Biomarkers of neurological tissue injury and inflammation in paediatric tuberculous meningitis

Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

In the Division of Neurosurgery

UNIVERSITY OF CAPE TOWN

March 2014

University of Cape Town

Candidate: Ursula Rohlwink

Supervisor: Professor Anthony Figaji

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

University of Cape Town

For my mother, Kerime

...who is my first, and greatest teacher, and is of such an exceptional nature that I am continuously convinced that I have met one of the world's most luminous beings in her. She is imbued in everything I am and in everything I do, including this project. I dedicate this project to her, in love and oneness

Acknowledgements

No project is the work of an individual alone, and this was no exception. A large part of the learning and the growing over the last four years has taken place at the bedside, in the laboratory or in front of the computer screen; but I have also had the opportunity to profit from the wisdom, the expertise and the support of many. I take this opportunity to acknowledge those people.

I wish to begin by acknowledging the patients who I had the privilege of studying for this project; their manner of managing their pain and illness with gentle surrender was utterly humbling. I thank their parents, who handed over their child's wellbeing with such trust and were so supportive of my presence during their child's treatment.

The individual to whom I owe the deepest gratitude is my supervisor, Tony Figaji; he has taught me everything I know about the brain, about science and about research, and has instilled in me the drive to do research that is meaningful to the patient, and to do so with integrity and belief. I consider him one of the revolutionaries in brain research today, and I have been tremendously privileged to work with him these last 5 years. I thank him for going beyond the call of duty on this project; he has been invested in it from its inception to the very last chapter of the thesis. Without him this project would not have been possible!

I also wish to thank

- Our Head of Department, Professor Graham Fieggen, for running a department in which the growth of each member is sincerely nurtured, and their triumphs joyously celebrated. I have been the fortunate beneficiary of his excitement for neuroscience and thank him for his support. I also thank Llewellyn Padayachy, Nthe Mjoli, Edwin Mogere, Ncedile Mankahla, Nico Enslin, Johan Malan, Dave Roytowski, Mohammed Ben-Husein, Emmanuel Wegoye, Chris Young, Tiago Morgado, Vincent Pretorius, and Nqobile Thango - my colleagues in the Neurosurgery Division, who managed these patients and were the ones to place the biological samples, upon which the bulk of this project is based, into my hands. Their support for the project in their already busy work schedules was greatly appreciated.
- My funders from the Clinical Infectious Diseases and Research Initiative and the Wellcome Trust who so generously supported this project and provided the laboratory space for sample analysis.
- All the paediatricians who were involved in sample collection and patient management, their cooperation was vital to the success of this project. I thank the ward nursing staff for their patience with me and my monitoring equipment in the wards, and the S19 Outpatients staff who were such a help with patients attending follow-up clinics.

- Lisa Ungerer who guided me through the logistics of sample processing and storage, and taught me my first laboratory techniques. The deep friendship that has emerged from our working relationship has been a gift. I am grateful to all the staff in the NHLS Chemistry lab for welcoming me into their working space and accommodating my work and consumables.
- Katalin Wilkinson for conducting all the ELISA and Luminex analysis with me, I learnt a great deal from her vast experience in the lab.
- The radiologists Tracy Kilborn, Nicole Wieselthaler and Ebrahim Banderker for the hours they spent reviewing and re-reviewing the imaging results of these patients.
- Kirsty Donald, who mentored me through the Griffiths Mental Scales assessments and was my sounding board in determining how best to analyse such qualitatively complex data. I'd also like to acknowledge Rosy Khethelo who assessed all the Xhosa speaking children with me; her insight and sweet nature were such a pleasure.
- Katya Mauff for her excellent statistical assistance with seemingly never-ending data.
- My colleagues on the 6th Floor for being so supportive and encouraging; in particular John Lazarus for sharing his office with me, and Noorunisa Vollenhoven for being a constant pillar of strength.
- Bronwyn Gavine for helping me gather and reformat some of the patient data, and for proof reading some of the final thesis chapters.
- Jo Wilmshurst for her valuable comment on several chapters.
- My beloved sister, Inge, for her unconditional love and support. And to her and my brother-in-law Levent for the great gift of my two beloved little ones, Emre and Lale
- My dear friends for their support and love, and for lending fresh eyes to several chapters of this thesis.

I feel enormously privileged to be able to work at an institution like Red Cross Children's Hospital, and I owe thanks to Professor Astrid Berg and Professor Heinz Rode for the instrumental role they played in paving my way to RXH. I thank them for their continued care and support of me in my work and in life – they are surrogate parents to me.

I have been smiled upon by a Force of infinite Majesty, and I am humbled

Biomarkers of Neurological Injury and Inflammation in Children with Tuberculous Meningitis

Candidate: Ursula Rohlwink, Supervisor: Professor Anthony Figaji

ABSTRACT

Background Tuberculous meningitis (TBM) in children has high mortality and neurological morbidity rates. The assessment of disease severity and prognostication are difficult because several factors influence initial presentation, and advanced tools for these are lacking. Biomarkers of neurological injury could help to assess severity and to prognosticate, but have not been assessed in paediatric TBM. This study examined serum and cerebrospinal fluid (CSF) biomarkers of neurological injury in paediatric TBM in association with clinical and physiological data, radiology, inflammatory markers, and outcome.

Methods Serum and CSF (ventricular and lumbar) samples were taken on admission and over 3 weeks in children with probable TBM and hydrocephalus. These were analysed with ELISA for neuromarkers S100B, neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP), and with Luminex multianalyte array assay for a panel of inflammatory markers. Results were compared with 2 controls groups. Computerized tomography was done on admission and magnetic resonance imaging (brain, spine and magnetic resonance angiography) at 3 weeks. Brain oxygenation was monitored invasively and non-invasively in selected patients. Clinical and neurodevelopmental outcomes were assessed at 6 months. Data were analysed with various statistical tools, including principal component analysis.

Results Data were collected from 44 children. Of these, 16% died and 36% had disability (25% mild-moderate, 11% severe). S100B, NSE, GFAP and inflammatory markers were elevated in CSF on admission and for up to 3 weeks, but not in serum. Elevated neuromarkers were significantly associated with poor outcome and increased over time in patients who died, although combined inflammatory biomarkers decreased. Cerebral infarcts occurred in 66% of patients and were associated with neuromarker elevation. Novel findings on spinal MRI were the high frequency of asymptomatic disease. Cerebral vascular pathology was documented frequently on imaging but did not predict infarcts. Low brain oxygenation was common and in keeping with physiological events and outcome.

Conclusion CSF neuro- and inflammatory markers are elevated in TBM. Neuromarkers were prognostic of clinical and radiological outcome and an increasing trend suggested ongoing injury. This does not appear to be related to ongoing inflammation as measured by cytokines but may reflect the ongoing secondary injury processes initiated by inflammation.

TABLE OF CONTENTS

Section D: Study results

List of Tables

Tables are numbered according to the chapter to which they belong and the order with which they appear in the chapter.

List of Figures

Figures are numbered according to the chapter to which they belong and the order with which they appear in the chapter.

List of abbreviations

ACA	Anterior cerebral artery
AEG	Air encephalogram
AFB	Acid fast bacilli
AIDS	Acquired immunodeficiency syndrome
ART	Anti-retroviral treatment
AUC	Area under the curve
BBB	Blood brain barrier
BCG	Bacillus Calmette–Guérin
BCH	Brooklyn Chest Hospital
CBF	Cerebral blood flow
CI	Confidence interval
CHCP	Communicating hydrocephalus
CNS	Central nervous system
Coef	Coefficient
CPP	Cerebral perfusion pressure
CSF	Cerebrospinal fluid
CSW	Cerebral salt wasting syndrome
CT	Computed tomography
CXR	Chest x-ray
DWI	Diffusion weighted imaging
ELISA	Enzyme-linked immunoassay
EMB	Ethambutol
ETH	Ethionamide
ETV	Endoscopic third ventriculostomy
EVD	External ventricular drain
FLAIR	Fluid-attenuated inversion-recovery
FiO2	Inspired fraction of oxygen
GCS	Glasgow comas score
GEE	General estimating equation
GFAP	Glial fibrillary acidic protein
GMDS-ER	Griffiths Mental Development Scales – extended version
GQ	General quotient
GRO	Growth regulated oncogene
Hb	Haemoglobin
HCP	Hydrocephalus
HIV	Human immunodeficiency virus
HLOD	Highest limit of detection
ICA	Internal carotid artery
ICP	Intracranial pressure
ICU	Intensive care unit
IFN-γ	Interferon-gamma
IL	Interleukin
INH	Isoniazid
IP-10	Interferon-inducible protein -10
IQR	Inter-quartile range (25^{th} -75^{th} percentile)
IRIS	Immune reconstitution inflammatory syndrome

LLOD	Lowest limit of detection
LOC	Level of consciousness
LP	Lumbar puncture
MAP	Mean arterial pressure
MCA	Middle cerebral artery
MCP-1	Monocyte chemoattractant protein -1
MIP-1α	Macrophage inflammatory protein 1-α
MRA	Magnetic resonance angiogram
MRC	Medical research council
MRI	Magnetic resonance imaging
Mtb	Mycobacterium tuberculosis
NCHCP	Non-communicating hydrocephalus
NHLS	National health laboratory service
NIRS	Near-infrared spectroscopy
NSE	Neuron specific enolase
NO	Nitric oxide
OP	Opening pressure
OR	Odds ratio
PbtO2	Brain tissue oxygen tension
PC	Principle component
PCA	Posterior cerebral artery
PCA	Principle component analysis
Pcomm	Posterior communicating artery
PCPS	Paediatric cerebral performance scale
PCR	Polymerase chain reaction
pTB	Pulmonary TB
PZA	Pyrazinamide
RANTES	Regulated upon-activation, normal T-cell-expressed and secreted
RMP	Rifampicin
ROC	Receiver operating curve
RXH	Red Cross War Memorial Children's Hospital
S100B	S100 beta
SaO2	Systemic saturation
SAS	Subarachnoid space
SES	Socio-economic status
SIADH	Syndrome of inappropriate anti-diuretic hormone
SO2	NIRS saturation
TB	Tuberculosis
TBH	Tygerberg Hospital
TBI	Traumatic brain injury
TBM	Tuberculous meningitis
TNF-α	Tumour necrosis factor- alpha
TST	Tuberculin skin test
VEGF	Vascular endothelial growth factor
VPS	Ventriculoperitoneal shunt
WCC	White cell count

List of Appendices: Included at the end of main thesis text

BIOMARKERS OF NEUROLOGICAL INJURY AND INFLAMMATION IN TBM

THESIS SUMMARY

Tuberculous meningitis (TBM) is the most lethal form of TB and is associated with unacceptably high rates of mortality and morbidity. It occurs commonly in children from high TB prevalence settings. However, TBM remains challenging for clinicians to discern early because of the nonspecific nature of its presentation and the lack of swift and definitive diagnostic tools. Consequently, treatment initiation is often delayed, which increases the risk of a poor outcome. The aetiology of neurological impairment in these patients is multifactorial – it may due to direct brain inflammation, vasculitis, hydrocephalus, hyponatremia, and seizures. Some factors may be reversible while others may lead to relentless deterioration. In keeping with this, some patients present in a poor condition and then improve, while others have a less severe presentation but then proceed to deteriorate and die. Clinical examination lacks the sensitivity and specificity to accurately assess injury severity and radiological examination only illustrates injury once it is irreversible. Advanced tools to assess cerebral injury severity, to direct limited resources to children at highest risk of poor outcomes, and prognosticate are lacking.

Inflammatory markers have been studied in TBM; however, the extent of inflammation as detected by these markers may not correlate with the extent of cerebral tissue injury. To date direct markers of cerebral tissue injury have not been widely examined in this pathology. Reliable markers of neurological tissue injury may serve as a means of assessing injury severity, predicting deterioration, directing treatment, prognosticating, and developing new strategies for treatment in TBM.

This project aimed to examine three markers of neurological tissue injury, S100B, neuron-specific enolase and glial-fibrillary acidic protein, as candidate biomarkers for assessing injury severity and predicting outcome in TBM. All children presenting to Red Cross War Memorial Children's Hospital with probable TBM and hydrocephalus from October 2010 to August 2013 were enrolled in this study. Hydrocephalus was a prerequisite because serial cerebrospinal fluid (CSF) sampling is common in this group and afforded the opportunity to examine biomarker trends. Serum, lumbar and ventricular CSF taken on admission and for the next three weeks were analysed for neuromarkers S100B, neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP) using enzyme-linked immunoassay (ELISA), and a panel of inflammatory markers analysed with Luminex multianalyte array assay. Results were compared with two controls groups: children with a fatty filum presenting for elective surgery (serum and CSF), and children with pulmonary TB (serum only). Data from brain computerized tomography scans at admission and magnetic resonance imaging (MRI - including spinal imaging and magnetic resonance angiography) at three weeks were collected, in addition to any

follow up imaging clinically indicated. Brain oxygenation was monitored invasively (brain tissue oxygenation, where clinically indicated) and non-invasively (near-infrared spectroscopy) in a subset of patients. Clinical outcome (according to the Paediatric Cerebral Performance Category Scale) and neurodevelopmental outcome (tested with the Griffiths Mental Scales) were assessed at six months. Data were analysed with various statistical tools, including principal component analysis.

Data were collected from 44 patients (median age 3.33 years). Of these, 16 % died, 36.4% suffered disability and 47.7% had no discernible neurological deficits on clinical assessment; however, neurodevelopmental deficits were present in all children assessed. In part, poor neurodevelopmental outcomes may reflect socio-economic and hospitalisation circumstances, but also highlight the more subtle deficits that these children may have. Biomarker analysis demonstrated that CSF S100B, NSE, GFAP and inflammatory markers were elevated in TBM patients on admission and for up to three weeks compared with controls. Ventricular CSF concentrations of neuromarkers exceeded lumbar concentrations, whereas inflammatory markers were more elevated in lumbar CSF, suggesting that the two compartments are not directly comparable. CSF Neuro- and inflammatory markers were not elevated in serum. Elevated neuromarkers were significantly associated with poor outcome and principle component analysis combining all three neuromarkers over time demonstrated increasing concentrations in patients who died, although combined inflammatory biomarkers decreased. Cerebral infarcts occurred in 66% of patients and showed significant associations with neuromarkers. Novel descriptions of findings on spinal MRI were the high frequency of asymptomatic disease, including arachnoiditis, intramedullary and extramedullary lesions, and thick exudates in the region of the cauda equina. Lumbar punctures on these children could not successfully draw CSF which has important clinical implications for diagnosis and differentiating between communicating and non-communicating hydrocephalus with air encephalography. Cerebral vascular pathology was documented frequently on imaging; these were not predictive of infarcts overall, but worse vascular pathology was observed in patients with large infarcts. Brain oxygenation monitors detected episodes of low brain oxygenation in keeping with physiological events and clinical and radiological outcome.

The main findings of this study indicate that CSF neuro- and inflammatory markers are elevated in TBM. Elevated neuromarkers are prognostic of poor clinical and radiological outcome, and an increasing temporal profile suggests ongoing cerebral injury despite treatment; this does not appear to be related to ongoing inflammation as measured by cytokines, but may be a consequence of the ongoing secondary injury processes initiated by inflammation. In addition to the biomarker results, several of our approaches in this study, including spinal and vascular imaging as well as brain oxygen monitoring, suggest that there are many aspects of TBM that need further investigation.

