2)**,** pp 193; author reply 193-4.

Klein, M., Koedel, U., Pfefferkorn, T., Zeller, G., Woehrl, B. & Pfister, H. W. 2011. Arterial cerebrovascular complications in 94 adults with acute bacterial meningitis. *Crit Care,* 15(6)**,** pp R281.

Kleines, M., Schiefer, J., Stienen, A., Blaum, M., Ritter, K. & Hausler, M. 2011. Expanding the spectrum of neurological disease associated with Epstein-Barr virus activity. *Eur J Clin Microbiol Infect Dis,* 30(12)**,** pp 1561-9.

Klugman, K. P., Walsh, A. L., Phiri, A. & Molyneux, E. M. 2008. Mortality in penicillin-resistant pneumococcal meningitis. *Pediatr Infect Dis J,* 27(7)**,** pp 671-2.

Knaus, W. A., Draper, E. A., Wagner, D. P. & Zimmerman, J. E. 1985. APACHE II: a severity of disease classification system. *Crit Care Med,* 13(10)**,** pp 818-29.

Kneen, R., Solomon, T. & Appleton, R. 2002. The role of lumbar puncture in suspected CNS infection--a disappearing skill? *Arch Dis Child,* 87(3)**,** pp 181-3.

Koenig, M. A., Bryan, M., Lewin, J. L., 3rd, Mirski, M. A., Geocadin, R. G. & Stevens, R. D. 2008. Reversal of transtentorial herniation with hypertonic saline.[see comment]. *Neurology,* 70(13)**,** pp 1023-9.

Koomen, I., Grobbee, D. E., Roord, J. J., Jennekens-Schinkel, A., van der Lei, H. D., Kraak, M. A. & van Furth, A. M. 2004. Prediction of academic and behavioural limitations in school-age survivors of bacterial meningitis. *Acta Paediatr,* 93(10)**,** pp 1378-85.

Kortgen, A., Niederprum, P. & Bauer, M. 2006. Implementation of an evidence-based "standard operating procedure" and outcome in septic shock. *Crit Care Med,* 34(4)**,** pp 943-9.

Koster-Rasmussen, R., Korshin, A. & Meyer, C. N. 2008. Antibiotic treatment delay and outcome in acute bacterial meningitis. *J Infect,* 57(6)**,** pp 449-54.

Kourtis, A. P., Bramson, B., van der Horst, C., Kazembe, P., Ahmed, Y., Chasela, C., Hosseinipour, M., Knight, R., Lugalia, L., Tegha, G., Joaki, G., Jafali, R. & Jamieson, D. J. 2005. Low absolute neutrophil counts in african infants. *J Int Assoc Physicians AIDS Care (Chic),* 4(3)**,** pp 73-6.

Krasinski, K., Nelson, J. D., Butler, S., Luby, J. P. & Kusmiesz, H. 1987. Possible association of mycoplasma and viral respiratory infections with bacterial meningitis. *Am J Epidemiol,* 125(3)**,** pp 499-508.

Kuan, W. S., Mahadevan, M., Tan, J. H., Guo, J. & Ibrahim, I. 2013. Feasibility of introduction and implementation of the Surviving Sepsis Campaign bundle in a Singapore emergency department. *Eur J Emerg Med,* 20(5)**,** pp 344-9.

Lacroix, S., Feinstein, D. & Rivest, S. 1998. The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations. *Brain Pathol,* 8(4)**,** pp 625-40.

Lamkanfi, M. & Dixit, V. M. 2014. Mechanisms and functions of inflammasomes. *Cell,* 157(5)**,** pp 1013-22.

Lazzarini, L., Toti, M., Fabris, P., Conti, E., Magni, G., Mazzotta, F. & De Lalla, F. 2008. Clinical features of bacterial meningitis in Italy: a multicenter prospective observational study. *J Chemother,* 20(4)**,** pp 478-87.

Lebel, M. H., Freij, B. J., Syrogiannopoulos, G. A., Chrane, D. F., Hoyt, M. J., Stewart, S. M., Kennard, B. D., Olsen, K. D. & McCracken, G. H., Jr. 1988. Dexamethasone therapy for bacterial meningitis. Results of two double-blind, placebo-controlled trials. *New England Journal of Medicine,* 319(15)**,** pp 964-71.

Leib, S. L., Leppert, D., Clements, J. & Tauber, M. G. 2000. Matrix metalloproteinases contribute to brain damage in experimental pneumococcal meningitis. *Infect Immun,* 68(2)**,** pp 615-20.

Leimkugel, J., Adams Forgor, A., Gagneux, S., Pfluger, V., Flierl, C., Awine, E., Naegeli, M., Dangy, J. P., Smith, T., Hodgson, A. & Pluschke, G. 2005. An outbreak of serotype 1 Streptococcus pneumoniae meningitis in northern Ghana with features that are characteristic of Neisseria meningitidis meningitis epidemics. *J Infect Dis,* 192(2)**,** pp 192-9.

Lepper, M. H. & Dowling, H. F. 1951. Treatment of pneumococcic meningitis with penicillin compared with penicillin plus aureomycin; studies including observations on an apparent antagonism between penicillin and aureomycin. *AMA Arch Intern Med,* 88(4)**,** pp 489-94.

Leppert, D., Leib, S. L., Grygar, C., Miller, K. M., Schaad, U. B. & Hollander, G. A. 2000. Matrix metalloproteinase (MMP)-8 and MMP-9 in cerebrospinal fluid during bacterial meningitis: association with blood-brain barrier damage and neurological sequelae. *Clin Infect Dis,* 31(1)**,** pp 80-4.

Lepur, D. & Barsic, B. 2007. Community-acquired bacterial meningitis in adults: antibiotic timing in disease course and outcome. *Infection,* 35(4)**,** pp 225-31.

Lepur, D., Kutlesa, M. & Barsic, B. 2011. Induced hypothermia in adult community-acquired bacterial meningitis--more than just a possibility? *J Infect,* 62(2)**,** pp 172-7.

Leverstein-van Hall, M. A., Hopmans, T. E., van der Sprenkel, J. W., Blok, H. E., van der Mark, W. A., Hanlo, P. W. & Bonten, M. J. 2010. A bundle approach to reduce the incidence of external ventricular and lumbar drain-related infections. *J Neurosurg,* 112(2)**,** pp 345-53.

Levy, M. M., Artigas, A., Phillips, G. S., Rhodes, A., Beale, R., Osborn, T., Vincent, J. L., Townsend, S., Lemeshow, S. & Dellinger, R. P. 2012. Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study. *Lancet Infect Dis,* 12(12)**,** pp 919-24.

Levy, M. M., Pronovost, P. J., Dellinger, R. P., Townsend, S., Resar, R. K., Clemmer, T. P. & Ramsay, G. 2004. Sepsis change bundles: converting guidelines into meaningful change in behavior and clinical outcome. *Crit Care Med,* 32(11 Suppl)**,** pp S595-7.

Lewis, D. K., Whitty, C. J., Walsh, A. L., Epino, H., Broek, N. R., Letsky, E. A., Munthali, C., Mukiibi, J. M. & Boeree, M. J. 2005. Treatable factors associated with severe anaemia in adults admitted to medical wards in Blantyre, Malawi, an area of high HIV seroprevalence. *Trans R Soc Trop Med Hyg,* 99(8)**,** pp 561-7.

Li, J., Wang, Y., Wang, X., Ye, L., Zhou, Y., Persidsky, Y. & Ho, W. 2013. Immune activation of human brain microvascular endothelial cells inhibits HIV replication in macrophages. *Blood,* 121(15)**,** pp 2934-42.

Liechti, F. D., Grandgirard, D., Leppert, D. & Leib, S. L. 2014. Matrix metalloproteinase inhibition lowers mortality and brain injury in experimental pneumococcal meningitis. *Infect Immun,* 82(4)**,** pp 1710-8.

Lindvall, P., Ahlm, C., Ericsson, M., Gothefors, L., Naredi, S. & Koskinen, L. O. 2004. Reducing intracranial pressure may increase survival among patients with bacterial meningitis. *Clin Infect Dis,* 38(3)**,** pp 384-90.

Lipshutz, A. K., Fee, C., Schell, H., Campbell, L., Taylor, J., Sharpe, B. A., Nguyen, J. & Gropper, M. A. 2008. Strategies for success: A PDSA analysis of three QI initiatives in critical care. *Jt Comm J Qual Patient Saf,* 34(8)**,** pp 435-44.

Loh, E., Kugelberg, E., Tracy, A., Zhang, Q., Gollan, B., Ewles, H., Chalmers, R., Pelicic, V. & Tang, C. M. 2013. Temperature triggers immune evasion by Neisseria meningitidis. *Nature,* 502(7470)**,** pp 237-40.

Lorenzl, S., Koedel, U. & Pfister, H. W. 1996. Mannitol, but not allopurinol, modulates changes in cerebral blood flow, intracranial pressure, and brain water content during pneumococcal meningitis in the rat. *Critical Care Medicine,* 24(11)**,** pp 1874-80.

Lovera, D. & Arbo, A. 2005. Risk factors for mortality in Paraguayan children with pneumococcal bacterial meningitis. *Trop Med Int Health,* 10(12)**,** pp 1235-41.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S. Y., Alvarado, M., Anderson, H. R., Anderson, L. M., Andrews, K. G., Atkinson, C., Baddour, L. M., Barker-Collo, S., Bartels, D. H., Bell, M. L., Benjamin, E. J., Bennett, D., Bhalla, K., Bikbov, B., Bin Abdulhak, A., Birbeck, G., Blyth, F., Bolliger, I., Boufous, S., Bucello, C., Burch, M., Burney, P., Carapetis, J., Chen, H., Chou, D., Chugh, S. S., Coffeng, L. E., Colan, S. D., Colquhoun, S., Colson, K. E., Condon, J., Connor, M. D., Cooper, L. T., Corriere, M., Cortinovis, M., de Vaccaro, K. C., Couser, W., Cowie, B. C., Criqui, M. H., Cross, M., Dabhadkar, K. C., Dahodwala, N., De Leo, D., Degenhardt, L., Delossantos, A., Denenberg, J., Des Jarlais, D. C., Dharmaratne, S. D., Dorsey, E. R., Driscoll, T., Duber, H., Ebel, B., Erwin, P. J., Espindola, P., Ezzati, M., Feigin, V., Flaxman, A. D., Forouzanfar, M. H., Fowkes, F. G., Franklin, R., Fransen, M., Freeman, M. K., Gabriel, S. E., Gakidou, E., Gaspari, F., Gillum, R. F., Gonzalez-Medina, D., Halasa, Y. A., Haring, D., Harrison, J. E., Havmoeller, R., Hay, R. J., Hoen, B., Hotez, P. J., Hoy, D., Jacobsen, K. H., James, S. L., Jasrasaria, R., Jayaraman, S., Johns, N., Karthikeyan, G., Kassebaum, N., Keren, A., Khoo, J. P., Knowlton, L. M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Lipnick, M., Lipshultz, S. E., Ohno, S. L., et al. 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet,* 380(9859)**,** pp 2095-128.

Lu, C. H., Chang, W. N. & Chang, H. W. 2001a. The prognostic factors of adult tuberculous meningitis. *Infection,* 29(6)**,** pp 299-304.

Lu, C. H., Chang, W. N. & Chang, H. W. 2001b. Streptococcal meningitis in adults: therapeutic outcomes and prognostic factors. *Clinical Neurology & Neurosurgery,* 103(3)**,** pp 137-42.

Lu, C. H., Chang, W. N., Chang, H. W. & Chuang, Y. C. 1999. The prognostic factors of cryptococcal meningitis in HIV-negative patients. *Journal of Hospital Infection,* 42(4)**,** pp 313-20.

Lucas, M. J., Brouwer, M. C. & van de Beek, D. 2013. Delayed cerebral thrombosis in bacterial meningitis: a prospective cohort study. *Intensive Care Med,* 39(5)**,** pp 866-71.

Ma, Y., Wen, X., Peng, J., Lu, Y., Guo, Z. & Lu, J. 2012. Systematic review and meta-analysis on the association between outpatient statins use and infectious disease-related mortality. *PLoS One,* 7(12)**,** pp e51548.

Macfarlane, J. T., Anjorin, F. I., Cleland, P. G., Hassan-King, M., Tor-Agbidye, S., Wali, S. S., Weir, W. R., Whittle, H. C., Yahaya, H. N. & Greenwood, B. M. 1979. Single injection treatment of meningococcal meningitis. 1. Long-acting penicillin. *Trans R Soc Trop Med Hyg,* 73(6)**,** pp 693-7.

Maconochie, I., Baumer, H. & Stewart, M. E. 2008. Fluid therapy for acute bacterial meningitis. *Cochrane Database Syst Rev,* 1)**,** pp CD004786.

Mai, N. T., Tuan, T. V., Wolbers, M., Hoang, D. M., Nga, T. V., Chau, T. T., Chuong, L. V., Sinh, D. X., Nghia, H. D., Phong, N. D., Phu, N. H., Diep, T. S., Hang, H. T., Chau, N., Farrar, J., Schultsz, C., Hien, T. T. & Simmons, C. P. 2009. Immunological and biochemical correlates of adjunctive dexamethasone in Vietnamese adults with bacterial meningitis. *Clin Infect Dis,* 49(9)**,** pp 1387-92.

