An Examination of Lumbar and Ventricular Cerebrospinal Fluid Findings in Children with Tuberculous Meningitis and Hydrocephalus

By

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This MMed is dedicated to my grandparents:

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ABSTRACT: AN EXAMINATION OF LUMBAR AND VENTRICULAR CEREBROSPINAL FLUID FINDINGS IN CHILDREN WITH TUBERCULOUS MENINGITIS AND HYDROCEPHALUS

Background: Childhood tuberculous meningitis (TBM) has poor outcomes. These are often associated with delayed diagnosis because early diagnosis and treatment is challenging. Existing diagnostic criteria use CSF characteristics to suspect TBM. However, lumbar and ventricular CSF may differ. These differences have not been well characterised Sometimes only ventricular CSF is available and decisions about surgical treatment may be influenced by CSF characteristics. This study examined CSF parameters from lumbar and ventricular compartments in patients with TBM and hydrocephalus who required neurosurgical procedures, their CSF temporal profiles, differentials between compartments, and factors that may influence these results.

Methodology: A descriptive cross-sectional study was conducted including data from two prospective TBM studies. Children treated for TBM and hydrocephalus at Red Cross War Memorial Children's Hospital with lumbar and/ or ventricular samples were selected. Pooled lumbar verses ventricular samples and paired time-linked samples in individual patients were analysed. Differences in CSF cell counts and biochemistry parameters across compartments were analyzed using Wilcoxon signed rank test, and temporal profiles graphically presented. Associations between laboratory, clinical and radiological data were analyzed using Mann-Whitney's U test. To test for associated factors, results of the nature of hydrocephalus (level of CSF obstruction) and spinal imaging were analyzed where available. Association between CSF parameters and morbidity was analyzed.

Results: Eighty-one patients were studied, 29 had time-linked paired CSF. The mean patient age was 36 months (2-156 months), 93% were HIV-uninfected, and the mortality rate was 13.6%. Seventy-two percent had communicating hydrocephalus, 16% non-communicating, and 12% uncertain (unable to demonstrate level of block). Medians of admission lumbar CSF showed low glucose (2.2 mmol/L), low chloride (112 mmol/L), raised protein (2g/L) and elevated white cell count (165 x 10^6/L). Corresponding values for admission ventricular CSF were minimally affected glucose (3mmol/L), mildly low to normal chloride (114.5mmol/L), normal to mildly raised protein (0.5g/L) and less elevated white cell count (22 x 10^6/L). In paired samples, all parameters were significantly different between lumbar and ventricular CSF. Ventricular CSF showed milder aberrations than lumbar CSF: lower protein and total white cell count, higher glucose and chloride. All paired samples showed higher lumbar CSF protein; lower lumbar CSF chloride in almost 80%; lower lumbar CSF glucose in 96%. Analysis of possible factors was limited by the small patient numbers who had full brain and spine imaging, and also paired CSF samples (n=17). However, maximum lumbar CSF protein was associated with severity of spinal disease on imaging. The lymphocyte ratio between lumbar and ventricular CSF was higher in patients with non-communicating and uncertain hydrocephalus. CSF parameters normalized slowly. White cell count and lymphocyte CSF differential were associated with favorable outcome in survivors.

Conclusion: Lumbar CSF depicted a typical TBM pattern. Ventricular CSF differed: CSF parameters were less abnormal in both pooled analysis and across individual paired samples. Spinal disease severity and nature of hydrocephalus may affect this differential. The CSF compartment sampled is therefore clinically relevant when interpreting CSF characteristics for diagnostic and treatment decisions. Studies of TBM diagnosis, pathophysiology, biomarkers and drug concentrations should consider these differences.

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ABBREVIATIONS

ADH	Anti-Diuretic Hormone
AEG	Air Encephalogram
AFB	Acid Fast Bacilli
BCG	*Bacillus Calmette-Guerin* vaccine
CI	Confidence Interval
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DNA PCR	Deoxy-Ribonucleic Acid Polymerase Chain Reaction
EPI	Expanded Programme of Immunization
ESR	Erythrocyte Sedimentation Rate
EVD	External Ventricular Drain
HCP	Hydrocephalus
Hib	*Haemophilus influenzae b*
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
HREC	Human Research Ethics Committee
IQR	Interquartile Range
ICP	Intracranial Pressure
MRI	Magnetic Resonance Imaging
Mtb	*Mycobacterium tuberculosis*
NAAT	Nucleic Acid Amplification Test
NHLS	National Health Laboratory System
OR	Odds Ratio
PCPCS	Paediatric Cerebral Performance Category Scale
PCR	Polymerase Chain Reaction

PCV	Pneumococcal Vaccine
RCWMCH	Red Cross War Memorial Children's Hospital
RSA	Republic of South Africa
SIADH	Syndrome of Inappropriate Antidiuretic Hormone Secretion
SPSS	Statistical Package for the Social Sciences
TB	Tuberculosis
TBM	Tuberculous meningitis
UCT	University of Cape Town
WCC	White Cell Count
WHO	World Health Organization
ZN-Stain	Ziehl-Neelsen stain

CHAPTER 1: BACKGROUND OF PAEDIATRIC TBM

1.1 Literature search strategy

A literature review of the subject was conducted with English articles identified using PubMed, ClinicalKey, SCOPUS, EBSCO Host and Google Scholar. There were no time limits set due to the known paucity of published literature on paediatric TBM.

Keywords used to search the literature included 'TB, tuberculo*, mycobact*'; 'Mening*, CNS, neur*, brain, nerv*'; 'Spin*, myelo*, myeli*'; 'Arachnoid*'; 'Lumba*'; 'ventric*'; 'CSF, cerebrospinal'.

SCOPUS and Google Scholar keyword search terms included "TB", "tuberculous", "tuberculosis"; "Meningitis", "CNS infection", "neurotuberculosis"; "arachnoiditis"; "CSF", "cerebrospinal fluid".

After exclusions for relevance, a total of 90 articles were reviewed.

1.2 The scope of the problem

Tuberculous meningitis (TBM) is responsible for 10% of tuberculosis (TB) cases in children, but is the most severe and lethal form of the disease (1–6). Children generally contract TB from an adult infected contact (3,5,7,8), and most cases (82%) occur under the age of 5 years (3). Despite treatment with antituberculous drugs and steroids, the disease has a high mortality (13%–50%) and high morbidity with poor neurological outcomes are common (50%) (3,5,9–12). Early commencement of anti-TBM treatment and steroids improves the odds of a good outcome (3,5,13), but delayed treatment often increases mortality (14).

1.3 Aetiology and Pathophysiology of TBM

TB is a granulomatous disease affecting multiple organ systems caused by *Mycobacterium tuberculosis* (*Mtb*) (6). TB granulomas are organised structures that form as a result of the cell mediated immunity to contain Mtb infected macrophages. Central nervous system (CNS) TB may occur individually as TBM, tuberculomas or tuberculous abscesses of the brain, TB of the spinal cord, TB of the bony spine, or together (2,15–18).

A "two step model" for the pathogenesis of TBM has been proposed (5,17,19). It has been suggested that *Mtb* spreads from a primary focus in the lungs via the lymphohematogenous route and crosses the blood brain barrier to form *Rich* foci (caseous granulomas/ tuberculomas) in the meninges and/or sub-pial area of the brain (19). The Rich focus then ruptures, releasing *Mtb* into the cerebrospinal fluid (CSF)-filled subarachnoid space surrounding the brain and spinal cord (3,20,21). Subsequently, this may progress to new tuberculomas developing, an inflammatory response of the meninges surrounding the brain and spinal cord, and thick inflammatory exudate in the basal cisterns that cause obstruction to CSF flow and vasculitis-induced brain infarction (3,6,21,22).

Varying descriptions for "TB spine" across literature include TB of the central nervous system, such as arachnoiditis (radiculomyelitis), intramedullary spinal cord tuberculomas, epidural phlegmon and TB spinal abscess, as well as involvement of the spinal column, such as TB discitis and TB infection of the vertebral bones (15,17,18,23). TB spondyloarthritis, described as spinal disease involving the spinal column and cord, occurs in 1% (18,23) and is a leading cause of paraplegia in developing countries (15,23,24). This MMed project excludes vertebral column bony involvement to focus on primary CNS involvement; TB

spine in this context refers to spinal involvement as subarachnoid and parenchymal involvement of the spinal nervous system.

TB arachnoiditis is thought to arise from haematogenous dissemination of *Mtb* seeding to the brain and spinal cord. It may occur primarily or develop secondary to vertebral or intracranial TB infection (15,17,25). TB spinal arachnoiditis may occur paradoxically following commencement of anti-TBM treatment, or evolve despite treatment (18), resulting in the formation of exudate, tuberculomas, abscesses, adhesions, and infarction or compression of the spinal cord (15,18). Symptomatic patients develop fever (70%), paraplegia (60%) or paraparesis (30%), monoparesis (10%), urinary retention (50%), and/or bowel incontinence (20%) (15). In one study, patients with spinal arachnoiditis more likely had a dry spinal tap (16).

1.4 Epidemiology of TBM in the Western Cape region of South Africa

TB is a global challenge: 10 million individuals develop TBM out of 2 to 3 billion infected with *Mtb* worldwide (3,14,26–28), up to 87% of which occurs in developing countries (27,29). The WHO Global TB Report (2018) (27) records that children accounted for 1 million of those newly infected with TB and 230,000 of the 1.6 million TB deaths. South Africa (SA) is amongst the hardest hit by TB worldwide, with an incidence of 567 (406 to 754) per 100,000 in 2017 (27). The Western Cape Province, where this MMed study was conducted, has had the highest incidence of TB in the country (26,30). TBM is the commonest cause of bacterial meningitis in adults and children (26,31) in the Western Cape. This has been influenced by the HIV pandemic and the successful reduction in other previously common causes of pediatric bacterial meningitis following an introduction of routine *H. Influenzae B (Hib)* and Pneumococcal vaccines (PCV) under the Extended Programme of Immunization (EPI) (31). The incidence of TBM varies with age; there

is a peak in infants (<12 months old) which decreases through to pre-adolescents and teens (3,6,21,22). A Western Cape study of paediatric TBM (3) recorded the following: most children were under 5 years old (82%), most were HIV negative (96%), and most had received routine BCG vaccine (97%). Almost half (47%) did not have a known TB contact. In a study by Rohlwink et al. (32) at RCWMCH results were similar: 84% were under 5 years old, 95% were HIV negative, but only 79% children were immunized with BCG. Their study had a high rate of CSF culture positivity (56%), which is higher than commonly reported, presumably because of larger amounts of CSF that were sent for culture. All cases were TB drug sensitive. Only 48% of patients had a recent positive TB contact. Half of the cohort (52%) had chest x-ray (CXR) findings suggestive of pulmonary TB, and most (76%) showed radiological evidence of concurrent TB spinal disease on spinal MRI, the vast majority of which was clinically asymptomatic. The authors identified subsets of pediatric spinal disease as extramedullary intradural plaque-like collections of exudate (9%), spinal tuberculomas (18%) and spinal arachnoiditis (72%) and no occurrence of vertebral TB. At the hospital, 24 children on average were admitted yearly with definite or probable TBM, with a mortality rate of 13.5% (32). Hydrocephalus with associated raised intracranial pressure (ICP) occurred in 72% of these children. Hydrocephalus signifies more severe TBM disease and is classified into two forms, namely communicating (where the block to CSF flow is in the basal cisterns and CSF in the ventricles communicates with CSF in the cisterns) and non-communicating (where there is a block to CSF egress from the ventricular system). The institutional protocol involves surgical treatment of non-communicating hydrocephalus in the first instance, and medical treatment (with or without temporary ventricular drainage) in the first instance for communicating hydrocephalus, with shunt surgery reserved for failed cases (33–35).

1.5 Clinical presentation and evaluation in TBM

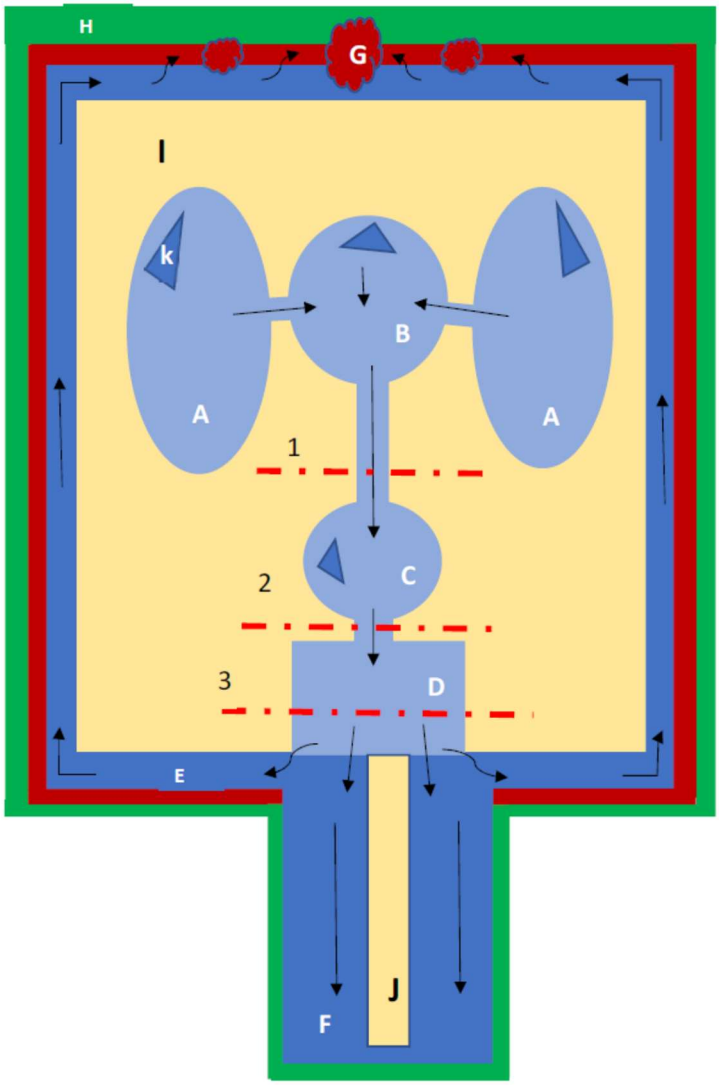
In children, TBM disease often develops within 2 – 6 months of a primary pulmonary TB infection (14). Common non-specific symptoms are cough, lethargy, apathy, restlessness, personality changes, inability to play, headaches, loss of appetite, nausea, diarrhoea, vomiting, fever and seizures (3,11,13,36). A clinical TBM staging system is routinely used by clinicians on admission and follow-up. It describes the range of neurological clinical signs exhibited on presentation depending upon severity of TBM disease and its impact on the CNS (3,10,11,37). Typical clinical neurological signs are depressed level of consciousness, cranial nerve palsies and other focal motor deficits, and abnormal movements; symptoms have usually been present for more than 6 days (38). Over time, several TBM severity grading systems have been developed for clinical use in children and adults across different resource settings, including the British Medical Research Council (MRC) staging system (39), the Vellore Grading System (40,41), the Tygerberg Children's Hospital Scale (37), Acute Physiology and Chronic Health Evaluation II score (42), and TBM Acute Neurological Score (43). Of these, the British MRC system has been most widely used (37). The original MRC staging system has undergone revisions to improve its effectiveness and application. The Revised TBM Medical Research Council (MRC) Scale (37) outlines four clinical stages (Appendix 1). When re-applied 1 week after admission, it is a good predictor of neurological outcome. The rationale for repeating staging after 1 week was to allow for resolution of any contributing secondary reversible clinical factors which are potentially treatable, such as hyponatremia, medications or seizures, which might give poorer staging on admission (32,37,44). Children with Stage 1 TBM have a normal level of consciousness (Glasgow Coma Scale [GCS] 15/15) and no focal neurological signs. Patients in Stage 2a TBM are alert (GCS 15) with a neurological deficit, or have a mild alternation of consciousness (GCS 13–14) with or without a focal neurological deficit. In stage 2b, children show moderate altered levels of consciousness (GCS 10-12) with/ without focal neurological deficits. Stage 3 TBM describes advanced disease with significantly depressed consciousness (GCS < 10) with/ without focal neurological deficits.

1.6 Important complications of TBM

Complications of TBM include hydrocephalus and raised intracranial pressure, cerebral ischemia and infarcts, hyponatremia, seizures, tuberculomas, and TB brain abscesses (5,6,17,38,44,45). Rare complications include mycotic cerebral vessel aneurysms, venous sinus thrombosis (5) and secondary bleeds (22,46). Most complications occur within the initial 3 months of anti-TBM treatment and contribute to poor clinical outcomes (5,6,45).

Hydrocephalus is a known factor independently contributing to poor outcome in TBM and is seen in the more severe spectrum of the disease (3,6,13,45). It occurs in 80-85% of TBM cases (5,6,44,47) and presents a life threatening neurosurgical emergency. It is more common in children (6,40,45). Two key mechanisms are described: tuberculous exudate in the basal subarachnoid space blocks cisternal CSF flow and may reduce absorption by the brain and arachnoid granulations (communicating hydrocephalus, approximately 80-85% of cases) and/or obstructs CSF flow at the outlet foramina of the fourth ventricle or cerebral aqueduct (non-communicating hydrocephalus, 15-20%) (6,45). The resultant rise in intracranial pressure reduces cerebral perfusion pressure and may reduce cerebral blood flow, increasing the risk of ischaemia. Hydrocephalus may also develop later when formation of scar tissue obstructs CSF outflow from the fourth ventricle (3,6,40). Currently available brain imaging techniques using MRI or CT cannot reliably differentiate between the two types of hydrocephalus (3,33,40,45,48). In older children, TBM hydrocephalus may show a 4-6 week delayed onset in contrast to children under 12 months old where it may present acutely within 5-10 days of the onset of TBM disease (6). A high index of suspicion is required by the attending clinician to seek urgent brain imaging and neurosurgical opinion regarding treatment options (44,45). Illustration 1 depicting the process of hydrocephalus is shown below.

Illustration 1: CSF Flow and Hydrocephalus in TBM



This illustration depicts brain, spinal cord, CSF flow and common points of CSF obstruction leading to hydrocephalus in TBM. Hydrocephalus causes increased ventricular size. CSF flows from the lateral ventricles (A) through their foramen, through the 3rd ventricle and the Aqueduct of Sylvius (B), into the 4th ventricle and it's foramen (C), which open into the Cisterns (pools of CSF) (D). (E) is the intracranial subarachnoid space. The spinal subarachnoid space (F) contains CSF, but may demonstrate a dry lumbar tap with the occurrence of spinal block. Subarachnoid granulations (G) absorb CSF into the venous sinus system. The skull (H), brain parenchyma (I) and spinal cord (J) are shown. Choroid plexus within the ventricles (K) produces CSF. CSF obstruction at points 1 and 2 cause non communicating hydrocephalus; and at 3 communicating hydrocephalus. *Source of diagram: Author.*

Other potential mechanisms that may increase intracranial pressure include cerebral oedema and obstruction of venous sinuses/ deep venous drainage of the brain.

Brain ischemia occurs due to compromised blood supply to the brain as a result of vasculitis and raised intracranial pressure. The middle cerebral artery and its perforators is most commonly involved (49,50). Reduced blood flow leads to brain infarction in 13% to 60% cases (16,49,50); infarction is associated with high mortality (5,6,22,49). Loss of autoregulatory capacity and brain oedema secondary to the injury may adversely contribute to the sequence of events. Common morbidities resulting from brain infarction include hemiplegia, spastic quadriplegia and cranial nerve palsies (5,22).

TB granulomas (tuberculomas) are focal space-occupying lesions that may develop in the parenchyma of the brain and spinal cord, subpial locations, or cisterns. As a result of mass effect and the underlying reaction of the brain, they may cause seizures and focal neurological deficits. Rarely, large intracranial tuberculomas or tuberculous abscesses may cause brain shift and reduced level of consciousness. Spinal cord tuberculomas may present with spinal cord compression and arm, leg, and even respiratory weakness (6). On CT or MRI scan with contrast, tuberculomas appear as ring-enhancing lesions with a variety of signal characteristics depending upon the histological stage of the lesion (5,6,18).

Hyponatremia occurs in 85% of pediatric TBM cases (44). The two causative mechanisms described are cerebral salt wasting and Syndrome of Inappropriate Anti-Diuretic Hormone secretion (SIADH) (44,45). The low serum osmolality accompanying hyponatremia may cause seizures and exacerbate brain oedema. An association has been suggested between presence of hyponatremia and worse outcome (45).

Rarely, TB abscesses form within the brain or spinal cord (6,44,51). They account for 0.5% of TB intracranial lesions and although they have suggestive imaging characteristics, occasionally they may be difficult to differentiate from tuberculomas, neoplasms, or other forms of intracranial abscesses (2). They have an outer capsule surrounding a liquefied caseating centre containing viable mycobacteria (6). Despite the start of anti-tuberculous therapy, they may progress in size and require surgical drainage. However, most resolve well with TBM treatment and steroids and surgical drainage or excision where needed (2,6,51). Alternative treatments such as thalidomide have been reported (52,53).

1.7 Clinical outcomes in children

TBM is associated with devastating consequences to the child and family (3,6,47). Even when appropriate TBM treatment is commenced or completed, poor outcomes are common (21,54). Although the results of the series by Van Well et al. (3)were relatively good compared to other series, still 13% of their cohort died and 71% were left with neurological sequelae. Similarly, Chiang et al. (54) reported that only around a third of patients survived without neurological sequelae. More severe pediatric TBM disease at presentation was associated with a higher occurrence of permanent neurological morbidity (3,37), exacerbated by cerebral vasculitis, infarcts, hyponatremia, raised intracranial pressure, and co-infection with HIV (3,37,45). Although treatment with steroids reduces mortality, it does not reduce long-term neurological sequelae in survivors.