This thesis presents the project findings and is structured to provide an introduction to TBM and biomarker analysis, followed by results of the analyses of neurological and inflammatory biomarkers and their association with clinical, radiological and physiological indicators of injury severity, as well as clinical and neurodevelopmental outcome:

Section A: Background of TBM

Chapter 1 discusses the dissemination of TB from the lung to the cranium and the pathophysiology of the meningitis disease that ensues.

Chapter 2 introduces the epidemiological burden of TBM and highlights the challenges facing treating clinicians. A summary of the current climate of TBM in the Western Cape, where this project was conducted, is also provided.

Chapter 3 outlines the radiological features associated with TBM, their role in diagnosis, evolution and contribution to outcome.

Chapter 4 discusses ischaemia in TBM and introduces two methods of brain oxygenation, brain tissue oxygen tension and near-infrared spectroscopy, which are not currently being used in this disease.

Section B: Introduction to Biomarkers

Chapter 5 provides an introduction to neurological biomarker analysis in cerebral infections, with a particular focus on S100B, NSE and GFAP in terms of normal reference ranges, and pathological elevations in adult and paediatric cerebral infections.

Chapter 6 summarises the inflammatory process underlying TBM with specific reference to inflammatory cytokines and chemokines which have been studied in this disease. This chapter concludes the introductory literature review of the thesis.

Section C: Study Outline

Chapter 7 defines the hypotheses and objectives of this project.

Chapter 8 introduces the inclusion and exclusion criteria and methods of patient recruitment.

Section D: Study Results

Chapter 9 presents the study patient cohort in terms of their demographic, clinical and outcome profile.

Chapter 10 is dedicated to biomarker analysis. The methods of sample collection and analysis are described, and the results are discussed in terms of comparisons with controls, temporal profile of

elevation, relationship between biomarkers, association with clinical characteristics and association with outcome.

Chapter 11 discusses the findings of admission and follow up scans, and introduces novel descriptions of spinal disease and vascular pathology on magnetic resonance angiograms. The association between radiology and biomarkers as well as outcome are discussed.

Chapter 12 provides a descriptive account of study findings with brain oxygenation monitors and discusses their potential utility in TBM.

Chapter 13 finally concludes this thesis with a summary of the overall findings and recommendations for future work.

The candidate was responsible for all aspects of this project, other than the clinical decisions made for patient management, including the recruitment of patients and controls, the gathering, storage and testing of samples in the laboratory, setting up and running the brain monitoring, collecting and analysing clinical, radiological and laboratory data, and liaising with the clinicians regarding patient follow-up and imaging. The candidate completed the necessary training and conducted the Griffiths Mental Scales neurodevelopmental assessments with the TBM patients and controls. Finally she was responsible for preparing this thesis.

SECTION A: BACKGROUND TO TBM

CHAPTER 1: PATHOGENESIS OF TUBERCULOUS MENINGITIS

The systemic immune response to tuberculous infection

Tuberculosis (TB) is spread through the inhalation of infectious droplets of aerosolised *mycobacteria tuberculosis (Mtb)* which colonise the alveolar macrophages in the lungs constituting the primary site of TB infection. Infected macrophages present TB antigens on major histocompatibility complex (MHC) Class II to T-cells and stimulate T-cell activation through the release of interleukin (IL)-12. A T-helper cell I (Th1) response is initiated and several key cytokines are released, including tumour necrosis factor alpha (TNF-α), IL-17 and various chemokines which stimulate the recruitment of additional macrophages and neutrophils. Interferon gamma (IFN-γ) is also released by Th1 cells in the lung and surrounding lymph nodes, resulting in activation of macrophages to kill the TB bacillus. The activated macrophages stimulate further T-cell activation and the initiation of an adaptive immune response to augment the functions of the innate immune macrophages. This inflammatory process results in the formation of a granuloma which encapsulates the infected cells. In healthy individuals this arrests the development of active TB disease. However, in the aged, immune compromised or very young, an on-going immune defensive may result in active disease associated with tissue destruction in the lung and dissemination of the TB bacillus to other organ systems [1,2].

Dissemination to the central nervous system (CNS)

Dissemination of TB commonly occurs hematogenously facilitating the seeding of *Mtb* to other sites including the liver, bones, kidney and CNS. In some cases, and often in children, exacerbated hematogenous spread of TB to the brain may occur in the context of miliary TB particularly in young children less than 2 years suggesting that the weak immune system predisposes young children to dissemination [3,4]. Dissemination probably occurs early after primary infection before the adaptive immune response is able to control the infection [3,5]. Several mechanisms by which the bacilli are able to migrate from the primary site of infection into the lymphatic or blood stream have been suggested. *Mtb* interact with epithelial cells in the alveoli and in vitro models have demonstrated that the bacilli are able to invade the epithelial cells, replicate intracellularly and initiate cell lysis and spread to neighbouring cells [6]. Bacterial proteins early secretory antigenic target 6kDa (ESAT-6) and culture filtrate protein 10kDA (CFP-10) are involved in cell lysis while heparin binding haemagglutinin adhesion (HBHA) aids in *Mtb* translocation across the epithelium without lysis [7]. *Mtb* may also invade and traverse vascular endothelial cells [8] and be trafficked to distant locations in phagocytes. Additionally mycobateria are able to survive in infected macrophages by preventing their fusion with lysosomes through the action of bacterial gene PknG [9] and dendritic cells, less efficient at destroying

bacilli, may also transport *Mtb* from the lungs to other organ systems and secondary lymphoid organs [7]. Furthermore, factors relating to host immunity and *Mtb* strain influence the degree to which dissemination may occur. Polymorphisms in the genes encoding for antigen recognition and macrophage activation [10-12] or impaired pro-inflammatory cytokine release may weaken the ability of the initial innate response to control infection [7]. Virulent TB strains, like the Beijing strain, which are able to compromise the innate immune response, promote survival, replication and more severe disease including TBM [11,13]. The association between decreased hours of sunshine and increased incidence of TBM suggest that vitamin D may have a role in preventing bacterial dissemination [14].

In order to maintain the crucial homeostasis of its microenvironment the CNS is protected from indiscriminate influx of potentially harmful blood-borne substances by 2 vascular barriers; the blood brain barrier (BBB) which forms the defence between the brain and the systemic circulation, and the blood –cerebrospinal fluid barrier (BCB) which separates the cerebrospinal fluid (CSF) from the systemic circulation at the level of the choroid plexus in the ventricles [15]. These barriers are formed by brain microvascular endothelial cells which are characterised by intercellular tight junctions which restrict paracellular transport, a paucity of endocytotic vesicles and fenestrae which limit transcellular movement and dedicated transport mechanisms to regulate influx and efflux of substances between the CNS and the blood compartment [15,16]. Despite these protective mechanisms *Mtb* bacilli are able to migrate across these barriers and enter the immune limited domain of the CNS. The mechanism by which the BBB is breached has been studied using in-vitro and animal models which demonstrate that *Mtb* is able to invade and traverse brain endothelial cells through rearrangement of the actin cytoskeleton of brain microvascular endothelial cells [8,17]. A number of bacterial genes are upregulated during the interaction between *Mtb* and the brain endothelium [8]; the gene *Rv0931c* (pknD) has been identified as a potential virulence factor responsible for promoting CNS infection in certain TB strains as it enables the bacilli to interact with extracellular factors on the brain endothelium facilitating bacillary endothelial adhesion [17]. Another potential route of entry is the Trojan horse mechanism by which *Mtb* are trafficked in infected macrophages across the BBB [9].

CNS inflammation

In order to protect the minimally regenerative brain from the damage of inflammation, the baseline immune characteristics of the brain are weak; antigen presentation is poor as microglia have poor antigen presenting capabilities, there is a dearth of antigen-presenting dendritic cells and low expression of MHC Class II molecules [15]. Traditionally it was believed that the CNS was an immune naïve domain but it is capable of mounting a local immune response manifested by the intrathecal production of inflammatory mediators. Unfortunately however, CNS inflammation is often ineffective in destroying the invading pathogen[18]. The presence of the BBB effectively reduces the access of

leukocytes and plasma components to the intracranial compartment and CNS inflammation is consequently dependent primarily on local production of inflammatory mediators although some systemically produced cytokines may gain access through specific transport systems on the BBB or through a leaky BBB [18].

Once the TB bacilli gain access to the brain, the poor innate local immunity allows their survival and replication and likely the development of silent tuberculous lesions. These lesions undergo either immediate or delayed progression to caseate and discharge their bacilliary contents. Based on post-mortem studies, Rich and McCordock contended that TBM stems from the rupture of one of these established tuberculous lesions located under the cortical pia or adjacent to the meninges or ventricles, the Rich's focus, which releases *Mtb* bacilli into the sub-arachnoid space resulting in a granulomatous infection of the meninges [3,19-21] may also result from the rupture of miliary tubercles of the brain [22], from a caseating focus in the choroid plexus [23] or rarely spread from the tuberculous infection of adjacent skull bones [23,24]. The cause of foci rupture is not clearly understood but the release of bacterial wall components into the CSF triggers a prolific inflammatory response characterised by the production of cytokines and the recruitment of leukocytes[25-27] (discussed in Chapter 6) and the formation of a thick inflammatory exudate. The inflammatory process is most prolific at the base of the brain, an important anatomical location that interfaces with the cerebral vasculature of the Circle of Willis, the pathways of CSF flow, and several cranial nerves, and that communicates inferiorly with the spine.

ANATOMICAL PATHOLOGY OF TBM

Exudate

The characteristic feature of TBM in post-mortem studies is the presence of inflammatory exudate in the basal cisterns and subarachnoid spaces of the brain [21,28,29]. It is described as thick and gelatinous [28,29] and is made up of lymphocytes, neutrophils, erythrocytes, monocytes, and epithelioid cells, which may merge to form Langhans' giant cells, and as well as *Mtb* bacilli [19,21,29]. The inflammatory exudate may organise into meningoencephalitis or progress to form intraparenchymal tuberculomas with caseous centres surrounded by cellular peripheries and associated generalised oedema and vascular congestion [21]. Where the exudate comes in contact with the brain tissue it causes a borderzone reaction which results in tissue damage [19]. Exudative material is seen primarily at the base of the brain but may also involve the cerebral convexity and cause ependymitis of the ventricle walls, and may extend downward to encase the spinal cord [19,21]. The basal localisation of the exudate is hypothesised to be as a result of normal CSF flow; although Rich foci are not exclusively distributed at the base of the brain [20]. The predominant location of exudate at the base of brain has a number of important implications; firstly, all the major cerebral vessels originate from the base of the brain and are at risk

of being encapsulated by the exudate. Secondly, the accumulation of exudate in the basal cisterns interferes with the circulation of CSF causing hydrocephalus. Thirdly, it envelopes and compresses the local cranial nerves including the optic and oculomotor resulting in cranial nerve palsies [21] .

Vasculitis

Exudate fills the interpeduncular fossa covering the optic chiasm anteriorly and extending posteriorly to the antero-superior surface of the pons. It spreads along the lateral extensions of the interpeduncular cistern into the Sylvian fissures along which run the middle cerebral arteries (MCA), anteriorly into the cisterna lamina terminalis where the anterior cerebral arteries are located (ACA) and postero-superiorly into the ambient cisterns where it surrounds the midbrain and comes into contact with the origins of the posterior cerebral artery (PCA), superior cerebellar arteries and the optic tracts. The origins of all the major cerebral vessels lie therefore in this main subarachnoid lake at the base of the brain and as the exudate spreads along the cisternal extensions it coats the large arteries and their small perforators causing subacute-chronic arteritis and vasculitis [28].

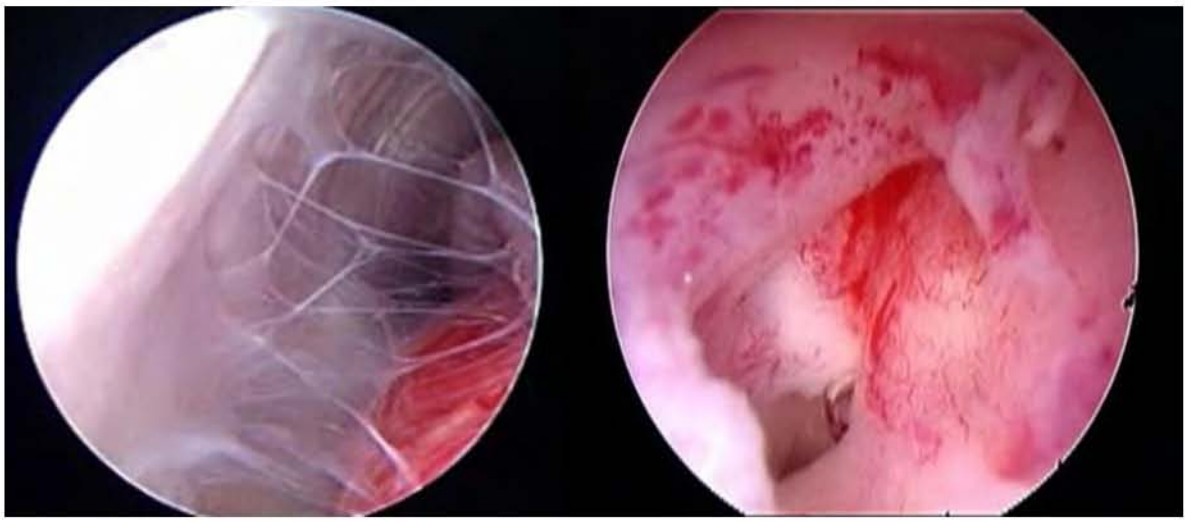
Vascular pathology includes infiltration of inflammation from the adventitia of arteries and veins inwards resulting in peri- or pan-arteritis involving segments or the full thickness of vascular wall tissue [30]. Infiltrative processes involve mostly vessels located at the base of the brain where the exudate is most prolific. Tubercles may also directly impinge on vessel walls [30]. Evolution of vascular inflammation may involve thickening of the vessel intima resulting in vessel stenosis or occlusion. This intimal proliferation is considered a feature of chronic or partially treated TBM at which juncture the exudate may have thickened and become more organised [30]. Necrosis of the vessel wall may involve partial or complete destruction of the vascular wall and is fibrinoid in nature. Necrosis may more commonly affect small calibre vessels and is demonstrated in both treated and untreated TBM [30]. Both cerebral and spinal vessels may be affected by any or all of these processes and this may reflect the duration of illness [30]. The role of vasospasm in initiating structural vessel wall damage and precipitating necrosis remains unclear, however, it is considered to play an important role in predisposing the brain to ischemic damage [30]. It has been suggested that TBM may be associated with a prothrombotic status which predisposes patients to an increased risk of infarction [31], however this too remains uncertain [30].