Maitland, K., Kiguli, S., Opoka, R. O., Engoru, C., Olupot-Olupot, P., Akech, S. O., Nyeko, R., Mtove, G., Reyburn, H., Lang, T., Brent, B., Evans, J. A., Tibenderana, J. K., Crawley, J., Russell, E. C., Levin, M., Babiker, A. G. & Gibb, D. M. 2011a. Mortality after fluid bolus in African children with severe infection. *N Engl J Med,* 364(26)**,** pp 2483-95.

Maitland, K., Molyneux, S., Boga, M., Kiguli, S. & Lang, T. 2011b. Use of deferred consent for severely ill children in a multi-centre phase III trial. *Trials,* 12(90.

Malawi, N. S. O. o. 1998. Malawi Population and Housing Census. *Malawi National Statistics*.

Manga, N. M., Ndour, C. T., Diop, S. A., Ka-Sall, R., Dia, N. M., Seydi, M., Soumare, M., Diop, B. M., Sow, A. I. & Sow, P. S. 2008. [Adult purulent meningitis caused by Streptococcus pneumoniae in Dakar, Senegal]. *Med Trop (Mars),* 68(6)**,** pp 625-8.

Marcos, M. A., Martinez, E., Almela, M., Mensa, J. & Jimenez de Anta, M. T. 2001. New rapid antigen test for diagnosis of pneumococcal meningitis. *Lancet,* 357(9267)**,** pp 1499-500.

Marriott, H. M., Mitchell, T. J. & Dockrell, D. H. 2008. Pneumolysin: a double-edged sword during the host-pathogen interaction. *Curr Mol Med,* 8(6)**,** pp 497-509.

Martin, N. G., Sadarangani, M., Pollard, A. J. & Goldacre, M. J. 2014. Hospital admission rates for meningitis and septicaemia caused by Haemophilus influenzae, Neisseria meningitidis, and Streptococcus pneumoniae in children in England over five decades: a population-based observational study. *Lancet Infect Dis,* 14(5)**,** pp 397-405.

Martinon, F., Burns, K. & Tschopp, J. 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell,* 10(2)**,** pp 417-26.

Martos, A., Cabellos, C., Martinez-Lacasa, J., Viladrich, P. F. & Gudiol, F. 1995. [Protective effect of dexamethasone and phenytoin in the treatment of experimental pneumococcal meningitis]. *Enfermedades Infecciosas y Microbiologia Clinica,* 13(3)**,** pp 146-50.

Marwick, C. & Davey, P. 2009. Care bundles: the holy grail of infectious risk management in hospital? *Curr Opin Infect Dis,* 22(4)**,** pp 364-9.

Mbelesso, P., Tatangba-Bakozo, A. & Fikouma, V. 2006. [Bacterial meningiditis in adult patients in Central African hospitals]. *Bull Soc Pathol Exot,* 99(4)**,** pp 261-3.

McCormick, D. W., Wilson, M. L., Mankhambo, L., Phiri, A., Chimalizeni, Y., Kawaza, K., Denis, B., Carrol, E. D. & Molyneux, E. M. 2012. Risk Factors for Death and Severe Sequelae in Malawian Children with Bacterial Meningitis, 1997-2010. *Pediatr Infect Dis J*.

McCullers, J. A. 2006. Insights into the interaction between influenza virus and pneumococcus. *Clin Microbiol Rev,* 19(3)**,** pp 571-82.

McIntyre, L. A., Fergusson, D., Cook, D. J., Rankin, N., Dhingra, V., Granton, J., Magder, S., Stiell, I., Taljaard, M. & Hebert, P. C. 2008. Fluid resuscitation in the management of early septic shock (FINESS): a randomized controlled feasibility trial. *Can J Anaesth,* 55(12)**,** pp 819-26.

McIntyre, P. B., O'Brien, K. L., Greenwood, B. & van de Beek, D. 2012. Effect of vaccines on bacterial meningitis worldwide. *Lancet,* 380(9854)**,** pp 1703-11.

Mendoza, S. A. 1976. Syndrome of inappropriate antidiuretic hormone secretion (SIADH). *Pediatric Clinics of North America,* 23(4)**,** pp 681-90.

Merkelbach, S., Rohn, S., Konig, J. & Muller, M. 1999. Usefulness of clinical scores to predict outcome in bacterial meningitis. *Infection,* 27(4-5)**,** pp 239-43.

Mertsola, J. 1991. Cytokines in the pathogenesis of bacterial meningitis. *Trans R Soc Trop Med Hyg,* 85 Suppl 1(17-8.

Mertsola, J., Kennedy, W. A., Waagner, D., Saez-Llorens, X., Olsen, K., Hansen, E. J. & McCracken, G. H., Jr. 1991. Endotoxin concentrations in cerebrospinal fluid correlate with clinical severity and neurologic outcome of Haemophilus influenzae type B meningitis. *Am J Dis Child,* 145(10)**,** pp 1099-103.

Mertsola, J., Ramilo, O., Mustafa, M. M., Saez-Llorens, X., Hansen, E. J. & McCracken, G. H., Jr. 1989. Release of endotoxin after antibiotic treatment of Gram-negative bacterial meningitis. *Pediatr Infect Dis J,* 8(12)**,** pp 904-6.

Michael, B., Menezes, B. F., Cunniffe, J., Miller, A., Kneen, R., Francis, G., Beeching, N. J. & Solomon, T. 2010. Effect of delayed lumbar punctures on the diagnosis of acute bacterial meningitis in adults. *Emerg Med J,* 27(6)**,** pp 433-8.

Michelow, I. C., Nicol, M., Tiemessen, C., Chezzi, C. & Pettifor, J. M. 2000. Value of cerebrospinal fluid leukocyte aggregation in distinguishing the causes of meningitis in children. *Pediatr Infect Dis J,* 19(1)**,** pp 66-72.

Milani, W. R., Antibas, P. L. & Prado, G. F. 2011. Cooling for cerebral protection during brain surgery. *Cochrane Database Syst Rev,* 10)**,** pp CD006638.

Miller, F., Phan, G., Brissac, T., Bouchiat, C., Lioux, G., Nassif, X. & Coureuil, M. 2014. The hypervariable region of meningococcal major pilin PilE controls the host cell response via antigenic variation. *MBio,* 5(1)**,** pp e01024-13.

Minns, R. A. & Engleman, H. M. 1988. The use of CSF pressure recordings in acute purulent meningitis. *Zeitschrift fur Kinderchirurgie,* 43 Suppl 2(28-9.

Moisi, J. C., Saha, S. K., Falade, A. G., Njanpop-Lafourcade, B. M., Oundo, J., Zaidi, A. K., Afroj, S., Bakare, R. A., Buss, J. K., Lasi, R., Mueller, J., Odekanmi, A. A., Sangare, L., Scott, J. A., Knoll, M. D., Levine, O. S. & Gessner, B. D. 2009. Enhanced diagnosis of pneumococcal meningitis with use of the Binax NOW immunochromatographic test of Streptococcus pneumoniae antigen: a multisite study. *Clin Infect Dis,* 48 Suppl 2(S49-56.

Molesworth, A. M., Cuevas, L. E., Connor, S. J., Morse, A. P. & Thomson, M. C. 2003. Environmental risk and meningitis epidemics in Africa. *Emerg Infect Dis,* 9(10)**,** pp 1287-93.

Moller, K., Hogh, P., Larsen, F. S., Strauss, G. I., Skinhoj, P., Sperling, B. K. & Knudsen, G. M. 2000. Regional cerebral blood flow during hyperventilation in patients with acute bacterial meningitis. *Clinical Physiology,* 20(5)**,** pp 399-410.

Moller, K., Strauss, G. I., Thomsen, G., Larsen, F. S., Holm, S., Sperling, B. K., Skinhoj, P. & Knudsen, G. M. 2002. Cerebral blood flow, oxidative metabolism and cerebrovascular carbon dioxide reactivity in patients with acute bacterial meningitis. *Acta Anaesthesiologica Scandinavica,* 46(5)**,** pp 567-78.

Molyneux, A. J. 2006. Changes in the treatment of patients with subarachnoid haemorrhage following publication of the International Subarachnoid Aneurysm Trial. *Clinical Neurology & Neurosurgery,* 108(2)**,** pp 115-6.

Molyneux, E. 1996. Managing meningitis and severe malaria. *Child Health Dialogue,* 3-4)**,** pp 6-7.

Molyneux, E. 2005. Human immunodeficiency virus infection and pediatric bacterial meningitis in developing countries. *J Neurovirol,* 11 Suppl 3(6-10.

Molyneux, E., Ahmad, S. & Robertson, A. 2006. Improved triage and emergency care for children reduces inpatient mortality in a resource-constrained setting. *Bull World Health Organ,* 84(4)**,** pp 314-9.

Molyneux, E., Nizami, S. Q., Saha, S., Huu, K. T., Azam, M., Bhutta, Z. A., Zaki, R., Weber, M. W. & Qazi, S. A. 2011. 5 versus 10 days of treatment with ceftriaxone for bacterial meningitis in children: a double-blind randomised equivalence study. *Lancet,* 377(9780)**,** pp 1837-45.

Molyneux, E. M., Kawaza, K., Phiri, A., Chimalizeni, Y., Mankhambo, L., Schwalbe, E., Kataja, M., Pensulo, P., Chilton, L. & Peltola, H. 2014. Glycerol and acetaminophen as adjuvant therapy did not affect the outcome of bacterial meningitis in malawian children. *Pediatr Infect Dis J,* 33(2)**,** pp 214-6.

Molyneux, E. M., Rochford, R., Griffin, B., Newton, R., Jackson, G., Menon, G., Harrison, C. J., Israels, T. & Bailey, S. 2012. Burkitt's lymphoma. *Lancet,* 379(9822)**,** pp 1234-44.

Molyneux, E. M., Tembo, M., Kayira, K., Bwanaisa, L., Mweneychanya, J., Njobvu, A., Forsyth, H., Rogerson, S., Walsh, A. L. & Molyneux, M. E. 2003. The effect of HIV infection on paediatric bacterial meningitis in Blantyre, Malawi. *Arch Dis Child,* 88(12)**,** pp 1112-8.

Molyneux, E. M., Walsh, A. L., Forsyth, H., Tembo, M., Mwenechanya, J., Kayira, K., Bwanaisa, L., Njobvu, A., Rogerson, S. & Malenga, G. 2002. Dexamethasone treatment in childhood bacterial meningitis in Malawi: a randomised controlled trial. *Lancet,* 360(9328)**,** pp 211-8.

Mook-Kanamori, B. B., Geldhoff, M., van der Poll, T. & van de Beek, D. 2011. Pathogenesis and pathophysiology of pneumococcal meningitis. *Clin Microbiol Rev,* 24(3)**,** pp 557-91.

Mook-Kanamori, B. B., Rouse, M. S., Kang, C. I., van de Beek, D., Steckelberg, J. M. & Patel, R. 2009. Daptomycin in experimental murine pneumococcal meningitis. *BMC Infect Dis,* 9(50.

Morton, C. C., Aitchison, A. J., Gehrig, K. & Ridgway, N. D. 2013. A mechanism for suppression of the CDP-choline pathway during apoptosis. *J Lipid Res,* 54(12)**,** pp 3373-84.

Mourvillier, B., Tubach, F., van de Beek, D., Garot, D., Pichon, N., Georges, H., Lefevre, L. M., Bollaert, P. E., Boulain, T., Luis, D., Cariou, A., Girardie, P., Chelha, R.,

Megarbane, B., Delahaye, A., Chalumeau-Lemoine, L., Legriel, S., Beuret, P., Brivet, F., Bruel, C., Camou, F., Chatellier, D., Chillet, P., Clair, B., Constantin, J. M., Duguet, A., Galliot, R., Bayle, F., Hyvernat, H., Ouchenir, K., Plantefeve, G., Quenot, J. P., Richecoeur, J., Schwebel, C., Sirodot, M., Esposito-Farese, M., Le Tulzo, Y. & Wolff, M. 2013. Induced hypothermia in severe bacterial meningitis: a randomized clinical trial. *JAMA,* 310(20)**,** pp 2174-83.

Mueller, J. E., Yaro, S., Madec, Y., Somda, P. K., Idohou, R. S., Lafourcade, B. M., Drabo, A., Tarnagda, Z., Sangare, L., Traore, Y., Fontanet, A. & Gessner, B. D. 2008. Association of respiratory tract infection symptoms and air humidity with meningococcal carriage in Burkina Faso. *Trop Med Int Health,* 13(12)**,** pp 1543-52.

Mueller, J. E., Yaro, S., Ouedraogo, M. S., Levina, N., Njanpop-Lafourcade, B. M., Tall, H., Idohou, R. S., Sanou, O., Kroman, S. S., Drabo, A., Nacro, B., Millogo, A., van der Linden, M. & Gessner, B. D. 2012. Pneumococci in the African meningitis belt: meningitis incidence and carriage prevalence in children and adults. *PLoS One,* 7(12)**,** pp e52464.