Delayed diagnosis and treatment lead to poorer TBM outcomes (3,5,49,55). Approximately 20% of children with TBM die while receiving anti-TBM treatment (56). The Revised MRC Scale applied 1 week after admission was shown to be an effective prognostication tool and was able to predict neurological outcome in 84% of cases who completed the standard 6 months of anti-TBM treatment. Patients who score worse

are more likely to have long term impaired CNS function and lower developmental quotient (37). Adverse outcomes include intellectual impairment (77%), motor deficits (44%), impaired hearing (16%) impaired vision (14%) and cerebral palsy (3,37).

1.8 Diagnosis

Pediatric TBM may be a diagnostic dilemma, often because the clinical presenting symptoms are non-specific (5,38,44,57). Prodromal symptoms (fever, vomiting, listlessness, headache, general ill health) may appear similar to a flu-like illness (44) and are easily overlooked, thus increasing the risk of diagnostic delay and progressive brain injury (3,6). Being typically a sub-acute meningitis, TBM may lack the classical feature of neck stiffness expected in meningitis (44). Consequently, almost 60% of children present with non-specific symptoms of greater than one week duration (3). Sometimes the diagnosis on CSF cannot be distinguished from viral or partially treated bacterial meningitis (3,58). Furthermore, approximately half of children have no known TB contacts (3). Although 57% of children with TBM also have PTB (12), sputum-based diagnosis has a low yield (8).

Given these difficulties, clinicians must have a raised awareness and a high index of suspicion for TBM (58), especially in endemic areas. Treatment delay proves the strongest risk factor for death (5,13,21). Sensitive, rapid and affordable methods for testing TBM are not readily available in resource-limited settings (4,59,60) and more needs to be done to develop better diagnostic approaches (5,47).

A positive microbiology identification of *Mtb* in CSF culture or Ziehl-Neelsen staining is the gold standard diagnostic test (57). However, the probability of culturing *Mtb* from CSF is often poor, owing to the paucibacillary nature of CSF in TBM (20,57,61), with only a 12% positive yield (3) requiring prolonged culture times of 14 to 42 days (57), although Rohlwink et al. (32) reported a higher culture positivity rate

of greater than 50%. CSF TB culture results have been regarded as too insensitive and slow to aid immediate clinical decision making (5,57,59,62), and function better as a 'rule-in' test rather than a 'rule-out' test (63,64). Alternative diagnostic tests have been investigated. *Mtb* DNA PCR methods have a sensitivity range of 33% to 90%, and specificity of 88% to 100% (65) but tend to be costly (29). *Mtb* DNA PCR testing methods such as Gene Xpert *Mtb*/RIF (Cepheid, USA) was originally developed and validated on sputum. Initial validation studies for use in CSF samples indicated lower sensitivity than CSF microbiological methods (60). Some recent evaluations show improvement in diagnostic sensitivity of GeneXpert as high as 72-88% in definite TBM cases (64,66). Further research into laboratory diagnosis of TBM are needed (63,64,66,67). Global TBM experts, including the WHO, recently provided strong recommendations for use of Gene Xpert testing on CSF as a useful initial, rapid diagnostic tool despite its limitations (57,64). Gene Xpert showed a sensitivity of 79.5% (62-90.2%) against a culture reference gold standard, and 55% against a clinical gold standard in a 2013 WHO pooled meta-analysis (57). Few studies have been conducted on nucleic acid amplification testing (NAAT) in pediatric TBM (62). Cost-benefit analysis studies on the use of NAAT for TBM diagnosis in resource limited settings are lacking.

A diagnosis of 'probable TBM' hinges upon a combination of clinical features, radiological features and CSF chemistry (4). Appendix 2 illustrates the Marais et al. (4) diagnostic criteria derived as a research tool to standardise TBM diagnoses across studies. A classical TBM CSF picture is characterized by lymphocyte predominance with less than 50% neutrophils; leukocyte counts of less than 500 x 10^6/L (50-1000 x 10^6/L); increased protein (0.5 – 2.5g/L or more); and low glucose (95% cases demonstrate a CSF to plasma glucose ratio less than 0.5) (4,38,63,68). However, deviations from this classical pattern may occur (1,3,5,65). Van Well et al. (3) reported, based on initial CSF results at presentation, that up to 11% of children with TBM were incorrectly treated as bacterial meningitis. Eighteen percent of cases presented with a CSF picture that might be misdiagnosed as viral meningitis because of lymphocyte predominance, relatively low cell count less than 400 – 500 x 10^6/L and relatively low protein less than 0.8g/L (69). Fungal meningitis and partially

treated bacterial meningitis could prove challenging to differentiate from TBM based on CSF parameters, particularly in early TBM stages when neutrophil predominance up to 36% may prevail confounding the diagnosis. Furthermore, in early TBM, CSF protein levels may still be low (29). A slow decrement in CSF abnormalities may help - pleocytosis and raised protein were found to persist longer in TBM (56 – 92 days) than in other forms of meningitis whose parameters normalized quickly (69–71). Serial lumbar punctures for CSF investigations may thus be helpful to distinguish TBM from other forms of meningitis. Schoeman et al. (70) highlighted the importance of understanding serial CSF changes over time during TBM treatment for clinical decision-making. They observed in their study of 131 children with TBM that a transient deterioration in lumbar CSF parameters during the early weeks of TBM treatment may occur and does not necessarily indicate incorrect TBM diagnosis or inappropriate treatment for TBM.

Contrasted brain CT scans (or MRI) are essential in the early radiological diagnosis of TBM. Radiological features include hydrocephalus (70-85%), pre-contrast hyperdensity in the cisterns (65.9%), basal meningeal enhancement (96%), infarcts (65.9%), tuberculomas (59%) (3,6,16). MRI spine is important in investigating TB spine. Spinal involvement has been reported in 76% of paediatric TBM patients, and included spinal tuberculomas (18%), enhancement of spinal cord meninges and nerve roots (spinal arachnoiditis, 73%) (16). Wasay et al. (15) and Rohlwink et al. (16) describe similar occurrence of spinal arachnoiditis in TBM (70% in adults and 72% in children respectively). Clues from CSF such as rising or very high protein values or a dry lumbar puncture tap (four-fold risk) are suggestive of TB arachnoiditis (16,18).

An absence of typically expected TBM radiological features on spinal imaging may not exclude TB myelitis (15). Vertebral lesions were not found in children presenting with TBM (16).

1.9 Parameters for treating TBM at Red Cross War Memorial Children's Hospital (RCWMCH)

Typically at RCWMCH, the decision to treat a patient for TBM is based on clinical judgment supported by a suggestive history, physical examination and laboratory/ imaging results. Suspected cases are started on four drug anti-TBM treatment (Rifampicin, Isoniazid, Pyrazinamide, Ethionamide) and steroids (Prednisolone). If there is evidence of pulmonary TB and not TBM, treatment would be down scaled to 3 drug pulmonary TB treatment. The parameters illustrating this clinical decision-making process are outlined in Appendix 3 based on the clinical protocols used at RCWMCH. Patients with hydrocephalus undergo lumbar air encephalography (5,33) and/or a CSF column test if an external ventricular drain (EVD) has been inserted (34) to establish whether the hydrocephalus is communicating or not. If the patient is acutely unstable with signs of raised ICP a temporary EVD is inserted before lumbar puncture and air encephalography. Non-communicating hydrocephalus is treated with a ventriculo-peritoneal shunt (VPS) or endoscopic third ventriculostomy (ETV) (33,34). Communicating hydrocephalus is medically treated in the first instance, with serial lumbar punctures in addition to Acetazolamide and Furosemide for 3-4 weeks or until the ICP normalises. Failed medical cases (progressive hydrocephalus and/or persistent increased intracranial pressure) undergo ventriculoperitoneal shunt insertion. Figaji & Fieggen (34) summarized the current treatment algorithm for raised intracranial pressure in pediatric TBM (Appendix 4).

1.10 Ventricular and lumbar CSF compartments in CNS infection

1.10.1 Comparisons between Ventricular and Lumbar CSF Compartments

Normal CSF flow is thought classically to follow a rostro-caudal direction in a pulsatile manner, although various theories of normal and abnormal CSF circulation have been proposed. A rostro-caudal gradient between ventricular and lumbar compartments of various CSF components, specifically protein, leucocytes and glucose is described in central nervous system (CNS) infection. Literature describing lumbar and

ventricular CSF compartments in TBM is scarce. Some data is available for bacterial meningitis. Gerber et al. (72) drew results from paired lumbar and ventricular CSF samples of a large patient cohort diagnosed with community acquired and post-neurosurgery acute bacterial meningitis. They demonstrated higher leukocyte counts in the lumbar CSF compared to ventricular CSF. This differential was attributed to inflammatory mechanisms mounted in response to meningeal infection that lead to an increased permeability of the blood-brain-barrier and blood-CSF barrier especially at the region of the CSF space with maximal inflammation. More importantly, it was noted that the wide variation in protein and leukocyte concentrations between lumbar and ventricular CSF in meningitis meant that the values of these two parameters could not be predicted for one compartment based on CSF samples taken from the other compartment. Naija et al. (73) described rostro-caudal gradients of analytes in paired ventricular and lumbar CSF samples in post-neurosurgical bacterial meningitis. Protein and leucocytes were higher and glucose lower in lumbar versus ventricular CSF samples. This differential was more marked during the acute stage of meningitis. They postulated that in human meningitis this differential picture reflects compartmentalized inflammation with more meningeal inflammation occurring in the lumbar than ventricular region. Torres-Corzo et al. (17) studied paired lumbar and ventricular CSF samples in patients with hydrocephalus secondary to neurocysticercosis. They too found a rostro-caudal gradient of protein and polymorphs with significantly higher levels in lumbar CSF. There was no significant difference between glucose levels in the two CSF compartments.

A study in adult TBM demonstrated a differential in *Mtb* bacteriological yields from CSF sampled in different CSF compartments: the cisternal CSF contained the highest yield (87.5%) when compared to ventricular CSF (75%) and lumbar CSF (11.5%) using standard *Mtb* microbiological methods (74). Normal ventricular CSF may not necessarily rule out TBM (75); therefore, lumbar CSF samples should be obtained for testing where TBM is suspected despite negative ventricular CSF findings. In TBM the cumulative addition of immune proteins and leukocytes as the CSF flows down the brain-spine axis may be increased

by arachnoiditis in the spinal canal which may hamper the CSF flow and lead to pooling in the lumbar space as described with spinal block in Froin's syndrome (76). Froin's syndrome is described as xanthrochromic (yellow tinged) and easily coagulable CSF resulting from raised CSF protein content from meningeal inflammation or blockage of CSF flow by tumours or spinal abscesses. Restricted CSF flow in the caudal spinal space is known to increase CSF protein. Therefore, lumbar CSF may reflect a combination of intracranial disease, intraspinal disease, and restricted flow (16).

Recently, Kamat et al. (77) reported surprising findings on lumbar and ventricular CSF: in their study there were no statistically significant differences between lumbar and ventricular CSF protein, contrary to expectation. CSF protein was remarkably similar in ventricular and lumbar CSF samples (2.471 g/L and 2.474 g/L respectively). With no differences between adults and children noted. No comment was made on other CSF parameters. The authors suggest that lumbar CSF protein levels can be used to predict ventricular CSF protein, the level of which should be a guide to whether a shunt should be placed or not, given their observation that patients with higher protein levels had an increased risk of shunt blockage. However, there are other studies that suggest differences between lumbar and ventricular CSF (78,79) and these differences would have important clinical implications for diagnosis and treatment; therefore further clarity is needed.

1.10.2 Temporal profiles of CSF parameters within the lumbar and ventricular compartments

There is little written about the temporal changes in ventricular and lumbar CSF parameters in TBM. Knowing these patterns of CSF cell count and biochemistry parameters and how they change in response to TBM treatment may help clinical decision-making (70,80). Where the diagnosis of TBM is in doubt, follow-up lumbar punctures to observe for CSF changes while on empirical TBM treatment may assist in strengthening the diagnosis of TBM and gauging the appropriateness of TBM treatment based on the typical treatment response.

Donald et al. (69) studied lumbar CSF changes over time in 99 children treated for TBM at Tygerberg Children's Hospital, Cape Town. No children were treated with adjuvant corticosteroids. Serial lumbar punctures over the first 4 weeks of anti-tuberculous drugs were performed. Typical cell count and biochemical findings with lymphocyte predominance, low glucose and high protein on initial CSF were described. In 9% of cases, an atypical picture of polymorph predominance raised to 2000 x 10^6/L similar to a bacterial meningitis was described. Fluctuations in cell count and biochemistry were seen throughout the first month of anti-tuberculous therapy; cell count and protein fluctuated more than glucose. A paradoxical transient pleocytosis in the range of 500 x10^6/L with a polymorph predominance often occurred in the first two weeks of treatment. Protein levels were high initially; only 18% of children had values less than 0.8g/L. Sixty-one percent of the children showed decreasing protein trends overall, either uninterrupted or fluctuating. The remaining 39% had higher proteins at 4 weeks than on admission. Twenty seven percent of cases had initially negative or trace result for Pandy's globulin test. By 4 weeks 39% had trace or no turbidity on performing Pandy's test. The authors recommend that during early anti-tuberculous therapy, fluctuations in CSF as described on follow up CSF studies are common and should not be a reason to stop TB treatment.

A later study at the same institution (70) reported on serial lumbar CSF changes in a randomized controlled study of adjunctive steroids in the treatment of paediatric TBM. One hundred and thirty one children with suspected or confirmed TBM again showed fluctuations over time in CSF protein, glucose and cell counts. Cell counts transiently worsened during the first month of combined TBM and steroid treatment, and then steadily decreased. Glucose rose steadily to reach normal levels only after the third week in the steroid treated arm. Protein and globulin levels remained high in the first month, gradually decreasing during the initial 4 weeks of combined TBM plus steroid treatment. CSF lactate and adenylate kinase were also monitored but did not show a significant difference in rates of normalizing in the group treated with

adjuvant steroids. Schoeman et al. (70) suggest that high CSF adenylate kinase may be associated with a poorer neurological outcome. CSF chloride was not reported on.

A Kosovan study on a largely BCG unimmunized (81%) paediatric population with TBM examined serial lumbar punctures up to the third month on TBM treatment to explore the duration and nature of cyto-biochemical changes in children treated for TBM (71). The authors found persistent CSF pleocytosis (50 – 500 $x10^6$/L) for a mean of 70 days (range 56 to 92 days) on TBM treatment. Lymphocyte predominance ranged from a mean of 67% in the first CSF sample to 98% in the fourth CSF sample taken 3 months after TBM treatment. They described a positive Pandy reaction for CSF globulin in 96% of cases on initial LP, which remained positive in 65% of cases after repeat LP after 3 months on TB treatment. Protein levels were normal or raised (mean 1.86 g/L) on the initial CSF sample but remained high in the three subsequent CSF samples. Glucose levels were lower than normal in CSF samples throughout TBM treatment across the 3-month period. These levels ranged from a mean of 1.49 mmol/L on the first CSF sample to low/ normal (mean 2.10 mmol/L) after 3 months. The CSF: Blood glucose ratio was depressed. Chloride was not reported on.

1.11 Knowledge gap

A knowledge gap exists from the lack of data on differences in CSF parameters between ventricular and lumbar CSF in TBM (81), and on how ventricular CSF parameters and the differential between ventricular and lumbar CSF change over time. Ventricular CSF has not been as systematically studied as lumbar CSF in TBM. These are important issues because decisions about surgery may be influenced by CSF characteristics, and if the results between ventricular and lumbar CSF are different, the source of the sample is important in those decisions. Furthermore, many studies in TBM are based primarily on lumbar CSF, the

characteristics of which might not only reflect production from the CNS. In fact, the characteristics of lumbar CSF may be influenced by flow dynamics and a contribution from systemic sources to a greater degree than ventricular CSF (78,79). Therefore, whether lumbar CSF adequately reflects ventricular CSF, or analytes of intrathecal origin, can be questioned.

1.12 Justification and significance of research

Knowing that spinal factors may influence lumbar CSF characteristics, and that asymptomatic spinal disease is common in TBM, better characterisation of the differences and similarities between ventricular and lumbar CSF may be helpful. This may also help clarify how well ventricular CSF parameters can be predicted from lumbar CSF results and what implications this has for decisions about VPS insertion. Where there is only a ventricular sample (for example when an emergent external ventricular drain is in place), a better understanding of the ventricular findings on admission may help avoid a missed diagnosis.

1.13 Aim and objectives

This study aimed to examine ventricular and lumbar CSF parameters in children with probable and definite TBM, on admission and over 3 weeks of treatment.

The *primary objective* was to describe the temporal profile of CSF cells, biochemistry, and microbiology in the lumbar and ventricular CSF compartments in TBM.

The *secondary objectives* were to: 1) identify possible predictors of the differential; and 2) determine association between that differential and patient outcome.

CHAPTER 2: METHODOLOGY

2.1 Study design

A descriptive retrospective cohort study was conducted on data collected as part of two ongoing TBM studies *(Ethics numbers HREC 318/2010 and 200/2014),* which provided a convenience sample from which data were derived for use to inform future projects. Patient data collection was conducted from October 2010 to March 2015.

2.2 Selection of patients

We screened all children treated at RCWMCH from October 2010 to March 2015 for suspected TBM and hydrocephalus and included those who had lumbar and/ or ventricular CSF sent for TB investigations and who met the research case definition of definite or probable TBM (4) (Appendix 2). Lumbar and ventricular CSF samples from day 1 to day 21 were included. Time-linked (paired) lumbar and ventricular CSF samples were taken for those patients who had received a lumbar puncture as well as neurosurgical interventions (ventricular CSF sampling) to treat raised ICP and hydrocephalus. These were typically taken in the operating room when an external ventricular drain (EVD) was placed as an emergency procedure and an air encephalogram and/or column test was done at the same time, or when a planned post-EVD air encephalogram/ column test were done in patients with EVDs in situ. Air encephalography and column tests have been previously described (33). Briefly, a lumbar air encephalogram involves the instillation of a small amount of air into the lumbar CSF space, sitting the patient upright thereafter, and performing a skull radiograph to determine if air is seen in the ventricular system, which indicates an open communicating CSF system. A column test is done only in patients with an EVD in situ; the patient is positioned for a lumbar puncture; a manometer is placed on the lumbar needle to check the opening pressure; this is compared to the opening pressure on a manometer attached to the ventricular drain (and zeroed at the same level) and then the cranial pressures are monitored as CSF is drained from the lumbar

needle. If the OP's are equal and the pressures decrease to the same degree after CSF drainage a diagnosis of communicating hydrocephalus in made. Occasionally, a lumbar puncture was performed for therapeutic purposes, and an EVD or VPS was placed soon thereafter. These were also included as paired samples if the samples were taken within 24 hours of each other. However, samples were excluded if the lumbar puncture followed a procedure: CSF samples within 48 hours after neurosurgical procedures or air encephalograms were excluded to avoid the potential artefact (inflammatory response) of the intracranial procedure or intracranial air on subsequent CSF findings. Patients were excluded if there was an infection of the EVD or shunt.

2.3 Data collection and sources

Demographic and clinical data were collected from patient clinical notes. Lumbar and ventricular CSF laboratory data including glucose, protein, chloride, cell count and microbiological/ *Mtb* diagnostics on admission and during the first 21 days of hospitalisation were collected from the National Health Laboratory Service (NHLS) database. Radiological data from admission and follow up CT and MRI scans were reviewed by three senior pediatric radiologists and a senior pediatric neurosurgeon according to criteria previously published by the reviewers (16), due to the paucity of standardised criteria. Reviewers were blinded to patient clinical characteristics and outcome. Disagreements were resolved through consultation and discussion until a consensus agreement was achieved. Specific features recorded included severity of hydrocephalus (mild, moderate, severe), presence of basal meningeal enhancement, presence of infarcts, presence of tuberculomas, presence and severity of spinal pathology (mild, moderate and severe), and CXR findings. Details of how these variables were recorded are included in Appendix 5.

Data were directly entered into an electronic Excel data collection spreadsheet on a secure password protected laptop. Patient identifiers were removed and re-coded to protect patient anonymity and privacy. Access to the anonymous dataset was restricted to the study investigators (MMed supervisors) and a UCT statistician as needed for data analysis purposes.

2.4 Data analysis

Data were exported from the electronic data collection instrument into the statistics package and coded. Before being analysed, the data was cleaned and verified. The data cleaning and verification process involved screening/ verifying missing data, and verifying if outlier CSF results were true values by reviewing patient clinical notes, scans and CSF patterns. STATA software version 14.1, R software version 3.3.2, and SPSS version 25 (IBM) were used to analyse the data.

2.5 Statistical analyses

2.5.1 Clinical descriptive statistics

Patient categorical baseline characteristics were described using number and percentage. Continuous variables were described using median, interquartile range (IQR), maximum to minimum or mean and standard deviations depending on data distribution. Variables studied were demographic, clinical and radiological features and CSF data obtained from both lumbar and ventricular compartments. Key cell count (polymorphonuclear cells, lymphocytes, total white cells) and biochemical (glucose, protein, chloride) parameters were analysed in the lumbar and ventricular CSF compartments. A p-value of <0.05 was considered significant.