Minor arteritis results in anoxia whereas severe cases lead to infarction of whole territories supplied by the afflicted vessel [28]. The exudate has a predilection for extension into the Sylvian fissures and therefore the MCA and its perforators around the floor of the 3rd ventricle are most commonly affected resulting in ischemic damage to the sub-thalamic nuclei and lower internal capsule [21]. Involvement of small vessels supplying the mid-brain may lead to infarction [28] and exudate in the interpeduncular cistern can compromise the vessels supplying the hypothalamic region and precipitate

hypothalamic symptoms in these patients [28]. Diffuse oedema is seen around the grey and white matter as a consequence of ischemia and borderzone encephalitis which often occurs in the brain tissue underlying vessels bathed in exudate [21]. In autopsy studies acute infarcts are associated with swelling of the infarcted area. When occurring in the basal ganglia they may be accompanied by compensatory diminution in the size of the lateral ventricle. Chronic infarcts however show shrinkage of the infarcted area and may therefore be associated with ex-vacuo dilatation of the ventricles [28]. Infarcts have been recorded in up to 41% of post-mortem TBM studies [32] and there is a correlation among neurological changes, antemortem angiography and post-mortem cerebral and vascular pathology [21]. Further discussion on cerebral vascular pathology and infarction follows in Chapter 3 on brain imaging.

Figure 1.1 below illustrates how the exudate fills the basal cisterns distorting normal anatomy and CSF flow, and coats the cerebral vessels.

Figure 1.1: Exudate in the basal cisterns in TBM



Endoscopic view of the interpeduncular cisterns of the brain in a non-TB infected patient, depicting clear subarachnoid space, visible vessels, free flow of CSF

Same endoscopic view of the interpeduncular cistern in a patient with TBM depicting thickened and inflamed ventricular walls, opaque cisterns obliterated with exudate, and vessels not visible

Hydrocephalus

Extension of exudative material into the basal cisterns including the ambient and pontine cistern results in a collar of exudate around the midbrain which blocks CSF flow around the upper brain stem. It may also cause narrowing of the cerebral aqueduct of the 3rd and 4th ventricles with resultant hydrocephalus (HCP). Rarely the occurrence of an intra-aqueductal tuberculoma may also precipitate HCP. Further discussion on communicating and non-communicating HCP follows in Chapter 2. The pressure of expanding ventricles and the opposing pressure of diffuse brain oedema as a result of pathological processes can negatively impact the grey and white matter leading to pallor and diffuse loss of myelin [21].

Granulomata/tubercles

Tuberculous encephalitis may occur deep in the sulci of the cerebrum and cerebellum. This is observed as small foci of necrosis in chronic cases which are not visible on the surface and only observed on cutting the brain. Encephalitis may also manifest as parenchymal tuberculomas which are identified in various stages of activity and healing in post-mortem investigations [28]. Tuberculomas may coexist with TBM or occur independently and are thought to originate from expanding tubercles in the parenchyma. These lesions are typically granulomatous consisting of a necrotic centre surrounded by epithelioid cells which may merge to form Langhans giant cells, lymphocytes and a reactive border containing astrocytes and associated surrounding oedema and vascular proliferation [19,21]. The vessel walls are characterised by infiltration and destruction by inflammatory cells [21]. Caseous necrosis may ensue resulting in liquefaction of the central caseous tissue and the formation of cysts containing a clear or straw-coloured liquid [33]. With time the tubercles become discretely organised and bound by a rim of connective tissue of reticulin fibres. They may occur throughout the brain in the cerebrum, cerebellum and brainstem [21].

Tuberculous brain abscess

TB brain abscesses may follow TBM or develop independently of a meningitic process [34,35], however, they are reported to occur infrequently [33]. Brain abscesses of tuberculous origin manifest as encapsulated collections of pus which contain tubercular bacilli in the absence of typical features of a TB granuloma including epithelioid and giant cells and mononuclear cell infiltrates [33]. These lesions must be distinguished from tuberculomas with central necrosis and liquefaction and Ziehl-Nelson staining or culture on the pus of surgically treated abscesses are preferable in confirming the diagnosis, although the culture yield may be poor [19,35]. The prolific exudative process promotes caseation which may soften and be associated with the infiltration of polymorphonuclear leukocytes and pus formation. The tuberculous abscess wall is typically thicker than that of pyogenic abscesses

and histological examination of the wall reveals predominantly vascular granulation tissue comprising a mix of acute and chronic inflammatory cells, particularly polymophonuclear leukocutes [33]. These abscesses have been reported in the cerebrum [33,34] and posterior fossa [34,36,37] occurring rarely in the brain stem [35], and may be singular or multiple [19].

Spinal TB

Spinal TB may develop as a primary tuberculous lesion, from the downward extension of intracranial TBM or secondary to vertebral TB [21,38,39]. It involves the cord, meninges and nerve roots and manifests as spinal arachnoiditis, intradural (extramedullary) tuberculomas or rarely as intramedullary tuberculomas [40-42]. Tuberculous leptomenigitis, also referred to as tuberculous radiculomyelitis, frequently accompanies TBM and is characterised by the extension of basal exudate into the spinal canal where it encases the spinal cord expanding the space between the dura and the leptomeninges causing compression but not infiltration of the spinal cord. It may be associated with areas of caseous necrosis, thickening of the dura and borderzone encephalitis of the cord. The nerve roots may become entrapped and matted in exudate filling the subarachnoid space precipitating the eventual adherence between the spinal nerve roots and thecal sac [21,24]. Exudate involvement of the lower lumbar segments is associated with difficulty in performing lumbar punctures and a high CSF protein [21]. The microscoscopic appearance of spinal exudate is identical to intracranial exudate, consisting of giant cells and caseous areas, and causing vasculitis of the spinal veins and arteries which may led to cord ischemia and infarction [21,43].

CHAPTER 2: TUBERCULOUS MENINGITIS: EPIDEMIOLOGY AND CLINICAL CONSIDERATIONS

Epidemiology

Tuberculosis continues to be a massive global health problem. According to the World Health Organisation (WHO) Global TB Report 2013 [44] the year 2012 saw 8.6 million incident TB cases and 1.3 million TB-related deaths. South Africa ranks as one of 2 countries with the highest incidence rate per capita with 1 new case of TB for every 100 people each year, and the Western Cape has the highest incidence in the country [45]. Although global mortality rates have been on the decline, mortality trends in South Africa appear less promising and it is estimated that approximately 119 000 South Africans died of TB in 2012. The global incidence of TB amongst children is estimated at 530 000 cases (6% of the total incident cases) and childhood TB mortality accounts for 8% of the global burden amongst human immunodeficiency virus negative (HIV-) individuals [44]. Long-standing challenges to the eradication of the disease include the lack of effective vaccination, poverty, lack of education, and poor access to early and effective health care. New challenges that have increased the global problem of TB include its association with HIV/ acquired immunodeficiency syndrome (AIDS) and the emergence of multidrug resistance.

Extrapulmonary TB constituted 15% of total global TB notifications [44]. TBM is the most severe form of extra-pulmonary tuberculosis and the most common form of neuro-tuberculosis [14,21] leading to death or severe disability in half of the affected individuals [45-52]. It is reported to occur more frequently in children [14,47], particularly in countries with high TB prevalence whereas adult cases are relatively more common in low TB prevalence regions [53]. The incidence in children is highest in the first 5 years, particularly between 1-3 years [45,48,54,55]. It is rarely observed before 3 months of age, however, the co-incidence of miliary TB and TBM occurs more commonly in younger children than TBM alone, and suggests that the weak immunity in young children may predispose them to disseminated TB [4]. TBM often develops in association with malnutrition, particularly protein-calorie malnutrition in children [21] and is most commonly seen in patients from rural or impoverished circumstances [48,56,57]. Risk factors for adults include corticosteroid treatment, alcoholism, diabetes mellitus, malignancy and HIV, and TBM has become the commonest form of meningitis in HIV infected (HIV+) adults [50,53]. In both adult and paediatric cohorts males are more commonly affected [45,47-49,51,52,58]. The disease peaks in late Winter/early Spring partly due to a seasonal increase in pulmonary TB during winter months of over-crowding, poor ventilation and poor housing conditions followed by a 3-4 month incubation period. The role of low vitamin D levels due to decreased

sunshine hours has also been suggested as the vitamin D receptor on microglia may play a role in the production of pro-inflammatory cytokines and immune activation [14]. The protective value of the Bacillus Calmette–Guérin (BCG) vaccination remains controversial, but it is reported to confer greater protection against severe forms of disseminated TB in children including miliary TB and TBM [59]. The comparison between vaccinated and unvaccinated paediatric TBM patients has generated mixed results in terms of the severity of presentation and outcome [60,61] but what is noteworthy is the predominance of BCG vaccination amongst children presenting with TBM (over 90% in some studies [45,62]). This raises important questions regarding the quality of vaccine administration and the composition of the BCG vaccine. Considerable effort has been dedicated to the development of more effective vaccines and important information about the immunology and genomics of TB is being generated [63].

Diagnosis and clinical presentation

TBM is considered a medical emergency requiring immediate treatment in all patients in whom it is suspected [53]. Diagnosing TBM remains challenging due to the non-specific nature of its presentation and the lack of clinical, laboratory or radiological tools to enable a swift and definitive diagnosis. Early diagnosis and commencement of treatment are considered the most important determinants of outcome [64]. Missed opportunities for timely diagnosis, late presentation, inaccessibility to medical care and poor health care seeking all contribute to delayed diagnosis [48,57,65].

Most institutions treat patients on the presumed diagnosis of TBM based on a combination of clinical, laboratory and radiological criteria [45,47,48,51]. However, there is considerable variability across these criteria and a lack of uniformity in defining TBM exists, particularly in defining probable versus possible TBM. A number of studies have attempted to develop diagnostic algorithms [66-68] but these have not been validated across different settings or patient age groups. A recent consensus statement set out to develop standardised diagnostic criteria to allow appropriate comparison between research studies. Relevant literature in both adult and paediatric TBM was reviewed by an international panel of experts from various disciplines involved with TBM management, and a case definition was derived that can be used independently of patient age, HIV status or clinical/research resources [69] (Appendix 1). International validation of this consensus case definition is still required.

Clinical presentation

In the early phases of disease patients may present with non-specific sub-acute symptoms like cough, fever, vomiting, malaise, lethargy, a worsening headache and weight loss/poor weight gain [5,47,48,50,51,69-72]. Meningism may be absent early in the disease and it is challenging to differentiate these symptoms from those of benign conditions like an upper respiratory tract infection [5,45]. This is

particularly the case in children in whom symptoms may be challenging to recognise; however, failure to thrive demonstrated by a declining percentile of mass for age relative to the original growth pattern recorded on the growth chart is one of the most sensitive early features of TBM in children [73]. With disease progression more severe symptoms develop including a decreased level of consciousness (LOC), meningism, signs of raised intracranial pressure (ICP), seizures and focal neurological fall-out, and it is often only at this point that the diagnosis of TBM is considered. Focal neurology may include paresis, pupillary abnormalities, aphasia and cranial nerve palsies. The cranial nerves may be compromised by nerve arachnoiditis, ischemia and hydrocephalus (HCP) and commonly involves cranial nerve II, as well as III and VI possibly due to their long subarachnoid course [47,48,52,74]. Headache, vomiting, poor weight gain or weight loss, meningism and an altered sensorium have been described among the commonest presenting symptoms [45,47-49,51,52,70-72]. Abnormal motor function is reported in over half of patients and cranial nerve palsies in one quarter [45,47]. Although raised ICP is common in these patients papilloedema is not often recorded [49,71] but a bulging anterior fontanelle may be reported in up to 70% of infants [75]. Presenting symptoms are not significantly related to sex [47]. History taking may reveal a recent positive TB contact in 20-66% of cases [45,48,71,72] and a previous history of TB in 13-27% of cases [71,72]. Patients are commonly symptomatic for more than 1 week before diagnosis but duration of symptoms may be over 1 month [45,47-50,72]. TB infection in other organs have been reported in over 30-50% of patients, including pulmonary, disseminated, abdominal and lymph node [47,52,71,72]. A number of studies have examined the clinical features most predictive of TBM; symptom duration of longer than 5 days [5] and recent poor weight gain [73] have shown strong predictive power.

Severity

Currently there is a deficit of tools to accurately assess injury severity and prognosticate. Clinical examination lacks the specificity to estimate disease severity as a number of factors not directly related to TBM may contribute to presenting symptoms. Radiological findings do not demonstrate evolving injury and brain damage is only evident once it is already irreversible. Currently the commonly used means of assessing the severity of disease on admission is the British Medical Council (BMC) TBM staging criteria [76]. This system was modified after its initial description to include the Glasgow Coma Score (GCS) and scores patients as Stage I with a GCS of 15/15 and no focal neurology, as Stage II with a GCS 11-14 or 15 with focal neurology, and as Stage III with a GCS $\leq$ 10. Although widely used this grading system does not account for the fact that a number of treatable factors other than TBM may be responsible for a decreased level of consciousness on admission including seizures, electrolyte disturbances, raised ICP and anti-convulsive medication. Consequently other grading systems including the Acute Physiology and Chronic Health Evaluation II [77], the TBM Acute Neurological Score (TBAN) [78], the Vellore grading system for patients with HCP

[79,80], and the Tygerberg Children's Hospital scale (TBH) [81] have been applied. Additionally a refined version of the MRC system was proposed which divides Stage II into 2 separate categories (Stage 2a: GCS 15 with neurological deficit/ GCS13-14 with/without neurological deficit, and Stage 2b: GCS 10-12 with/without focal neurological deficit) [81]. A recent paediatric study demonstrated that all these various scales had adequate power in predicting outcome and that using the refined MRC staging one week after admission significantly enhanced its prognostic power [81]. However, the MRC system applied at admission still remains the most widely used [45,47,69] and the majority of presenting cases fall within Stage II (≈50%) or Stage III (≈40%) [45,47-50,69-71,75]. A longer duration of symptoms is reported in patients with more severe disease spanning sometimes more than 3 weeks before admission and diagnosis [48]. Table 2.1 summarises the MRC stages of TBM severity.

Table 2.1: MRC stages for classifying the severity of TBM

MRC staging		**Refined MRC staging**	
Stage I: mild	GCS 15/15 No focal neurology	**Stage I: mild**	GCS 15/15 No focal neurology
Stage II: moderate	GCS 11-14 or GCS 15 With focal neurology	**Stage IIa: moderate**	GCS 15 with neurological deficit/ GCS13-14 with/without neurological deficit
Stage III: severe	GCS ≤ 10	**Stage IIb: moderate**	GCS 10-12 with/without focal neurological deficit
		Stage III: severe	GCS < 10 with/without neurological deficit

This table includes the criteria for mild – severe stages of TBM using the MRC Staging and a refined version of the MRC Staging described in Van Toorn et al [81].

Laboratory Diagnosis

The current gold-standard for TB diagnosis is the culture of *Mtb* or the visualisation of acid-fast bacilli (AFB) in cerebrospinal fluid (CSF). CSF culture positivity yields are notoriously poor ranging from 0-47% [45,47-49,52,70,72,75] and results can take longer than 40 days. Consequently treatment must be initiated based on clinical suspicion to mitigate the devastating outcomes associated with treatment delay. Factors that may improve culture yields include larger volumes of CSF (>6ml), repeated CSF investigation in patients undergoing multiple lumbar punctures (LPs) and liquid culture media [82,83]. CSF smear is the most accurate rapid investigation but the visualisation of AFBs is variable (0- 50% of patients) [47,48,51,70,71,75] and highly dependent of the volume of CSF and the expertise with which it is examined [53]. The sensitivity may also be improved with larger volumes and repeated CSF's and with

the use of fluorescent microscopy [69]. A recent laboratory study found that modifying the laboratory procedure by crytospinning the CSF to collect the cells, fixing and staining them with TritonX-100 dye and then staining them with Ziehl-Neelsen dye allowed for 100% AFB visualisation. The method allowed the visualisation of not only extracellular bacilli but also intracellular bacilli located in monocytes, neutrophils and lymphocytes [84]. Further validation studies for this innovative technique are required.