Muhammad, R. D., Oza-Frank, R., Zell, E., Link-Gelles, R., Narayan, K. M., Schaffner, W., Thomas, A., Lexau, C., Bennett, N. M., Farley, M. M., Harrison, L. H., Reingold, A., Hadler, J., Beall, B., Klugman, K. P. & Moore, M. R. 2013. Epidemiology of invasive pneumococcal disease among high-risk adults since the introduction of pneumococcal conjugate vaccine for children. *Clin Infect Dis,* 56(5)**,** pp e59-67.

Muhlemann, K., Alexander, E. R., Weiss, N. S., Pepe, M. & Schopfer, K. 1996. Risk factors for invasive Haemophilus influenzae disease among children 2-16 years of age in the vaccine era, Switzerland 1991-1993. The Swiss H. Influenzae Study Group. *Int J Epidemiol,* 25(6)**,** pp 1280-5.

Murad, A., Ghostine, S. & Colohan, A. R. 2008. Controlled lumbar drainage in medically refractory increased intracranial pressure. A safe and effective treatment. *Acta Neurochir Suppl,* 102(89-91.

Mustafa, M. M., Lebel, M. H., Ramilo, O., Olsen, K. D., Reisch, J. S., Beutler, B. & McCracken, G. H., Jr. 1989a. Correlation of interleukin-1 beta and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis. *Journal of Pediatrics,* 115(2)**,** pp 208-13.

Mustafa, M. M., Ramilo, O., Mertsola, J., Risser, R. C., Beutler, B., Hansen, E. J. & McCracken, G. H., Jr. 1989b. Modulation of inflammation and cachectin activity in relation to treatment of experimental Hemophilus influenzae type b meningitis. *Journal of Infectious Diseases,* 160(5)**,** pp 818-25.

Mustafa, M. M., Ramilo, O., Saez-Llorens, X., Mertsola, J., Magness, R. R. & McCracken, G. H., Jr. 1989c. Prostaglandins E2 and I2, interleukin 1-beta, and tumor necrosis factor in cerebrospinal fluid in infants and children with bacterial meningitis. *Pediatr Infect Dis J,* 8(12)**,** pp 921-2.

Mustafa, M. M., Ramilo, O., Saez-Llorens, X., Mertsola, J. & McCracken, G. H., Jr. 1989d. Role of tumor necrosis factor alpha (cachectin) in experimental and clinical bacterial meningitis. *Pediatr Infect Dis J,* 8(12)**,** pp 907-8.

Mustafa, M. M., Ramilo, O., Syrogiannopoulos, G. A., Olsen, K. D., McCracken, G. H., Jr. & Hansen, E. J. 1989e. Induction of meningeal inflammation by outer membrane vesicles of Haemophilus influenzae type b. *Journal of Infectious Diseases,* 159(5)**,** pp 917-22.

Mutalima, N., Molyneux, E., Jaffe, H., Kamiza, S., Borgstein, E., Mkandawire, N., Liomba, G., Batumba, M., Lagos, D., Gratrix, F., Boshoff, C., Casabonne, D., Carpenter, L. M. & Newton, R. 2008. Associations between Burkitt lymphoma among children in Malawi and infection with HIV, EBV and malaria: results from a case-control study. *PLoS One,* 3(6)**,** pp e2505.

Mutoh, T., Kazumata, K., Ajiki, M., Ushikoshi, S. & Terasaka, S. 2007. Goal-directed fluid management by bedside transpulmonary hemodynamic monitoring after subarachnoid hemorrhage. *Stroke,* 38(12)**,** pp 3218-24.

Mutoh, T., Kazumata, K., Ishikawa, T. & Terasaka, S. 2009. Performance of bedside transpulmonary thermodilution monitoring for goal-directed hemodynamic management after subarachnoid hemorrhage. *Stroke,* 40(7)**,** pp 2368-74.

Mutoh, T., Kazumata, K., Terasaka, S., Taki, Y., Suzuki, A. & Ishikawa, T. 2014. Early intensive versus minimally invasive approach to postoperative hemodynamic management after subarachnoid hemorrhage. *Stroke,* 45(5)**,** pp 1280-4.

Myburgh, J. A. 2011. Fluid resuscitation in acute illness--time to reappraise the basics. *N Engl J Med,* 364(26)**,** pp 2543-4.

Na, S., Kuan, W. S., Mahadevan, M., Li, C. H., Shrikhande, P., Ray, S., Batech, M. & Nguyen, H. B. 2012. Implementation of early goal-directed therapy and the surviving sepsis campaign resuscitation bundle in Asia. *Int J Qual Health Care,* 24(5)**,** pp 452-62.

Nadel, S., Britto, J., Booy, R., Maconochie, I., Habibi, P. & Levin, M. 1998. Avoidable deficiencies in the delivery of health care to children with meningococcal disease. *J Accid Emerg Med,* 15(5)**,** pp 298-303.

Naranbhai, V., Chang, C. C., Durgiah, R., Omarjee, S., Lim, A., Moosa, M. Y., Elliot, J. H., Ndung'u, T., Lewin, S. R., French, M. A. & Carr, W. H. 2014. Compartmentalization of innate immune responses in the central nervous system during cryptococcal meningitis/HIV coinfection. *AIDS*.

Nathan, N., Borel, T., Djibo, A., Evans, D., Djibo, S., Corty, J. F., Guillerm, M., Alberti, K. P., Pinoges, L., Guerin, P. J. & Legros, D. 2005. Ceftriaxone as effective as long-acting chloramphenicol in short-course treatment of meningococcal meningitis during epidemics: a randomised non-inferiority study. *Lancet,* 366(9482)**,** pp 308-13.

Nau, R. & Eiffert, H. 2005. Minimizing the release of proinflammatory and toxic bacterial products within the host: a promising approach to improve outcome in life-threatening infections. *FEMS Immunol Med Microbiol,* 44(1)**,** pp 1-16.

Nazifi, S., Rezakhani, A. & Badran, M. 1997. Evaluation of hematological, serum biochemical and cerebrospinal fluid parameters in experimental bacterial meningitis in the calf. *Zentralblatt fur Veterinarmedizin - Reihe A,* 44(1)**,** pp 55-63.

Ndlovu, R. J. & Sihlangu, R. H. 1992. Preferred sources of information on AIDS among high school students from selected schools in Zimbabwe. *J Adv Nurs,* 17(4)**,** pp 507-13.

Neal, J. W. & Gasque, P. 2013. How does the brain limit the severity of inflammation and tissue injury during bacterial meningitis? *J Neuropathol Exp Neurol,* 72(5)**,** pp 370-85.

Nguyen, H. B., Kuan, W. S., Batech, M., Shrikhande, P., Mahadevan, M., Li, C. H., Ray, S. & Dengel, A. 2011. Outcome effectiveness of the severe sepsis resuscitation bundle with addition of lactate clearance as a bundle item: a multi-national evaluation. *Crit Care,* 15(5)**,** pp R229.

Nguyen, T. H., Tran, T. H., Thwaites, G., Ly, V. C., Dinh, X. S., Ho Dang, T. N., Dang, Q. T., Nguyen, D. P., Nguyen, H. P., To, S. D., Nguyen v, V., Nguyen, M. D., Campbell, J., Schultsz, C., Parry, C., Torok, M. E., White, N., Nguyen, T. C., Stepniewska, K. & Farrar, J. J. 2007. Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis. *N Engl J Med,* 357(24)**,** pp 2431-40.

Nielsen, H., Kvinesdal, B., Benfield, T. L., Lundgren, J. D. & Konradsen, H. B. 1998. Rapid loss of specific antibodies after pneumococcal vaccination in patients with human immunodeficiency virus-1 infection. *Scand J Infect Dis,* 30(6)**,** pp 597-601.

Nigrovic, L. E., Malley, R. & Kuppermann, N. 2012. Meta-analysis of bacterial meningitis score validation studies. *Arch Dis Child,* 97(9)**,** pp 799-805.

Nijman, R. G., Vergouwe, Y., Thompson, M., van Veen, M., van Meurs, A. H., van der Lei, J., Steyerberg, E. W., Moll, H. A. & Oostenbrink, R. 2013. Clinical prediction model to aid emergency doctors managing febrile children at risk of serious bacterial infections: diagnostic study. *BMJ,* 346(f1706.

Nisanevich, V., Felsenstein, I., Almogy, G., Weissman, C., Einav, S. & Matot, I. 2005. Effect of intraoperative fluid management on outcome after intraabdominal surgery. *Anesthesiology,* 103(1)**,** pp 25-32.

Nolan, C. M., McAllister, C. K., Walters, E. & Beaty, H. N. 1978. Experimental pneumococcal meningitis. IV. The effect of methyl prednisolone on meningeal inflammation. *J Lab Clin Med,* 91(6)**,** pp 979-88.

Noritomi, D. T., Ranzani, O. T., Monteiro, M. B., Ferreira, E. M., Santos, S. R., Leibel, F. & Machado, F. R. 2014. Implementation of a multifaceted sepsis education program in an emerging country setting: clinical outcomes and cost-effectiveness in a long-term follow-up study. *Intensive Care Med,* 40(2)**,** pp 182-91.

Nunes, M. C. & Madhi, S. A. 2012. Safety, immunogenicity and efficacy of pneumococcal conjugate vaccine in HIV-infected individuals. *Hum Vaccin Immunother,* 8(2)**,** pp 161-73.

Nunes, M. C., von Gottberg, A., de Gouveia, L., Cohen, C., Kuwanda, L., Karstaedt, A. S., Klugman, K. P. & Madhi, S. A. 2011. Persistent high burden of invasive pneumococcal disease in South African HIV-infected adults in the era of an antiretroviral treatment program. *PLoS One,* 6(11)**,** pp e27929.

Nyasulu, P., Cohen, C., De Gouveia, L., Feldman, C., Klugman, K. P. & von Gottberg, A. 2011. Increased risk of death in human immunodeficiency virus-infected children with pneumococcal meningitis in South Africa, 2003-2005. *Pediatr Infect Dis J,* 30(12)**,** pp 1075-80.

Oakley, A., Strange, V., Bonell, C., Allen, E. & Stephenson, J. 2006. Process evaluation in randomised controlled trials of complex interventions. *BMJ,* 332(7538)**,** pp 413-6.

Oates-Whitehead, R. M., Maconochie, I., Baumer, H. & Stewart, M. E. 2005. Fluid therapy for acute bacterial meningitis. *Cochrane Database Syst Rev,* 3)**,** pp CD004786.

Ogutu, B. R., Newton, C. R., Muchohi, S. N., Otieno, G. O. & Kokwaro, G. O. 2002. Phenytoin pharmacokinetics and clinical effects in African children following fosphenytoin and chloramphenicol coadministration. *Br J Clin Pharmacol,* 54(6)**,** pp 635-42.

Okike, I. O., Ribeiro, S., Ramsay, M. E., Heath, P. T., Sharland, M. & Ladhani, S. N. 2014. Trends in bacterial, mycobacterial, and fungal meningitis in England and Wales 2004-11: an observational study. *Lancet Infect Dis,* 14(4)**,** pp 301-7.

Okoromah, C. A. & Afolabi, B. B. 2004. Mannitol and other osmotic diuretics as adjuncts for treating cerebral malaria. *Cochrane Database Syst Rev,* 4)**,** pp CD004615.

Okubadejo, N. U. & Danesi, M. A. 2004. Diagnostic issues in cerebral malaria: a study of 112 adolescents and adults in Lagos, Nigeria. *Niger Postgrad Med J,* 11(1)**,** pp 10-4.

Oliveira, C. R., Morriss, M. C., Mistrot, J. G., Cantey, J. B., Doern, C. D. & Sanchez, P. J. 2014. Brain Magnetic Resonance Imaging of Infants with Bacterial Meningitis. *J Pediatr*.

Olivetta, E., Tirelli, V., Chiozzini, C., Scazzocchio, B., Romano, I., Arenaccio, C. & Sanchez, M. 2014. HIV-1 Nef impairs key functional activities in human macrophages through CD36 downregulation. *PLoS One,* 9(4)**,** pp e93699.

Opatowski, L., Varon, E., Dupont, C., Temime, L., van der Werf, S., Gutmann, L., Boelle, P. Y., Watier, L. & Guillemot, D. 2013. Assessing pneumococcal meningitis association with viral respiratory infections and antibiotics: insights from statistical and mathematical models. *Proc Biol Sci,* 280(1764)**,** pp 20130519.

Opravil, M., Fierz, W., Matter, L., Blaser, J. & Luthy, R. 1991. Poor antibody response after tetanus and pneumococcal vaccination in immunocompromised, HIV-infected patients. *Clin Exp Immunol,* 84(2)**,** pp 185-9.

Ostergaard, C., Yieng-Kow, R. V., Knudsen, J. D., Frimodt-Moller, N. & Espersen, F. 2003. Evaluation of fusidic acid in therapy of experimental Staphylococcus aureus meningitis. *Journal of Antimicrobial Chemotherapy,* 51(5)**,** pp 1301-5.