To compare the difference between lumbar and ventricular CSF we first examined data for each compartment across all patients and all time points. Next, we identified patients who had paired samples of lumbar and ventricular CSF (taken within 24 hours of each other), and further analysed the difference between the compartments for these paired samples. Analysis of distribution of data demonstrated that data was not normally distributed therefore all statistical tests used were non-parametric tests.

2.5.2 Comparisons between lumbar and ventricular CSF compartments overall (pooled)

Temporal profiles of lumbar and ventricular CSF were analysed across time epochs of 4 days as not all patients had samples taken each day. This time frame would allow for adequate resolution to identify short term changes in CSF parameters.

2.5.3. Comparison between CSF compartments for paired samples

Using the paired, time-linked samples we conducted a Wilcoxon signed rank test to establish whether CSF chemistry (glucose, protein, chloride) and cell counts (polymorphonuclear cells, lymphocytes and WCC) were significantly different between the 2 compartments. Thereafter, the *differential* between paired lumbar and ventricular CSF parameters was calculated as lumbar minus ventricular CSF values for each parameter. Differenial was used as an index of the degree of difference between compartments. Next, a *ratio* of lumbar to ventricular samples was also calculated. Ratio was used to account for variation in absolute values. Data on the differentials and ratios are presented for each patient in whom paired samples were collected.

2.5.4 Analysis of lumbar or ventricular CSF analytes with radiology characteristics

To examine the impact of the nature of hydrocephalus (communicating, non-communicating and uncertain), the presence of infarcts, tuberculomas and spinal disease, and the severity of spinal disease (mild, moderate-severe) may have on perturbations of CSF parameters we analyzed the association between these radiology characteristics (in patients who had full brain and spine MRIs) and 1) the lowest glucose and chloride, and the highest protein and cell count in lumbar and ventricular CSF, 2) the ratios of lumbar and ventricular CSF chemistry and cytology, and 3) the differentials in lumbar and ventricular CSF chemistry and cytology. This included 108 analyses.

According to published and clinically used thresholds for CSF composition at our institution, low glucose and chloride were defined as less than 2.2 mmol/L and 116 mmol/L respectively, high lymphocyte count as more than 5 $x10^6$/L, high polymorphonuclear count as any cells found $x10^6$/L, and high total white cell count as more than 10 $x10^6$/L (82,83). Based on different thresholds used for elevated protein, we categorized protein as increased according to 1) the general CSF threshold of more than 0.4g/L (83), and 2) the threshold of 0.8 g/L that has been found to have high specificity in the diagnosis of TBM (3,32,82). Analyses were conducted using Mann-Whitney's U or Kruskall Wallis tests, significance was set at 0.05, and significant results are accompanied by Box and Whisker Plots.

2.5.5 Analysis of associations between patient outcomes and CSF compartment

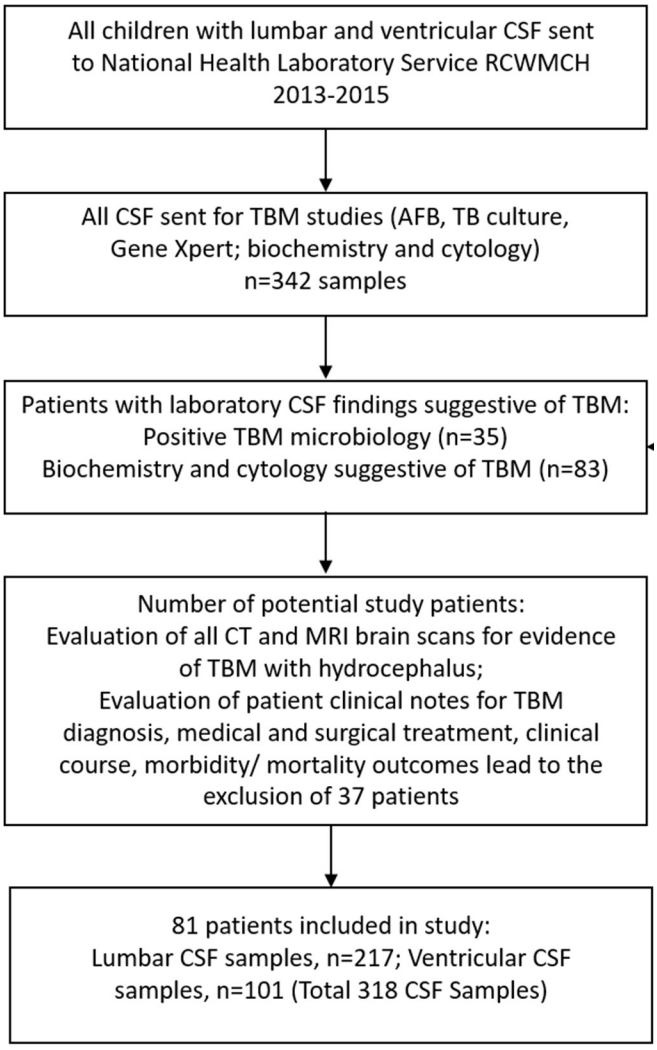
Patient outcome was assessed using the Pediatric Cerebral Performance Category Scale (84) (briefly, this is a 6 point scale comprising 1, normal; 2, mild disability; 3, moderate disability; 4, severe disability; 5, deep coma or vegetative state; 6, death) – Appendix 6. The PCPCS score was dichotomized for favorable outcome in survivors (PCPCS 1-3) and unfavorable outcome in survivors (PCPCS 4-5). Mortality at 12

months after admission was also recorded. The association between morbidity and mortality with CSF differentials and ratios was examined using Mann Whitney's U. Repeated measures were excluded (n=4 samples).

CHAPTER 3: RESULTS

A total of 81 children were enrolled in the study selected from all children treated for suspected TBM and hydrocephalus attending RCWMCH from October 2010 to March 2015. A flow diagram outlining patient selection is shown in Figure 1.

Figure 1: Flowchart of Patient Selection Process



The figure outlines the steps taken in selecting study patients. Exclusions were made based on evaluation of clinical, radiological and laboratory data.

3.1 Descriptive data of patient admission clinical characteristics

Clinical features were reported based on availability of data recorded in the clinical records. Table 1 outlines patient demographic admission characteristics. The median age was 36 (2 – 156) months. Forty-nine children (61%) were male. Fifty seven percent (n=41) of the children were underweight for age. Most patients (n=74, 92%) were HIV negative, few were positive (n=6, 7%) and in one patient HIV status was unknown. Three (50%) of HIV co-infected patients were on anti-retroviral therapy.

Table 1: Demographic Characteristics of Children with TBM and Hydrocephalus on Admission

Patient parameter	
Age	36 (2-156) months
Male	49 (60.5)
Nutritional Status [a] (n=72)	
Normal weight ≥ -1 SD	31 (43)
Underweight < -1 SD	41 (56.9)
HIV positive [b] (n=80)	6 (7.5)
TB Contact[c] (Total n=74)	30 (40.5)

Demographic variables are presented as median (minimum – maximum), or number (percent). [a] not recorded in n=9 (11%), [b] investigated in n=80, [c] recorded in n=74 (91%).

Most children had constitutional symptoms (n=69, 88%) – Table 2. Fever was reported in 51 children (63%), weight loss in 44 (54%), and night sweats in 8 (10%). Most (n=58, 72%) had no cough. Mantoux tuberculin skin tests were positive in 40 (82%) of those tested.

Table 2: General Clinical Features of TB on Admission

Patient parameter	n (%)
Constitutional symptoms [a] (Total n=78)	69 (88.5)
Fever	51 (63)
Weight loss	44 (54.3)
Night sweats	8 (9.9)
Cough	23 (28.4)
Positive Mantoux Result [b]	40 (81.6)
Evidence of TB at other sites	29 (35.8)

This table illustrates the clinical features related to TB infection in number (percentage). [a] Only recorded in n=78 (96%), [b] Mantoux tests were only conducted in n=49 (61%) of patients due to a nationwide shortage of tests.

Revised MRC TBM staging on initial presentation showed 37% of patients in stage 3 (n=30), 3.6% (n=28) in stage 2b, and 21% (n=17) in stage 2a. Only 6 (7%) children had no neurological deficits. The mortality rate was 13.6%. Definite TBM was diagnosed in 35 (43.2%) of patients, and probable TBM in 46 (56.8%) patients.

Table 3 shows the nature of hydrocephalus in the cohort. Fifty-eight patients (71.6%) had communicating hydrocephalus; 13 (16%) had non-communicating hydrocephalus; 10 (12.3%) had uncertain results due to a failed study (lumbar puncture needle was in the subarachnoid space but no CSF could be collected and/or air was not visible on the skull radiograph). Two patients had an EVD placed emergently because of their poor clinical condition, but died before an air encephalogram or column test could be performed.

Table 3: Classification of Children with TB Meningitis and Hydrocephalus

Patient parameter	**n (%); n=81**
Revised MRC TBM Stage	
Stage 1	6 (7.4)
Stage 2a	17 (21)
Stage 2b	28 (34.6)
Stage 3	30 (37)
TB Meningitis (TBM) Diagnosis	
Definite TBM	35 (43.2)
Probable TBM	46 (56.8)
Nature of Hydrocephalus (Air Encephalogram and/or column test)	
Communicating	58 (71.6)
Non-communicating	13 (16)
Uncertain (Failed study/ study not performed)	10 (12.3)

Results are illustrated as number (percentage). Results for 81 study patients are reported. Revised MRC TBM Stage is a system of staging severity of TBM based on level of consciousness and neurological fallout (van Toorn et al. , 2012).

Table 4 displays the neurological condition of the cohort. Percentages are given either for the full cohort, where the data were collected for all patients, or for the number of patients in whom that information was documented. The median admission GCS score was 11 (range 5-15). Most patients (n=72, 88.9%) presented with reduced consciousness. Focal neurological signs were documented in 58 (71.6%) patients (including cranial nerve palsies and hemiparesis). Only 58 patients had their lumbar opening pressure recorded, 38 (66%) of whom had raised intracranial pressure (defined according to RCWMCH Neurosurgery Department Clinical Protocols as opening pressure >27cmH2O/ 20mmHg). Papilledema was present in 15 (40.5%) of the 37 patients in whom fundoscopy was done. Cushing's reflex is a physiological response to raised intracranial pressure resulting in high blood pressure and low heart rate, detectable on clinical

examination. Thirty-two (57.1%) of the 56 patients whose blood pressure was documented on admission had high blood pressure. Twenty-seven (45.8%) of the 59 patients whose heart rate was documented on admission had a low heart rate for age. Headache was recorded in 26 (61.9%) children over 5 years old (n=42). Irritability was reported in 34 (41.8%) patients, vomiting in 42 (51.9%), neck stiffness in 58 (71.6%), and seizures were documented in 32 children (39.5%). A bulging fontanelle was found in 11 children with an open fontanelle (52.4%).

Table 4: Neurological Features of Children with TB Meningitis and Hydrocephalus

Patient parameter	n (%)
Focal neurological signs	58 (71.6)
Raised Intracranial Pressure [a]	38 (65.5)
Raised Blood Pressure for age [b]	32 (57.1)
Bradycardia [c]	27 (45.8)
Headache [d]	26 (61.9)
Irritability	34 (41.8)
Vomiting	42 (51.9)
Neck stiffness	58 (71.6)
Seizures	32 (39.5)
Abnormal posturing	15 (18.5)
Altered level of consciousness (GCS<15)	72 (88.9)
Lethargy	72 (88.9)
Not walking [e]	12 (21.4)
Bulging anterior fontanelle [f]	11 (52.4)
Papilledema [g]	15 (40.5)
Sun setting sign	3 (3.7)

Data are presented as absolute numbers (percentages) for signs and symptoms. Raised blood pressure was determined using standard pediatric vital parameter tables and is reported from recordings within the first 48 hours of admission. [a] Raised ICP was defined as an opening CSF pressure of > 27cmH2O measured using a manometer, the opening pressure was reported in 58 patients, [b] Blood pressure for age was reported in 56 patients, [c] Low heart rate for age was

reported in 59 patients, [d] Headache was only applicable to children > 5years old (n=42). [e] This was determined in children ≥ 18 months who had previously been walking (n=56). [f] Applicable to children ≤ 18 months, n=21). [g] Fundoscopy was only performed in 37 patients.

3.1.2 CNS imaging in TBM with hydrocephalus

Findings from all scans, admission and follow-up, are presented in Table 5. All patients included in the study had hydrocephalus. Severity of hydrocephalus and basal enhancement was graded using criteria previously defined by our institution (16) (Appendix 5). Twenty-two patients (27.2%) had mild hydrocephalus, 32 (38.8%) moderate, and 27 (33.8%) severe. Seventy-six patients (97.4%) demonstrated basal meningeal enhancement on admission in keeping with TBM, graded as mild in 33 (47.1%) patients, moderate in 32 (45.7%), and severe in 5 (7.1%). Fifty-one (63%) patients had brain infarcts; most of these had multiple infarcts (n=31, 60.8% of all infarcts). Thirty-seven patients (47.4%) had tuberculomas. Spinal imaging does not form part of standard imaging protocol at our institution; only thirty-nine patients (48.2%) had spinal scans that were done as part of a prospective study. Thirty-one of these scans (79.5%) were abnormal: most (n=30, 76,9%) showed features of spinal arachnoiditis, three (7.7%) had a single spinal tuberculoma and another three (7.7%) multiple spinal tuberculomas. Spinal disease severity was defined by institutional criteria (Appendix 5). Seventeen patients (54.8%) had mild, 8 (25.8%) moderate and 6 (19.4%) severe spinal disease. Severe spinal disease was characterised by extensive involvement of the spinal cord and subarachnoid space.

Of the 81 children enrolled in this study, 73 (90.1%) had lumbar CSF and 55 (67.9%) had ventricular samples. Twenty-nine patients (35.8%) had paired lumbar – ventricular samples taken at the same time or within 24 hours of each other; 4 patients (13.8%) had 2 CSF pairs.

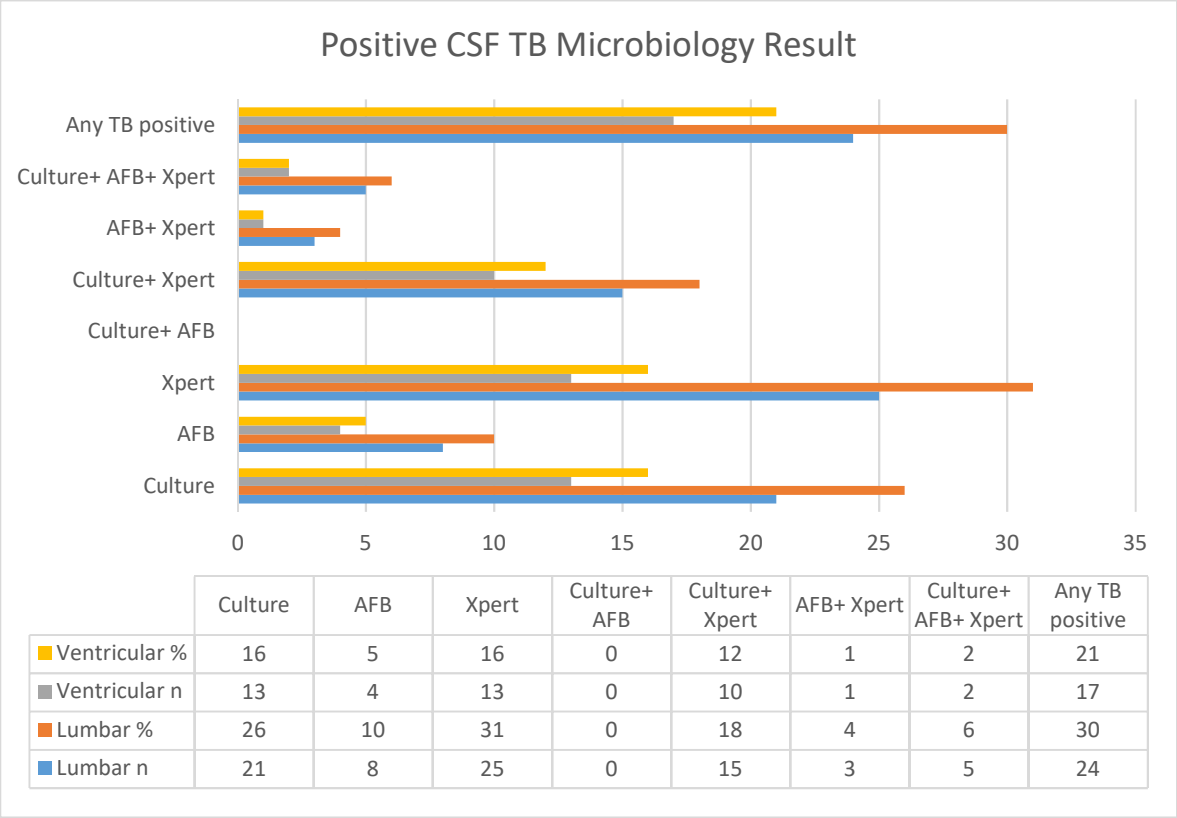
Table 5: CT and MRI Brain and Spine Features of TBM with Hydrocephalus

Scan Feature	n (%)
Hydrocephalus[a]	81 (100)
Non-communicating	13 (16)
Communicating	58 (71.6)
Uncertain	9 (11.1)
Admission enhancement present[b]	76 (97.4)
Infarcts present	51 (63)
Tuberculoma present[b]	37 (47.4)
Spinal disease present[c]	31 (79.5)
Spinal disease severity	
Mild	17 (54.8)
Moderate	8 (25.8)
Severe	6 (19.4)

Data are presented as number (percentage). [a] One patient was shunted immediately and did not have an air encephalogram or column test. [b] Three patients did not receive contrast on admission scan. [c] Spinal imaging was performed in 39 patients.

Figure 2 shows a breakdown of the 35 patients (43.2%) whose CSF yielded a positive result from one of the 3 TB microbiological tests performed. Relative to the entire cohort, 29 (36%) had positive TB culture, 12 (15%) had positive AFB, 34 (42%) had positive Gene Xpert. No patient (0%) had combined culture positivity plus AFB. Twenty-two (27%) patients had combined positive results for TB culture and Gene Xpert. Four (5%) were both AFB and Gene Xpert positive. The CSF TB microbiology results reported are disaggregated according to lumbar or ventricular CSF compartment.

Figure 2: TBM Confirmed on CSF Microbiology



	Culture	AFB	Xpert	Culture+ AFB	Culture+ Xpert	AFB+ Xpert	Culture+ AFB+ Xpert	Any TB positive
Ventricular %	16	5	16	0	12	1	2	21
Ventricular n	13	4	13	0	10	1	2	17
Lumbar %	26	10	31	0	18	4	6	30
Lumbar n	21	8	25	0	15	3	5	24

The graph illustrates positive CSF microbiology: TB culture (gold standard), AFB (Acid Fast Bacilli) and DNA Gene Xpert. Relative to the total cohort of 81 patients, 35 patients had a positive TB CSF microbiology result. The data table shows percentages and absolute numbers for lumbar and ventricular CSF analytes. Yellow bars represent ventricular CSF percentage. Grey bars represent ventricular CSF absolute number. Orange bars represent lumbar CSF percentage, Blue bars represent lumbar CSF absolute number.

3.2 CSF temporal profiles in lumbar versus ventricular CSF compartments (pooled samples)

The following tables 6 - 7 and figures 3 - 8 indicate overall temporal profiles of lumbar CSF and ventricular CSF parameters measured over the first 3 weeks post-admission. All data points for each CSF compartment were analyzed.

3.2.1 Lumbar CSF results

Over the first 21 days post-admission lumbar CSF glucose had a median of 2.2 mmol/L (IQR 1.5-2.9 mmol/L), protein values had a median of 2 g/L (IQR 1.2-2.3 g/L), and chloride had a median of 112 g/L (IQR 107-119 g/L). Lumbar CSF polymorphonuclear cells had a median of 15 x10^6/L (IQR 4-50 x10^6/L), lymphocytes had a median of 135 x10^6/L (IQR 63-286 x10^6/L), and total white cells had a median of 165 x10^6/L (IQR 86-340 x10^6/L). Lumbar CSF median glucose and chloride fell below the normal limits. CSF protein was above the normal value of 0.4 g/L in almost all (n=195, 97.5%) lumbar samples, and above 0.8 g/L in 87% (n=174) throughout the first 3 weeks of hospitalization. Cell counts were always above normal. The lumbar CSF analytes over 4-day time epochs are shown below (Table 6) and trends described under their corresponding graphs.

3.2.2 Ventricular CSF results

Values are shown in Table 7. Over the first 21 days post-admission, the medians and IQR for ventricular CSF biochemistry were: glucose 3 mmol/L (IQR 2.6–3.6 mmol/L), protein 0.5 g/L (IQR 0.3-0.9 g/L), and chloride 114.5 g/L (IQR 109-120 g/L). For cells, medians and IQR were: polymorphonuclear cells 2 x10^6/L (IQR 0-5.5 x10^6/L), lymphocytes 21 x10^6/L (IQR 9-40 x10^6/L), and total white cells 22 x10^6/L (IQR 10-45 x10^6/L). Ventricular CSF median glucose fell within normal limits, chloride was below normal, and protein was > 0.4 g/L in n=58 (65.2%), and > 0.8 g/L in n=28 (31.5%) of samples.