Molecular technologies involving nucleic acid amplification like real-time polymerase reaction (PCR) are being investigated for TBM diagnosis as they potentially offer tests which are rapid and highly sensitive and specific. Studies in the clinical setting have demonstrated high specificities but sensitivities have not been adequate for these to be used as rule-out tests, but they may confirm TBM in patients presenting with signs and symptoms suggestive of TBM [85,86]. This is largely as a result of the small number of bacilli and the presence of endogenous amplification inhibitors in the CSF [85]. A novel nested PCR assay which dramatically increases the sensitivity and specificity of DNA amplification is in development, but widespread routine use is not yet feasible due to the complex and laborious techniques involved and the risk of cross-contamination; research is ongoing [85]. However, unlike culture and microscopy these molecular tests may remain sensitive for up to 1 month after the initiation of treatment [83,87]. The Gene Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) which uses real time PCR to allow for rapid near-patient diagnosis and drug sensitivity testing has shown high sensitivities and specificities in the diagnosis of pulmonary TB from sputum samples [88] and can effectively be applied in resource-limited settings [89]. Studies on CSF samples in HIV+ TBM patients have found this method to be 11 times more sensitive than fluorescent microscopy, although the technique is currently still expensive [90]. A study investigating the potential of this test in paediatric extra-pulmonary TB including TBM is currently underway.

Diagnostic techniques that detect *Mtb* antibodies and antigens have demonstrated poor sensitivities and specificities and are consequently still considered experimental [69,91]. Adenosine deaminase levels are elevated in the CSF and serum of TBM patients [92,93] and offer a useful diagnostic test which can differentiate TBM from other meningitides in resource limited areas as the assay is quick, easy and inexpensive to perform. Cut-off values suggestive of TBM are however varied across studies making ADA levels challenging to interpret and this test is not routinely being used [91].

The mantoux or tuberculin skin test (TST) is frequently carried out in paediatric cases and is positive in up to 60% of paediatric patients [45,47], defined by an induration of $\geq$ 10mm or $\geq$ 5mm in HIV-infected or malnourished children. Repeat TST's are recommended in children who test negative as improved nutrition may lead to improved results [94]. The specificity of TST may be mitigated by BCG vaccination and a positive result cannot make the distinction between infection and active disease [69].

Similarly, interferon-gamma release assay (IGRA) may demonstrate higher accuracy in diagnosing latent TB [95] but fail to differentiate infection from disease [69].

Laboratory investigations

CSF microscopy and chemistry are essential in the presumptive diagnosis of TBM.

CSF white cell count: Elevated leukocytes (10 - 100 cells/μL) occur in the overwhelming majority of patients (>80%) [45,48]; lymphocytic predominance of >50% is common [47,48,51] and may be > 90% [47,49].

CSF Chemistry: Glucose is commonly low often with a CSF/blood glucose ratio of < 60%, protein is elevated [45,47-49,51,71,75].

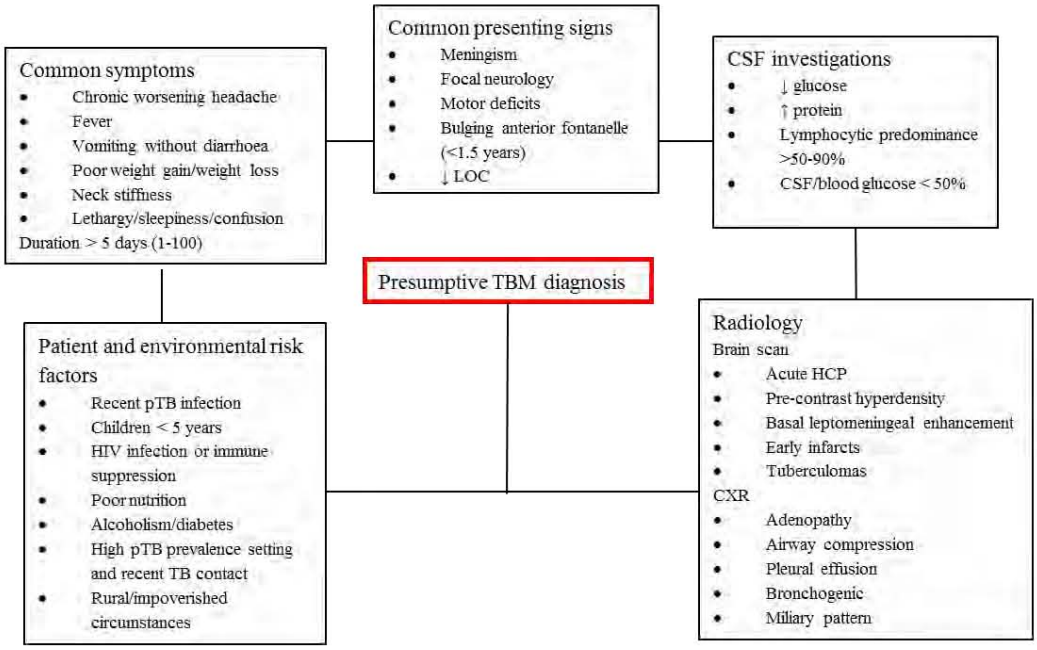
Atypical findings have been described in children and HIV+ adults in whom CSF may be acellular or demonstrate neutrophil predominance similar to bacterial meningitis. Glucose levels may be normal or elevated, and protein levels may be low [51,96].Therefore, while these investigations contribute to the presumptive diagnosis, they are not considered rule-out tests.

Radiology

Radiological findings indicative of TBM include hydrocephalus (HCP), basal and leptomeningeal enhancement, infarcts and tuberculomas. These features are a vital component in building a presumptive TBM diagnosis and are discussed in detail in Chapter 3.

The combination of clinical, laboratory and radiological criteria upon which the presumptive diagnosis of TBM is made are outlined in Figure 2.1

Figure 2.1: Components of presumptive TBM diagnosis



This flow diagram illustrates the various components which contribute to the presumptive diagnosis of TBM

TREATMENT

Medical management

The WHO TB treatment guidelines for children recommend 12 months of antibiotic treatment consisting of a 2 month regimen of isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA) and ethambutol (EMB) followed by 10 months of INH and RMP. Hospitalisation for the initial 2 months is recommended [97]. Treatment doses for adults are lower but 12 months is also the recommended duration of treatment [53]. A systematic review and meta-analysis suggested that 6 months of treatment were sufficient although a formal study to compare 6 month with longer treatment regimens has not been conducted [98]. Longer treatment durations are encouraged due to concerns regarding relapse, the unclear influence of disease severity, undetected drug resistance and poor CSF penetration of some of the drugs [5,53].

In resource limited contexts like South Africa administering and controlling such a long duration of treatment may not be feasible and hospital stays are curtailed by limited beds and staffing. Short-course intensive treatment regimens over 6 months are less expensive and less labour intensive and may improve patient compliance which is vital as treatment interruption is an independent risk factor for death in TBM [46,58]. Consequently, health care institutions in the Western Cape have adopted the 6

month intensified short course regimen introduced at TBH, comprising INH (20 mg/kg), RMP (20mg/kg), PZA (40mg/kg) and ethionamide (ETH – 20mg/kg) for 6 months in HIV- children and 9 months in HIV+ patients with drug-susceptible disease [46,99]. Studies evaluating this institution's treatment outcomes using this regimen over the last 15 years have consistently reported the regimen to be safe, sufficient and well tolerated [46,99]. This group has also demonstrated successful home-based care for the last 5 months after hospital discharge with an absence of relapses in 90% of children treated at home [46]. An adult study using high dose intravenous RMP and moxifloxacin for 2 weeks in conjunction with standard treatment for 6 months demonstrated higher drug exposure without increased adverse effects and a 50% reduction in 6 month mortality, although this study was not powered as an outcome study [100].

There is some debate about the choice of fourth drug and there are a number of options. Ethambutol has poor CSF penetration in the absence of meningeal inflammation and is associated with side effects including optic neuropathy [53,91]. Streptomycin is frequently associated with toxicities and resistance has become common [5,91]. Ethionamide shows good CSF penetration under conditions of meningeal pathology and health but may cause nausea and is consequently administered at night. Studies with fluoroquinolones suggest that certain agents including levofloxacin and moxifloxacin penetrate the CSF adequately and contribute to antituberculosis activity and improved outcome [5,100]. This group of antibiotics form an essential component of 2nd line treatment for multidrug-resistant (MDR) TBM [5]. They are, however, not recommended for women who are pregnant or lactating and may be associated with seizure activity at high doses [5,53,91].

Adjunctive therapy

A randomised controlled trial on adjunctive steroid use in paediatric TBM concluded that steroids significantly reduced mortality, tuberculoma incidence and meningeal enhancement on CT and improved cognitive outcome. They had no effect on infarct size or incidence or on permanent motor deficits [101]. An adult study found similarly that steroids reduced the risk of death across all stages of TBM but had no mitigating effect on the proportion of patients who died or were left severely disabled by 9 months and the benefit to mortality in HIV+ individuals was less certain [58]. A Cochrane Review on the subject found that overall corticosteroids reduce mortality and disabling residual neurological deficits [102]. Steroids are recommended as part of the routine treatment for TBM for the first 4-6 weeks [5,53]. The mechanism by which steroids work is still unclear; there is some evidence in animal and human studies to suggest a role for the polymorphism in LTA4H which encodes for leukotriene A4 hydrolase, an enzyme which controls the balance of pro- and anti-inflammatory eicosanoids and the expression of tumour necrosis factor α (TNF-α). Variability in the expression of LTA4H renders individuals prone to either a hypo- or hyper-inflammatory state; corticosteroids may

be suitable only for the latter phenotype suggesting that individualisation of treatment may be important.

A safety and efficacy trial using thalidomide in paediatric TBM found that it demonstrated immuno-modulating effects including a decrease in pro-inflammatory mediators, reduced enhancement on head CT scans, decreased incidence of tuberculomas and improved resolution of established tuberculomas [103]. However, the occurrence of adverse effects in the treatment arm of the randomised controlled trial necessitated the premature termination of the study [104]. Adjunctive aspirin use has been considered owing to its anti-thrombotic, anti-ischemic and anti-inflammatory action. A randomised controlled trial in adult TBM found that the combination of adjunctive steroids and aspirin led to a decreased frequency of death at 3 months and a 19% decreased risk of ischemic stroke [105]. This was followed by a paediatric study which found that low and high dose aspirin were not associated with a significant decrease in mortality or motor disability. Their high dose aspirin group were, however, younger and had more severe disease; the fact that their outcomes were no different from the other treatment groups may suggest some benefit of high dose aspirin [106]. Currently aspirin does not form part of formal treatment recommendations and further studies are required.

Drug resistance

Detecting drug resistance early so as to initiate an appropriate alternative line of therapy is key to preventing death and the spread of drug-resistant TB strains [5,107]. CSF culture is currently the only standard way to establish drug susceptibility [91]and results can take as long as 10 weeks, increasing the risk of severe disease, mortality and morbidity [108]. PCR techniques which allow rapid drug sensitivity testing are urgently needed but are limited by poor sensitivities in CSF and uncertainty around which mutations denote resistance for some drugs [5,91]. Diagnosis relies on suspicion, patient history and knowledge of the local drug resistance patterns. Mono-resistant TB demonstrates resistance to INH or RMP and MDR TB denotes resistance to both INH and RMP [107]. Globally MDR TB accounted for 3.6% of newly diagnosed cases and 20% of previously treated cases in 2012 [44]. South African studies found that 8-10 % of adult and paediatric TBM cases demonstrated MDR as well as resistance to second-line drugs and the authors suggest that this may be an underestimate due to the difficulty in culturing TB from CSF. Two of the adult patients were resistant to 7 of the available TB drugs [4,108]. Studies from other locations have reported resistance (MDR and mono-resistance) in 11-15% of TBM patients [52,70]. MDR TBM is associated with high mortality; delay in diagnosis and initiation of appropriate treatment likely contribute to poor outcomes [108,109]. A general consensus on treatment regimens for drug-resistant TBM has not been established and regimens should be tailored according to the drug susceptibility profile of individual patients. Second-line drugs used to treat drug-resistant TB include 1) fluoroquinolones (moxifloxacin, gatifloxacin, oflofloxacin, levofloxacin,

ciprofloxacin), 2) injectable agents including aminoglycosides (amikacin, kanamycin and streptomycin) and the glycopolypeptide capreomycin, 3) oral bacteriostatic agents (ethionamide, protionamide, cycloserine, para-aminosalicyclic acid, thioacetazone) and 4) drugs with unclear efficacy (clofazimine, amoxicillin, cluvulanate, clarithromycin and linezolid) [107].

HIV co-infection

Treatment for TBM patients co-infected with HIV is complicated by the risk of potential drug-drug interactions and paradoxical TB associated immune reconstitution inflammatory syndrome (IRIS) after the initiation of anti-retroviral therapy (ART) [46]. This condition occurs in HIV+, ART-naive patients who respond well to anti-TB treatment until the introduction of ART. Patients present with recurrent, new or worsening symptoms or deterioration of radiological manifestations of TB disease. Risk factors for TB-IRIS include more advanced HIV disease with lower CD4 cell count, extrapulmonary TB, earlier initiation of ART, and a more vigorous viral response to ART [110]. A recent adult study of HIV+ TBM patients found that TB-IRIS occurred in 47% of patients manifesting as worsening headache, confusion, meningism, seizures and other signs of focal neurology. CSF neutrophil counts were higher at admission and poor outcome was seen in 44% of TBM-IRIS cases [111]. The recommended time to commence ART in adults is 2 weeks after the initiation of anti-TB treatment, recommendations for paediatric TBM have not been established but treatment may be delayed 2-4 weeks after the initiation of anti-TB medication [46]. Paradoxical worsening of TBM after treatment initiation is also seen in HIV- individuals or HIV+ individuals not on ART and is attributed to a hypersensitivity reaction of the immune system to bacterial antigen release as a result of drug induced *Mtb* destruction [24,91]. These reactions are however more severe and frequent in ART-receiving patients and may involve multiple organ systems [110].