Ota, M. O., Roca, A., Bottomley, C., Hill, P. C., Egere, U., Greenwood, B. & Adegbola, R. A. 2012. Pneumococcal antibody concentrations of subjects in communities fully or partially vaccinated with a seven-valent pneumococcal conjugate vaccine. *PLoS One,* 7(8)**,** pp e42997.

Otero, R. M., Nguyen, H. B., Huang, D. T., Gaieski, D. F., Goyal, M., Gunnerson, K. J., Trzeciak, S., Sherwin, R., Holthaus, C. V., Osborn, T. & Rivers, E. P. 2006. Early goal-directed therapy in severe sepsis and septic shock revisited: concepts, controversies, and contemporary findings. *Chest,* 130(5)**,** pp 1579-95.

Ovstebo, R., Brandtzaeg, P., Brusletto, B., Haug, K. B., Lande, K., Hoiby, E. A. & Kierulf, P. 2004. Use of robotized DNA isolation and real-time PCR to quantify and identify close correlation between levels of Neisseria meningitidis DNA and lipopolysaccharides in plasma and cerebrospinal fluid from patients with systemic meningococcal disease. *J Clin Microbiol,* 42(7)**,** pp 2980-7.

Pagliano, P., Fusco, U., Attanasio, V., Rossi, M., Pantosti, A., Conte, M. & Faella, F. S. 2007. Pneumococcal meningitis in childhood: a longitudinal prospective study. *FEMS Immunol Med Microbiol,* 51(3)**,** pp 488-95.

Palmu, A. A., Kaijalainen, T., Saukkoriipi, A., Leinonen, M. & Kilpi, T. M. 2012. Nasopharyngeal carriage of Streptococcus pneumoniae and pneumococcal urine antigen test in healthy elderly subjects. *Scand J Infect Dis,* 44(6)**,** pp 433-8.

Pankey, G. A. & Sabath, L. D. 2004. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clin Infect Dis,* 38(6)**,** pp 864-70.

Paris, M. M., Hickey, S. M., Trujillo, M., Ahmed, A., Olsen, K. & McCracken, G. H., Jr. 1997. The effect of interleukin-10 on meningeal inflammation in experimental bacterial meningitis. *Journal of Infectious Diseases,* 176(5)**,** pp 1239-46.

Paris, M. M., Hickey, S. M., Uscher, M. I., Shelton, S., Olsen, K. D. & McCracken, G. H., Jr. 1994. Effect of dexamethasone on therapy of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. *Antimicrob Agents Chemother,* 38(6)**,** pp 1320-4.

Park, W. S. & Chang, Y. S. 2000. Effects of decreased cerebral perfusion pressure on cerebral hemodynamics, brain cell membrane function and energy metabolism during the early phase of experimental Escherichia coli meningitis in the newborn piglet. *Journal of Korean Medical Science,* 15(2)**,** pp 203-10.

Park, W. S., Chang, Y. S., Ko, S. Y., Kang, M. J., Han, J. M. & Lee, M. 1999. Efficacy of anti-tumor necrosis factor-alpha antibody as an adjunctive therapy in experimental Escherichia coli meningitis in the newborn piglet. *Biology of the Neonate,* 75(6)**,** pp 377-87.

Park, W. S., Chang, Y. S. & Lee, M. 2001. Effect of hypothermia on brain cell membrane function and energy metabolism in experimental Escherichia coli meningitis in the newborn piglet. *Neurochemical Research,* 26(4)**,** pp 369-74.

Park, W. S., Chang, Y. S., Shim, J. W., Kim, M. J., Ko, S. Y., Kim, S. S., Hwang, J. H., Choi, C. W. & Lee, M. 2003. Effects of dopamine infusion on cerebral blood flow, brain cell

membrane function and energy metabolism in experimental Escherichia coli meningitis in the newborn piglet. *Journal of Korean Medical Science,* 18(6)**,** pp 869-75.

Parkes-Ratanshi, R., Wakeham, K., Levin, J., Namusoke, D., Whitworth, J., Coutinho, A., Mugisha, N. K., Grosskurth, H., Kamali, A. & Lalloo, D. G. 2011. Primary prophylaxis of cryptococcal disease with fluconazole in HIV-positive Ugandan adults: a double-blind, randomised, placebo-controlled trial. *Lancet Infect Dis,* 11(12)**,** pp 933-41.

Patel, V. B., Theron, G., Lenders, L., Matinyena, B., Connolly, C., Singh, R., Coovadia, Y., Ndung'u, T. & Dheda, K. 2013. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous meningitis in a high burden setting: a prospective study. *PLoS Med,* 10(10)**,** pp e1001536.

Pecco, P., Pavesio, D. & Peisino, M. G. 1991. [Rational bases of current etiopathogenetic therapy of bacterial meningitis. Review of the literature and personal experience in 122 pediatric cases]. *Minerva Pediatrica,* 43(12)**,** pp 753-75.

Pelkonen, T., Roine, I., Anjos, E., Kaijalainen, S., Roivainen, M., Peltola, H. & Pitkaranta, A. 2012. Picornaviruses in cerebrospinal fluid of children with meningitis in Luanda, Angola. *J Med Virol,* 84(7)**,** pp 1080-3.

Pelkonen, T., Roine, I., Anjos, E., Maki, M., Peltola, H. & Pitkaranta, A. 2013. Herpesviruses in cerebrospinal fluid of children with meningitis in Luanda, Angola. *Acta Paediatr,* 102(6)**,** pp e281-3.

Pelkonen, T., Roine, I., Cruzeiro, M. L., Pitkaranta, A., Kataja, M. & Peltola, H. 2011. Slow initial beta-lactam infusion and oral paracetamol to treat childhood bacterial meningitis: a randomised, controlled trial. *Lancet Infect Dis,* 11(8)**,** pp 613-21.

Pelkonen, T., Roine, I., Monteiro, L., Correia, M., Pitkaranta, A., Bernardino, L. & Peltola, H. 2009. Risk factors for death and severe neurological sequelae in childhood bacterial meningitis in sub-Saharan Africa. *Clin Infect Dis,* 48(8)**,** pp 1107-10.

Peltola, H. 2001. Burden of meningitis and other severe bacterial infections of children in africa: implications for prevention. *Clin Infect Dis,* 32(1)**,** pp 64-75.

Peltola, H. & Roine, I. 2009. Improving the outcomes in children with bacterial meningitis. *Curr Opin Infect Dis,* 22(3)**,** pp 250-5.

Peltola, H., Roine, I., Fernandez, J., Zavala, I., Ayala, S. G., Mata, A. G., Arbo, A., Bologna, R., Mino, G., Goyo, J., Lopez, E., de Andrade, S. D. & Sarna, S. 2007. Adjuvant glycerol and/or dexamethasone to improve the outcomes of childhood bacterial meningitis: a prospective, randomized, double-blind, placebo-controlled trial.[see comment]. *Clinical Infectious Diseases,* 45(10)**,** pp 1277-86.

Perea-Milla, E., Olalla, J., Sanchez-Cantalejo, E., Martos, F., Matute-Cruz, P., Carmona-Lopez, G., Fornieles, Y., Cayuela, A. & Garcia-Alegria, J. 2009. Pre-hospital antibiotic treatment and mortality caused by invasive meningococcal disease, adjusting for indication bias. *BMC Public Health,* 9(95.

Petrara, M. R., Freguja, R., Gianesin, K., Zanchetta, M. & De Rossi, A. 2013. Epstein-Barr virus-driven lymphomagenesis in the context of human immunodeficiency virus type 1 infection. *Front Microbiol,* 4(311.

Pfister, H. W., Koedel, U., Haberl, R. L., Dirnagl, U., Feiden, W., Ruckdeschel, G. & Einhaupl, K. M. 1990. Microvascular changes during the early phase of experimental bacterial meningitis. *J Cereb Blood Flow Metab,* 10(6)**,** pp 914-22.

Phiri, K. S., Calis, J. C., Faragher, B., Nkhoma, E., Ng'oma, K., Mangochi, B., Molyneux, M. E. & van Hensbroek, M. B. 2008. Long term outcome of severe anaemia in Malawian children. *PLoS One,* 3(8)**,** pp e2903.

Pido-Lopez, J., Kwok, W. W., Mitchell, T. J., Heyderman, R. S. & Williams, N. A. 2011. Acquisition of pneumococci specific effector and regulatory Cd4+ T cells localising within human upper respiratory-tract mucosal lymphoid tissue. *PLoS Pathog,* 7(12)**,** pp e1002396.

Power, R., Langhaug, L. F., Nyamurera, T., Wilson, D., Bassett, M. T. & Cowan, F. M. 2004. Developing complex interventions for rigorous evaluation--a case study from rural Zimbabwe. *Health Educ Res,* 19(5)**,** pp 570-5.

Prasad, K., Kumar, A., Gupta, P. K. & Singhal, T. 2007. Third generation cephalosporins versus conventional antibiotics for treating acute bacterial meningitis. *Cochrane Database Syst Rev,* 4)**,** pp CD001832.

Prasad, K., Singhal, T., Jain, N. & Gupta, P. K. 2004. Third generation cephalosporins versus conventional antibiotics for treating acute bacterial meningitis. *Cochrane Database Syst Rev,* 2)**,** pp CD001832.

Pryde, K., Walker, W. T., Hollingsworth, C., Haywood, P., Baird, J., Hussey, M., Freeman, A., Gawne-Cain, M., Harms, B., Kirkham, F. J. & Faust, S. N. 2013. Stroke in paediatric pneumococcal meningitis: a cross-sectional population-based study. *Arch Dis Child,* 98(8)**,** pp 647-9.

Quist-Paulsen, P. 2010. Statins and inflammation: an update. *Curr Opin Cardiol,* 25(4)**,** pp 399-405.

Radetsky, M. 1992. Duration of symptoms and outcome in bacterial meningitis: an analysis of causation and the implications of a delay in diagnosis. *Pediatr Infect Dis J,* 11(9)**,** pp 694-8; discussion 698-701.

Ragin, A. B., Wu, Y., Storey, P., Cohen, B. A., Edelman, R. R. & Epstein, L. G. 2006. Monocyte chemoattractant protein-1 correlates with subcortical brain injury in HIV infection. *Neurology,* 66(8)**,** pp 1255-7.

Rajam, G., Bangert, M., Hammons, G. M., Melnick, N., Carlone, G. M., Sampson, J. S. & Ades, E. W. 2010. P4 peptide therapy rescues aged mice from fatal pneumococcal sepsis. *Clin Vaccine Immunol,* 17(11)**,** pp 1823-4.

Ramakrishnan, M., Ulland, A. J., Steinhardt, L. C., Moisi, J. C., Were, F. & Levine, O. S. 2009. Sequelae due to bacterial meningitis among African children: a systematic literature review. *BMC Med,* 7(47.

Ramsuran, V., Kulkarni, H., He, W., Mlisana, K., Wright, E. J., Werner, L., Castiblanco, J., Dhanda, R., Le, T., Dolan, M. J., Guan, W., Weiss, R. A., Clark, R. A., Karim, S. S., Ahuja, S. K. & Ndung'u, T. 2011. Duffy-null-associated low neutrophil counts influence HIV-1 susceptibility in high-risk South African black women. *Clin Infect Dis,* 52(10)**,** pp 1248-56.

Rangachari, P., Madaio, M., Rethemeyer, R. K., Wagner, P., Hall, L., Roy, S. & Rissing, P. 2014. Role of communication content and frequency in enabling evidence-based practices. *Qual Manag Health Care,* 23(1)**,** pp 43-58.

Rankin, J. 1957. Cerebral vascular accidents in patients over the age of 60. III. Diagnosis and treatment. *Scott Med J,* 2(6)**,** pp 254-68.

Rello, J., Lisboa, T., Lujan, M., Gallego, M., Kee, C., Kay, I., Lopez, D. & Waterer, G. W. 2009. Severity of pneumococcal pneumonia associated with genomic bacterial load. *Chest,* 136(3)**,** pp 832-40.

Reyburn, H., Mbatia, R., Drakeley, C., Carneiro, I., Mwakasungula, E., Mwerinde, O., Saganda, K., Shao, J., Kitua, A., Olomi, R., Greenwood, B. M. & Whitty, C. J. 2004. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ,* 329(7476)**,** pp 1212.

Ribes, S., Taberner, F., Domenech, A., Cabellos, C., Tubau, F., Linares, J., Fernandez Viladrich, P. & Gudiol, F. 2005. Evaluation of ceftriaxone, vancomycin and rifampicin alone and combined in an experimental model of meningitis caused by highly cephalosporin-resistant Streptococcus pneumoniae ATCC 51916. *J Antimicrob Chemother,* 56(5)**,** pp 979-82.

Richards, G., Levy, H., Laterre, P. F., Feldman, C., Woodward, B., Bates, B. M. & Qualy, R. L. 2011. CURB-65, PSI, and APACHE II to assess mortality risk in patients with severe sepsis and community acquired pneumonia in PROWESS. *J Intensive Care Med,* 26(1)**,** pp 34-40.