Table 6: Description of Lumbar CSF Biochemistry and Cell Count Over Time

Time Epochs	Days	1-4	5-8	9-12	13-16	17-21	Overall
Glucose (mmol/L)	Median	1.6	2.6	2.3	2.4	2.8	2.2
	Minimum	0.3	1.1	1.2	0.8	1.2	0.3
	Maximum	4.8	4.5	3.9	4.3	4.4	4.8
	25th % (Q1)	1.1	2.1	1.8	1.7	2.4	1.5
	75th% (Q2)	2.3	3.1	3.1	3.0	3.2	2.9

Chloride (mmol/L)	Median	109	114.5	114	114.5	119	112
	Minimum	93	95	100	100	101	93
	Maximum	147	130	128	140	172	172
	25th % (Q1)	105	107	110	111.3	113.5	107
	75^{tho}% (Q2)	113	121.8	120	123	127	119
Protein (g/L)	Median	2	2	1.7	1.5	1.3	2
	Minimum	0.3	0.7	0.4	0.3	0.2	0.2
	Maximum	48.9	35.4	14.7	9.7	6.1	48.9
	25th % (Q1)	1.4	1.3	0.9	0.7	0.9	1.2
	75^{tho}% (Q2)	2.8	2.5	2.1	2.1	2.2	2.3
Polymorphonuclear cells (x10^6/L)	Median	18	17	7	11	8	15
	Minimum	0	0	0	0	0	0
	Maximum	635	270	121	184	405	635
	25th % (Q1)	6	3	1	4	2	4
	75^{tho}% (Q2)	57	55	35	50	31	50
Lymphocytes (x10^6/L)	Median	137	268	138	100	68	135
	Minimum	8	21	11	0	20	0
	Maximum	621	2275	870	1855	270	2275
	25th % (Q1)	56	118	56	64.5	40	63
	75^{tho}% (Q2)	286	455	199	329	112.5	286
TWCC (x10^6/L)	Median	170	305	176	116	73	165
	Minimum	9	23	11	5	23	5
	Maximum	995	2280	920	1960	575	2280
	25th % (Q1)	86	132	90	68	59	86
	75^{tho}% (Q2)	330	615	242	455.5	144.5	340

This table depicts descriptive statistics for lumbar CSF compartment analytes in time epochs (4 days) and overall values across the 21 days post admission. The overall values are the values calculated for the full dataset (all time points). Biochemistry analytes were glucose, protein and chloride. Cellular analytes were polymorphonuclear cells , lymphocytes and total white cell count (TWCC).

Table 7: Description of Ventricular CSF Biochemistry and Cell Count Over Time

Time Epochs	Days	1-4	5-8	9-12	13-16	17-21	Overall
Glucose (mmol/L)	Median	3.1	3.5	3	3.5	2.5	3
	Minimum	1.2	1.4	2.3	2.8	2.1	1.2
	Maximum	5.4	5.2	4.4	3.9	3.2	5.4
	25th % (Q1)	2.6	2.6	2.4	2.6	2.1	2.6

	75th% (Q2)	3.6	3.7	3.3	3.9	3.2	3.6
Chloride (mmol/L)	Median	112	116	116	117.5	118	114.5
	Minimum	89	93	106	114	115	89
	Maximum	148	149	133	121	118	149
	25th % (Q1)	108	110	112	114.3	115	109
	75th% (Q2)	119	121	121.5	120.8	118	120
Protein (g/L)	Median	0.5	0.8	0.4	0.8	1.4	0.5
	Minimum	0.1	0.1	0.2	0.2	0.9	0.1
	Maximum	2	2.7	2	1	2	2.7
	25th % (Q1)	0.3	0.2	0.3	0.4	0.9	0.3
	75th% (Q2)	0.8	1.2	1.3	1	2.1	0.9
Polymorphonuclear cells (x10^6/L)	Median	3	2	2	5.5	3	2
	Minimum	0	0	0	1	0	0
	Maximum	74	15	13	13	10	74
	25th % (Q1)	0	0.3	0	2	0	0
	75th% (Q2)	5	5.5	5	11.3	10	5.5
Lymphocytes (x10^6/L)	Median	13	25.5	22	36	98	21
	Minimum	0	4	5	17	93	0
	Maximum	100	480	84	57	136	480
	25th % (Q1)	6	12.3	9	19.8	93	9
	75th% (Q2)	35	36.5	67	53.8	136	40
TWCC (x10^6/L)	Median	15	29	23	41.5	103	22
	Minimum	0	0	5	18	101	0
	Maximum	139	495	89	70	136	495
	25th % (Q1)	9	11.5	11	22	101	10
	75th% (Q2)	39	40.5	73	64.8	136	45

This table depicts descriptive statistics for ventricular CSF compartment analytes over 21 days post admission. Biochemistry analytes were glucose, protein and chloride. Cells were polymorphonuclear cells, lymphocytes and total white cell count (TWCC).

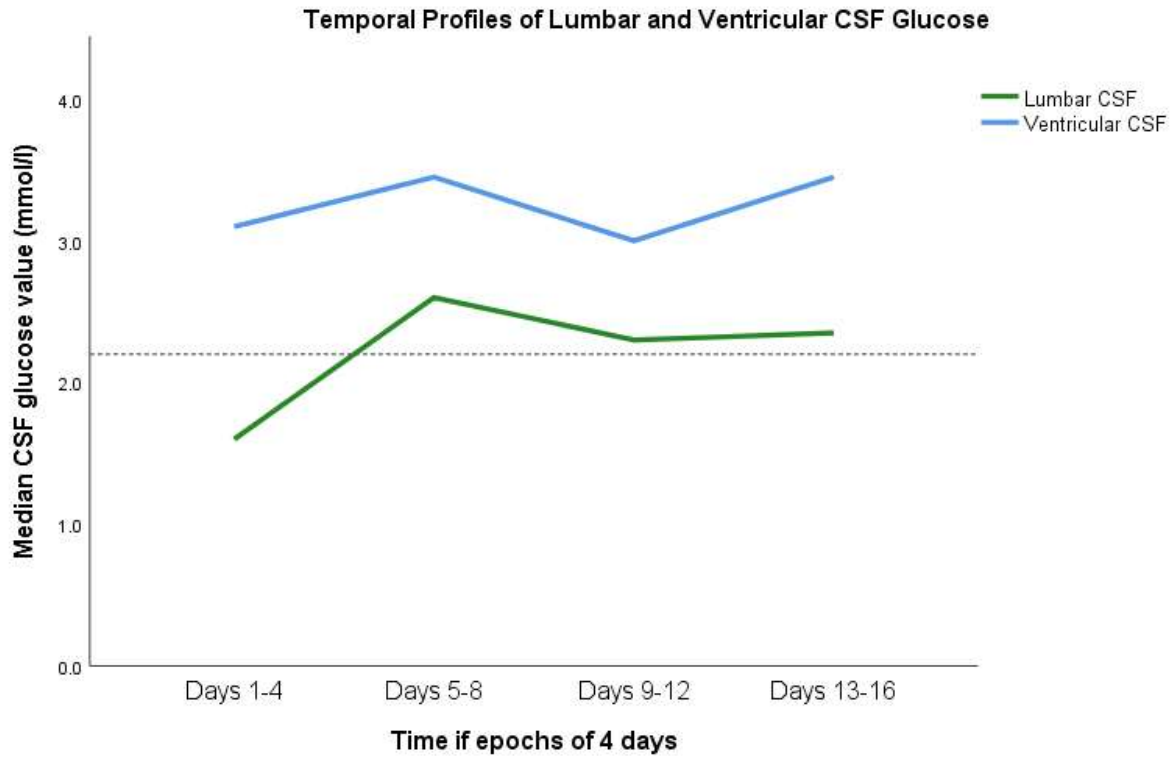
3.3 Temporal profiles of lumbar and ventricular CSF samples

Time point 17-21 days was excluded from the plots because the numbers were too few. Profiles including this time point are shown in Appendix 9.

3.3.1 CSF glucose temporal profile

Lumbar CSF glucose began low and normalised by the start of week 2 (Figure 3). Ventricular CSF glucose remained within the normal range throughout the 21-day period. Ventricular CSF glucose concentrations were greater than in lumbar CSF.

Figure 3:

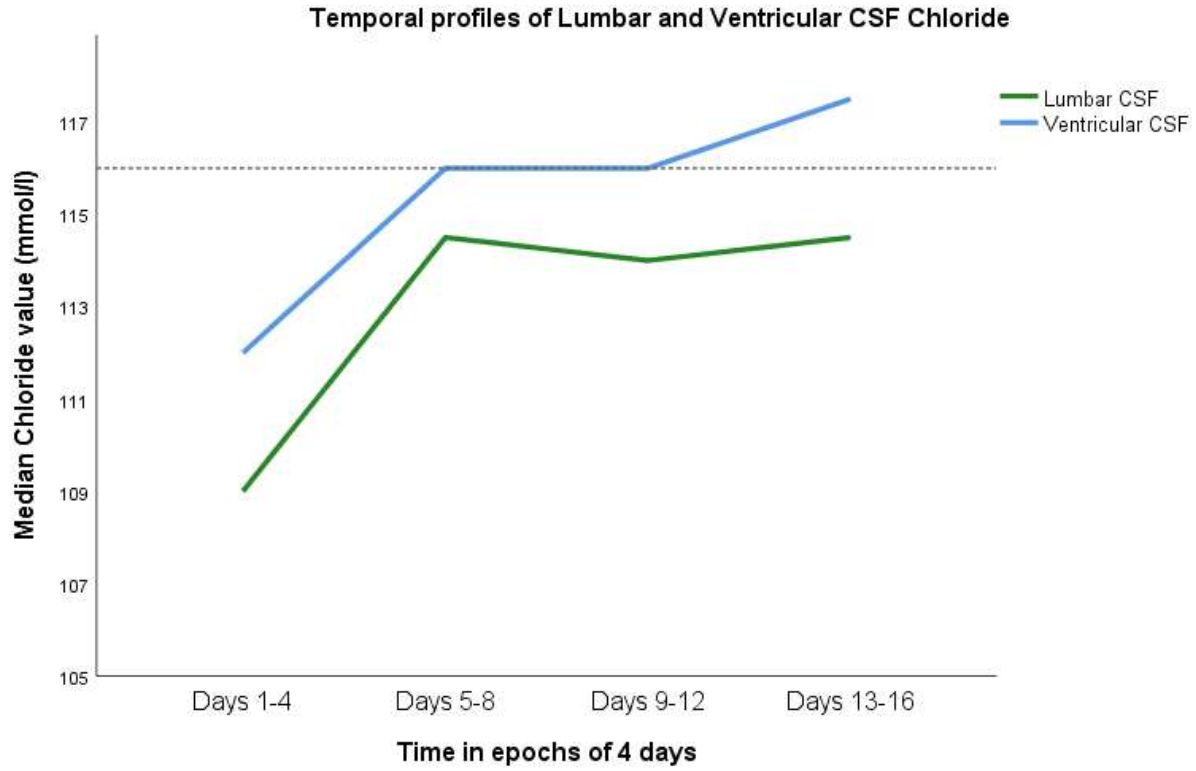


Temporal changes in overall lumbar and ventricular CSF glucose over time epochs of 4 days for the first 16 days after admission in children with TBM and hydrocephalus. The horizontal dotted line represents the minimum normal glucose value of 2.2 mmol/L. Median lumbar CSF values are shown as a green line. Median ventricular CSF values are shown as a blue line. Number of samples per time point with lumbar CSF in green and ventricular CSF in blue: Day 1-4 (n=89, 59), Day 5-8 (n=39, 14), Day 9-12 (n=31, 10), Day 13-16 (n=20, 4)

3.3.2 CSF chloride temporal profile

Chloride concentrations in both lumbar and ventricular CSF began low, but increased by the end of the first week. Ventricular CSF chloride concentrations were greater than those in lumbar CSF (Figure 4).

Figure 4:

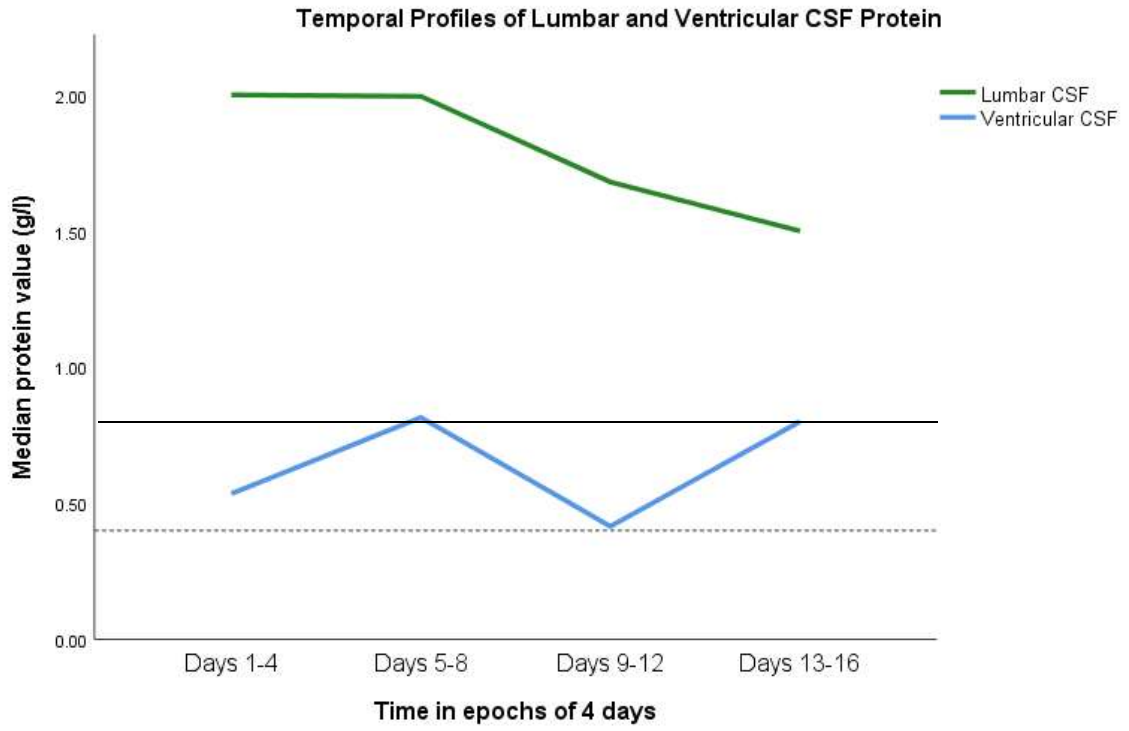


Temporal changes in lumbar and ventricular CSF chloride over time epochs of 4 days for the first 16 days after admission in children with TBM and hydrocephalus. The horizontal dotted line represents the minimum normal chloride value of 116 mmol/L. Median lumbar CSF values are shown as a green line. Median ventricular CSF values are shown as a blue line. Number of samples per time point with lumbar CSF in green and ventricular CSF in blue: Day 1-4 (n=85, 59), Day 5-8 (n=36, 14), Day 9-12 (n=31, 9), Day 13-16 (n=20, 4).

3.3.3 CSF protein temporal profile

Lumbar CSF protein was greater than ventricular CSF protein. Lumbar CSF protein remained above the limits of 0.4 and 0.8 g/L throughout the first 3 weeks of hospitalization. Median ventricular CSF protein was above the limit of 0.4 g/L (Figure 5) but not the limit of 0.8 g/L (Figure 5).

Figure 5:

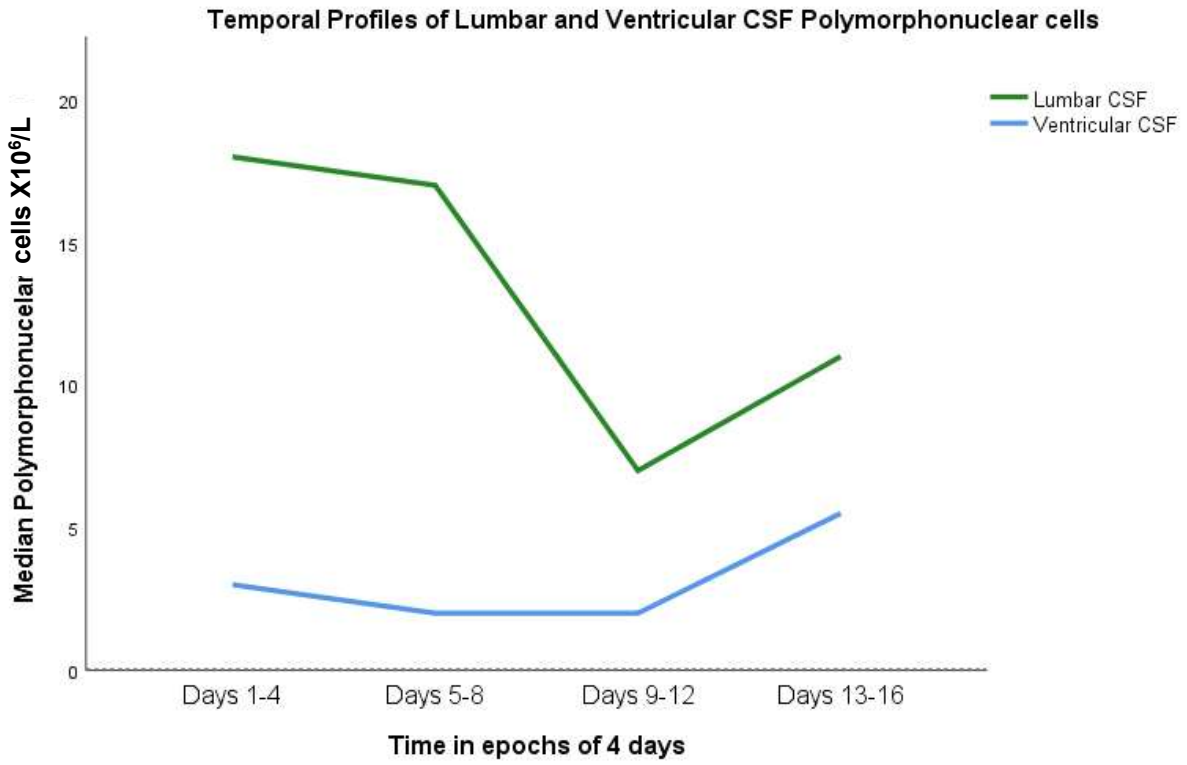


Temporal changes in lumbar and ventricular CSF protein over specific time epochs of 4 days for the first 16 days after admission in children with TBM and hydrocephalus. The horizontal dotted line represents the maximum protein value of 0.4 g/L and the horizontal solid line represents 0.8 g/L respectively. Median lumbar CSF values are shown as a green line. Median ventricular CSF values are shown as a blue line. Number of samples per time point with lumbar CSF in green and ventricular CSF in blue: Day 1-4 (n=89, 58), Day 5-8 (n=38, 14), Day 9-12 (n=30, 10), Day 13-16 (n=18, 4).

3.3.4 CSF polymorphonuclear cells temporal profile

Lumbar and ventricular CSF polymorphonuclear cell counts remained above zero for the 3 weeks of hospitalisation. Lumbar CSF counts were higher than ventricular counts, Figure 6.

Figure 6:

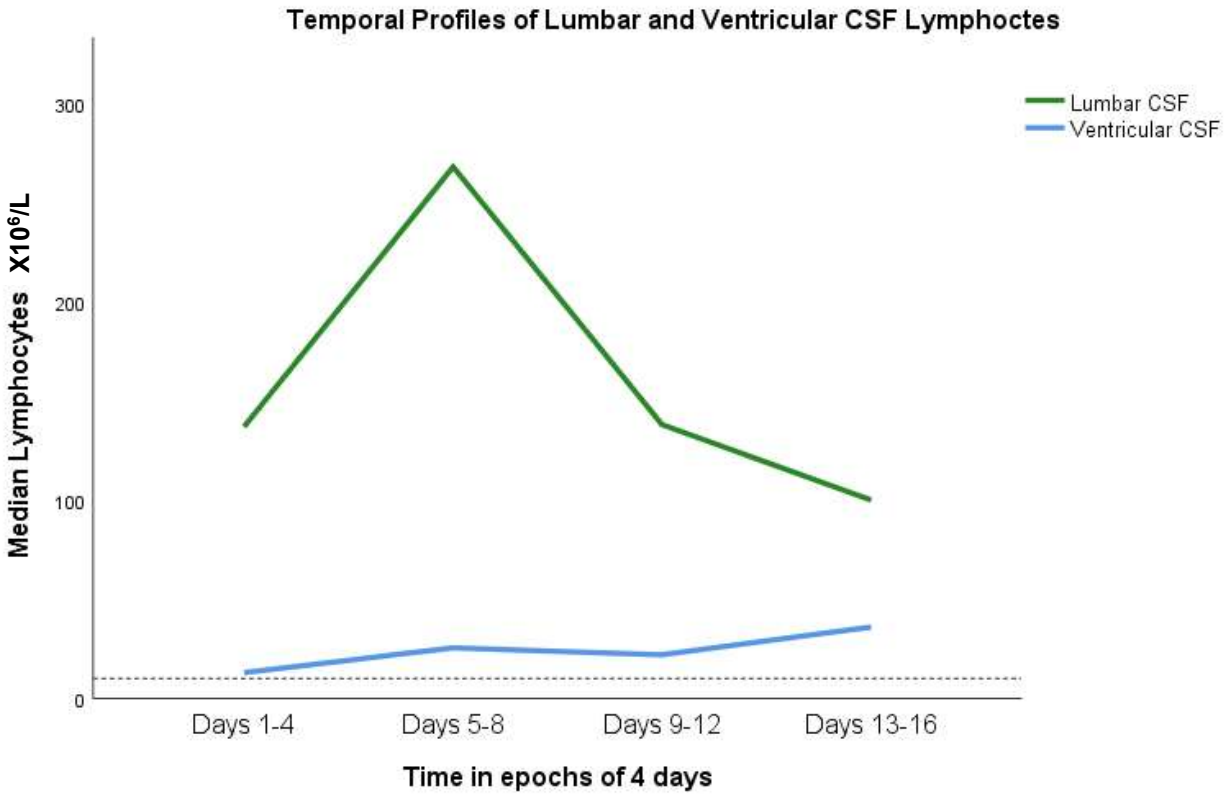


Temporal changes in lumbar and ventricular CSF polymorphonuclear cells over specific time epochs of 4 days for the first 16 days after admission in children with TBM and hydrocephalus. The horizontal dotted line represents the maximum normal polymorphonuclear cell value of 0 x10^6/L. Median lumbar CSF values are shown as a green line. Median ventricular CSF values are shown as a blue line. Number of samples per time point with lumbar CSF in green and ventricular CSF in blue: Day 1-4 (n=91, 59), Day 5-8 (n=39, 16), Day 9-12 (n=31, 11), Day 13-16 (n=21, 4).