Supportive and surgical management

Raised ICP

Raised ICP may be owing to cerebral oedema, mass occupying tuberculomas or abscesses, and HCP. Treatment with hypertonic saline is recommended for brain swelling [112] and a combination of medical and surgical management for HCP, tuberculomas and abscesses. Invasive continuous ICP monitoring is not currently used in TBM and further research into protocols for monitoring and managing ICP which are appropriate to the pathophysiological processes of TBM are required [112]. The benefits of ICP monitoring in meningitis have, however, been demonstrated in patients with bacterial meningitis [113]. HCP occurs in as many as 80% of patients with TBM [45,47,49,64,114-118] and can be more common and more severe in children [24,118]. It may evolve rapidly or progress slowly; however, when the

compensatory capacity for increased CSF volume is reached, even small additional increases in volume will lead to precipitous increases in ICP. Raised ICP compromises cerebral blood flow (CBF) and increases the risk of ischemia, left untreated raised ICP can result in brain herniation and death [112]. Raised ICP and HCP are consequently significantly associated with poor outcome, but can be relatively effectively treated if appropriately diagnosed. HCP in TBM is unusual as it is of two forms; communicating HCP (CHCP) if the obstruction to CSF flow by the exudate occurs in the subarachnoid space, mostly at the level of basal cisterns, and non-communicating HCP (NCHCP) when the obstruction occurs at the outlet foramina of the 4th ventricle at the cerebral aqueduct, caused by the circumferential compression of meningeal exudates, mesencephalic oedema, intraventricular exudate or the presence of a tuberculoma [22,112]. Occasionally exudate occludes the foramen of Monro, which leads from the lateral ventricles to the third ventricle. Although NCHCP occurs less frequently (14-25%) [45,64,101] determining the nature of the HCP has important implications for the appropriate management of ICP. Accurately differentiating between the two forms of HCP is not usually possible by examining brain imaging. Red Cross War Memorial Children's Hospital (RXH) and TBH follow a protocol of investigating the communicating nature of HCP by air encephalography (AEG) and/or column tests [64,101,112,119,120]. As there have been no controlled trials to validate methods of treating HCP considerable variability across centres exists, with some centres advocating shunts in all patients with hydrocephalus [80,121-125]. Paediatric studies report that up to 70% of TBM patients receive neurosurgical intervention for HCP [48,49,75].

CHCP is either treated medically [22,45,112,126], or using venticuloperitoneal shunts (VPS) determined by the severity of HCP regardless of HCP communication on the assumption that early CSF diversion decreases the risk of brain damage and improves outcome [47,127]. Medical treatment proposes the use of diuretics acetazolamide (50-100mg/kg/day) and furosemide (1mg/kg/day) in combination with serial LPs. The frequency of LPs is variable across different centres ranging from daily to weekly [22,112]. It has been argued that weekly LPs are unable to assess the trend in ICP between LPs and may miss ongoing raised ICP, whereas daily LPs allow more regular ICP monitoring and reduction, as the ICP returns to baseline within 12-24 hours after an LP [112]. If by 3-4 weeks ICP has not normalised as determined by an elevated LP opening pressure, clinical deterioration or progressive HCP on computed tomography (CT) brain scan, a VPS is inserted for these patients who have failed medical treatment [22,112]. Medical treatment for CHCP is successful in 75-82% of patients [22,45] and patients who are shunted due to failed medical treatment may suffer poorer outcomes [22]. In cases where patients demonstrate severe HCP and a very depressed level of consciousness an external ventricular drain (EVD) may be used as a temporising measure until the ICP comes sufficiently under control and medical treatment can take over [112].

NCHCP is always treated surgically as an LP in this context would create a pressure gradient across the foramen magnum and result in hindbrain herniation (coning) [112]. Surgical options include a VPS which is generally a lifelong device and can be prone to repetitive complications in nearly one third of patients [22,127]. These include shunt infections reported in 13-15% of patients [22,127] and shunt blockage in 13-40% of cases [22,127] often due to the high protein CSF content [128]. Other complications may include over draining and wound breakdown [22]. Multiple shunt revisions are required in almost 20% of patients [127]. Variation in complication rates is a reflection of surgical expertise, shunts used, and patient variables including age, nutritional status, concomitant skin infections and immune compromise [22]. Outcomes in shunted patients are subject to the severity of disease and ischemic processes [121,122].

The alternative is an endoscopic third ventriculostomy (ETV), a procedure first described in the context of TBM [120]. This minimally invasive procedure involves making a stoma in the floor of the third ventricle allowing communication between the ventricles and the SAS and bypassing the blockage of the ventricular system. ETVs may be challenging due to the presence of proteinacious exudate in the ventricles which mars visibility and renders the floor of the third ventricle thick and difficult to perforate. Unrecognisable anatomy of the third ventricle floor may occur in 20-30% of patients and consequently the rate of procedural abandonment is high [127,129,130]. The long term success of ETVs may be compromised by exudate blockage in the SAS which may prevent egress and reabsorption of CSF over the cortical surface, and predispose closure of the stoma in the inflamed ventricular floor [127,130,131]. ETV success rates for NCHCP in terms of technical completion are reported to be approximately 60% [129]. Medical treatment is recommended after an ETV as cisternal obstruction and CSF malabsorption may still occur and the HCP has not been cured but converted from NCHCP to CHCP [112,132]. ETVs are also performed in patients with CHCP [133,134]. In these cases ETVs performed later in the disease course, even in chronic and burnt out cases, are reported to have higher success than those performed during the early acute phase [131,133,135]. Due to the potential complications of operative procedures for HCP coupled with the high rate of success with medical treatment for CHCP some centres recommend avoiding ETVs and VPS unless absolutely required [130].

Tuberculomas and TB abscesses may manifest with signs of raised ICP depending on their location [33,35]. Neurosurgical resection is only required for tuberculomas with mass effect [49] or intramedullary lesions causing progressive neurological deficit [136]. The role of steroids in the treatment of tuberculomas is inconclusive: dexamethasone did not alter their evolution in adults [114] but a trial on corticosteroids in children demonstrated enhanced tuberculoma resolution and reduced incidence of delayed tuberculoma development [101]. It is suggested that the nature of the necrotic centre, whether caseous or coagulative, may influence the varying degree to which these lesions respond to treatment

[137]. Abscesses often present acutely, they may respond poorly to standard medical treatment and can present months after the commencement of TBM management [138]. A paediatric case series suggested clinical and radiological improvement with adjunctive use of thalidomide [34]. In cases of neurological deterioration a combination of medical and neurosurgical treatment may be preferable, this may involve stereotactic aspiration of the pus or surgical excision and drainage of the lesion depending on the abscess location with brain stem lesions posing the greatest risks [24,33,35,138].

Hyponatraemia

Hyponatraemia is a common finding in TBM occurring in 60 -90% of patients [112,139] it is associated with poorer outcome [140] and may exacerbate cerebral oedema [112]. Perturbations in sodium levels may be the result of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) or cerebral salt wasting (CSW). Elucidating the cause is important as the two syndromes require different treatment. SIADH is characterised by excessive release of antidiuretic hormone (ADH) which leads to increased water absorption in the renal tubules leaving patients euvolemic or fluid overloaded. Total body sodium levels are unaltered but fluid levels are increased. Treatment requires fluid restriction to counter inappropriate water retention [112,141]. Inappropriate release of ADH may be due to ischemic or inflammatory damage to the hypothalamus [112]. CSW involves increased secretion of atrial natriuretic peptide (ANP) which leads to increased loss of urine sodium and subsequent extracellular fluid loss due to the renal fluid loss. When urine loss outweighs fluid loss hyponatremia ensues and total body sodium is reduced [141,142]. Treatment in this case requires salt and fluid replacement rather than fluid restriction which would exacerbate an already depleted intravascular volume in patients at risk of cerebral ischemia [112]. It is therefore important to correctly ascertain the cause for hyponatremia; however, this is challenging and necessitates an accurate assessment of intravascular volume. Furthermore, CSW may result in secondary compensatory secretion of ADH [112]. Both SIADH and CSW have been reported in TBM [139,143] yet uncertainty about the prevalence and diagnosis of each condition remains and it is recommended that fluid restriction be avoided and cautious treatment with hypertonic saline and slow correction of sodium levels be pursued [112].

Outcome

Mortality rates range from 3.8 - 43% in children [46-49,75] and 17 - 79% in adults with higher mortality seen in HIV+ cohorts [50-52,71,72]. MRC TBM stage demonstrates a significant relationship with mortality and morbidity with a high rate of death and disability seen with Stage II (≈ 30%) and III (≈ 80%) and little to no neurological morbidity seen with Stage I [22,45,47,49,50,70,72]. Mortality may be higher

in younger children [48], in HIV+ individuals with lower CD4 counts [50] and may commonly occur during the acute phase of disease [48-50,72].

Good outcomes are reported in 16 - 50 % of patients based on the severity of disease on admission and co-infection with HIV [45,47,52,71,72]. The majority of patients are left with residual neurological deficit including visual impairment [45,47,144], motor deficits in 28 – 44 % [45,47] hearing loss in 16% [45] and cognitive deficits (75%). Motor and cognitive morbidities are as a result of cerebral infarction [145] and optochiasmatic arachnoiditis and optochiasmal tuberculomas give rise to visual deficits [144].

Predictors of outcome are numerous and varied across studies and include a delayed initiation of treatment, old age, low admission GCS, race, TBM stage, convulsions, headaches, motor function, brainstem dysfunction, CD4 count, basal exudate, cerebral infarction and the presence of comorbidities on multivariate analysis [45,47,52,70,72,146]. Poorer outcomes have been reported in patients with confirmed TBM in some [4,70] but not all studies [50].

PAEDIATRIC TBM IN THE WESTERN CAPE

Three retrospective studies examining the profile of paediatric meningitis at one of the 2 major tertiary institutions in the Western Cape have revealed that over the period from 1981 to 2009 the incidence of TBM has continued to rise and this disease now accounts for the majority of cases of bacterial meningitis [54,55,147]. Prior to 1995 TBM was not yet the forerunning causative agent, but rates have doubled from 10% to 22% of all paediatric meningitis cases in the recent decade [54]. The increased presence of TBM may in part be attributed to the rising incidence of pulmonary TB throughout this province from 689 cases/100 000 population in 1997 to 1000 cases/100 000 population in 2012 [44]; this augments the risk of primary infection in children vulnerable to CNS dissemination. Another contributing factor may be the increasing national HIV seropositivity from approximately 4.5% in 1995 to 10.3% total population in 2009 [148]. Recently published studies have found relatively low rates of 3.8 - 14 % HIV co-infection in paediatric TBM cohorts [14,45,46,54]; however, studies on children admitted for long-term hospitalisation due to severe TB or poor social circumstances have found an increase in HIV rates between 1998 and 2001 [94,149]. Notably, the frequency of HIV-testing is variable with some studies reporting testing in only 40-80% of cases [14,45,46,54]. It is possible, therefore, that the rate of HIV infection in children with TBM may be underestimated, and the opportunity to commence appropriate antiretroviral treatment may be missed [54]. The prevalence of HIV in the Western Cape is, however, the lowest nationally [4].

Incidence rates of TBM in the Western Cape in 2006 were estimated to be 31.5 per 100 000 in children <1 year and 0.7 per 100 000 in children from 10 – 14 years [45]. Studies spanning from 1985 to the present day demonstrate that the majority of cases continue to occur in children under the age of 5 years and of indigenous African ancestry or mixed ancestry [45,54,150]. The vast majority of these children stem from impoverished families who live in overcrowded dwellings lacking in basic amenities [56,150]. Contact with adult pulmonary TB source cases is reported in almost 60% of children with pulmonary and extra-pulmonary TB and drug susceptibility patterns mirror those of adult contacts [94]. Drug susceptibility testing is limited to culture positive CSF samples and has not always been routinely performed. Current estimates of overall childhood drug resistance at Tygerberg Hospital suggest 12% prevalence [45]. In a selective cohort of children with severe TB admitted to Brooklyn Chest Hospital (BCH) 19% of cases (pTB and TBM) had confirmed drug resistance with an almost even spread between mono-INH and multi-drug resistant TB. A history of previous TB treatment was recorded in 22% of the study cohort; only half of these patients had been compliant, and 15% of defaulters had drug resistance suggesting acquired drug resistance [94]. This finding parallels national results on MDR TB which confirm the significant association between previous

treatment and drug resistance [151]. According to the WHO 2012 Global TB Report South Africa recorded only 77% treatment success rate and is one of the three countries with increasing rates of MDR which occurs in 1.8% of new cases and 6.7% of retreatment cases. Poor treatment completion is likely to contribute to future increases in MDR TB and TBM prevalence in adults and children, who are as likely as adults to develop MDR [44].

Mortality rates reported for TBM in the last 5 years have been promisingly low (3.8-12%) [45,46] and this may be attributed to the better adherence to drug regimens, active treatment of HCP and raised ICP and a low rate of HIV co-infection and multi-drug resistant TB [45,46]. The morbidity rates, however, remain high with only 16-20% of children returning to their pre-TBM baseline [45,46]. Motor deficits, cognitive impairment and neurodevelopmental delay are among the commonest morbidities and almost 20% of children are left severely disabled [45,145].

Although focussed care may be provided during the acute in-hospital period, in a resource limited country like South Africa access to adequate rehabilitation, outpatient follow-up and special schooling is poor. A study on the long term outcomes of children with TBM demonstrated that less than half of post-TBM children with moderate to severe sequelae had access to specialist follow-up and the majority of patients in rural settings relied on private general practitioners due to poor accessibility to state hospital services [56]. The majority of children attended mainstream schooling and more than half of these school-going children had failed at least once. The acute and long-term repercussions of TBM afflict not only the child, but also their family. Up to 35% of mothers cease to work in order to care for disabled children and those who maintain employment lose income for days spent taking their children for medical treatment. Family earnings are further compromised by additional expenses incurred directly due to the illness and its consequences. Sadly, most children presenting with TBM come from impoverished households to begin with and there is little capacity for the additional burdens this disease poses [56]. Over 15% of paediatric TB admissions for long-term hospitalisation are due to poor social circumstances where parents are unable to care for their children or comply with treatment [94]. Poor access to clinics, difficulty finding supervision for other children and fear of losing income make regular clinic attendance challenging and contribute to poor compliance, potential relapse and acquired drug resistance [94]. The cost to the country in the form of medical expenses for acute and outpatient care, welfare grants as well as the loss of economic productivity due to disability is another consideration.

CHAPTER 3: RADIOLOGICAL FEATURES OF TUBERCULOUS MENINGITIS

Due to the limitations inherent in CSF TB culture, the decision to initiate treatment rests on the presumptive diagnosis, which is based on a combination of history, clinical examination, CSF chemistry and radiography. Due to the risk of transtentorial herniation associated with lumbar punctures in patients with non-communicating HCP, imaging prior to a lumbar puncture is clinically prudent and may aid in safe patient management as well as diagnosis [112]. The value of computed tomography (CT) brain scans and magnetic resonance imaging (MRI) in the diagnosis of TBM is well recognised [49,64,69,152,153] and a recent consensus document includes radiographic features of TBM as a key factor in the presumptive diagnosis [69]. Several radiographic studies of TBM have identified features highly suggestive of the disease on CTB and MR imaging, including hydrocephalus (HCP), periventricular lucency, basal and leptomeningeal enhancement, infarcts, and tuberculomas. The presence of HCP, enhancement and infarcts is considered a diagnostic triad in this disease [152]. The role of vasculitis in TBM has led to angiographic studies which detail pathology in the major vessels of the Circle of Willis and deep perforators that arise from these vessels. Dissemination of TB into the spinal canal also occurs in association with TBM and commonly reported radiological features include spinal arachnoiditis and tuberculomas. Radiological follow up in the weeks and months after the initiation of treatment provides valuable information about the resolution or progression of this disease and the development of new complications that may require intervention.

The definitions and severity of radiological features in TBM vary across studies and currently no uniform guidelines exist. Some groups have adopted detailed objective criteria while others have relied on the experience of the reviewing radiologists. Additionally, some groups have access to more sophisticated imaging technologies than others. While this makes the generalizability of specifics challenging across studies, the overwhelming presence of classic radiological features allows for an adequate overview of TBM radiology combining the available literature. Although this project focuses specifically on paediatric TBM, much of the literature on the radiology of TBM reports on combined adult and paediatric cohorts. Consequently, this review chapter covers both adult and paediatric data with an emphasis on children where possible. Table 3.1 outlines the frequency of key radiological features across studies discussed in this review.