Righetti, E., Celani, M. G., Cantisani, T., Sterzi, R., Boysen, G. & Ricci, S. 2004. Glycerol for acute stroke. *Cochrane Database Syst Rev,* 2)**,** pp CD000096.

Ritchie, N. D., Mitchell, T. J. & Evans, T. J. 2012. What is different about serotype 1 pneumococci? *Future Microbiol,* 7(1)**,** pp 33-46.

Rivers, E., Nguyen, B., Havstad, S., Ressler, J., Muzzin, A., Knoblich, B., Peterson, E. & Tomlanovich, M. 2001. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med,* 345(19)**,** pp 1368-77.

Robb, E., Jarman, B., Suntharalingam, G., Higgens, C., Tennant, R. & Elcock, K. 2010. Using care bundles to reduce in-hospital mortality: quantitative survey. *BMJ,* 340(c1234.

Roberts, K., Whalley, H. & Bleetman, A. 2005. The nasopharyngeal airway: dispelling myths and establishing the facts. *Emerg Med J,* 22(6)**,** pp 394-6.

Robertson, M. A. & Molyneux, E. M. 2001. Triage in the developing world--can it be done? *Arch Dis Child,* 85(3)**,** pp 208-13.

Roca-Feltrer, A., Kwizombe, C. J., Sanjoaquin, M. A., Sesay, S. S., Faragher, B., Harrison, J., Geukers, K., Kabuluzi, S., Mathanga, D. P., Molyneux, E., Chagomera, M., Taylor, T., Molyneux, M. & Heyderman, R. S. 2012. Lack of decline in childhood malaria, Malawi, 2001-2010. *Emerg Infect Dis,* 18(2)**,** pp 272-8.

Roca, A., Bottomley, C., Hill, P. C., Bojang, A., Egere, U., Antonio, M., Darboe, O., Greenwood, B. M. & Adegbola, R. A. 2012. Effect of age and vaccination with a pneumococcal conjugate vaccine on the density of pneumococcal nasopharyngeal carriage. *Clin Infect Dis,* 55(6)**,** pp 816-24.

Roine, I., Pelkonen, T., Bernardino, L., Lauhio, A., Tervahartiala, T., Lappalainen, M., Kataja, M., Pitkaranta, A., Sorsa, T. & Peltola, H. 2014a. Predictive value of cerebrospinal fluid matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 concentrations in childhood bacterial meningitis. *Pediatr Infect Dis J,* 33(7)**,** pp 675-9.

Roine, I., Pelkonen, T., Bernardino, L., Leite, M., Kataja, M., Pitkaranta, A. & Peltola, H. 2014b. Factors Affecting Time to Death from Start of Treatment among Children Succumbing to Bacterial Meningitis (213-979). *Pediatr Infect Dis J*.

Roine, I., Peltola, H., Fernandez, J., Zavala, I., Gonzalez Mata, A., Gonzalez Ayala, S., Arbo, A., Bologna, R., Mino, G., Goyo, J., Lopez, E., Dourado de Andrade, S. & Sarna, S. 2008. Influence of admission findings on death and neurological outcome from childhood bacterial meningitis. *Clin Infect Dis,* 46(8)**,** pp 1248-52.

Roine, I., Saukkoriipi, A., Leinonen, M. & Peltola, H. 2009. Microbial genome count in cerebrospinal fluid compared with clinical characteristics in pneumococcal and

Haemophilus influenzae type b meningitis in children. *Diagn Microbiol Infect Dis,* 63(1)**,** pp 16-23.

Ronco, C. 2004. Early goal directed therapy and early goal ultrafiltration therapy for critically ill patients with acute kidney injury. *Int J Artif Organs,* 27(11)**,** pp 911-2.

Rosch, J. W., Boyd, A. R., Hinojosa, E., Pestina, T., Hu, Y., Persons, D. A., Orihuela, C. J. & Tuomanen, E. I. 2010. Statins protect against fulminant pneumococcal infection and cytolysin toxicity in a mouse model of sickle cell disease. *J Clin Invest,* 120(2)**,** pp 627-35.

Rossoni, A. M. d. O., Dalla Costa, L. M., Berto, D. B., Farah, S. S., Gelain, M., Brandileone, M. C. d. C., Ramos, V. H. M. & Almeida, S. M. d. 2008. Acute bacterial meningitis caused by Streptococcus pneumoniae resistant to the antimicrobian agents and their serotypes. *Arquivos de Neuro-Psiquiatria,* 66(3A)**,** pp 509-15.

Rouphael, N. G. & Stephens, D. S. 2012. Neisseria meningitidis: biology, microbiology, and epidemiology. *Methods Mol Biol,* 799(1-20.

Rowin, M. E., Xue, V. & Irazuzta, J. 2001. Hypothermia attenuates beta1 integrin expression on extravasated neutrophils in an animal model of meningitis. *Inflammation,* 25(3)**,** pp 137-44.

Sacchi, C. T., Fukasawa, L. O., Goncalves, M. G., Salgado, M. M., Shutt, K. A., Carvalhanas, T. R., Ribeiro, A. F., Kemp, B., Gorla, M. C., Albernaz, R. K., Marques, E. G., Cruciano, A., Waldman, E. A., Brandileone, M. C. & Harrison, L. H. 2011. Incorporation of real-time PCR into routine public health surveillance of culture negative bacterial meningitis in Sao Paulo, Brazil. *PLoS One,* 6(6)**,** pp e20675.

Saez-Llorens, X., Jafari, H. S., Severien, C., Parras, F., Olsen, K. D., Hansen, E. J., Singer, I. I. & McCracken, G. H., Jr. 1991. Enhanced attenuation of meningeal inflammation and brain edema by concomitant administration of anti-CD18 monoclonal antibodies and dexamethasone in experimental Haemophilus meningitis. *Journal of Clinical Investigation,* 88(6)**,** pp 2003-11.

Saha, S. K., Darmstadt, G. L., Yamanaka, N., Billal, D. S., Nasreen, T., Islam, M. & Hamer, D. H. 2005. Rapid diagnosis of pneumococcal meningitis: implications for treatment and measuring disease burden. *Pediatr Infect Dis J,* 24(12)**,** pp 1093-8.

Samra, Z., Shmuely, H., Nahum, E., Paghis, D. & Ben-Ari, J. 2003. Use of the NOW Streptococcus pneumoniae urinary antigen test in cerebrospinal fluid for rapid diagnosis of pneumococcal meningitis. *Diagn Microbiol Infect Dis,* 45(4)**,** pp 237-40.

Sanjoaquin, M. A., Allain, T. J., Molyneux, M. E., Benjamin, L., Everett, D. B., Gadabu, O., Rothe, C., Nguipdop, P., Chilombe, M., Kazembe, L., Sakala, S., Gonani, A. & Heyderman, R. S. 2013. Surveillance Programme of IN-patients and Epidemiology (SPINE): Implementation of an Electronic Data Collection Tool within a Large Hospital in Malawi. *PLoS Med,* 10(3)**,** pp e1001400.

Scarborough, M., Gordon, S. B., Whitty, C. J., French, N., Njalale, Y., Chitani, A., Peto, T. E., Lalloo, D. G. & Zijlstra, E. E. 2007. Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. *N Engl J Med,* 357(24)**,** pp 2441-50.

Scarborough, M. & Njalale, Y. 2004. Bacterial meningitis in a high HIV prevalence setting in sub-Saharan Africa--challenges to a better outcome. *Trop Doct,* 34(4)**,** pp 203-5.

Scarborough, M. & Thwaites, G. E. 2008. The diagnosis and management of acute bacterial meningitis in resource-poor settings. *Lancet Neurol,* 7(7)**,** pp 637-48.

Schaftenaar, E., Verjans, G. M., Getu, S., McIntyre, J. A., Struthers, H. E., Osterhaus, A. D. & Peters, R. P. 2014. High Seroprevalence of Human Herpesviruses in HIV-Infected Individuals Attending Primary Healthcare Facilities in Rural South Africa. *PLoS One,* 9(6)**,** pp e99243.

Scheld, W. M., Dacey, R. G., Winn, H. R., Welsh, J. E., Jane, J. A. & Sande, M. A. 1980. Cerebrospinal fluid outflow resistance in rabbits with experimental meningitis. Alterations with penicillin and methylprednisolone. *J Clin Invest,* 66(2)**,** pp 243-53.

Scheld, W. M. & Sande, M. A. 1983. Bactericidal versus bacteriostatic antibiotic therapy of experimental pneumococcal meningitis in rabbits. *J Clin Invest,* 71(3)**,** pp 411-9.

Schut, E. S., Brouwer, M. C., de Gans, J., Florquin, S., Troost, D. & van de Beek, D. 2009a. Delayed cerebral thrombosis after initial good recovery from pneumococcal meningitis. *Neurology,* 73(23)**,** pp 1988-95.

Schut, E. S., Brouwer, M. C., Scarborough, M., Mai, N. T., Thwaites, G. E., Farrar, J. J., Reitsma, J. B. & van de Beek, D. 2012. Validation of a Dutch risk score predicting poor outcome in adults with bacterial meningitis in Vietnam and Malawi. *PLoS One,* 7(3)**,** pp e34311.

Schut, E. S., Westendorp, W. F., de Gans, J., Kruyt, N. D., Spanjaard, L., Reitsma, J. B. & van de Beek, D. 2009b. Hyperglycemia in bacterial meningitis: a prospective cohort study. *BMC Infect Dis,* 9(57.

Schutte, C. M., Van der Meyden, C. H. & Magazi, D. S. 2000. The impact of HIV on meningitis as seen at a South African Academic Hospital (1994 to 1998). *Infection,* 28(1)**,** pp 3-7.

Scott, J. A., Hall, A. J., Muyodi, C., Lowe, B., Ross, M., Chohan, B., Mandaliya, K., Getambu, E., Gleeson, F., Drobniewski, F. & Marsh, K. 2000. Aetiology, outcome, and risk factors for mortality among adults with acute pneumonia in Kenya. *Lancet,* 355(9211)**,** pp 1225-30.

Seehusen, D. A., Reeves, M. M. & Fomin, D. A. 2003. Cerebrospinal fluid analysis. *Am Fam Physician,* 68(6)**,** pp 1103-8.

Shander, A. 2004. Anemia in the critically ill. *Crit Care Clin,* 20(2)**,** pp 159-78.

Shehabi, Y., Bellomo, R., Reade, M. C., Bailey, M., Bass, F., Howe, B., McArthur, C., Murray, L., Seppelt, I. M., Webb, S. & Weisbrodt, L. 2013. Early goal-directed sedation versus standard sedation in mechanically ventilated critically ill patients: a pilot study*. *Crit Care Med,* 41(8)**,** pp 1983-91.

Short, K. R., Reading, P. C., Brown, L. E., Pedersen, J., Gilbertson, B., Job, E. R., Edenborough, K. M., Habets, M. N., Zomer, A., Hermans, P. W., Diavatopoulos, D. A. & Wijburg, O. L. 2013. Influenza-induced inflammation drives pneumococcal otitis media. *Infect Immun,* 81(3)**,** pp 645-52.

Siddiqi, O. K., Ghebremichael, M., Dang, X., Atadzhanov, M., Kaonga, P., Khoury, M. N. & Koralnik, I. J. 2014. Molecular Diagnosis of Central Nervous System Opportunistic Infections in HIV-Infected Zambian Adults. *Clin Infect Dis,* 58(12)**,** pp 1771-7.

Singhi, S., Jarvinen, A. & Peltola, H. 2008. Increase in serum osmolality is possible mechanism for the beneficial effects of glycerol in childhood bacterial meningitis. *Pediatric Infectious Disease Journal,* 27(10)**,** pp 892-6.

Sloan, D. J. 2012. *Malawi Standard Treatment Guidelines*, College of Medicine, University of Malawi.

Sloan, D. J., van Oosterhout, J. J., Malisita, K., Phiri, E. M., Lalloo, D. G., O'Hare, B. & MacPherson, P. 2013. Evidence of improving antiretroviral therapy treatment delays: an analysis of eight years of programmatic outcomes in Blantyre, Malawi. *BMC Public Health,* 13(490.

Sobanski, M. A., Barnes, R. A. & Coakley, W. T. 2001. Detection of meningococcal antigen by latex agglutination. *Methods Mol Med,* 67(41-59.

Stamatovic, S. M., Shakui, P., Keep, R. F., Moore, B. B., Kunkel, S. L., Van Rooijen, N. & Andjelkovic, A. V. 2005. Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. *J Cereb Blood Flow Metab,* 25(5)**,** pp 593-606.

Stocker, R. & Biro, P. 2005. Airway management and artificial ventilation in intensive care. *Curr Opin Anaesthesiol,* 18(1)**,** pp 35-45.

Sudarsanam, T. D., Rupali, P., Tharyan, P., Abraham, O. C. & Thomas, K. 2013. Pre-admission antibiotics for suspected cases of meningococcal disease. *Cochrane Database Syst Rev,* 8(CD005437.