3.3.5 CSF lymphocytes temporal profile

Lumbar CSF lymphocytes were elevated overall; they peaked at day 5-8. Ventricular CSF lymphocytes counts were raised, but remained lower than lumbar CSF counts, Figure 7.

Figure 7:

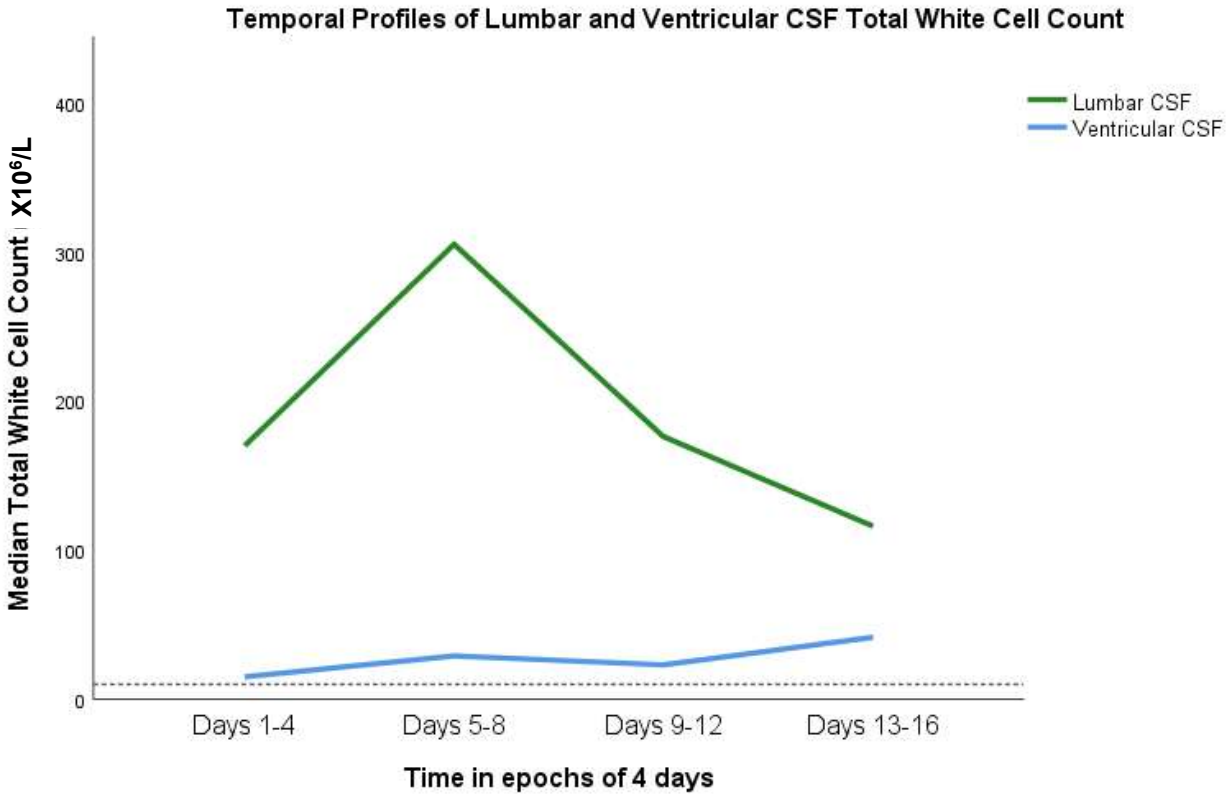


The graph shows changes in lumbar and ventricular CSF lymphocytes over specific time epochs of 4 days for the first 16 days after admission in children with TBM and hydrocephalus. The horizontal dotted line represents the maximum normal lymphocyte value of 5 x10^6/L. Median lumbar CSF values are shown as a green line. Median ventricular CSF values are shown as a blue line. Number of samples per time point with lumbar CSF in green and ventricular CSF in blue: Day 1-4 (n=91, 59), Day 5-8 (n=39, 16), Day 9-12 (n=31, 11), Day 13-16 (n=21, 4).

3.3.6 CSF total white cell count temporal profile

The total white cell count reflected the patterns seen for polymorphonuclear cells and lymphocytes. Lumbar and ventricular CSF values were elevated, lumbar values to a greater degree. Lumbar values peaked on days 5-8.

Figure 8:



The graph shows changes in unpaired lumbar and ventricular CSF total white cell count over specific time epochs of 4 days for the first 16 days after admission in children with TBM and hydrocephalus. The horizontal dotted line represents the maximum normal total white cell count value of 10 x10^6/L. Total white cells are derived from the sum of polymorphonuclear cells and lymphocytes. Median lumbar CSF values are shown as a solid green line. Median ventricular CSF values are shown as a solid blue line. Number of samples per time point with lumbar CSF in green and ventricular CSF in blue: Day 1-4 (n=91, 59), Day 5-8 (n=39, 16), Day 9-12 (n=31, 11), Day 13-16 (n=21, 4).

3.4 Paired (time-linked) lumbar and ventricular CSF parameters

3.4.1 Paired lumbar and ventricular CSF biochemistry

A total of 33 paired lumbar and ventricular CSF samples were collected from 29 patients; most of whom only had 1 sample pair (n=25, 86%). Most (81.8%) pairs were collected in days 1 to 3 post-admission. One patient did not have lumbar CSF tested for biochemistry parameters and was excluded from analysis of biochemistry values but included for cell count comparison. Summary statistics of paired lumbar and ventricular CSF biochemistry results are summarised in Table 8. Table 9 shows the individual values for each paired lumbar-ventricular CSF sample, and the differential and ratio between the concentrations.

Glucose: Only 32 paired samples had glucose concentrations analysed. Most ventricular CSF glucose values (n= 30/32, 93.8%) fell within the normal range of 2.2 to 4.0 mmol/L, whereas most (n= 25/32, 78.1%) lumbar glucose values were low. The vast majority (n=30/32, 93.8%) of the ventricular CSF glucose values were higher than lumbar glucose values. The differences between the compartments was significant ($p<0.001$). The median differential between the compartments (calculated as lumbar-ventricular) was -1.8 (IQR 2.1 to 1.4). The median ratio (lumbar/ ventricular) was 0.4 (IQR 0.2 to 0.6).

Protein: Only 31 paired samples had protein concentrations analysed. All lumbar protein values were > 0.8 g/L (n=31/31, 100%), whereas only 19 (61.3%) ventricular protein values were > 0.4 g/L, and 7 (22.6%) were > 0.8 g/L. All lumbar CSF protein values were significantly greater than corresponding paired ventricular protein values ($p<0.001$). The median protein differential was 1.5 g/L (IQR 1.1 to 2.8). The median protein ratio was 4 (4.7 to 4.5).

Chloride: Only 31 paired samples had chloride concentrations analysed. In most patient (n=24/31, 77.4%) lumbar chloride was below the normal limit; 71% (n=22/31) of ventricular values were abnormal. Most patients (n=23, 74.2%) had higher ventricular than lumbar values; the difference between the compartments

was statistically significant (*p=0.01*). The median chloride differential was -4 (IQR -3.5 to -4.5). The median chloride ratio was 0.96 (IQR 0.97 to 0.96).

Table 8: Summary Statistics for Lumbar and Ventricular Paired CSF Biochemistry Analytes

	Glucose (mmol/L)		Protein (g/L)		Chloride (mmol/L)	
CSF	**Lumbar**	**Ventricular**	**Lumbar**	**Ventricular**	**Lumbar**	**Ventricular**
MIN	0.3	1.2	0.9	0.2	95	93
MAX	3	4.5	48.9	1.5	131	126
MEDIAN	1.2	3	2	0.5	108	112
p-value (of medians)	*p<0.001		*p<0.001		*p=0.01	
25th (Q1)	0.7	2.8	1.4	0.3	104	107.5
75th (Q3)	2.1	3.5	3.6	0.8	114	118.5

Descriptive data for paired lumbar and ventricular CSF biochemistry analytes glucose (n=32), protein (n=31), chloride (n=31). P-values of medians between lumbar and ventricular compartments are indicated. '*' denotes statistical significance.

Table 9: Paired Lumbar and Ventricular CSF Biochemical Parameters

Patient No	CSF Biochemistry											
	Glucose (mmol/L) (Normal 2.2-4.0)				Protein (g/L) (Normal 0.2-0.8)				Chloride (mmol/L) (Normal 116-130)			
	Lumbar	**Vent**	**Diff**	**Ratio**	**Lumbar**	**Vent**	**Diff**	**Ratio**	**Lumbar**	**Vent**	**Diff**	**Ratio**
2	0.3	2.4	-2.1	0.1	1.1	0.7	0.4	1.6	109	110	-1	1
4	0.5	3	-2.5	0.2	48.9	0.5	48.4	97.8	102	123	-21	0.8
5	2.7	2.8	-0.1	1	11.8	0.5	11.3	23.6	97	94	3	1

6	0.6	2.8	-2.2	0.2	1	0.3	0.7	3.3	117	121	-4	1
11	0.7	2.9	-2.2	0.2	7.8	0.8	7.1	9.8	113	126	-13	0.9
11	1.2	2.8	-1.6	0.4	15.5	1.1	14.5	14.1	95	93	2	1
12	1.3	3.4	-2.1	0.4	5.4	0.9	4.5	6		109		0
16	3	3	0	1	1.4	0.8	0.7	1.8	104	107	-3	1
19	1	4.3	-3.3	0.2	2	0.2	1.9	10	121	102	19	1.2
20	1.2	3.2	-2	0.4	13.5	0.3	13.2	45	101	111	-10	0.9
20	2.8	4.4	-1.6	0.6	35.4	0.2	35.2	177	104	120	-16	0.9
22	2.1	3.5	-1.4	0.6	1.2	0.5	0.8	2.4	108	114	-6	1
22	3	1.5	1.5	2	2.3	0.4	1.8	5.8	118	106	12	1.1
23	0.7	1.2	-0.5	0.6	3.7	1.5	2.2	2.5	131	108	23	1.2
25	2.1	3.7	-1.6	0.6	3.6	0.4	3.2	9	109	115	-6	1
29	1.6	3.6	-2	0.4	2.4	0.3	2.1	8	104	103	1	1
31	0.3	3.3	-3	0.1	1.4	0.2	1.2	7	104	109	-5	1
35	0.8	3	-2.2	0.3	1.4	0.6	0.8	2.3	109	112	-3	1
36	1.3	2.5	-1.2	0.5	1.6	0.4	1.2	4	105	110	-5	1
41	0.7	3.7	-3	0.2	2	1.4	0.7	1.4	106	107	-1	1
43	1.8	3.9	-2.1	0.5	3	1.3	1.7	2.3	107	113	-6	1
48	2.9	3	-0.1	1	2	0.8	1.6	2.7	113	116	-3	1
52	0.8	4.5	-3.7	0.2	2	0.4	1.6	5	111	119	-8	0.9
53	1	2.8	-1.8	0.4	2	0.7	1.3	2.8	116	120	-4	1
55	1.2	2.3	-1.1	0.5	2	0.4	1.6	5	106	112	-6	1
57	1.2	3.5	-2.3	0.3	0.9	0.2	0.6	4.5	107	113	-6	1
57	1.6	3.3	-1.7	0.5	2	0.3	1.7	6.7	111	113	-2	1
58	2	3.5	-1.5	0.6	1.3	0.8	0.5	1.6	121	122	-1	1
63	2.5	3	-0.5	0.8		1			106	121	-15	0.9

72	1.1	3	-1.9	0.4	1.3	0.5	0.8	2.6	114	112	2	1
73	2.5	3.2	-0.7	0.8	1.1	0.9	0.1	1.2	119	118	1	1
81	0.6	2.6	-2	0.2	2.3	0.8	1.5	2.9	107	109	-2	1

The table shows 28 of the 29 patients with paired lumbar-ventricular CSF (n=32) biochemical analytes (glucose, protein, chloride), their differentials and ratios (%) – patient 1's data are not shown as lumbar chemistry tests were not performed. Normal ranges according to standard National Health Laboratory Services parameters are shown in brackets. The abbreviations "Vent" = ventricular. "Diff" = Differential. Differentials were calculated as lumbar-ventricular value. Ratios were calculated as lumbar/ ventricular value. Lumbar and ventricular paired samples were collected within 24hrs of each other. Blank space represents missing data when investigations were not performed.

3.4.2 Paired lumbar and ventricular CSF cell counts

Table 10 depicts summary statistics and Table 11 depicts individual absolute values for paired lumbar and ventricular CSF cell results in the 29 patients with CSF pairs. In general, values for all cells were higher in lumbar compared to ventricular samples, and these differences were statistically significant ($p<0.001$ for polymorphonuclear cells, lymphocytes, and total white cell count). In 5 patients (15.2%) the ventricular polymorphonuclear cell counts was higher than the lumbar CSF. Nine ventricular CSF samples (27.3%) and 4 (12.1%) lumbar CSF samples had normal polymorphonuclear cell counts of 0 $x10^6$/L. All lumbar CSF samples (n=33, 100%) had raised lymphocyte and total white cell counts. Four (12.1%) ventricular samples had lymphocyte counts of </= 5 $x10^6$/L and 8 (24.2%) had total white cell counts of </= 10 $x10^6$/L. The median polymorphonuclear cell differential (lumbar minus ventricular value) was 9 (IQR 3 to 47); lymphocyte differential was 128 (IQR 40 to 237), and for total white cell count this was 179 (IQR 63 to 252) $x10^6$/L.

Table 10: Summary Statistics for Lumbar and Ventricular Paired CSF Cell Counts

	Polymorphonuclear cells ($x10^6$/L)		Lymphocytes ($x10^6$/L)		Total white cell count ($x10^6$/L)	
	Lumbar	**Ventricular**	**Lumbar**	**Ventricular**	**Lumbar**	**Ventricular**
MIN	0	0	10	0	21	0
MAX	280	74	2275	136	2280	139
MEDIAN	12	3	149	21	202	23
p-value (of medians)	*p<0.001		*p<0.001		*p<0.001	
25th (Q1)	3	0	48	8	74	11
75th (Q3)	53	6	282	45	309	57

This table shows descriptive statistical findings for paired lumbar and ventricular CSF cytology analytes polymorphonuclear cells (n=33), lymphocytes (n=33), total white cell count (n=33). Paired CSF were taken on admission and during subsequent weeks. P-values of medians for paired lumbar and ventricular compartments are indicated. '*' denotes statistical significance.

Table 11: Paired Lumbar and Ventricular CSF Cell Count Parameters

Patient No	CSF cell count											
	Polymorphonuclear cells ($x10^6$/L) (normal = $x10^6$/L)				Lymphocytes ($x10^6$/L) (normal </= $5x10^6$/L)				Total white cell count ($x10^6$/L) (normal </=10 $x10^6$/L)			
	Lumbar	**Vent**	**Diff**	**Ratio**	**Lumbar**	**Vent**	**Diff**	**Ratio**	**Lumbar**	**Vent**	**Diff**	**Ratio**
1	11	3	8	3.7	205	24	181	8.5	216	27	189	8
2	4	2	2	2	411	100	311	4.1	415	102	313	4.1
4	15	1	14	15	360	8	352	45	375	9	366	41.7
5	0	0	0	0	270	27	243	10	270	27	243	10

6	4	0	4	0	22	11	11	2	26	11	15	2.4
11	280	0	280	0	40	6	34	6.7	320	6	314	53.3
11	50	0	50	0	60	5	55	12	110	5	105	22
12	1	2	-1	0.5	74	24	50	3.1	75	26	49	2.9
16	74	5	69	14.8	71	8	63	8.9	145	13	132	11.2
19	12	0	12	0	286	0	286	0	298	0	298	0
20	0	0	0	0	443	10	433	44.3	443	10	433	44.3
20	5	4	1	1.2	2275	38	2237	59.9	2280	42	2238	54.3
22	63	20	43	3.2	139	45	94	3.1	202	65	137	3.1
22	30	15	15	2	425	95	330	4.5	455	110	345	4.1
23	12	74	-62	0.2	158	65	93	2.4	170	139	31	1.2
25	150	3	147	50	115	9	106	12.8	265	12	253	22.1
29	12	7	5	1.7	149	21	128	7.1	161	28	133	5.8
31	59	10	49	5.9	10	1	9	10	69	11	58	6.3
35	38	3	35	12.7	35	5	30	7	73	8	65	9.1
36	19	3	16	6.3	22	17	5	1.3	41	20	21	2
41	1	0	1	0	258	45	213	5.7	259	45	214	5.8
43	58	5	53	11.6	277	51	226	5.4	335	56	279	6
48	90	2	88	45	100	10	90	10	190	12	178	15.8
52	8	1	7	8	176	37	139	4.8	184	38	146	4.8
53	1	0	1	0	37	6	31	6.2	38	6	32	6.3
55	0	2	-2	0	286	21	265	13.6	286	23	263	12.4
57	6	3	3	2	15	6	9	2.5	21	9	12	2.3
57	0	2	-2	0	55	9	46	6.1	55	11	44	5
58	16	7	9	2.3	201	51	150	3.9	217	58	159	3.7
63	55	13	42	4.2	1705	57	1648	29.9	1760	70	1690	25.1

72	3	27	-24	0.1	71	40	31	1.8	74	67	7	1.1
73	2	0	2	0	202	136	66	1.5	204	136	68	1.5
81	17	3	14	5.7	40	16	24	2.5	57	19	38	3

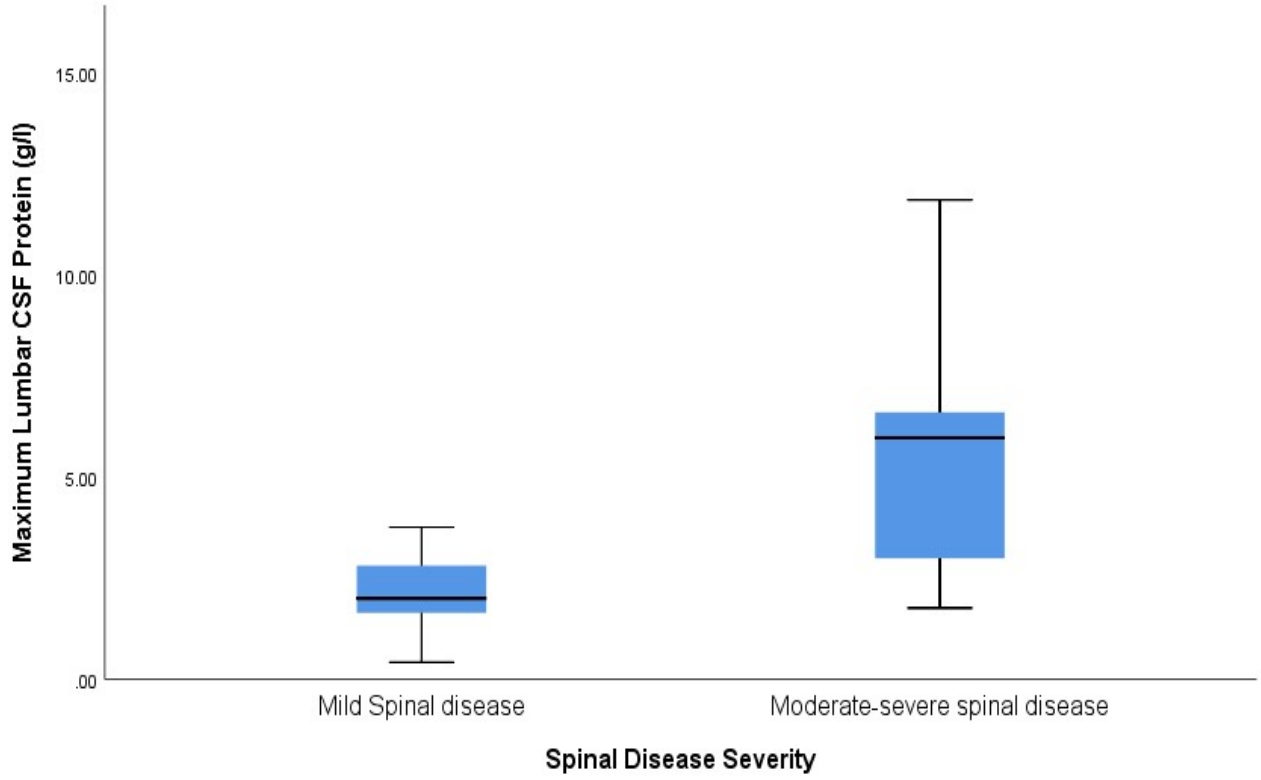
Descriptive data for patients (n=29) with paired lumbar-ventricular CSF (n=33) cytology analytes (polymorphonuclear cells, lymphocytes and total white cell count), their differentials and ratios (presented as %). The abbreviations "Vent" = ventricular. "Diff" = Differential. Normal ranges according to standard RCWMCC NHLS Laboratory parameters are shown in brackets. Differentials were calculated as lumbar-ventricular value. Ratios were calculated as lumbar/ ventricular value. Lumbar and ventricular paired samples were collected within 24hrs of each other. Blank space represents missing data when investigations were not performed.