CT and MRI

CT scans provide valuable information in TBM and are widely used in TBM diagnosis and in planning acute HCP management. In most centres it remains the first imaging modality in the clinical

management of patients. MRI has increasingly become part of routine care in many centres internationally for several reasons. Unlike CT, MRI does not involve radiation, and there is a growing body of literature that has raised concerns about the long term risk of inducing cancer and cognitive deficits after exposure to excessive CT-radiation in children [154,155]. A study comparing CT and MRI scans in paediatric TBM found that MRI is more sensitive in detecting the number, location and temporal resolution of infarcts with DWI demonstrating the greatest sensitivity for acute infarcts [156]. MRI also better detects enhancement involving the basal cisterns, leptomeninges and cranial nerves due to better contrast resolution, multiplanar imaging and the lack of bony artifact. More tuberculomas are also detected [156,157]. Specialised MR sequences including fluid-attenuated inversion-recovery (FLAIR), diffusion weighted imaging (DWI) and apparent diffusion coefficient (ADC) mapping add further value. For these reasons, MRI is the preferred imaging technique in these patients; however, limited access to MR technology as well as the need for sedation or anaesthesia in children limits access to this imaging modality. Therefore, CT scans remain an important tool, especially in resource-limited settings where TBM occurs most frequently. It performs equally well in detecting HCP which requires the most urgent attention in acute TBM management [156].

RADIOLOGICAL FEATURES

Hydrocephalus (HCP)

HCP occurs in as many as 80% of patients with TBM [45,47,49,64,114-118] and can be more common and more severe in children [24,118]. HCP is of two forms; communicating HCP (CHCP) if the obstruction to CSF flow by the exudate occurs in the subarachnoid space, mostly at the level of basal cisterns, and non-communicating HCP (NCHCP) when the obstruction occurs at the outlet foramina of the 4th ventricle at the cerebral aqueduct, caused by the circumferential compression of meningeal exudates, mesencephalic oedema, intraventricular exudate or the presence of a tuberculoma [22,112]. Occasionally exudate occludes the foramen of Monro, which leads from the lateral ventricles to the third ventricle. Although NCHCP occurs less frequently (12-25%) [45,64,158], determining the nature of the HCP has important implications for the appropriate management of intracranial pressure (ICP): lumbar punctures (LPs) in the context of NCHCP can create a pressure gradient across the foramen magnum and result in hindbrain herniation (coning) [112]. These patients cannot be treated medically with serial LPs and require neurosurgical intervention. Accurately differentiating between the two forms of HCP is not usually possible by examining brain imaging and requires further investigations including an air encephalogram or column test [112,120,159] and the ICP cannot be estimated based on ventricular size in childhood TBM [64]. Periventricular lucency indicates interstitial oedema as a result of the transependymal movement of CSF in response to increased intraventricular pressure [24], and is

considered a marker of acute HCP. It occurs commonly in TBM [160], usually in association with severe HCP [118]. Several definitions of HCP focus primarily on ventricular dilatation [114,160], others have incorporated periventricular lucency and surface sulcal effacement [64] as well as clinical signs of raised ICP [161], allowing for a broader characterisation of HCP severity.

Evolution

HCP in TBM is characterised by its progressive nature [24]. At admission it may range from mild to severe [47,118] and is more commonly seen in patients with a long duration of symptoms before the onset of treatment [118,160,161]. Medical treatment for CHCP has been shown to resolve HCP in approximately two thirds of cases but more than one third of patients may fail medical treatment and require ventriculoperitoneal shunting (VPS) by 1 month post-admission [22,64,114,152]. HCP may persist or develop where there was no baseline HCP in up to 20% of patients for 6 months after treatment commencement [160,161]. This highlights the importance of follow-up imaging to identify patients in need of surgical intervention to control HCP and raised intracranial pressure. Complete HCP resolution may take from 3-6 months or longer [160,161] and steroids do not significantly improve HCP in adults or children [101,114].

Associations

Markers of inflammation have shown mixed associations with HCP. In a study by Thwaites et al CSF protein, interferon-γ (IFN-γ), interleukin-6 (IL-6) and interleukin-10 (IL-10) concentrations were significantly associated with HCP after 60 days [114]. However, in a study by Misra et al their panel of cytokines did not demonstrate a relationship with HCP at baseline or after 3 months, although the sample size of this study was small [162]. HCP is associated with a longer duration of illness and advanced TBM stage (according to the British Medical Research Council [BMC][76]) [118,160,161]. It contributes to a depressed level of consciousness due to raised ICP and is significantly associated with focal neurology and visual impairment [160]. Unresolved HCP and raised ICP are also important causes of seizures in paediatric TBM [163]. Radiologically it is associated with the presence of basal exudate, infarcts and tuberculomas [160].

Prognostic marker

The association between HCP and outcome is mixed; several studies have concluded that no association exists [47,64,158]. In part this may be explained by the varying descriptions of the severity of hydrocephalus, the difficulty in predicting ICP from ventricular size, and variable approaches in treating hydrocephalus. However, two studies focused specifically on HCP have found that patients with HCP on admission had a significantly higher incidence of death and severe disability [160,161]. In

both these studies patients with HCP relative to those without HCP were characterised by a longer delay in the initiation of treatment and more severe disease including a greater number of infarcts. Neither study stratified outcomes as a function of the severity of HCP at admission or during the disease course. In a paediatric study covering 20 years of TBM admissions, HCP and periventricular lucency were associated with outcome in univariate but not multivariate analyses [45]. In another study spanning 10 years, mortality was significantly higher in those patients for whom HCP was not successfully treated [164]. The delay in presentation, severity of HCP, degree of success in HCP treatment [22] and the severity of illness including the concomitant presence of infarcts [165] likely all have some bearing on this relationship.

Enhancement

Enhancement is present in up to 93% of admission scans and may be visible in the basal cisterns, the Sylvian fissure, the tentorium, around the optic chiasm, over the cerebral convexity, and (with extension of disease into the ventricular system) in the ependyma and choroid plexus [24,45,114,115,117,118,158,166,167]. Occasionally it affects the cerebellar sulci and cranial nerves [166]. Enhancement is likely due to meningeal inflammation and the presence of an enhancing gelatinous exudate or granulation tissue in the subarachnoid space [168]. Enhancement is best identified on contrast-enhanced CT and T1-weighted MR scans and the typical pattern is considered one of the most sensitive features of TBM [153]. Pre-contrast hyperdensity in the basal cisterns is a highly specific feature of TBM and is identified as obliterated basal cisterns on non-contrast CT scans [24,153]. To overcome the subjective variability in characterising basal enhancement objective criteria have been proposed by Przybojewski et al: the most useful criteria include enhancement at the suprasellar and middle cerebral artery (MCA) cistern, linear enhancement in the MCA cistern, obliteration of the basal cisterns and asymmetrical enhancement [168]. Enhancement in TBM is generally diffuse but may be focal in less than 10% of cases and involves the Sylvian fissure, cistern of the lateral fossa, ambient and quadrigeminal cisterns [115].

Evolution

The evolution of enhancement is variable; some follow-up scans show improvement or no change but up to 28% of scans may demonstrate new or progressive enhancement after the first month of treatment [64,152,158] usually with resolution by 6 months [64,158]. Early enhancement may be limited to the basal meninges while obliteration of the basal cisterns and extension into the Sylvian and hemispheric fissures and over the cortical surface of the brain may signify progressive disease [153,168]. The presence and progression of enhancement is considered to add confidence to the diagnosis of TBM as this is such a characteristic feature of this illness [152]. The beneficial effect of steroids on enhancement is

uncertain: a significant decrease has been reported in children [101] but no change in the degree or duration of enhancement has been found in adults [114].

Associations and marker of prognosis

Basal enhancement is seen more commonly in children [118] and is associated with a poorer response to medical treatment for raised ICP and more severe stage of TBM [64]. Moderate to severe enhancement has been associated with more basal ganglia infarcts and poorer clinical (motor and visual) and cognitive outcome [47,64,118,158]. It is suggested that basal enhancement may therefore reflect the underlying vasculitis which is the primary cause of poor outcome [64]; however, the relationship between exudate and angiographic abnormalities is variable and may reflect timing in the disease course [116,118,169]. Furthermore, the intensification of enhancement is contended to signify an ongoing inflammatory response [64]; however, a significant association between exudate and inflammatory cytokines and chemokines has also not been demonstrated [114,162]. Cytokine associations with various features of TBM are, however, poor and they may not represent the ideal markers of ongoing inflammation [170-172].

Infarction

Infarcts are reported in 30 to 60% of adult and paediatric patients with TBM, ranging from small lacunar infarcts to global infarction [45,114,116,118,160,167,173-175].

Location

Ischemia resulting in infarcts is the major source of irreversible damage in TBM. Periarteritis of the cerebral vessels surrounded by exudate progresses to panarteritis and vascular occlusion, most commonly affecting the medial branches of the middle cerebral artery (MCA). This results in infarcts in the head of the caudate nucleus, anterior limb of the internal capsule and the anteromedial thalamus – considered the 'tubercular zone' [176] due to the high incidence of infarction in these areas [24,49,64,114,116,118,166,169,173,174,177]. The large vascular distribution territories of the MCA and anterior cerebral artery (ACA) are less frequently affected but complete occlusion leads to grey and white matter infarcts [64,166,169,178]. In both adult and paediatric studies most infarcts occur within the basal ganglia, although extension of infarcts to cortical and watershed areas is also seen. The posterior circulation is thought to be less affected by ischemia ; however, infarcts in the cerebellum and midbrain have been described in adults [166,169,173,174] and have been reported in 37- 47% children with TBM in whom they occur in the cerebral peduncles, pons, brachium ponti and midbrain [117,179]. These lesions are likely also due to exudate coating the basilar artery and its branches and appear to be

associated with more severe clinical presentation and poorer outcome [117,179]. Infarcts may be single or multiple, unilateral or bilateral [169,174,175,178].

DWI detects acute infarcts earlier and estimates infarct size with greater accuracy than conventional T2-weighted MRI. It is suggested that this technique is superior in detecting evolving ischemia within hours after the onset of the ischemic process [167]. It has also been used to detect borderzone necrosis - areas of infarction immediately adjacent to or underlying areas of severe exudate or cisternal and meningeal inflammation [180]. Acute and subacute infarcts appear hyper-intense on DWI and T2-weighted imaging with possible hypointensity on T1-weighted images. They may be associated with peri-lesional oedema or mass effect. Chronic infarcts appear iso- to hypo-intense on DWI and T1-weighted and hyperintense on T2-weighted imaging [167]. Fluid-attenuated inversion-recovery (FLAIR) imaging assists in characterising old infarcts which have a central hypointense area surrounded by a hyperintense rim [24]. On CT old infarcts appear as hypodense lesions. Chronic infarcts are often associated with ipsilateral dilatation of the anterior horn of the lateral ventricle, due to tissue loss [28,118]. Infarcts may undergo hemorrhagic transformation [166,175]; however, this is less common and rarely seen in children. More commonly a pattern of luxury perfusion in the infarcted territory may be seen.

Evolution

Infarcts may be poorly demonstrated on admission scans [114,152], especially on CT; MRI and DWI may be more sensitive in this early phase [152,176]. Infarcts not present on admission imaging are identified on follow- up scans in 22-48% of children and adults, suggesting that the disease process is ongoing despite treatment and that the injury is evolving [64,114,152,152,158,175]. There are little data available on the temporal profile of infarct development in the acute phase when patients are at greatest risk. A paediatric study using CT showed infarct development within one week of the initial scan [152] and a DWI study in adults found that 90% of infarcts within the first 7 days were of the acute/subacute nature [167]. In a study of imaging at 6 weeks, 3 months and 6 months after admission, new infarcts not present on initial imaging were detected on scans at 6 weeks with no further development observed on the 3 and 6 month scans [158] suggesting that patients are at greatest risk during the early weeks of illness. Trials with adjunctive steroids and aspirin in adults and children have not shown a statistically significant reduction in infarct size or number, nor any change in infarct location [101,105,114].

Associations

Infarcts have been associated with other radiological features of TBM including HCP [175] and exudate [166,173,175], although there is some variability across different studies [174,177]. Infarcts have also shown mixed associations with markers of inflammation; significant associations are reported with a reduced CSF/blood glucose ratio, elevated IL-8 and IL-10 concentrations and greater impairment in the blood

brain barrier (BBB) [114]; however, this is not consistent across studies [162]. Among CSF findings only neutrophil levels demonstrate an association with infarction and it is suggested that this may be indicative of occult vascular thrombosis in the early stages of disease [173]. They are associated with hypertension [175], and more severe disease on admission according to BMC staging [167,173-176], and raised intracranial pressure [177]. The association with admission Glasgow Coma Score (GCS) is mixed [174,177]. While infarcts are significantly associated with focal neurology [174,177] and may result in seizures and EEG abnormalities [163], they may in some patients be clinically silent [117,169]. The association with age in adult and paediatric studies is mixed with some evidence supporting an increased risk with age >25 years [174] and other evidence finding no association at all [173,177]. Infarcts and sex are not related in adults or children [173,174,177].

Prognostic marker

Infarcts have a strong association with poor outcome, including mortality, neurological deficits, cognitive impairment, and neurodevelopmental delay [45,47,101,158,167,169,173,175,177,178145]. This relationship may vary based on regional infarct localisation (with poorer outcome seen with subcortical MCA and posterior circulation infarcts [174,175,178] and focal motor deficit correlating well with infarct location [177]), infarct size (with lacunar infarcts correlating with better outcome than large lesions [176]), infarct number (with multiple territory infarcts heralding a worse prognosis [174,178]) and as a function of time (with some patients improving over the long term). Factors other than infarction, such as duration of illness, HCP and raised ICP also have significant roles to play in determining outcome [175]. Infarcts, however, remain an important prognostic indicator in TBM.

Vascular pathology

The vessels of the Circle of Willis traversing the basal cisterns are at greatest risk of injury due to the accumulation of exudate at the base of the brain; the supraclinoid portion of the internal carotid (ICA), the MCA and ACA are commonly affected with the MCA and its lenticulostriate perforators being most compromised, likely due to the high prevalence of exudate in the basal cisterns and Sylvian fissure. Involvement of the posterior circulation, namely the posterior cerebral artery (PCA), cerebellar, basilar and vertebral arteries, occurs less frequently but may occur in conjunction with or independently of pathology in the anterior circulation [116,166,169,177,178]. Vascular abnormalities detected on MR and CT angiograms include paucity of terminal branches, irregular beading and segmental attenuation of major vessels, complete vascular occlusion and very rarely aneurysms [167,176,177]. This is as a consequence of the inflammatory exudate which subjects the arteries to evolving stages of arteritis of the vessel wall (infiltrative, proliferative and necrotising) followed by fibrinoid necrosis of the vascular endothelium and secondary thrombus formation or aneurysmal dilatation [176,177]. Infarcts are considered a result of cerebral hypoperfusion due to a variable combination of vasospasm,

proliferation of the vessel intima and thrombosis [176]. HCP may further compromise vascular integrity by precipitating stretching of already compromised vessels [116,169,176,181]. Abnormalities on MR and CT angiography are seen in 46-70% of TBM patients [116,166,169,182].