Swann, O., Everett, D. B., Furyk, J. S., Harrison, E. M., Msukwa, M. T., Heyderman, R. S. & Molyneux, E. M. 2014. Bacterial Meningitis in Malawian Infants Less Than 2 Months of Age: Etiology and Susceptibility to World Health Organization First-Line Antibiotics. *Pediatr Infect Dis J*.

Swartz, M. N. 2004. Bacterial meningitis--a view of the past 90 years. *N Engl J Med,* 351(18)**,** pp 1826-8.

Sydenham, E., Roberts, I. & Alderson, P. 2009. Hypothermia for traumatic head injury. *Cochrane Database Syst Rev,* 2)**,** pp CD001048.

Szelenyi, J. 2001. Cytokines and the central nervous system. *Brain Res Bull,* 54(4)**,** pp 329-38.

Tamune, H., Takeya, H., Suzuki, W., Tagashira, Y., Kuki, T., Honda, H. & Nakamura, M. 2013. Cerebrospinal fluid/blood glucose ratio as an indicator for bacterial meningitis. *Am J Emerg Med*.

Tauber, M. G., Khayam-Bashi, H. & Sande, M. A. 1985. Effects of ampicillin and corticosteroids on brain water content, cerebrospinal fluid pressure, and cerebrospinal fluid lactate levels in experimental pneumococcal meningitis. *Journal of Infectious Diseases,* 151(3)**,** pp 528-34.

Tauber, M. G., Sachdeva, M., Kennedy, S. L., Loetscher, H. & Lesslauer, W. 1992. Toxicity in neuronal cells caused by cerebrospinal fluid from pneumococcal and gram-negative meningitis. *Journal of Infectious Diseases,* 166(5)**,** pp 1045-50.

Tauber, M. G. & Sande, M. A. 1984. Pathogenesis of bacterial meningitis: contributions by experimental models in rabbits. *Infection,* 12 Suppl 1(S3-10.

Teasdale, G. & Jennett, B. 1976. Assessment and prognosis of coma after head injury. *Acta Neurochir (Wien),* 34(1-4)**,** pp 45-55.

Theilen, U., Wilson, L., Wilson, G., Beattie, J. O., Qureshi, S. & Simpson, D. 2008. Management of invasive meningococcal disease in children and young people: summary of SIGN guidelines. *BMJ,* 336(7657)**,** pp 1367-70.

Thigpen, M. C., Whitney, C. G., Messonnier, N. E., Zell, E. R., Lynfield, R., Hadler, J. L., Harrison, L. H., Farley, M. M., Reingold, A., Bennett, N. M., Craig, A. S., Schaffner, W., Thomas, A., Lewis, M. M., Scallan, E. & Schuchat, A. 2011. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med,* 364(21)**,** pp 2016-25.

Thomas, K. E., Hasbun, R., Jekel, J. & Quagliarello, V. J. 2002. The diagnostic accuracy of Kernig's sign, Brudzinski's sign, and nuchal rigidity in adults with suspected meningitis. *Clin Infect Dis,* 35(1)**,** pp 46-52.

Thompson, M. J., Ninis, N., Perera, R., Mayon-White, R., Phillips, C., Bailey, L., Harnden, A., Mant, D. & Levin, M. 2006. Clinical recognition of meningococcal disease in children and adolescents. *Lancet,* 367(9508)**,** pp 397-403.

Thwaites, G. E., Nguyen, D. B., Nguyen, H. D., Hoang, T. Q., Do, T. T., Nguyen, T. C., Nguyen, Q. H., Nguyen, T. T., Nguyen, N. H., Nguyen, T. N., Nguyen, N. L., Vu, N. T., Cao, H. H., Tran, T. H., Pham, P. M., Nguyen, T. D., Stepniewska, K., White, N. J. & Farrar, J. J. 2004. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. *N Engl J Med,* 351(17)**,** pp 1741-51.

Tjalsma, H., Bolhuis, A., Jongbloed, J. D., Bron, S. & van Dijl, J. M. 2000. Signal peptide-dependent protein transport in Bacillus subtilis: a genome-based survey of the secretome. *Microbiol Mol Biol Rev,* 64(3)**,** pp 515-47.

Too, L. K., Ball, H. J., McGregor, I. S. & Hunt, N. H. 2014. The pro-inflammatory cytokine interferon-gamma is an important driver of neuropathology and behavioural sequelae in experimental pneumococcal meningitis. *Brain Behav Immun*.

Townsend, G. C. & Scheld, W. M. 1996. The use of corticosteroids in the management of bacterial meningitis in adults. *J Antimicrob Chemother,* 37(6)**,** pp 1051-61.

Trachtenberg, J. D., Kambugu, A. D., McKellar, M., Semitala, F., Mayanja-Kizza, H., Samore, M. H., Ronald, A. & Sande, M. A. 2007. The medical management of central nervous system infections in Uganda and the potential impact of an algorithm-based approach to improve outcomes. *Int J Infect Dis,* 11(6)**,** pp 524-30.

Traore, Y., Tameklo, T. A., Njanpop-Lafourcade, B. M., Lourd, M., Yaro, S., Niamba, D., Drabo, A., Mueller, J. E., Koeck, J. L. & Gessner, B. D. 2009. Incidence, seasonality, age distribution, and mortality of pneumococcal meningitis in Burkina Faso and Togo. *Clin Infect Dis,* 48 Suppl 2(S181-9.

Trivedi, K., Tang, C. M. & Exley, R. M. 2011. Mechanisms of meningococcal colonisation. *Trends Microbiol,* 19(9)**,** pp 456-63.

Tromp, M., Hulscher, M., Bleeker-Rovers, C. P., Peters, L., van den Berg, D. T., Borm, G. F., Kullberg, B. J., van Achterberg, T. & Pickkers, P. 2010. The role of nurses in the recognition and treatment of patients with sepsis in the emergency department: A prospective before-and-after intervention study. *Int J Nurs Stud*.

Tsai, H.-C., Liu, S.-F., Wu, K.-S., Liu, Y.-C., Shi, M.-H., Chen, E.-R., Yen, C.-M., Lee, S. S.-J., Huang, Y.-L. & Chen, Y.-S. 2008. Dynamic changes of matrix metalloproteinase-9 in patients with Klebsiella pneumoniae meningitis. *Inflammation,* 31(4)**,** pp 247-53.

Tunkel, A. R. 2004. Reducing Intracranial Pressure may Increase Survival among Patients with Bacterial Meningitis. *Curr Infect Dis Rep,* 6(4)**,** pp 296-297.

Tunkel, A. R., Hartman, B. J., Kaplan, S. L., Kaufman, B. A., Roos, K. L., Scheld, W. M. & Whitley, R. J. 2004. Practice guidelines for the management of bacterial meningitis. *Clin Infect Dis,* 39(9)**,** pp 1267-84.

Tunkel, A. R. & Scheld, W. M. 1989. Therapy of bacterial meningitis: principles and practice. *Infect Control Hosp Epidemiol,* 10(12)**,** pp 565-9.

Tuomanen, E. I., Saukkonen, K., Sande, S., Cioffe, C. & Wright, S. D. 1989. Reduction of inflammation, tissue damage, and mortality in bacterial meningitis in rabbits treated with monoclonal antibodies against adhesion-promoting receptors of leukocytes. *J Exp Med,* 170(3)**,** pp 959-69.

Tureen, J. 1995. Effect of recombinant human tumor necrosis factor-alpha on cerebral oxygen uptake, cerebrospinal fluid lactate, and cerebral blood flow in the rabbit: role of nitric oxide. *Journal of Clinical Investigation,* 95(3)**,** pp 1086-91.

Ulanova, M., Hawdon, N., Nix, E., Ferroni, G. & McCready, W. 2011. Immune response of adults with secondary immunodeficiency to pediatric Haemophilus influenzae type b (Hib) vaccine. *Allergy Asthma Clin Immunol,* 7 Suppl 2(A23.

Ume, E. O. & Gelb, A. W. 1988. Reversal of iatrogenic diazepam overdose with flumazenil. *Clin Pharm,* 7(8)**,** pp 614-6.

Vaina, C. L., Paul, S. P., Heaton, P. A., Routley, C. & Mazhar, H. 2013. Diagnosing and managing invasive meningococcal disease in children. *Emerg Nurse,* 21(2)**,** pp 24-7.

van de Beek, D. 2012. Progress and challenges in bacterial meningitis. *Lancet,* 380(9854)**,** pp 1623-4.

van de Beek, D., Brouwer, M. C., Thwaites, G. E. & Tunkel, A. R. 2012. Advances in treatment of bacterial meningitis. *Lancet,* 380(9854)**,** pp 1693-702.

van de Beek, D., de Gans, J., McIntyre, P. & Prasad, K. 2007. Corticosteroids for acute bacterial meningitis. *Cochrane Database Syst Rev,* 1)**,** pp CD004405.

van de Beek, D., de Gans, J., Spanjaard, L., Vermeulen, M. & Dankert, J. 2002. Antibiotic guidelines and antibiotic use in adult bacterial meningitis in The Netherlands. *J Antimicrob Chemother,* 49(4)**,** pp 661-6.

van de Beek, D., de Gans, J., Spanjaard, L., Weisfelt, M., Reitsma, J. B. & Vermeulen, M. 2004. Clinical features and prognostic factors in adults with bacterial meningitis. *N Engl J Med,* 351(18)**,** pp 1849-59.

van de Beek, D., Farrar, J. J., de Gans, J., Mai, N. T., Molyneux, E. M., Peltola, H., Peto, T. E., Roine, I., Scarborough, M., Schultsz, C., Thwaites, G. E., Tuan, P. Q. & Zwinderman, A. H. 2010. Adjunctive dexamethasone in bacterial meningitis: a meta-analysis of individual patient data. *Lancet Neurol,* 9(3)**,** pp 254-63.

van der Poll, T. & Opal, S. M. 2008. Host-pathogen interactions in sepsis. *Lancet Infect Dis,* 8(1)**,** pp 32-43.

van Hoek, A. J., Andrews, N., Waight, P. A., George, R. & Miller, E. 2012. Effect of serotype on focus and mortality of invasive pneumococcal disease: coverage of different vaccines and insight into non-vaccine serotypes. *PLoS One,* 7(7)**,** pp e39150.

van Oosterhout, J. J., Laufer, M. K., Graham, S. M., Thumba, F., Perez, M. A., Chimbiya, N., Wilson, L., Chagomerana, M., Molyneux, M. E., Zijlstra, E. E., Taylor, T. E. & Plowe, C. V. 2005. A community-based study of the incidence of trimethoprim-sulfamethoxazole-preventable infections in Malawian adults living with HIV. *J Acquir Immune Defic Syndr,* 39(5)**,** pp 626-31.

Varon, E. 2009. [Epidemiology of acute bacterial meningitis in adult patients in France]. *Med Mal Infect,* 39(7-8)**,** pp 432-44.

Vengerov, I. I., Nagibina, M. V., Chentsov, V. B., Migmanov, T. E., Menushenkova, S. A. & Molotilova, T. N. 2008. [Clinical implication of lactate-acidosis in purulent meningitis]. *Terapevticheskii Arkhiv,* 80(11)**,** pp 33-6.

Vetsika, E. K. & Callan, M. 2004. Infectious mononucleosis and Epstein-Barr virus. *Expert Rev Mol Med,* 6(23)**,** pp 1-16.

Wali, S. S., Macfarlane, J. T., Weir, W. R., Cleland, P. G., Ball, P. A., Hassan-King, M., Whittle, H. C. & Greenwood, B. M. 1979. Single injection treatment of meningococcal meningitis. 2. Long-acting chloramphenicol. *Transactions of the Royal Society of Tropical Medicine & Hygiene,* 73(6)**,** pp 698-702.

Walker, A. S., Prendergast, A. J., Mugyenyi, P., Munderi, P., Hakim, J., Kekitiinwa, A., Katabira, E., Gilks, C. F., Kityo, C., Nahirya-Ntege, P., Nathoo, K. & Gibb, D. M. 2012. Mortality in the year following antiretroviral therapy initiation in HIV-infected adults and children in Uganda and Zimbabwe. *Clin Infect Dis,* 55(12)**,** pp 1707-18.

Walker, O., Sowunmi, A. & Salako, L. A. 1992. Pitfalls in the diagnosis of malaria: a-parasitaemic severe malaria. *J Trop Pediatr,* 38(5)**,** pp 268.

Wall, E., Carwright, K., Scarborough, M., Ajdukiewicz, K., Goodson, P., Mwambene, J., Zijlstra, E., Gordon, S., French, N., Faragher, B., Heyderman, R. & Lalloo, D. 2013a. High mortality amongst adolescents and adults with bacterial meningitis in Sub-Saharan Africa: an analysis of 715 cases from Malawi *PLoS One,* in press(

Wall, E. C., Ajdukiewicz, K. M., Heyderman, R. S. & Garner, P. 2013b. Osmotic therapies added to antibiotics for acute bacterial meningitis. *Cochrane Database Syst Rev,* 3(CD008806.