3.5 Analysis of unpaired lumbar and ventricular CSF biochemistry and cell count against radiology characteristics

3.5.1 Minimum and Maximum lumbar and ventricular CSF chemistry and cell counts

In unpaired, non time-linked CSF samples, only maximum lumbar CSF protein demonstrated a significant association with spinal disease severity (*p=0.04*), with higher protein concentrations observed in patients with moderate-severe spinal disease (Figure 9). There were no significant associations found between ventricular CSF and radiology characteristics.

Figure 9: Box and Whisker Plot for Highest Lumbar CSF Protein Based on Spinal Disease Severity



The box plot demonstrates the maximum lumbar CSF protein reported across the study cohort. Protein for 200 lumbar CSF samples were analysed. Mild spinal disease (n=17) was defined as a thin layer of exudate surrounding spinal cord and nerve roots, moderate as the presence of plaques or clumping of spinal nerve roots, and severe as the presence of extensive exudate in surrounding spinal cord and nerve roots or in subarachnoid space (moderate-severe spinal disease n=14).

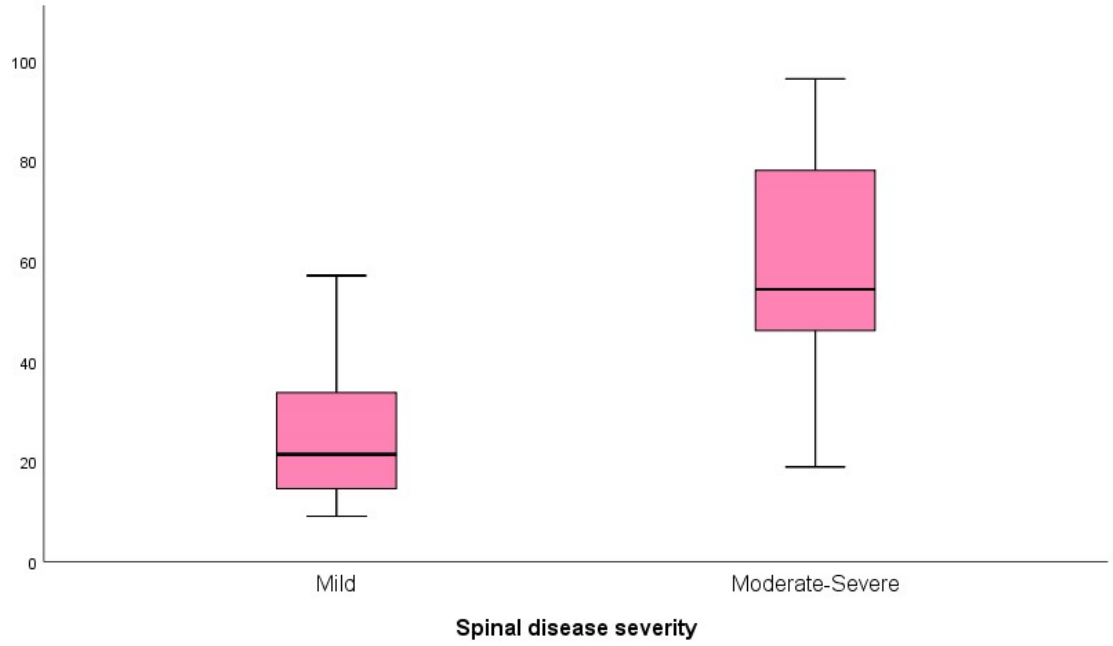
3.5.2 Lumbar and ventricular CSF chemistry and cell count differentials

There were no other significant relationships with CSF differentials and radiology characteristics.

3.5.3 Lumbar and ventricular CSF chemistry and cell count ratios

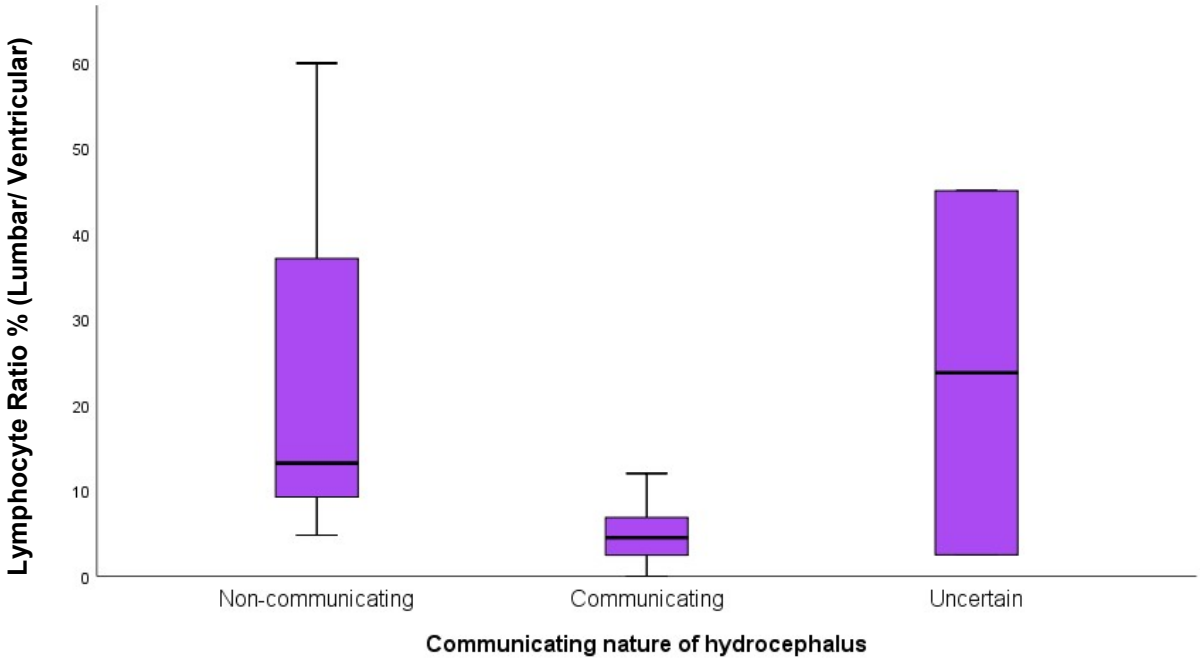
The ratio of CSF glucose between the lumbar and ventricular compartments was significantly greater in patients with mild spinal disease compared to those with moderate-severe disease ($p=0.046$) – Figure 10. The lymphocyte ratio was significantly different based on the communicating nature of hydrocephalus ($p<0.013$), with the lowest ratio demonstrated in patients with communicating hydrocephalus – Figure 11. There were no other significant relationships with CSF ratios and radiology characteristics.

Figure 10: Box and Whisker Plot for the Ratio of CSF Glucose Based on Spinal Disease Severity



The box plot demonstrates the ratio of lumbar and ventricular glucose in patients with paired lumbar and ventricular CSF samples. Glucose for 32 CSF pairs were analysed. The ratio was calculated as lumbar/ventricular, and presented as a percentage. Mild spinal disease (n=4) was defined as a thin layer of exudate surrounding spinal cord and nerve roots, moderate as the presence of plaques or clumping of spinal nerve roots, and severe as the presence of extensive exudate in surrounding spinal cord and nerve roots or in subarachnoid space (moderate-severe spinal disease n=24).

Figure 11: Box and Whisker Plot for the Ratio of CSF Lymphocyte Count Based on the Communicating Nature of Hydrocephalus

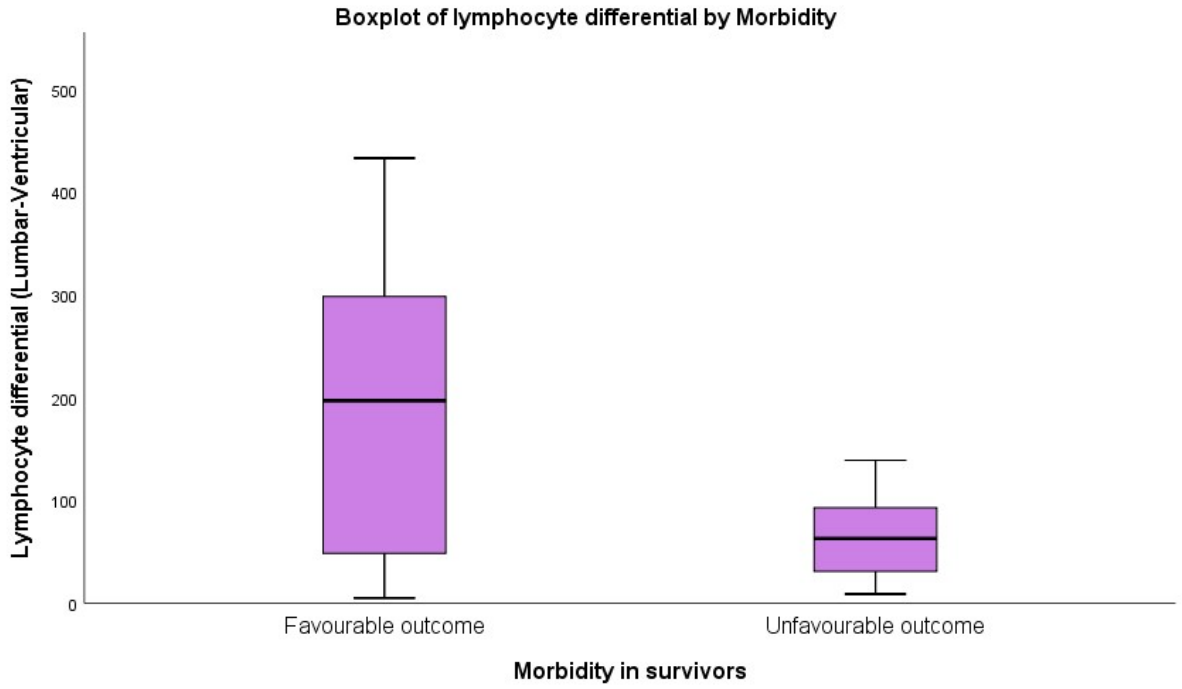


The box plot demonstrates the ratio of lumbar and ventricular glucose in patients with paired lumbar and ventricular CSF samples. Lymphocyte counts for 33 CSF pairs were analysed. The ratio was calculated as lumbar/ventricular, and presented as a percentage. Hydrocephalus was defined as communicating (n=20) if air was observed in the ventricular system after an air encephalogram, non-communicating (n=7) if air is observed at the base of the brain only, and uncertain (n=2) when the test could not be performed for patients in whom no CSF could be obtained through a lumbar puncture, or in patients for whom the test was not performed.

3.6 Analysis of association between morbidity, mortality and CSF lumbar and ventricular differentials and ratios

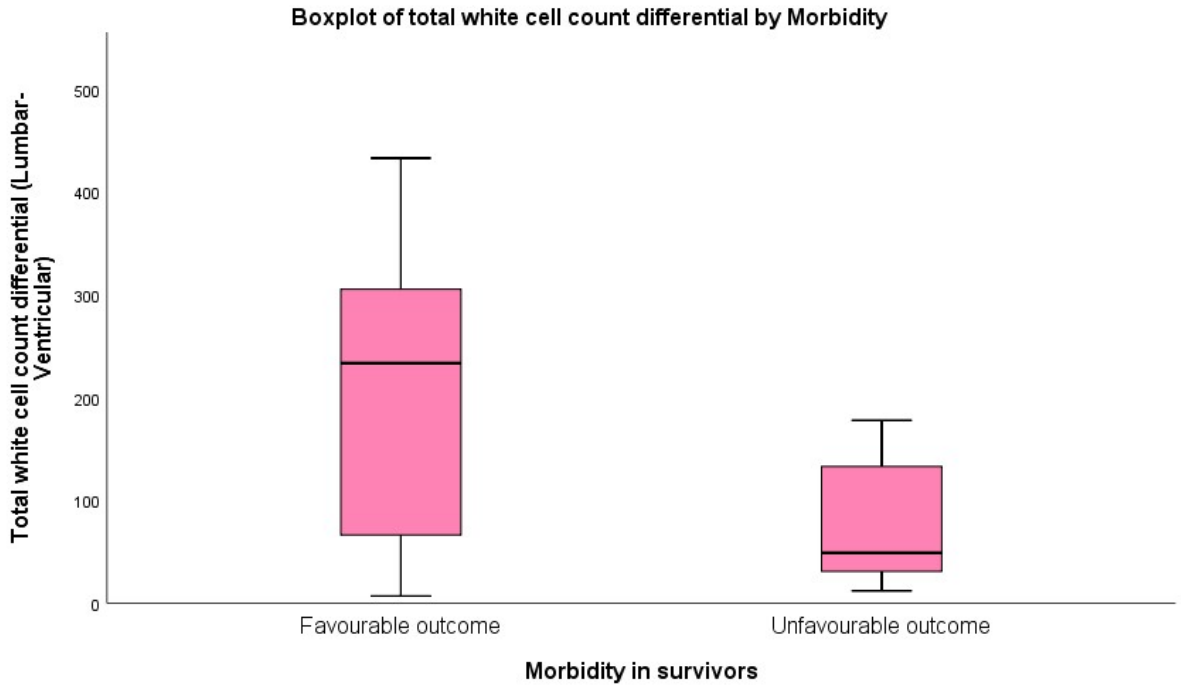
Lymphocyte differential (p=0.04) and total white cell count differential (p=0.02) were significantly associated with favorable outcome in survivors. Box and whisker plots (Figure 12 and 13 respectively) of these findings are highlighted below. There were no other significant associations obtained.

Figure 12: Box and Whisker Plot of CSF Lymphocyte Differential by Morbidity



The box plot demonstrates the association between CSF lymphocyte differential and morbidity outcome in survivors. The differential was calculated as lumbar – ventricular CSF lymphocyte count. Lymphocyte count for 33 CSF pairs were analysed. Good outcome (n=16) is categorized as PCPCS score of 1-3. Unfavourable outcome (n=8) is categorized as PCPCS score of 4-5.

Figure 13: Box and Whisker Plot of CSF Total White Cell Count Differential by Morbidity



The box plot demonstrates the association between CSF total white cell count differential and morbidity outcome in survivors. Total white cell count for 33 CSF pairs were analysed. The differential was calculated as lumbar – ventricular CSF total white cell count. Good outcome (n=16) is categorized as PCPCS score of 1-3. Unfavourable outcome (n=8) is categorized as PCPCS score of 4-5.

CHAPTER 4: DISCUSSION AND CONCLUSION

4.1 Discussion

This study examined ventricular and lumbar CSF in children with TBM in a pooled analysis of samples from 81 patients and in time-linked paired samples from 29 patients. Paired samples were examined to avoid any bias in the collection of pooled ventricular and lumbar CSF. In summary, the overall results show the persistent perturbations in CSF chemistry (glucose, chloride, protein) and cell count (polymorphonuclear cells, lymphocytes, total white cell count) in the first 3 weeks of TBM treatment in children with TBM and hydrocephalus. Across all parameters, lumbar CSF was significantly different to ventricular CSF, with derangements being more marked in the lumbar compartment. Lumbar CSF protein and white cell count typically were elevated and glucose and chloride were reduced. In contrast, ventricular CSF often only demonstrated mild disturbances. Analysis of pooled samples and of time-linked samples were similar. Knowledge of CSF trends and differences between compartments adds value for early diagnosis and treatment, as well as for clinical decision-making about surgical options for hydrocephalus treatment. Particularly where CSF biochemistry and cytology analytes diverge from traditionally expected patterns associated with TBM, or early in the presentation when TB microbiology results are not yet available, and empirical clinical decisions must be emergently instituted during routine clinical care to prevent possible TBM mortality/ morbidity. There are several implications of these findings: 1) If only ventricular CSF is available, it is important to note that it may demonstrate only mild features and this should not exclude the diagnosis of TBM. A lumbar CSF should be obtained when safe to do so; 2) For reporting and for clinical studies it is important that the compartment from which CSF is obtained is specified and that comparisons should only be done on samples from the same compartment; 3) For decisions about whether VP shunts are reasonable with reference to CSF protein values, the lumbar CSF does not reflect the ventricular CSF values and should not be used as a surrogate as such.

In the analysis of causative factors for the differences between the two compartments, the study is limited by smaller numbers of patients who had time-linked paired samples as well as MRIs; only 17 had full brain and spine MRIs in addition to time-linked paired samples. However, the results suggest some factors that need further exploration, in particular the nature of hydrocephalus and spinal disease, both of which may influence the flow of CSF between the ventricles and the spinal subarachnoid space.

4.1.1 Clinical characteristics

The results of patient demographics in this study are similar to those that have been reported by other TBM studies in the Western Cape (4,11,32,69). Patients typically are young and HIV positivity is low. Only 6 patients in this study were HIV positive; the numbers were too small to analyse differences between the groups. Although the Western Cape is notorious for a high TB prevalence (82), only 37% of patients reported a TB contact. This low percentage may reflect caregiver under-reporting.

In keeping with other studies (32,37,50), most patients presented late. The study cohort presented with advanced TBM disease and neurological deficits (MRC Stage 2 and 3). Most patients typically presented with nonspecific fever, weight loss, neck stiffness, depressed level of consciousness and focal neurology. Notably, our study reported higher proportions of confirmed *Mtb* in lumbar or ventricular CSF (43.2%) than reported in the literature (12%) (3). This may have been due to larger CSF volumes sent for analysis. Large volumes of CSF above 10ml are known to increase microbiological yield (5,57).

The literature reports mortality in TBM ranging from 13-69% (12). This study demonstrated mortality rates on the lower end (13.6%), which is notable given the more advanced presentation of patients: almost 71.6% of the group were in Stage 2b or 3 at presentation, and 63% had brain infarction. The disease severity is consistent with the referral pattern to the hospital (the only dedicated paediatric intensive care and paediatric

neurocritical care hospital) and the presentation with hydrocephalus. Of interest, just over half the patients had documented high blood pressure for age and just under half had bradycardia, which may reflect a Cushing's response to high ICP or brainstem ischemia. By 6 months, 77.5% patents had good neurological outcome (PCPCS). Several factors in the study setting could contribute to good neurological outcomes, including a well-resourced tertiary pediatric intensive care unit, early availability of MRI/CT scan, neurosurgical interventions, active in-hospital rehabilitation programs and possibly superior routine anti-TBM drug therapy using Ethionamide which crosses blood brain barrier better than Ethambutol used elsewhere outside the Western Cape (5). Of course, the PCPCS is a clinical score and does not take into account more subtle deficits that may affect neurodevelopmental outcomes.

4.1.2 Lumbar and ventricular CSF compartments

The study analysed the pooled differences between lumbar and ventricular CSF samples (n= 217 and 101 respectively) as well as individual differences in time-linked paired samples in 29 patients. Overall, lumbar CSF results were typical of TBM patients, while ventricular CSF results were less abnormal. Broadly, protein and cells were significantly lower, and glucose and chloride higher in ventricular than in lumbar CSF. This highlights the relevance of interpreting CSF results in a context of the compartment from where it is drawn when attempting to fit results to existing diagnostic TBM criteria or interpreting various perturbations (4,68,75,82).

Several possible reasons may explain the differences (6,16,85,32,40,45,69,70,78,79,81). First, tuberculous inflammation is more marked in the subarachnoid space of the basal cisterns than the ependyma of the ventricles. Given that the bulk flow theory of CSF flow is from ventricular space to subarachnoid space, and the spinal subarachnoid space is continuous with the cisterns of the brain, this may contribute to the

observed difference. Also, the main site of CSF production is in the ventricles so this may lead to a dilutional effect. Second, asymptomatic spinal disease (which was present in 80% of the unselected patients who underwent MRI) may contribute to the increased abnormalities in the lumbar CSF (16). Third, there is a physiological rostral-to-caudal differential in CSF biochemistry that may be exacerbated when there is a relative or absolute spinal block to CSF flow. A normal rostro-caudal CSF flow from ventricles to spinal subarachnoid space is described (86) triggered by the cardiac cycle (85). Lumbar CSF protein is subject to a cumulative effect of inflammatory proteins being added progressively as CSF flows caudally in the subarachnoid space by the addition of proteins due to the subarachnoid inflammatory process, particularly where there is spinal arachnoiditis, possibly caused by increased permeability of the inflamed lumbar meninges to blood-derived proteins and cells, and stagnation of CFS flow due to the relative block to CSF flow (78,79,85). Lower glucose in the lumbar space is suggested to represent cumulative glucose consumption along the rostro-caudal gradient and perturbed lumbar meningeal glucose transport as a result of local inflammation (73,78,87). The features of spinal block in Froin's syndrome are well described (76). The typical features of this are an overall increase in lumbar CSF protein, but specifically a decrease in brain-derived proteins with an increase in (predominant) blood-derived proteins, such as albumin (78) . This is consistent with the findings of the current study. In the study by Rohlwink et al. (88), the concentrations of brain-derived proteins such as S100B was higher in ventricular CSF, but concentrations of blood-derived proteins such as inflammatory mediators were higher in lumbar CSF. Albumin accounts for most of the protein in CSF and is blood-derived. The increased differential in cases of non-communicating hydrocephalus and 'uncertain' hydrocephalus are also consistent with this: there is a block to CSF communication between the ventricles and the spinal subarachnoid space. The 'uncertain' hydrocephalus group likely reflects failure of air from the air encephalogram test to ascend through the spinal subarachnoid space due to arachnoiditis and exudate. This also would explain why the highest protein levels were found in this group.