Evolution

There is little available literature on follow up angiograms. A study by Sing et al demonstrated that angiographic abnormalities persisted for longer than 6 months in 82% of patients who had baseline vascular pathology and new abnormalities developed in 20% of patients who had normal baseline angiograms [116].

Associations

Evidence of vascular pathology is present on the angiograms of 70-90% of TBM patients with infarcts [169,182]. Therefore, approximately 10-30% of these patients with infarcts do not demonstrate vascular abnormality; this may due to limitations of angiography or highlights that other factors are also responsible for brain tissue injury, these are discussed in Chapter 4. Additionally, up to one third of abnormal angiograms are not associated with infarction. It has been suggested that vessel narrowing in these patients may be related to vasospasm early in the pathophysiology of the disease [118]. Timely intervention to treat the vascular pathology in this group of patients could prevent the development of infarcts [166,169,182]. However, although MR and CT angiography offer a non-invasive means of assessing vascular abnormalities they are not routinely performed in TBM. Furthermore, how these angiographically-detected pathologies should be treated is uncertain.

MRA abnormalities are also associated with HCP [116,169,182], visual impairment [116], hemiparesis [116,181] and leptomeningeal enhancement [116]. Variable associations have been demonstrated with exudate [116,118,169]. Early vasospasm may not be associated with exudate but more progressive disease often involves increased accumulation of exudate and vasculitis [118]. Associations with BMC stage have also been mixed [116,169,182]; small vessels may be involved during the early phase of disease and larger vessels in later phases, although this requires further investigation [169]. This variability may be due to patient heterogeneity and the diversity of factors which could precipitate vascular injury [169]. In largely adult studies no associations have been demonstrated with age or sex, nor with admission GCS, CSF characteristics or duration of illness [116,169,181]. Angiographic abnormalities have been reported more frequently in patients who suffer a poor outcome at 6 months, but this did not reached statistical significance [116,169].

Prognostic marker

Angiographic abnormalities have not shown a significant relationship with outcome. The fact that not all infarcts are associated with angiographic abnormality and not all angiographic abnormalities are related to infarcts suggests that there are other factors responsible for infarction and an isolated angiographic abnormality is not necessarily indicative of a bad outcome. Where disturbed angiography is associated with poor outcome like motor impairment or focal neurology this is probably mediated by infarction. Neither infarcts nor vascular pathology on angiography are associated with admission GCS; this may be due to their evolving nature whereas a depressed level of consciousness on admission may more likely be a consequence of more immediate factors like raised ICP and electrolyte imbalance.

Parenchymal Tuberculomas

Cerebral tuberculomas are the most common extra-pulmonary tuberculous lesion that present clinically [183]. These may account for the genesis of TBM through the rupture of the Rich's focus. These intracranial granulomas account for a large portion of intracranial non-neoplastic lesions in TB endemic areas and sometimes can be difficult to discern from brain tumours [184].

Location

Tuberculomas present as single or more frequently as multiple lesions of varying sizes [163,185]. They are reported to most commonly arise supratentorially in adults and infratentorially in children [163,185] and may occur in the brain parenchyma, ventricular ependyma and frequently in association with areas of meningeal inflammation including the region of the basal cisterns, surrounding the vessels of the Circle of Willis and in the Sylvian fissures [137]. A paediatric study found that all parenchymal tuberculomas present on admission were associated with a miliary TB pattern on chest x-ray which suggests seeding to the central nervous system, while the vascular distribution of tuberculomas supported hematogenous spread of TB bacilli to the brain [137]. Their radiological appearance differs depending on whether they are solid, noncaseating or caseating with a solid or liquefied centre. Non-caseating lesions appear hypo/isodense and enhance homogenously with contrast on CT and T1-weighted imaging, and hyperintense on T2-weighted images. Solid, caseating tuberculomas are usually iso- to hypointense on CT and T1-weighted images, identified by ring enhancement with contrast or gadolinium MR and appear iso/hypointense on T2. Liquified centres demonstrate marked ring enhancement on contrasted CT and T1-weighted images and are hyperintense on T2-weighted images. Differentiating these tuberculomas from abscesses of tuberculous or pyogenic origin can be challenging, DWI has shown promise in differentiating between cerebral abscesses and tuberculomas [42]. Tuberculomas may be associated with surrounding oedema [24,136,184].

Evolution

The presence of tuberculomas on admission scans varies from 11-64% of adults and children with TBM [45,49,64,101,114,137,158,160,185]. These may resolve within the first month of treatment [64] but tuberculomas are commonly observed several weeks and up to 6 months after the initiation of treatment, during which time established tuberculomas may enlarge and new tuberculomas develop [49,114,137,183,185]. These have been termed as 'paradoxical' tuberculomas and described as a phenomenon which may occur as a result of antimicrobial destruction of the mycobacteria and the consequent release of bacillary debris which precipitates a hypersensitivity reaction and an excessive ongoing inflammatory response as part of immune reconstitution[24,183]. They usually do not present symptomatically except in the rare circumstance where their size and location may precipitate CSF obstruction, localising signs or seizures, and are consequently often incidentally discovered during follow-up imaging [64,114,137,163]. Tuberculomas may take 6 months [64] or longer to resolve [137,158,185].

Associations

Tuberculomas have been associated with IL-6 and IL-10 concentrations after 60 -90 days of treatment which may indicate enhanced immune reactivity [114,185]. An association with elevations in CSF white cell count and protein has been reported but findings are mixed [162,183]. Tuberculomas may precipitate seizures and abnormalities on EEG [163,185] and are associated with focal deficits [185]. An association with the presence [185] and persistence of basal enhancement has been observed, suggesting that meningeal tuberculomas appear to develop in areas of meningeal inflammatory intensity [137]. No association with infarction has been demonstrated [177].

Prognostic marker

Generally tuberculomas resolve without many significant residual signs on CT and little bearing on mortality after 6-9 months [47,64,185]. An association with cognitive impairment and tuberculomas at 6 months has been reported [158] and disability may be significantly worse when they are located in the frontal and temporal lobes, the brainstem or optic chiasm [185].

Parenchymal TB abscesses

TB abscesses are rare complications of CNS TB. They are radiologically very similar to caseating tuberculomas although they may be larger with thinner walls, tend to be singular, and follow a more rapid disease course. They often present acutely with symptoms of fever, headache and neurological signs and may require urgent neurosurgical intervention [24,138].

A summary of intracranial radiological features reported in the literature is provided in Table 3.1 at the end of this chapter.

Spinal TB associated with TBM

The literature on intradural spinal disease associated with TBM is sparse. Studies comprise predominantly case reports or small series of less than 10 patients and no data on spinal disease in paediatric TBM has been published [186]. What data exists suggests spinal involvement accompanying TBM in 18-24% of cases [40,187]. However, most studies have focused on patients presenting with neurological deficits and investigation of asymptomatic TBM patients has not been widely conducted although spinal disease may be clinically silent. The prevalence of spinal disease in TBM patients may therefore be under appreciated. Spinal arachnoiditis is optimally identified on MRI with gadolinium as nodular, thick or linear enhancement with obliteration of the subarachnoid space, loss of the cord outline and clumping of nerve roots in the lumbar region [40]. Spinal lesions, like intracranial tuberculomas, typically appear isointense on T1 and iso- to hypointense on T2 MRI images, depending on the stage of tuberculoma formation, and frequently occur in the thoracic region [42,186].

Evolution and prognosis

Intradural spinal disease may develop weeks to years after the onset of TBM. Analogous to the appearance of 'paradoxical' intracranial tuberculomas, expansion of TBM to the spinal compartment may occur weeks after antimicrobial and steroid commencement and it is suggested that this too may be the result of a strengthened immune response and increased antigen release following treatment [186]. Spinal arachnoiditis is often asymptomatic, possibly because the exudate does not always disturb cord and nerve root function [40]. However, in some cases more severe spinal disease may progress to motor and sensory deficits: mass lesions may cause spinal cord compression [42], chronic arachnoiditis may result in axonal damage, peripheral nerve injury and neuropathies, and vasculitis may cause infarction [188]. Response to treatment in these cases is often poor [38,40]. Syringomyelia can develop as a consequence of focal scarring in the subarachnoid space which inhibits CSF flow and forces CSF into the central canal. Focal cystic dilatations may amalgamate to form a CSF-filled syrinx [24,189]. These patients may present with limb weakness and sphincter dysfunction [189]. The benefit of steroids for intradural spinal disease remains inconclusive and surgical intervention is only recommended under circumstances of clinical deterioration [42,186]

Chest X-ray

Chest x-rays are suggestive of TB in 29-72% of patients with TBM [45,49,67,161,163,187,190] and demonstrate mediastinal lymphadenopathy, consolidation, collapse, cavitation and pleural effusion [45,49]. A miliary pattern has been reported in 12-60% of TBM patients [4,45,49,187,191] and occurs more commonly in young children less than 2 years of age [4].

Effect of HIV status

Studies in adults and children comparing the CT and MRI features of TBM patients based on their HIV status have found that basal enhancement, non-communicating HCP, and parenchymal tuberculomas occur more frequently in HIV-uninfected (HIV-) patients [192-194]. HIV-infected (HIV+) patients demonstrated little exudate formation after the initiation of anti-TB medication and a greater frequency of ventricular dilatation secondary to cerebral atrophy, [192-194]. The paucity of basal enhancement and parenchymal tuberculomas on imaging has been confirmed in post-mortem brain studies, and it has been suggested that the relative absence of these features in HIV+ individuals may represent an impaired granulomatous inflammatory response as a result of immune suppression [193,195]. However, even in the context of no immune suppression (age-appropriate CD4 count) in HIV infection, basal enhancement may still be absent suggesting that other factors may exist [194]. The influence of HIV status on the number of infarcts remains unclear [193-196]. HIV presents the additional challenge of immune reconstitution syndrome (IRIS) whereby the recovery of the immune system after anti-retroviral treatment initiation results in paradoxical worsening of TB. Neuroradiological manifestations include tuberculomas, radiculomyeltits, and spondylitis; however, sample sizes studied have been too small to identify definitive radiological markers of TBM-IRIS [197].

Summary

Radiological features of TBM including HCP, basal enhancement, infarcts and tuberculomas occur commonly and aid in swifter diagnosis and initiation of appropriate management strategies. They also serve as indicators of injury severity. Common admission features include acute HCP with peri-ventricular lucency and basal meningeal enhancement on contrasted scans. Imaging findings illustrate the evolving and chronic nature of TBM with baseline pathology persisting over time accompanied by the evolution of new signs of disease. Follow up scans within the weeks and months following admission are required by the frequent occurrence of new pathology and evolution of existing pathology. Even in the face of clinical improvement radiological pathology may persist. The value of radiology is, however, limited by temporal resolution and usually demonstrates parenchymal injury once it is already irreversible.

Variability in the prevalence and severity of radiological features of TBM across the literature is a function of several factors: firstly, currently no widely accepted review criteria exist; secondly, the progressive nature of this disease and variable timing of imaging relative to the onset of disease makes comparisons across patients challenging; and thirdly, access to imaging techniques like MRI is limited and direct comparisons between CT and MRI findings are limited. While MR technologies offer greater sensitivity and specificity in recognising and quantifying the features of TBM, CT scans remain a valuable tool in the diagnosis and long term management of these patients, especially in

resource-limited settings. Similarly, the large variability in the prevalence of radiological features within adult and paediatric cohorts makes it difficult to highlight particular radiological differences between these two patient groups. Perhaps the most striking difference in contexts where HIV and TB are endemic is the substantially lower HIV infection rate in children in whom less radiological variability is seen as a result of differing levels of immune suppression and reconstitution.

Potential radiological sequelae of TBM include encephalomalcia, syringomyelia and syringobulbia. It is challenging to identify the prognostic significance of individual radiological features as each feature is the result of the same disease process and may be temporally and causally linked to other features.

Table 3.1: Frequency of radiological features of TBM

Authors	N	Scan	FU	Hydrocephalus			Basal meningeal enhancement			Infarcts			Tuberculoma		
				Adm	FU	Total	Adm	FU	Total	Adm	FU	Total	Adm	FU	Total
Paediatric cohort															
Schoeman et al (1988)	27	MRI	x			100			100			74			11
Leiguarda et al (1988)*	65	CT	x			89			69			38			28
Schoeman et al (1994)	198	CT	Variable	83	x		75	28[A]		31	22		11	2	13
Patwari et al (1996)	136	CT	x			32						13			27
Andronikou et al (2004)*	37	CT	x			68			89			62			13.5
Farinha et al (2000)	33	33	Not specified	94	x	94	93	x	93	33	17	50	15	18[†]	x
Paganini et al (2000)*	40	CT	x			78			25			5			x
Ravenscroft et al (2001)*	202	CT													17
Andronikou et al (2005) [†]	50	CT	1 week	x	25		88	10		60	40				
			1 month	x	28		88	20		60	48				
Theron et al (2006)*	130	CT	x			73			x			x			x
Przybojewski et al (2006)	34														
Andronikou et al (2006)*	118	CT	x									57			
Van Well et al (2009)*	553	CT	x			70			75			32			13
Van der Merwe et al (2009)*	30	MRI	x									47[B]			
Pienaar et al (2009)*	30	CT	x			77			70			70			17
		MRI	x			77			97			83			40
Ramzan et al (2013)*	65	CT	x			72			60			29			9
Mixed cohort															
Bhargava et al (1982)	60	CT	x			83			82			28			10
Kinglsey (1987)	25	CT	Variable	72	12	84	64	32[†C]	x	12	20[D]	32	16	12[E]	28
Gupta et al (1994)	26	MRI	x	73			85			54			65		
Ranjan et al (2003) [†]	31	CT	6 weeks	48	26		48	23		32	19		42	10	
			3 months	48	16		48	45		32	19		42	6	
			6 months	48	3		48	-23		32	19		42	-13	
Ahmadinejad et al (2004)	96	CT	x	80											
Kalita et al (2009)	122	MRI	x	39			26			45			49		
Adult cohort															
Chan et al	31	CT/MRI	3 months	29	3		26	x		29	x		10	x	

(2003)															
Koh et al (2007)	43	CT/MRI	x							21[F]					
Thwaites et al (2007)	43	MRI	60 days	77	-10		82[G]	x		9	32		64	10	
Shukla et al (2008)*	30	MRI/DWI	x			47			90			57			73
Anuradha et al (2010)	100	MRI	Not specified	31			94			27	3	30	38		
Anuradha et al (2011)	110	MRI	X	31			88			25			39		
Singh et al (2012)	47	CT		77			96[H] 62[I]			41			17		
Raut et al (2013)	80	MR	6 months	65	28[†]		93[H] 53[I]	x		33	x		39	x	

Adm = admission scan, FU = follow-up scan, Total = combination of admission and FU scans where features on FU were new and not present on the admission scan. Data presented as percentages rounded up to the nearest whole number, follow-up percentages calculated based on the number of patients with follow up scans. Adults defined as > 13 years.