Wall, E. C., Cartwright, K., Scarborough, M., Ajdukiewicz, K. M., Goodson, P., Mwambene, J., Zijlstra, E. E., Gordon, S. B., French, N., Faragher, B., Heyderman, R. S. & Lalloo, D. G. 2013c. High Mortality amongst Adolescents and Adults with Bacterial

Meningitis in Sub-Saharan Africa: An Analysis of 715 Cases from Malawi. *PLoS One,* 8(7)**,** pp e69783.

Wall, E. C., Everett, D. B., Mukaka, M., Bar-Zeev, N., Feasey, N., Jahn, A., Moore, M., van Oosterhout, J. J., Pensalo, P., Baguimira, K., Gordon, S. B., Molyneux, E. M., Carrol, E. D., French, N., Molyneux, M. E. & Heyderman, R. S. 2014a. Bacterial Meningitis in Malawian Adults, Adolescents, and Children During the Era of Antiretroviral Scale-up and Haemophilus influenzae Type b Vaccination, 2000-2012. *Clin Infect Dis*.

Wall, E. C., Gordon, S. B., Hussain, S., Goonetilleke, U. R., Gritzfeld, J., Scarborough, M. & Kadioglu, A. 2012. Persistence of pneumolysin in the cerebrospinal fluid of patients with pneumococcal meningitis is associated with mortality. *Clin Infect Dis,* 54(5)**,** pp 701-5.

Wall, E. C., Gritzfeld, J. F., Scarborough, M., Ajdukiewicz, K. M., Mukaka, M., Corless, C., Lalloo, D. G. & Gordon, S. B. 2014b. Genomic pneumococcal load and CSF cytokines are not related to outcome in Malawian adults with meningitis. *J Infect*.

Wang, X., Theodore, M. J., Mair, R., Trujillo-Lopez, E., du Plessis, M., Wolter, N., Baughman, A. L., Hatcher, C., Vuong, J., Lott, L., von Gottberg, A., Sacchi, C., McDonald, J. M., Messonnier, N. E. & Mayer, L. W. 2012. Clinical validation of multiplex real-time PCR assays for detection of bacterial meningitis pathogens. *J Clin Microbiol,* 50(3)**,** pp 702-8.

Wang, Z., Xiong, Y., Schorr, C. & Dellinger, R. P. 2013. Impact of sepsis bundle strategy on outcomes of patients suffering from severe sepsis and septic shock in china. *J Emerg Med,* 44(4)**,** pp 735-41.

Wartenberg, K. E. & Mayer, S. A. 2006. Medical complications after subarachnoid hemorrhage: new strategies for prevention and management. *Curr Opin Crit Care,* 12(2)**,** pp 78-84.

Watera, C., Nakiyingi, J., Miiro, G., Muwonge, R., Whitworth, J. A., Gilks, C. F. & French, N. 2004. 23-Valent pneumococcal polysaccharide vaccine in HIV-infected Ugandan adults: 6-year follow-up of a clinical trial cohort. *AIDS,* 18(8)**,** pp 1210-3.

Watson, D. A., Musher, D. M., Jacobson, J. W. & Verhoef, J. 1993. A brief history of the pneumococcus in biomedical research: a panoply of scientific discovery. *Clin Infect Dis,* 17(5)**,** pp 913-24.

Weisfelt, M., de Gans, J. & van de Beek, D. 2007a. Bacterial meningitis: a review of effective pharmacotherapy. *Expert Opin Pharmacother,* 8(10)**,** pp 1493-504.

Weisfelt, M., de Gans, J., van der Poll, T. & van de Beek, D. 2006a. Pneumococcal meningitis in adults: new approaches to management and prevention. *Lancet Neurol,* 5(4)**,** pp 332-42.

Weisfelt, M., Determann, R. M., de Gans, J., van der Ende, A., Levi, M., van de Beek, D. & Schultz, M. J. 2007b. Procoagulant and fibrinolytic activity in cerebrospinal fluid from adults with bacterial meningitis. *J Infect,* 54(6)**,** pp 545-50.

Weisfelt, M., van de Beek, D. & de Gans, J. 2006b. Dexamethasone treatment in adults with pneumococcal meningitis: risk factors for death. *Eur J Clin Microbiol Infect Dis,* 25(2)**,** pp 73-8.

Weisfelt, M., van de Beek, D., Hoogman, M., Hardeman, C., de Gans, J. & Schmand, B. 2006c. Cognitive outcome in adults with moderate disability after pneumococcal meningitis. *J Infect,* 52(6)**,** pp 433-9.

Weisfelt, M., van de Beek, D., Spanjaard, L., Reitsma, J. B. & de Gans, J. 2006d. Attenuated cerebrospinal fluid leukocyte count and sepsis in adults with pneumococcal meningitis: a prospective cohort study. *BMC Infect Dis,* 6(149.

Weisfelt, M., van de Beek, D., Spanjaard, L., Reitsma, J. B. & de Gans, J. 2006e. Clinical features, complications, and outcome in adults with pneumococcal meningitis: a prospective case series. *Lancet Neurol,* 5(2)**,** pp 123-9.

Weisfelt, M., van de Beek, D., Spanjaard, L., Reitsma, J. B. & de Gans, J. 2006f. Community-acquired bacterial meningitis in older people. *J Am Geriatr Soc,* 54(10)**,** pp 1500-7.

Weisfelt, M., van de Beek, D., Spanjaard, L., Reitsma, J. B. & de Gans, J. 2008. A risk score for unfavorable outcome in adults with bacterial meningitis. *Ann Neurol,* 63(1)**,** pp 90-7.

Wells, J., Shetty, A. K., Stranix, L., Falkovitz-Halpern, M. S., Chipato, T., Nyoni, N., Mateta, P. & Maldonado, Y. 2006. Range of normal neutrophil counts in healthy zimbabwean infants: implications for monitoring antiretroviral drug toxicity. *J Acquir Immune Defic Syndr,* 42(4)**,** pp 460-3.

Werno, A. M. & Murdoch, D. R. 2008. Medical microbiology: laboratory diagnosis of invasive pneumococcal disease. *Clin Infect Dis,* 46(6)**,** pp 926-32.

White, K., Ostrowski, K., Maloney, S. & Norton, R. 2012. The utility of cerebrospinal fluid parameters in the early microbiological assessment of meningitis. *Diagn Microbiol Infect Dis,* 73(1)**,** pp 27-30.

White, N. J., Warrell, D. A., Looareesuwan, S., Chanthavanich, P., Phillips, R. E. & Pongpaew, P. 1985. Pathophysiological and prognostic significance of cerebrospinal-fluid lactate in cerebral malaria. *Lancet,* 1(8432)**,** pp 776-8.

WHO. 2004. Intergrated management of adolescent and adult illnesses. *WHO online publications* [Online]. Available: http://dosei.who.int/uhtbin/cgisirsi/XmxkjFVTqd/36200016/5/0 [Accessed 26/8/2010].

WHO. 2009. The clinical use of blood handbook. *Blood transfusion safety* [Online]. Available: http://www.who.int/bloodsafety/clinical_use/en/.

WHO. 2010. Guidlines for managing meningitis epidemics in Africa, WHO

WHO. 2013a. Control of epidemic meningococcal disease. *World Health Organisation practical guidelines* [Online]. [Accessed 12/8/2013].

WHO 2013b. Meningococcal disease in countries of the African meningitis belt, 2012 - emerging needs and future perspectives. *Wkly Epidemiol Rec,* 88(12)**,** pp 129-36.

Wiersinga, W. J., van Dellen, Q. M., Spanjaard, L., van Kan, H. J., Groen, A. L. & Wetsteyn, J. C. 2004. High mortality among patients with bacterial meningitis in a rural hospital in Tanzania. *Ann Trop Med Parasitol,* 98(3)**,** pp 271-8.

Wijewardana, I., Jarvis, J. N., Meintjes, G., Harrison, T. S. & Bicanic, T. 2011. Large volume lumbar punctures in cryptococcal meningitis clear cryptococcal antigen as well as lowering pressure. *J Infect*.

Williams, E. H., Hayes, R. J. & Smith, P. G. 1986. Admissions to a rural hospital in the West Nile District of Uganda over a 27 year period. *J Trop Med Hyg,* 89(4)**,** pp 193-211.

Winkler, F., Angele, B., Pfister, H. W. & Koedel, U. 2009. Simvastatin attenuates leukocyte recruitment in experimental bacterial meningitis. *Int Immunopharmacol,* 9(3)**,** pp 371-4.

Wip, C. & Napolitano, L. 2009. Bundles to prevent ventilator-associated pneumonia: how valuable are they? *Curr Opin Infect Dis,* 22(2)**,** pp 159-66.

Wippel, C., Maurer, J., Fortsch, C., Hupp, S., Bohl, A., Ma, J., Mitchell, T. J., Bunkowski, S., Bruck, W., Nau, R. & Iliev, A. I. 2013. Bacterial Cytolysin during Meningitis Disrupts the Regulation of Glutamate in the Brain, Leading to Synaptic Damage. *PLoS Pathog,* 9(6)**,** pp e1003380.

Wolter, N., Cohen, C., Tempia, S., Madhi, S. A., Venter, M., Moyes, J., Walaza, S., Malope Kgokong, B., Groome, M., du Plessis, M., Pretorius, M., Dawood, H., Kahn, K., Variava, E., Klugman, K. P. & von Gottberg, A. 2014a. HIV and influenza virus infections are associated with increased blood pneumococcal load: a prospective, hospital-based observational study in South Africa, 2009-2011. *J Infect Dis,* 209(1)**,** pp 56-65.

Wolter, N., Tempia, S., Cohen, C., Madhi, S. A., Venter, M., Moyes, J., Walaza, S., Kgokong, B. M., Groome, M., du Plessis, M., Magomani, V., Pretorius, M., Hellferscee, O., Dawood, H., Kahn, K., Variava, E., Klugman, K. P. & von Gottberg, A. 2014b. High nasopharyngeal pneumococcal density, increased by viral co-infection, is associated with invasive pneumococcal pneumonia. *J Infect Dis*.

Wu, H. M., Cordeiro, S. M., Harcourt, B. H., Carvalho, M., Azevedo, J., Oliveira, T. Q., Leite, M. C., Salgado, K., Reis, M. G., Plikaytis, B. D., Clark, T. A., Mayer, L. W., Ko, A. I., Martin, S. W. & Reis, J. N. 2013. Accuracy of real-time PCR, Gram stain and culture for Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae meningitis diagnosis. *BMC Infect Dis,* 13(26.

Yan, J. 2010. [The effect of early goal-directed therapy on treatment of critical patients with severe sepsis/septic shock: a multi-center, prospective, randomized, controlled study]. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue,* 22(6)**,** pp 331-4.

Yaro, S., Lourd, M., Traore, Y., Njanpop-Lafourcade, B. M., Sawadogo, A., Sangare, L., Hien, A., Ouedraogo, M. S., Sanou, O., Parent du Chatelet, I., Koeck, J. L. & Gessner, B. D. 2006. Epidemiological and molecular characteristics of a highly lethal pneumococcal meningitis epidemic in Burkina Faso. *Clin Infect Dis,* 43(6)**,** pp 693-700.

Yealy, D. M., Kellum, J. A., Huang, D. T., Barnato, A. E., Weissfeld, L. A., Pike, F., Terndrup, T., Wang, H. E., Hou, P. C., LoVecchio, F., Filbin, M. R., Shapiro, N. I. & Angus, D. C. 2014. A randomized trial of protocol-based care for early septic shock. *N Engl J Med,* 370(18)**,** pp 1683-93.

Zachariah, R., Harries, K., Moses, M., Manzi, M., Line, A., Mwagomba, B. & Harries, A. D. 2009. Very early mortality in patients starting antiretroviral treatment at primary health centres in rural Malawi. *Trop Med Int Health,* 14(7)**,** pp 713-21.

Zhang, Q., Leong, S. C., McNamara, P. S., Mubarak, A., Malley, R. & Finn, A. 2011. Characterisation of regulatory T cells in nasal associated lymphoid tissue in children: relationships with pneumococcal colonization. *PLoS Pathog,* 7(8)**,** pp e1002175.

Zoons, E., Weisfelt, M., de Gans, J., Spanjaard, L., Koelman, J. H., Reitsma, J. B. & van de Beek, D. 2008. Seizures in adults with bacterial meningitis. *Neurology,* 70(22 Pt 2)**,** pp 2109-15.

Zweigner, J., Jackowski, S., Smith, S. H., Van Der Merwe, M., Weber, J. R. & Tuomanen, E. I. 2004. Bacterial inhibition of phosphatidylcholine synthesis triggers apoptosis in the brain. *J Exp Med,* 200(1)**,** pp 99-106.