Paired samples tended to be collected early after admission. CSF analyte patterns from unpaired independent ventricular and lumbar compartments showed widest differences during the first 1-2 weeks of admission upon attaining their peak or trough levels. A similar pattern was expected within CSF pairs taken in the first week accounting for a wide analyte differential. Therefore, having a majority of CSF pairs drawn within the initial week of admission and a paucity of repeat CSF pairs over time was not expected to significantly affect study conclusions.

4.1.2.1 CSF GLUCOSE

Literature associates TBM with low CSF glucose <2.2 mmol/L (82), classically in reference to lumbar CSF. This study showed that lumbar and ventricular CSF glucose profiles distinctly differed overall and across time. The median differential between lumbar and ventricular CSF glucose was -1.9 mmol/L in paired samples. A delay in normalization of lumbar CSF glucose is described in fully and partially treated populations (69–71). In this study, lumbar CSF glucose was initially decreased as expected and normalized in the second week. In contrast, ventricular CSF glucose remained within normal limits most of the time (94%) and was higher than lumbar CSF glucose in 94% of cases.

4.1.2.2 CSF CHLORIDE

There are few studies on chloride in paediatric TBM. Ramkissoon & Coovadia (89) identified an association between low CSF chloride (less than 110 mmol/L) and TBM, provided bacterial meningitis was excluded. Rohlwink et al. commented on RCWMCH TBM clinical diagnostic criteria, which includes CSF chloride less than 116 mmol/L (32). Our study also used a normal CSF chloride lower limit of 116 mmol/L for both compartments in keeping with standard pediatric text values (83). Interestingly, CSF chloride is not included as a parameter in the globally accepted TBM diagnostic criteria (4,68). Ventricular CSF chloride

was low only on admission and then normalized whereas lumbar CSF chloride remained low. Chloride was lower in lumbar than ventricular CSF in almost 75% of the paired cases.

4.1.2.3 CSF PROTEIN

For pooled and time-linked samples, the median protein concentration was lumbar CSF was 2g/L and for ventricular CSF 0.5 g/L. Regardless of the threshold used, lumbar CSF protein was consistently higher. For paired samples, all of the lumbar CSF results showed an abnormally high protein (based on the higher threshold of 0.8g/L), whereas only 22% of ventricular CSF samples showed elevated protein. The median difference between lumbar and ventricular CSF protein concentrations in individual paired cases was 1.5 g/L (IQR 1.1 to 2.8 g/L). These results are contrary to the results of Kamat et al. who found equivalent concentrations of protein in ventricular and lumbar CSF (77). In their study, CSF protein was remarkably similar in ventricular and lumbar CSF samples (2.471g/L and 2.474g/L respectively). The authors suggest that lumbar CSF protein levels can be used to predict ventricular CSF protein, the level of which should be a guide to whether a shunt should be placed or not, given their observation that patients with higher protein levels had an increased risk of shunt blockage. However, there are several potential limitations and possible explanations of their data. Only mean values were reported; when reporting on the same paediatric cohort of the patients at the same institution, Van Well (3) reported a much lower median concentration of protein (1.6 g/L). Also, the analysis was only done on pooled samples, which may mask the true differences between ventricular and lumbar samples in individual patients. Further clarity is needed on the subject because studies of protein levels in CSF point to a clear differential between ventricular and lumbar CSF and the known physiological and pathological factors that may influence this (78,79). There are important implications to this – access to ventricular CSF is not standard and so in most patients lumbar CSF studies are used as a proxy for diagnosing and interpreting brain pathological processes, and in some cases directing therapies including surgery. Based on our results, this should be done with caution.

Protein levels in the lumbar CSF slowly decreased from the second week, but in general remained elevated. All study patients received standard steroid-containing TBM therapy expected to yield normalization of protein across all CSF compartments (32,69,70). We demonstrated steady lumbar protein decline, approaching normal during days 17-21. It is difficult to comment on the temporal profile of ventricular CSF values beyond the first two weeks because the numbers are small and may reflect bias from patients with ongoing disease processes including unresolved hydrocephalus. Neurosurgical interventions (EVD, VPS) were unlikely to contribute to persistent raised ventricular CSF protein because CSF derangements immediately post-neurosurgical interventions were excluded and any concomitant bacterial meningitis was ruled out.

4.1.2.4 CSF WHITE CELLS

The study results concurred with literature descriptions of elevated CSF white cells with lymphocyte predominance (4,32,68,82), particularly in the lumbar CSF compartment. Ventricular white cells were markedly less than the lumbar compartment, only mildly elevated with the highest values on admission. White cell concentrations were 170 $x10^6$/L (lumbar CSF) versus 15 (ventricular CSF) for pooled samples and 202 versus 23 for paired samples. The difference between the compartments may be due to a greater inflammatory process occurring in the subarachnoid space or the influence of spinal disease, as discussed above. Although there was a progressive decrease over time (late in the first week), white cells remained elevated and failed to normalise by day 21 in both lumbar and ventricular compartments. The persistent elevation of white cells may be clinically useful in diagnosing paediatric TBM particularly in the context of repeated CSF sampling after 2-3 weeks. This study also demonstrates that TBM should not be excluded based on normal to mildly raised polymorphonuclear cells or lymphocytes in ventricular CSF. Over time, there was a slight increase in cells and protein in the ventricular CSF samples. This reflects a small number of samples from patients (n=3) who likely do not reflect the average (and therefore required external

ventricular drainage and/ or ventricular shunts which enabled access to ventricular CSF). This is also seen in lumbar CSF polymorphonuclear cell count in the third week. We excluded patients with any obvious bacterial infection as a consequence of the external ventricular drain, but it is possible that these patients reflect outliers or a cellular reaction to a persistent external ventricular drain. Regardless, these small numbers late in the disease course probably do not reflect a typical pattern in TBM.

4.1.3 Lumbar and ventricular CSF analytes, radiology and factors influencing CSF differentials

4.1.3.1 RADIOLOGY FINDINGS IN TBM WITH HYDROCEPHALUS

With respect to the level of CSF obstruction, 16% had non-communicating hydrocephalus, consistent with previously published results; 11% had an uncertain level of obstruction. The latter was the result of a strict definition and reflects failure to demonstrate air in cranium in the air encephalography study. Strictly speaking, this cannot be described as non-communicating and must be due to a technical failure or because of an obstruction to CSF in the spinal canal. Although the numbers are small, the highest protein levels are seen in this group, in keeping with the possibility that this reflects a spinal block. Five of the nine patients with uncertain hydrocephalus had spinal imaging, and all of them showed evidence of spinal disease. In keeping with this, maximum lumbar CSF protein was significantly associated with the severity of spinal disease on imaging. Rohlwink et al. (16) also demonstrated that spinal exudate is associated with higher lumbar CSF protein and can cause near or complete obliteration of the thecal sac. In an open system, the rostro-caudal CSF flow (86) may facilitate an equilibration of biochemical analytes and leukocytes and, consequently, less difference between the ventricular and lumbar compartments. In these cases, the high concentration of protein in the spinal canal likely represents an accumulation of inflammatory exudate and a stagnation of CSF flow. This likely contributes to a dry tap and inability to perform the AEG or column test, and could hinder the course of air up the spinal canal if the AEG could be performed, leading to an uncertain result. Consistent with this, the lymphocyte ratio between lumbar and ventricular CSF was also

higher in patients with non-communicating and uncertain hydrocephalus. In these situations (very high lumbar CSF protein and marked differences between lumbar and ventricular CSF results), rational use of MRI spine may be indicated. Inability to obtain lumbar CSF (possibly due to very high protein concentrations) should raise suspicion of spinal arachnoiditis, tuberculoma or exudate.

With respect to brain imaging, typical radiological findings were demonstrated (16,18,22,50,90). The results showed almost universal basal meningeal enhancement on contrasted CT brain (97.4%), although it was scored as mild in almost half of admission scans. This highlights the need for routine use of contrast in the radiological diagnosis of TBM and importance of a trained radiologist eye to detect mild radiological changes. Brain infarcts (63%) and brain tuberculomas (47.4%) were common but sometimes occurred on subsequent scans over time. This emphasises the value of access to repeat diagnostic brain imaging in endemic areas, which are unfortunately often the poorest resourced. The development of disease manifestations over time is also consistent with the persistent protein and white cell perturbations found in lumbar and ventricular CSF, reflecting an ongoing inflammatory process despite commencement of TBM treatment.

4.1.4 Lumbar and ventricular CSF analyte association with morbidity and mortality

In our results, patients with a favorable outcome had a greater differential between lumbar and ventricular CSF for lymphocytes and total white cell count. The reason for this is not apparent as a similar association was not found for differentials of other parameters. Therefore, this may be a spurious result, a reflection of higher white cell counts in ventricular CSF, or a proxy for differences in the way patients were treated if a CSF block was suspected. Patterns of host intracranial inflammatory response in TBM may play a role. In view of a dearth of literature on the subject, further research is required to confirm this finding.

4.1.5 Limitations

Completeness of demographic, clinical and outcome data was influenced by quality and detail of clinical notes documented by attending doctors. However, it did not have a substantial effect on the quality or completeness of laboratory CSF data, which was the study's primary focus. Although various data sources were interrogated to identify patients, a few patients may have been missed due to poor record keeping and patient notification.

Many TBM patients do not receive follow up at RCWMCH after discharge on TBM treatment but are referred to secondary level hospitals. Data on serial CSF samples, therefore, was limited to patients with clinical follow up and repeat CSF studies done at RCWMCH. Similarly, outcome data was limited to patients who were followed up at RCWMCH, and collection of secondary data to assign a PCPCS score was tied to the quality and completeness of clinical notes recorded by the attending doctor. There was a likelihood of bias from patients lost-to-follow-up which could affect serial sampling and outcome. The researchers aimed to look at association of outcome, the small sample size is acknowledged. There would be benefit conducting more studies in future based on a larger sample size.

As this study analysed samples taken as part of routine clinical care, the timing of serial CSF lumbar and/ or ventricular samples sent for laboratory analysis did not follow a prescribed interval. CSF samples were collected at the discretion of the attending doctor in line with the patient's clinical progress and condition. The trend analysis was therefore biased by different numbers of CSF samples available at the different time points. However, as all of these patients had hydrocephalus, we were able to examine several samples in each patient over the 3-week period. Similarly, tracking functional neurological outcome using the PCPCS

score (Appendix 6) could only be done based on when patients were routinely followed up which did not follow a strict pre-set time protocol. Samples for analysis were biased towards those patients who survived the acute stages of TBM, therefore this study was limited to patients who survived.

Spinal imaging was performed in less than half the study group, preventing full examination of the impact of spinal disease on CSF parameters and the differential between compartments. Spinal imaging in TBM is not a routine part of investigative protocols, but was rather the focus of one of the existing studies. Not all patients had MRI brain and spine given that CT imaging is standard for admission imaging and those patients who died early did not receive follow up MRI imaging. Furthermore, there were only 6 patients with severe spinal disease. In patients with the most severe spinal disease, it was often not possible to obtain CSF.

Finally, these data apply to children, adult CSF characteristics may differ: typically, slower CSF flow rates with age correspond to physiologically higher protein concentrations.

4.2 Conclusion and Recommendations

This study illustrated that CSF chemistry and cell count differ between the ventricular (rostral) and lumbar (caudal) compartments in TBM with hydrocephalus across time. Importantly, the data highlights the fact that: 1) lumbar and ventricular CSF in TBM are significantly different; 2) ventricular CSF may not demonstrate the typical picture of perturbed CSF expected in TBM, that this should not dismiss suspicions of TBM, and that the CSF compartment of sampling must be taken into account when diagnosing TBM; 3)

spinal disease, and the level of CSF block causing hydrocephalus, may influence the ability to sample lumbar CSF and the analyte concentrations in the lumbar CSF. In addition to the impact on clinical decisions, these differences should also be considered in studies usually based on lumbar CSF, including diagnostics, disease biomarkers or drug recovery. Further clinical utilization of these results and determination of reasons for the differences require additional research.

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APPENDICES

Appendix 1: Revised Medical Research Council (MRC) Scale for Staging TBM (37)

TBM Stage	Clinical Criteria
1	GCS 15, patient alert and oriented with no focal neurological deficits
2a	GCS 15, patient alert and oriented but with neurological deficit; OR GCS 13-14 with/ without neurological deficit
2b	GCS 10-12 with/ without focal neurological deficits
3	GCS < 10 with/ without focal neurological deficits
	**GCS = Glasgow Coma Scale*

Adapted from van Toorn, R., Springer, P., Laubscher, J. & Schoeman, J. 2012. Value of different staging systems for predicting neurological outcome in childhood tuberculous meningitis. International Journal of Tuberculous Lung Disease. 16(5):628-632.

Appendix 2: Consensus Case Definition of TBM

a) Table 1: TBM Case Definition Classification, TBM Case Definition in Research Settings, (4)

TBM CASE DEFINITION CLASSIFICATION	SUMMARY OF COMPONENTS
Definite TBM	Isolation of Mtb in CSF (AFB/ culture/ DNA PCR); histopathological diagnosis
Probable TBM	Higher diagnostic score with or without brain imaging plus exclusion of alternative diagnosis
Possible TBM	Lower diagnostic score with or without brain imaging plus exclusion of alternative diagnosis; CSF or brain imaging required
Not TBM	Alternative diagnosis established and absence of co-morbidity with TBM
Clinical entry criteria	Headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered level of consciousness, lethargy

Adapted from *Marais, S., Thwaites, G., Schoeman, J., Torok, M., Misra, U., Prasad, K., Donald, P., Wilkinson, R. et al. 2010. Tuberculous meningitis: A uniform case definition for use in clinical research. Lancet. (10):803-812. DOI:10.1016/S1473-3099(10)70138-9***.**

b) Table 2: Scoring Table, TBM Case Definition in Research Settings, (4)

	CRITERIA	DIAGNOSTIC SCORE
1	**Clinical Criteria**	**Maximum category score = 6**
	• Symptom duration > 5 days;	4
	• Systemic symptoms suggestive of TB – one or more of: Weight loss; night sweats; cough > 2 weeks;	2
	• In children < 10yrs old, history of recent (< 1yr) close contact with person diagnosed positive pulmonary TB/ positive TST/ positive IGRA;	2
	• Focal neurological deficit (excluding cranial nerve palsies);	1

	• Cranial nerve palsies • Altered level of consciousness	 1 1
2	**CSF Criteria**	**Maximum category score = 4**
	• Clear appearance; • 10-500 x10^6/L; • Lymphocyte predominance > 50%; • Protein level > 1g/L; • CSF:Plasma glucose ratio < 50% OR an absolute CSF glucose < 2.2 mmol/L	1 1 1 1 1
3	**Cerebral Imaging Criteria**	**Maximum category score = 6**
	• Hydrocephalus; • Basal meningeal enhancement; • Tuberculoma; • Infarct; • Pre-contrast basal hyperdensity	1 2 2 1 2
4	**Evidence of TB elsewhere**	**Maximum category score = 4**
	• CXR suggestive of active TB where signs of TB = 2, miliary TB = 4 • CT/ MRI/ Ultra sound evidence of TB outside the CNS • Positive AFB seen OR Mtb cultured from another source i.e. Sputum, lymph node, gastric washings, urine, blood culture • Positive commercial Mtb NAAT from an extra neural specimen	2 OR 4 2 4 4
5	**Exclusion of Alternative Diagnosis**	

	• An alternative diagnosis must be confirmed via microbiology, serology or histopathology • Consider alternative diagnosis (depending on age, immunological status, geographic region) e.g. CNS infections (bacterial, viral, parasitic e.g. cerebral malaria or opportunistic infections), brain abscess and CNS malignancy	
	Abbreviations: AFB – Acid Fast Bacilli; CNS – Central Nervous System; CSF- Cerebrospinal Fluid; CT - Computer Tomography Scan; IGRA- Interferon-Gamma Release Assays; TB – Tuberculosis; TST – Tuberculin Skin Test; MRI – Magnetic Resonance Imaging; Mtb – Mycobacterium tuberculosis; NAAT – Nucleic Acid Amplification Test	

Adapted from *Marais, S., Thwaites, G., Schoeman, J., Torok, M., Misra, U., Prasad, K., Donald, P., Wilkinson, R. et al. 2010. Tuberculous meningitis: A uniform case definition for use in clinical research. Lancet. (10):803-812. DOI:10.1016/S1473-3099(10)70138-9*.

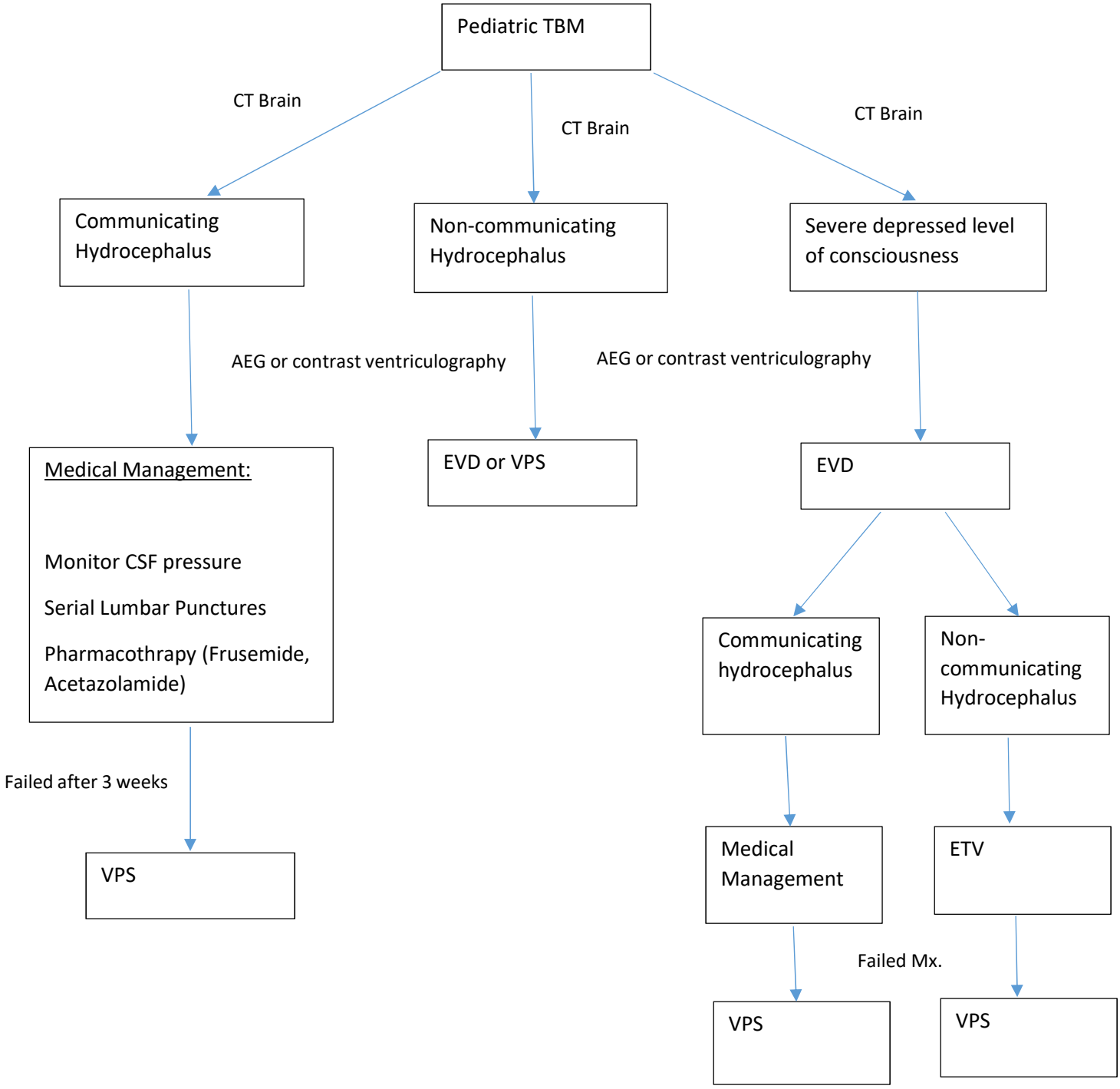
Appendix 3: Parameters for Treating TBM at RCWMCH