Appendices

Appendix 1.1 BAM study phase 2 clinical care bundle checklist

Date…….. Study number ……

Care bundle element	Physiological abnormality	Intervention	Required for this patient? Y = Yes N = No
Airway support	All participants GCS < 8 require NP airway	NP airway inserted	
Head tilt 30 degrees	All participants GCS < 11 require head tilt	Elevate head of bed or tilt trolley to 30 degrees	
Oxygenation	Sp02 <94%	Nasal flow 02 from concentrator	
Glucose	RBS <4	S/L , NG or IV dextrose	
Antibiotic therapy	1st dose within 1 hour of arrival	Drug chart timing	
Perfusion :- **shock**	CRT*>3 sec, BP <90 syst, MAP <70, postural hypotension UOP<0.5ml/kg	IV fluid bolus 20ml/kg Ringer's lactate. Repeated if required. Total volume of fluid given: ……..	
Perfusion :– **no shock**	Normal BP/heart rate/CRT	Maintenance fluids with IV RL (see drug chart)	
Seizures	1 or more seizure in AETC	Acute prompt treatment of seizures with IV/PR benzodiazepines and IV phenobarbitone if seizures persist	Diazepam Y / N Chlorpromazine Y / N Phenobarbitone Y / N

*CRT = Capillary Refill Time

To all ward clinicians

Your patient has been enrolled in the BAM study and has received a clinical care bundle of early goal directed therapy for suspected adult bacterial meningitis while in the AETC during admission. The care bundle was delivered over a 6 hour time period. The elements of the care bundle your patient received are documented above. We will follow up the patient daily and monitor their progress. Please call the BAM team if you have any questions or concerns about the care your patient received.

Appendix 1.2 BAM phase 2: Fluid bolus protocol for shocked participants

Shock = CRT>3 seconds, Systolic BP <90, Mean arterial BP < 70, pulse rate > 100, blood lactate >4mmol/L, drop of >40mmHg BP from chronic hypertensive BP.

If any one or more features are present then start resuscitation protocol

1. If not present already site an intravenous cannula

2. Assess if evidence of chronic cardiac, liver or renal disease. Each of these will make bolus fluid more hazardous to the participant, and will need an alteration to this protocol to be prescribed by a clinician. Inform the PI if pre-existing cardiac disease/

3. Prescribe and administer a fluid bolus of either Ringer's Lactate, or Saline 0.9% over 30 minutes.
4. Calculate your fluid bolus based on the estimated patient weight of 20ml/kg using the chart below. If you are unhappy with your estimated weight calculation use a 1000ml bolus for a 50-60kg individual.

40kg	800ml
50kg	1000ml
60kg	1200ml
70kg	1400ml
80kg	1600ml
90kg	1800ml

5. Check the observations carefully at the next hourly time period.

6. If the participant remains shocked, administer a second bolus over 60 minutes and follow points 2-4. This can be repeated until the participant is no longer shocked. If > 2 boluses are given call the PI or the medical registrar for a careful clinical assessment.

7. Document in the participant clinical care bundle sheet in the patient notes how many fluid boluses the participant received.

8. Once the clinical observations are normal and the patient is not shocked, administer IV fluid in standard 3l/24 hours protocol and prescribe this in the clinical notes and discharge the participant to the ward at the end of the 6 hour time period.

9. Recheck and document the blood lactate at the end of the 6 hour care bundle period.

10. If the participant is anaemic Hb < 6g/dL on hemocue, organise a unit transfusion of blood. Do not stop fluid resuscitation while waiting for blood. There is no evidence that you will make a participant more anaemic by giving fluid while waiting for blood. The aim is to increase the tissue perfusion, particularly the brain. Substitute blood for fluid in the protocol when blood is available. 1 unit of blood has approximately the same volume in terms of resuscitation as a litre of saline.

11. **STOP the intravenous fluid protocol if any of the following happen**:
    a) Sudden increase in respiratory rate of > 5 breaths/minute
    b) Drop in oxygen saturations of more than 3% (e.g. from 94% to 91%)
    c) New chest crackles

d) Acute chest pain
e) Drop in GCS of more than 2 points
f) New oxygen requirement
g) A medical doctor diagnoses pulmonary oedema or cerebral oedema.

12. **If any features in (11) occur, you MUST inform the PI and complete a SAE form**

Appendix 1.3 BAM phase 2: seizure treatment protocol

Introduction

A seizure is defined as one or more jerking movements of any body part caused by abnormal electrical activity in the brain. It can be either associated with a loss of consciousness (generalised seizure) or no loss of consciousness (partial seizure where the patient is awake).

Bacterial meningitis is associated with seizures in severe infection and the presence of seizures is associated with a significantly worse outcome. Therefore seizure treatment is an essential part of the BAM care bundle. Seizures are commonly associated with hypoglycaemia, hypoxia or hypo-perfusion of the brain, all of which must be corrected for the seizure to stop.

Protocol

If a seizure is observed, check the following:

1. Airway is open or protected using an NP airway. If suctioning is required move the participant to the resuscitation area in AETC.
2. Oxygen saturations are above 94%. If lower than this or unreadable, give oxygen immediately.
3. Site an IV cannula if one is not present already. Check if the blood pressure has been stable and >90mmgHg. If the patient is unstable give IV fluids.
4. Check the blood sugar. If it is lower than 4mmol/L give a bolus of 50% dextrose IV
5. If the seizure continues despite the manoeuvres in 1-4, give a bolus of IV diazepam or Lorazepam if available. Titrate the dose between 1-10mg. Start with 1mg and flush through the line with saline. Gradually increase the dose in 1-2mg increments until the seizures stop with the smallest dose you can give.
6. Monitor for respiratory depression with respiratory rate and Sp02.
7. Re-assess the participant. If the seizures have stopped, continue to monitor.
8. If the seizure continues or re-occurs check steps 1-4 again quickly.
9. Give a further IV bolus of diazepam or lorazepam. **Watch for respiratory depression (p2)**
10. If the seizures fail to terminate or re-occur load with IV phenytoin 15mg/kg or phenobarbitone 600mg loading dose followed by 90mg IV.
11. **Watch for respiratory depression (p2)**
12. **If the seizures do not terminate with this treatment, refer to ICU asap for anaesthetic management of the seizure.**

Loading protocol for phenobarbitone

1. Make up 600mg of phenobarbitone in 10-20 mls of saline 0.9%.
2. Give as a slow push IV over 15-20 minutes.
3. Watch BP and puse rate using a cardiac monitor if possible. Caution risk of hypotension or arrythmias if given too quickly.
4. Monitor for respiratory depression with respiratory rate and Sp02.

Respiratory depression

This is a drop in the respiratory rate of > 5 breaths/minute from baseline or a drop in the Sp02 of more than 3%. This will often be accompanied by a significant drop in the GCS or a prolonged post ictal period. If respiratory depression is apparent, stop the bolus infusion immediately and insert an NP airway. Refer to ICU for respiratory support until the respiratory rate improves. Use a bag and mask if necessary to support ventilation until ICU or anaesthetics are available to assist.

Appendix 1.4 BAM protocol for management of agitated patients

Protocol:

1. Good lighting levels to avoid agitation
2. Use repeated orientation like time and familiar objects
3. Repeated reassurance

4. Avoidance of physical, emotional, or chemical restraints
5. Minimal distractions, calm environment
6. Gentle and handle approach
7. Check RBS to rule out hypoglycaemia

Sedation should be avoided if at all possible as it may cause worsening of the condition

Indications for sedation:

In order to carry out essential investigations i.e., lumbar puncture
To prevent patient endangering himself or other patients
To relieve distress in highly agitated

Restless and irritability
Give lorazepam 0.5-1mg po or im

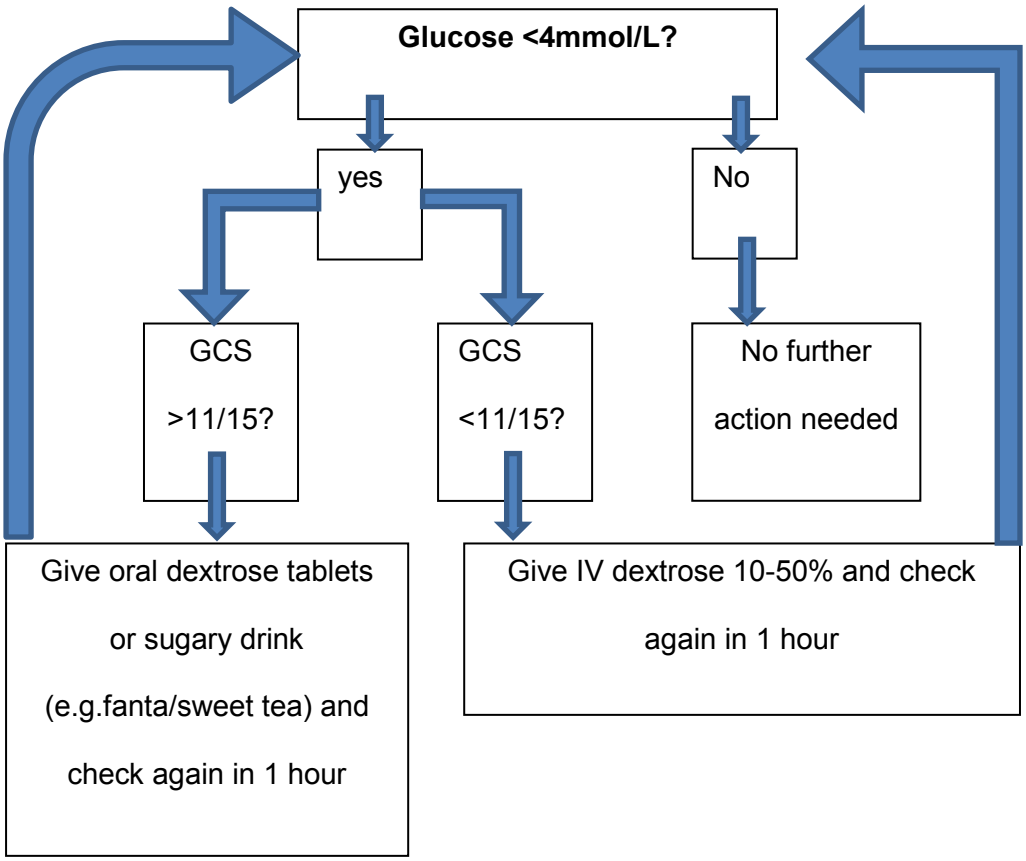
Appendix 1.5 BAM treatment of hypoglycaemia protocol Phase 2

Introduction

Hypoglycaemia can be defined as a random blood glucose measurement of less than 4mmol/L. This can lower the seizure threshold in the brain and cause serious cognitive difficulties. It is important to correct hypoglycaemia as soon as it is detected to optimise brain function and reduce the risk of further seizures.

Protocol

1. Take a random blood sugar (RBS) on every study participant
2. If the RBS is greater than 4mmol/L no action is needed
3. If it is lower than 4mmol/L and the GCS is over 11/15, correct with oral dextrose and food/sweet tea.
4. If however the GCS is less than 11/15 the glucose must be corrected with intravenous dextrose. Use the flowchart below.



5. At the end of the 6 hour care bundle if the participant is stable and well there is no need to check the blood sugar again. If it is still low ensure a handover is given to the HDU the participant is being transferred to, and the medical registrar is aware of the persistent hypoglycaemia.
6. Protocol for making up 10% dextrose. Use a 50 ml syringe if available. Take 10mls of 50% dextrose solution into the syringe. Add 40mls of saline or ringer's lactate and mix. Give the entire 50ml as a slow push IV .

Appendix 1.6 BAM nasopharyngeal airway protocol

Introduction

In critical illness, a patient may lose involuntary control over the airway. This most commonly happens when the patient becomes comatose (GCS<8). A nasopharyngeal (NP) airway provides support to the upper airway to keep oxygen flowing between the nose and the back of the pharynx. It is not a replacement for a full endotracheal tube which secures the airway but it may prevent common causes of airway obstruction in coma such as tongue displacement, mucous, food boluses and vomitus. The use of NP airways is recommended in patients who are unable to tolerate a guedel

(oropharayngeal) airway but who require airways support, or where a guedel airway is not appropriate. It will also induce less gagging than an oro-pharyngeal airway.

A NP airway insertion is contraindicated in suspected cases of facial trauma or basal skull fracture where the airway may pass through the skull. It is also contraindicated if CSF is seen to be draining from the nose, eyes, ears or mouth of the patient. In other situations the NP airway is very safe and usually very well tolerated.

Procedure

1. Determine the Glasgow coma score of the participant. If it is less than 8/15 then an airway is indicated as per the BAM protocol
2. Using suction if necessary ensure the upper airway through the nose is clear
3. Insert the airway at 90 degrees to the face and push it directly into the nasopharynx. Do not angle the airway up or down, but push it directly backwards into the participant's nose.
4. Follow the curve of the airway as you push into the nasopharynx as per the image below.

5. Insert the airway until only the flared tip remains outside of the nose. Apply oxygen through the airway if indicated.
6. Side effects from long term use of the airway include the following:

- Mucosal irritation
- Sinusitis
- Retropharyngeal ulcers
- Temporary vocal cord paralysis
- Temporary deafness

7. To remove the airway, simply pull it out of the nose towards you. Discard it immediately and warn the participant that they may cough as it is removed.
8. Ensure that you warn the participant's guardian that they may suffer side effects, if the airway is required for more than 2 days.