PARAMETER	EXPECTED YIELD
HISTORY	
Constitutional symptoms of TB – fever, weight loss, night sweats, loss of appetite	
History of a recent TB contact. (Investigate contact's TB information)	
History of positive Mantoux	
Growth faltering/ crossing weight centiles on Road to Health Card	
PHYSICAL EXAMINATION	
Wasting, lymphadenopathy	
Clinical features of TB elsewhere e.g. pulmonary TB, abdominal TB, TB adenitis	
Features consistent with Meningitis	
Clinical findings in keeping with the classification of stage 1, 2 and 3 TBM STAGE 1 – meningitis, no neurological fallout, no depression of level of consciousness STAGE 2 – depressed level of consciousness, cranial nerve fallout, seizures STAGE 3 – comatose	
LABORATORY	
CSF (lumbar CSF and where hydrocephalus present ventricular CSF) Cytology (hours) – moderately raised white cells with a lymphocyte predominance, Biochemistry (hours) - low glucose, markedly high protein, low chloride	
Microbiology – evidence of *Mtb* in CSF a) Standard practice: acid fast bacilli on Ziehl-Neelsen stain - hours; TB culture – up to 42 days b) Selected cases at doctor's discretion: TB PCR Cephin Gene Xpert – days	40% (Schoeman, 2001) Use of Gene Xpert not yet validated on CSF and is not yet standard practice.
TBM with no hydrocephalus: Repeat lumbar CSF study 3 - 4 weeks later looking for persistent CSF changes which points towards a likely diagnosis of TBM (assessment and decision made by pediatrician team with/ without consultation with neurosurgeons)	
TBM with hydrocephalus requiring neurosurgical decompression interventions (e.g. Endoscopic Third Ventriculostomy, Ventroperitoneal Shunt) or conservative medical management with Acetazolamide and Lasix: Repeat ventricular CSF at intervals	

determined by neurosurgeons and clinical condition of the patient (assessment and decision made by neurosurgeons)	
RADIOLOGY SUGGESTIVE OF TBM	
Uncontrasted & contrasted CT brain (up to 24 hours) – basal meningeal enhancement; acute hydrocephalus; tuberculomas	83% (Schoeman 2001)
SUPPORTIVE INVESTIGATIONS	
Chest X-Ray suggestive of Pulmonary TB	60% (Schoeman 2001)
Positive Mantoux (72hrs)	65% (Schoeman 2001)
Induced sputum (days for microscopy and Ziehl-Neelsen stain, weeks for culture and Cephin Gene Xpert)	
Gastric washings (days for microscopy and Ziehl-Neelsen stain, weeks for culture and Cephin Gene Xpert)	40% (Schoeman 2001)
Blood – raised Erythrocyte Sedimentation Rate; wide globulin fraction	
Ultrasound of abdomen (days to week)	
Fine Needle Aspiration Biopsy of suspected TB lymph node (days for histology and microbiology for Ziehl-Neelsen stain)	

Source: Author and Co-Authors Personal Experience and Practice Protocols at RCWMCH

Appendix 4: Treatment Algorithm for Raised Intracranial Pressure in Pediatric TBM with Hydrocephalus (34)



**ETV-Endoscopic Third Ventriculostomy; EVD-External Ventricular Drain; TBM-Tuberculous Meningitis; VPS-Ventriculo-Peritoneal Shunt (Adapted from Figaji, A., Fieggen, A. & Peter, J. 2003. Endoscopic third ventriculostomy in tuberculous meningitis. Child's Nervous System. 19:217-225)*

Appendix 5: Brain/ Spinal Imaging Grading Criteria RCWMCH

a) Table 1: Description of Brain Imaging (with Contrast) Parameters in TBM with Hydrocephalus

	CT Brain Imaging	Parameter Definition and Grading
1	Basal meningeal enhancement	Focal: enhancement localised Diffuse: enhancement not localised
2	Hydrocephalus	Mild: rounding of 3rd ventricle, visible temporal horns Moderate: all ventricles dilated, absence of transepyndymal fluid shift Severe: transepyndymal fluid shift, ventricles dilated, loss of sulcal markings
3	Pre-contrast hyperdensity	Assessed on uncontrasted CT brain scan. Sylvian fissures or basal cisterns hyperdense and obliterated
4	Tuberculomas and TB abscess	Number: single or multiple Anatomical location Unilateral Vs bilateral occurrence
5	Infarcts	Number: single or multiple Size: small - lacunar infarcts Medium - multiple or large size Global - extensive infarction present in all vascular regions

Source: Adapted from RCWMCH Radiology Department (2015)

b) Table 2: Description of MRI Spinal Imaging (With Contrast) Parameters in TBM and Hydrocephalus

	Spinal Imaging	**Parameter Definition and Grading**
1	Spinal Arachnoiditis	Definition: Contrast enhancement of spinal roots or spinal cord. Presence of intradural or extra-dural exudate collections (plaques), nodules, clumping of spinal nerve roots was assessed. Grading: Mild - thin layer of exudate surrounding spinal cord and nerve roots Moderate - presence of plaques or clumping of spinal nerve roots Severe - presence of extensive exudate in surrounding spinal cord and nerve roots or in subarachnoid space
2	Tuberculomas or TB abscesses	Divided into two anatomical categories namely intra-medullary or surface

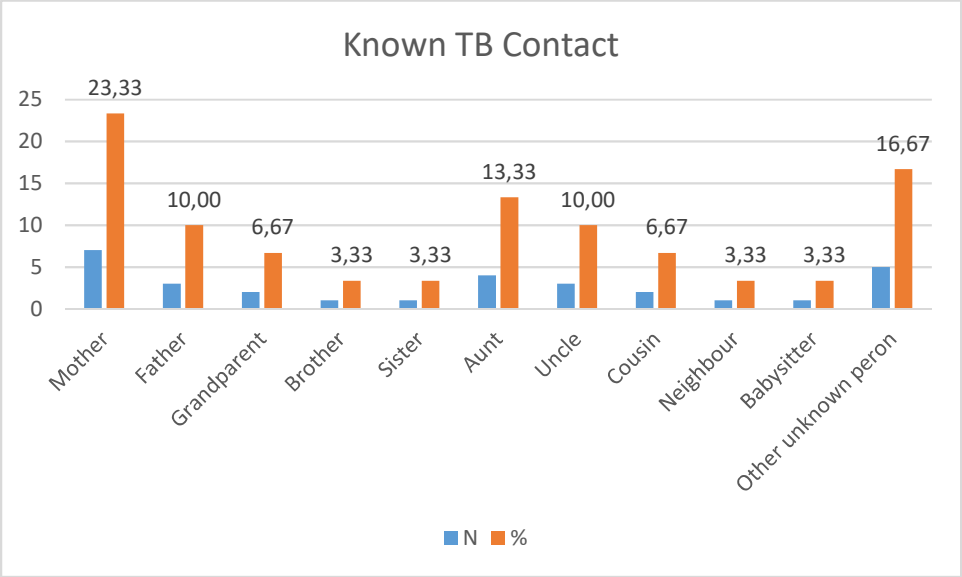
Source: Adapted from RCWMCH Radiology Department (2015)

Appendix 6: Pediatric Cerebral Performance Cognitive Scale [PCPCS], (84)

Clinical Features	Category	Score
Normal at age appropriate level; School age child attends regular school classroom	Normal	1
Conscious alert and able to interact at an age appropriate level; School age child attending regular school classroom but grade perhaps not appropriate for age; May have a mild neurologic deficit	Mild disability	2
Conscious; Sufficient cerebral function for age-appropriate independent activities of daily life; School age child attending special education classroom; may have learning deficit	Moderate disability	3
Conscious; Dependent on others for daily support because of impaired brain function	Severe disability	4
Any degree of coma without any of the criteria for brain death; Unawareness even if awake in appearance without interaction with the environment; Cerebral unresponsiveness; No evidence of cortical function and not aroused by verbal stimuli; Possibly some reflexive responses spontaneous eye opening and/ or sleep-wake cycles	Coma/ vegetative state	5
Apnea; OR Areflexia; OR electroencephalographic (EEG) silence	Brain death	6

Adapted from Fiser, D. 1992. Assessing the outcome of pediatric intensive care. Journal of Pediatrics. 121(1):68-74

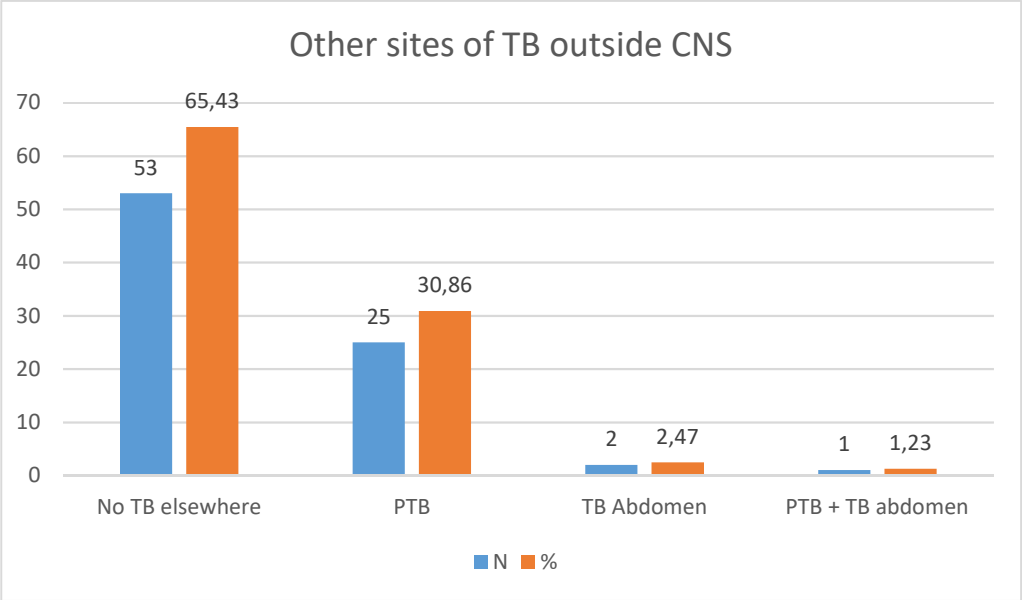
Appendix 7: TB Contacts In Study Patients Treated for TBM with Hydrocephalus at RCWMCH



Graph showing known TB contacts. Blue bars represent absolute number. Red bars represent percentage.

Appendix 8: Sites of TB Outside the CNS

One third patients (n=29, 36%) had co-existing clinical features of TB outside the nervous system. Twenty five (30.86%) demonstrated radiological evidence of PTB and 2 (2.47%) of TB abdomen. One patient (1.23%) had TB disseminated to both the lung and abdomen.



Sites of TB outside the central nervous system. Blue bar represents absolute number, orange bar represents percentage. PTB = pulmonary TB, TB = tuberculosis

Appendix 9: Profiles of Lumbar and Ventricular CSF Parameters timepoints day 17-21

Pt No.	Day of collection	Location	Glucose (mmol/L)	protein value (g/L)	Chloride (g/L)	Polymorphonuclear cells ($x10^6$/L)	Lymphocytes ($x10^6$/L)	TWCC ($x10^6$/L)
27	18	2	2,4	1,42	124	1	36	37
44	20	2	2,5	2,09	118	10	93	103
70	17	2	2,1	2	115	3	98	101
73	19	2	3,2	0,92	118	0	136	136
3	20	1	3,1	0,23	121	0	29	29
5	16	1	2,6	2,55	111	50	135	185
5	21	1	2,6	3,25	112	14	73	87
6	17	1	2,9	0,61	120	17	20	37
9	19	1	3,5	0,49	136	1	61	62
9	21	1	3,1	0,77	143	0	71	71
11	19	1	2,8	6,13	114	0	68	68
13	20	1	1,2	2,22	110	20	270	290
21	21	1	3,2	2,25	116	405	170	575
23	19	1	3,2	0,38	116	8	87	95
25	17	1	2,9	1,02	119	41	21	62
25	19	1	1,2	3,32	113	99	268	367
30	17	1	2,6	1,1	128	3	125	128
31	19	1	3,6	1,34	172	11	39	50
32	18	1	3,6	0,91	129	21	52	73
33	17	1	2,7	2,16	122	2	85	87
36	18	1	4,4	2,31	101	3	20	23
37	21	1	1,5	1,62	112	46	65	111
55	18	1	1,9	2	121	2	63	65
59	18	1	3	2	115	49	100	149
68	17	1	2,5	2	129	5	156	161
71	18	1	2,7	0,78	118	4	66	70
72	17	1	2,3	0,93	124	15	41	56
73	18	1	2,5	1,05	119	2	202	204
77	18	1	1,8	3	109	62	78	140
78	18	1	3	1	126	4	20	24

The table illustrates CSF datapoints excluded from analysis due to small sample numbers taken post-admission on day 17-21 time epoch. Under the column "location": 1=Lumbar CSF. 2=Ventricular CSF.

Appendix 10: Ethics Review Board Study Approval: HREC REF: 566/2015

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05 August 2015

HREC REF: 566/2015

Prof A Figaji
Neurosurgery
Room 617, 6th Floor
ICH Building
Red Cross War Memorial Children's Hospital
Rondebosch

Dear Prof Figaji

PROJECT TITLE: LUMBAR AND VENTRICULAR CEREBROSPINAL FLUID CHANGES IN CHILDREN WITH TUBERCULOUS MENINGITIS AND HYDROCEPHALUS - Sub-study linked to 318/2010 & 200/2014 (MMED Candidate - Dr L Mwenda)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has formally approved the above-mentioned study.

Approval is granted for one year until the 30th August 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the following student: Dr Lona Mwenda is also involved in this project

Please quote the HREC reference no in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637

Hrec/ref:566/2015

Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix 11: Red Cross War Memorial Hospital Research Approval Letter

Western Cape Government
Health

DR AN PARBHOO
Manager: Medical Services
Red Cross War Memorial Children's Hospital
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03 December 2018

Dr L Mwenda
Department of Paediatrics

Dear Dr Mwenda,

RESEARCH: RXH: RCC 165

PROJECT TITLE: An Examination of Lumbar and Ventricular Cerebrospinal Fluid Findings in children with Tuberculous Meningitis and Hydrocephalus

It is a pleasure to inform you that approval is hereby granted to conduct above-mentioned study at Red Cross War Memorial Children's Hospital.

Yours sincerely,

DR AN PARBHOO
MANAGER: MEDICAL SERVICES

Appendix 12: Study Data Collection Tool

A	Sheet 1 - TBM Cases, Clinical Notes	Clinical Data Variables: Response (code)
	DEMOGRAPHICS	
1	Ref code	
2	Patient Name (First name, Surname)	
3	Folder number	
4	Date of Birth (dd/mm/yyyy)	
5	Age (in months)	
6	Gender	Male (1) Female (2)
7	Admission date (dd/mm/yyyy)	
	HIV AND NUTRITION STATUS	
8	HIV status	Positive (1) Negative (0) Unknown (3)
9	If HIV positive, is patient on HAART	Yes on HAART (1) No not yet on HAART (0)
10	Nutritional status	Normal weight for age, >/= -1SD (1) Malnourished, < -1SD (0) Not recorded (9)
11	Other co-morbid disease	Yes (1) No (0) Not stated (2)
	TB INFORMATION	
12	TBM diagnosis (Marais et al. (4) definition*)*	Definite TBM (1) Probable TBM (2) No TBM (3) Not recorded (9)
13	Known TB Contact	Yes (1) No (0)
14	List who is the known TB contact	
	CONSTITUTIONAL SYMPTOMS	
15	Any constitutional symptoms	Yes (1) No (0) Not stated (2)
16	Fever	Yes (1) No (0) Not stated (2)

17	Weight loss	Yes (1)	No (0)	Not stated (2)
18	Night sweats	Yes (1)	No (0)	Not stated (2)
19	Cough	Yes (1)	No (0)	Not stated (2)
20	Mantoux result	Positive (1)	Negative (0)	Not done (2)
21	TB elsewhere (outside of CNS)	Yes (1)	No (0)	Not stated (2)
22	List other sites of TB infection outside CNS, Date and results of investigations done	Pulmonary TB (1) TB abdomen (2) Other site: state (4)		
	TBM IMAGING			
23	Brain imaging done	Yes (1)	No (0)	Not stated (2)
24	Type of brain/ spinal imaging done	CT brain/ spine (1) MRI brain/ spine (2) Both CT and MRI brain/spine (3)		
25	Brain imaging results and date (dd/mm/yyyy)	Hydrocephalus (yes, no) Hydrocephalus severity (mild, moderate, severe) Meningeal enhancement (yes, no) Tuberculoma or TB abscess (yes, no) Number of tuberculoma/ TB abscess (single, multiple) Infarcts (yes, no) Number of infarcts (single, multiple) Spinal arachnoiditis (yes, no) Severity of arachnoiditis (mild, moderate, severe)		
26	TBM diagnosis (Marais et al. (4) definition*)*	Definite TBM (1) Probable TBM (2) No TBM (3) Not recorded (9)		

	HYDROCEPHALUS INFORMATION	
27	Hydrocephalus	Yes (1) No (0) Undefined (9)
28	Type of hydrocephalus	Communicating (1) Non-communicating (0) Undefined (failed test/ unspecified) (9)
29	Air encephalogram result	Communicating HCP (1) Non-communicating HCP (2) Failed test (5) Not done (9)
30	Air encephalogram date, (dd/mm/yyyy)	Communicating HCP (1) Non-communicating HCP (2) Failed test (5) Not done (9)
31	Column test result	Communicating HCP (1) Non-communicating HCP (2) Failed test (5) Not done (9)
32	Column test date, (dd/mm/yyyy)	
	TBM STAGING AND FOCAL NEUROLOGICAL SIGNS	
33	Revised MRC TBM Staging – (van Toorn et al. (37))	Stage 1 (GCS 15 without focal neurology) Stage 2a (GCS 15 with neurological deficit/ GCS13-14 with/without neurological deficit) Stage 2b (GCS 10-12 with/without focal neurological deficit) Stage 3 (GCS < 10 with/without neurological deficit)
34	Focal neurological signs	Yes (1) No (0) Not stated (2)
35	Pupils	Bilaterally reactive (1) Unilaterally non-reactive (2)

		Bilaterally not reactive (3) Sluggish (4) Not stated (9)		
36	Paresis	No paresis (1) Hemiparesis (2) Quadriparesis (3) Not stated (9)		
37	Raised intracranial pressure	Yes (1)	No (0)	Not stated (2)
38	Raised blood pressure	Yes (1)	No (0)	Not stated (2)
39	Bradycardia	Yes (1)	No (0)	Not stated (2)
40	Headache	Yes (1)	No (0)	Not stated (2)
41	Irritability	Yes (1)	No (0)	Not stated (2)
42	Vomiting	Yes (1)	No (0)	Not stated (2)
43	Neck stiffness	Yes (1)	No (0)	Not stated (2)
44	Seizures	Yes (1)	No (0)	Not stated (2)
45	Abnormal posturing	Yes (1)	No (0)	Not stated (2)
46	Altered level of consciousness (Glasgow Coma Scale <15)	Yes (1)	No (0)	Not stated (2)
47	Lethargy	Yes (1)	No (0)	Not stated (2)
48	Not walking	Yes (1)	No (0)	Not stated (2)
49	Bulging anterior fontanelle	Yes (1)	No (0)	Not stated (2)
50	Papilledema	Yes (1)	No (0)	Not stated (2)
51	Sun-setting eyes	Yes (1)	No (0)	Not stated (2)

B	Sheet 2 - CSF Results, NHLS	Response: value, units
1	Ref code	
2	Patient Name (First name, Surname)	
3	Folder No.	
52	Date of CSF sample (dd/mm/yyyy)	
53	Time of CSF sample (hr/ min)	
54	CSF sample site	Lumbar puncture (1) Ventricular compartment (2)
55	CSF Appearance	Clear & colorless Turbid Xanthrochromic Blood stained
	CSF BIOCHEMISTRY RESULTS	
56	Glucose measurement	mmol/L
57	Lower glucose limit	Glucose </= 2.2 mmol/L; Yes (1) No (0)
58	Protein measurement	g/dL
59	Upper protein limit	Protein > 0.4 g/L Yes (1) No (0) Protein > 0.8 g/L Yes (1) No (0) Protein > 1 g/L Yes (1) No (0)
60	Chloride measurement	mmol/L
61	Lower chloride limit	Chloride < 116 mmol/L Yes (1) No (0)
	CSF CYTOLOGY RESULTS	
62	Polymorphonuclear cell count	$x10^6$/L
63	Upper polymorph limit	Polymorph > 0 $x10^6$/L Yes (1) No (0)
64	Lymphocyte count	$x10^6$/L
65	Upper lymph limit	Lymph >5 $x10^6$/L Yes (1) No (0)
66	Total White Cell count	$x10^6$/L

67	Upper TWCC limit	TWCC > 10 x10^6/L Yes (1) No (0)
	CSF MICROBIOLOGY RESULTS	
68	Gram stain	Positive (1) Negative (0) Not done (9)
69	Bacterial culture	Positive (1) Negative (0) Not done (9)
70	CSF Ziehl-Neelsen Stain (AFB)	Positive AFB (1) Negative AFB (2) Not done (9)
71	TB culture	Positive (1) Negative (2) Not done (9)
72	TB PCR GeneXpert	Positive (1) Negative (0) Not done (9)

C	**Sheet 3 - Outcome Data: morbidity in survivors; and mortality**	**Response (code)**
1	Ref code	
2	Patient Name (First name, Surname)	
3	Folder number	
73	Mortality	Alive (1); date (dd/mm/yyyy) Dead (0); date (dd/mm/yyyy)
74	Morbidity in survivors (Pediatric Cerebral Performance Cognitive Scale)	Normal (1) Mild disability (2) Moderate disability (3) Severe disability (4) Coma/ Vegetative state (5) Brain dead (6)