* Timing of scans not specified, [†] Follow up percentages reported as a combination of deterioration of baseline features and new feature development and are therefore a representation of change over time. Negative values indicate improvement

[A] Only represents deterioration of baseline enhancement, no new enhancement reported, [B] Brainstem infarcts, [C] Follow up 1-96 months, [D] Follow up at 2 weeks, [E] Follow up 5-7 months, [F] Within first week post-admission, [G] includes enhancement of basal meninges, Sylvian fissure, convexity, posterior fossa and ventricles, [H] Refers to meningeal enhancement, [I] refers to basal enhancement

CHAPTER 4: ISCHAEMIA AND BRAIN OXYGENATION IN TBM

Ischaemia plays a pivotal role in the poor outcomes associated with TBM. Infarction is common in these patients and a strong prognostic indicator for poor outcomes including disability and death [45,47,101,145,158,167,169,173,175,177,178]. Currently, no effective diagnosis or treatment of threatened infarction exists, and ongoing brain injury occurs despite full treatment with anti-TB medication and adjunctive steroids. Other cerebral pathologies like traumatic brain injury have incorporated monitors of brain oxygenation into patient management and have demonstrated the value of such monitors in detecting and potentially mitigating secondary ischaemic brain injury [198-200]. This chapter outlines briefly the factors contributing to ischaemia and poor brain oxygenation in TBM and introduces two monitors of brain oxygenation which may have utility in diagnosing and monitoring brain oxygenation compromise in TBM.

Factors precipitating ischaemia and poor brain oxygenation

Ischaemia is the common end-point of two important complications resulting from TBM; HCP associated with raised ICP and cerebrovascular injury. Both of these complications are thought to result from the formation of a thick basal exudate which blocks the flow of CSF precipitating HCP, and coats the cerebral vessels resulting in vessel pathology (discussed in Chapter 1). Elevated ICP can cause brain herniation due to pressure gradients created within the cranial cavity and compromises cerebral perfusion [201,202]. Brain oxygenation decreases as cerebral perfusion compromise leads to progressively reduced CBF (especially below 18mls/100g/min) and subsequent metabolic dysfunction [203-205]. Exudative coating of the cerebral vessels causes a local inflammatory response in the vascular wall resulting in infiltrative, proliferative and necrotising vascular pathologies which may result in vasospasm in the early stages of disease and vascular occlusion in more advanced cases [30]. The basal location of the exudate and predilection for the Sylvian fissures places the major vessels of the Circle of Willis, their branches and perforators at risk of vasculitis. Cerebrovascular injury is likely to result in either focal ischaemia, due to individual vessel involvement, or general ischemia as a result of decreased cerebral perfusion pressure respectively [112].

Other factors which may also contribute to compromised CBF or poor brain oxygenation include hemodynamic disturbances like the loss of pressure autoregulation and vascular reactivity due to cerebral infection [206]. When pressure autoregulation is intact, an increase in mean arterial pressure (MAP) within the autoregulatory range will result in cerebral vasoconstriction and maintenance of a relatively constant CBF. Conversely, cerebral vessels vasodilate in response to a drop in MAP thereby allowing for increased flow. However, when pressure autoregulation is impaired cerebral vessels fail

to adjust their calibre to changes in systemic blood pressure and passively distend with increase blood pressures, causing increased cerebral blood volume and therefore raised ICP. Cerebral cell death due to bacterial toxins, the accumulation of oxygen free radicals and inflammatory cytokines, and poor cerebral perfusion is associated with cytotoxic oedema [18,26,27,207]. Additionally, cerebral inflammation may disrupt the BBB, allowing the influx of proteins and water thereby facilitating vasogenic oedema [18]. Both cytotoxic and vasogenic oedema further contribute to raised ICP as well as compromised brain oxygenation as transport of oxygen from the vessels into tissues takes place through diffusion, driven by partial pressure gradients. Increases in the diffusion distance of oxygen between the vessels and tissues due to oedema may compromise the diffusion of oxygen into the cells and potentiate ischemia [208]. Inflammatory cytokines alter CBF, cerebral oxygen uptake, cerebral metabolism and lead to the uncoupling of metabolic demand and CBF [209,210]. These patients are also at risk of a hypercoagulable state which may increase the risk of thrombosis [106,176].

Brain oxygenation monitoring

Several techniques are available for monitoring various aspects of brain oxygenation, including 'snapshot' imaging of the brain like positron emission tomography (PET) scanning, which is the gold-standard for measuring brain perfusion, and continuous methods including brain tissue oxygen tension (PbtO2) and near-infrared spectroscopy (NIRS). The ideal brain oxygenation monitor would provide accurate real time data on brain oxygenation which allows clinicians to respond immediately to episodes of hypoxia or ischaemia, would be responsive to interventions and enable the tailoring of treatment to suit the individual patient's needs, and demonstrate sensitivity to the dynamics of other physiological variables like ICP [211]. Available brain oxygenation monitors measure various aspects of brain oxygenation and have variable strengths and limitations, and none has achieved widespread acceptance as yet. PbtO2 and NIRS differ in the physiological variables they measure and the physical principles used to measure them, but both have been used in a number of domains of neurocritical care and will be briefly introduced here.

Brain tissue oxygen tension (PbtO2)

PbtO2 is frequently used in the management of cerebral ischaemia [200,211]. It involves inserting a thin catheter into the parenchyma and is considered safe, efficient, and sensitive to cerebral ischaemia [200,212]. There are various suggestions of what PbtO2 represents; however, it may be considered a measure of the factors that affect the perfusion and diffusion characteristics of oxygen in brain tissue [211]. Consequently, it is influenced by a number of factors including: 1) the systemic partial pressure of oxygen (PaO2 – also a measure of oxygen tension) in that increases in PaO2 will lead to increases in PbtO2 even if blood is fully saturated; 2) tissue barriers to diffusion, like oedema; 3) raised ICP which compromises cerebral perfusion pressure (CPP) and exerts local tissue pressure effects; and 4) arterial

carbon dioxide (CO2) which is positively associated with PbtO2 if vascular CO2 activity is preserved [211].

Threshold values

Normal and threshold values have not been established in healthy humans; however, animal studies demonstrating normal values ranging from 25-30 mmHg have been confirmed in patients with normal ICP and CPP, in normal tissue of patients with brain tumours, and in patients who underwent temporary clipping of cerebral vessels [212-218]. The risk of poor outcome increases as PbtO2 falls below 20 mmHg [219] and values between 15 and 20 mmHg may be indicative of oligemia or cell damage [198]. A PbtO2 threshold of 10mmHg has demonstrated the strongest association with outcome [198,212,220,221] and is associated with ischaemic CBF thresholds and perturbations in brain function [222-226]. Low PbtO2 values are independent and strong prognosticators of poor outcome and death after adult and paediatric head trauma [198,227]. Currently there are no formal guidelines detailing indications for PbtO2 monitoring in either adults or children, however maintaining PbtO2 >10mmHg is recommended for paediatric neurocritical care [228].

Clinical applications

PbtO2 has been used extensively in paediatric and adult TBI and is associated with reduced mortality in these patients [198-200,229,230]. Other applications have included subarachnoid haemorrhage [231-233] and stroke [234]. The only available literature on PbtO2 in meningitis includes a case report of PbtO2-guided decompressive craniectomy in a patient with bacterial meningitis [235], and a case report from our institution on PbtO2 monitoring in TBM [236]. In this case report one of the two TBM patients presented with severe TBM on admission and demonstrated a dramatic decline in PbtO2 levels from >25mmHg to 0mmHg within 17 hours after admission and catheter insertion. This occurred in spite of ICP control with an EVD and full treatment, suggesting that vascular insults may continue despite adequate patient management. The second patient presented with less severe TBM and also experienced a decline in PbtO2; however, this responded to immediate interventions aimed at increasing oxygen delivery and diffusion. These included adjusting ventilator settings to increase the inspired fraction of oxygen (FiO2) and the arterial CO2, fluid resuscitation to augment the CPP and blood transfusion to improve haemoglobin (Hb). The PbtO2 recovered to levels above 20mmHg within 5-6 hours and the patient went on to make a good recovery with no evidence of infarction on follow-up CT brain scan. This study suggests a role for PbtO2 as a monitor of brain oxygenation and the response to interventions in TBM. Although PbtO2 is a focal monitor and focal ischaemia in other locations may not be detected, the diffuse distribution of exudate and the large territory infarcts in TBM suggest that ischaemia is likely diffuse rather than exclusive to discrete foci [236]. Furthermore, when placed in normal white matter PbtO2 offers a good approximation of global brain oxygenation

[200,224,237]. PbtO2 does not, however, only reflect the balance between the supply and demand of CBF and brain oxygenation as it is influenced by other factors, such as diffusion through tissues. These need to be considered in the interpretation of PbtO2 data.

Near-infrared spectroscopy (NIRS)

NIRS provides an attractive means of non-invasive continuous brain oxygenation assessment available at the bedside [238,239,239]. It has been used for somatic monitoring, for monitoring the normal brain at risk of injury during cardiac surgery [240], and in neurocritical care [241]. NIRS uses optical technology based on the transmission and absorption of near-infrared light of multiple wavelengths as it passes through tissues. Oxygenated and deoxygenated Hb have different absorptive capacities for near-infrared light and, therefore, their relative concentrations can be used to calculate brain oxygenation. The NIRS sensors are applied to the scalp and the near-infrared beam investigates an area of approximately 2cm below the skull.

Threshold values

Research with an animal model established that a NIRS saturation (SO2) of 35% for longer than 2-3 hours was associated with permanent neurological deficits [238,239]. Normal values in humans are thought to be between 60% and 80% [238,242,243], however, baseline variation is as high as 10%. This high variability may be due to the fact that thresholds of SO2 at which ischemia develop seem to be individual and disease specific [238]. However, little human data exists to support particular absolute values as treatment thresholds and, therefore, fluctuations within an established individual or population range as well as trend analysis have been used as measures of compromised SO2 [244]. Computed indices of cerebral oxygenation obtained using NIRS, like the tissue oxygen index (TOI - the ratio of oxygenated to total tissue hemoglobin) have been used as measures of intracranial oxygenation, with their own defined thresholds [240]. Normal values for TOI are considered to be in the range of 65% to 85% in adults. Studies in patients undergoing elective carotid endarterectomies showed that a change in TOI of -13% reflected a threshold for cerebral ischemia with a high degree of sensitivity and specificity [245]. However, findings pertaining to TOI still require validation in other clinical contexts. NIRS is also used intra-operatively, frequently with cardiac surgery, where 20% deviation from an established baseline SO2 alerts surgeons to impending cerebral injury [246,247].

Clinical application

NIRS has been used as a monitor of brain oxygenation and CBF in paediatric TBI [248], impending cerebral ischaemia during surgery [245-247], cerebral autoregulation [241], in functional studies [249], in neonates with birth asphyxia [244,250], in assessing CBF changes in response to reductions in raised ICP associated with HCP treatment [251], and as a means of characterizing ICP waves in hydrocephalic or

TBI patients [252]. There are no known published studies on the use of NIRS in meningitis in humans, however, research in experimental models of meningitis have found NIRS to be a useful tool to study cerebral hemodynamics in response to cerebral infection [253,254].

Limitations

Concerns regarding the validity of NIRS as a monitoring device center on technical limitations. Near-infrared light appears to penetrate the cortex only superficially, suggesting that the tissue sampled does not extend beyond grey matter. Penetration of infrared light may be distorted by the skull and its interface with multiple underlying layers which may create an optic channel, by contamination from ambient light or extracranial tissue perfusion, by light scatter, CSF, brain swelling, and skull thickness, although this is less of a concern in children with thinner skull bones [238,255,256]. Additionally, defining the relative contributions of the venous, arterial and capillary compartments is challenging. Spatial–resolved spectroscopy has been developed to accommodate for the contribution of multiple vascular compartments [245,255], and further advances in NIRS technology have demonstrated improved NIRS reliability, including frequency domain NIRS which allows the calculation of light absorption and scattering, and facilitates the determination of oxygenated and deoxygenated Hb in absolute concentrations excluding extracranial contamination [257].

The potential utility of PbtO2 and NIRS as monitors of brain oxygenation in TBM was explored as part of this study, and findings are presented in Chapter 12.

SECTION B: INTRODUCTION TO BIOMARKERS

CHAPTER 5: BIOMARKERS OF NEUROLOGICAL TISSUE INJURY IN CEREBRAL INFECTION

Central nervous system (CNS) infections, including TBM, are an important public health concern worldwide as they occur commonly and are associated with high rates of mortality and morbidity [258,259]. The resulting cerebral tissue injury accounts for most deaths related to CNS infections and stems from direct bacterial toxicity and consequences of the host inflammatory response including ischaemia [26,27]. However, several uncertainties about the disease process remain because the pathogenetic mechanisms of tissue injury are still not fully understood. Quantification of the degree of cerebral injury is inexact: disease severity is commonly evaluated by the clinical status of the patient and radiological manifestations. Yet several factors, both reversible and irreversible, may contribute to the presenting neurological status, and imaging findings manifest late in the disease course once the damage is usually already permanent. Biomarkers to diagnose disease, quantify injury, and monitor progress are used frequently for other organ systems but their use in CNS pathology is in its infancy. Several studies have examined biomarkers of neurological tissue injury in different CNS pathologies which shed light on the underlying pathophysiological processes while they are occurring; however, data are sparse. This chapter reviews the available literature on neuromarkers S100B, NSE and GFAP in cerebral infection in general, as there are only a handful of studies published on TBM.

Role of Biomarkers in CNS pathology

Biomarkers are measurable objective indicators of normal function or pathology. They provide information about dynamic processes and pathogen activity that assist diagnosis, prognostication, and evaluation of treatment safety and efficacy. They can also act as surrogate markers for clinical or research end-points, such as the effectiveness of novel treatments [260]. Their quantification is user-independent and several biological fluids like blood and urine are easily obtained for investigation [261-264].

Biomarkers for CNS pathologies are gaining increasing attention and are being investigated across a spectrum of acute and chronic CNS diseases [265]. CNS infection, trauma, hypoxia, inflammation or degeneration results in cell damage and a collection of breakdown products in the cerebral extracellular fluid as well as increased permeability of the BBB. Diffusing along concentration gradients into the CSF and through a leaky BBB into the blood stream, these products become accessible measurable indicators of brain injury. The degree to which these biomarker levels are elevated reflects the severity of injury, the cell-specificity hints at the nature and potentially the

location of injury, and sequential sampling provides information about the evolution of the damage [260,266,267].

The ideal biomarker for brain injury should demonstrate high sensitivity and specificity for the brain, their release should be associated with irreversible brain injury and reflect the temporal profile of that injury, they should appear rapidly in serum, demonstrate limited variability based on age and sex, and should be easily and speedily quantified by reliable assays [268]. However, finding such an ideal biomarker for the brain presents many challenges. The brain is a highly complex and heterogeneous organ with multiple cell types, and disease varies both in form and severity. CSF is better than serum in reflecting changes in the brain, but is not always accessible. The size and amount of the biomarker infiltrating the blood stream is limited by the blood brain barrier (BBB), and so serum values may be a function of cell injury as well as the degree of BBB disruption, which commonly occurs in brain injury [261]. Even in CSF variability exists; biomarker levels may be influenced by the distance between the affected area and the CSF compartment, regional variability of biomarker proteins in the brain and degradation by proteinases in the parenchyma or CSF [269]. In addition, biomarker analysis is purely a quantitative measure which cannot reflect both the qualitative and quantitative functions of the brain [270]. One way of overcoming some of these limitations is by using a panel rather than individual biomarkers and by combining these with clinical and radiological tools [261,271].

Studies in traumatic brain injury (TBI) [270,272], subarachnoid haemorrhage [267,273], dementia [274], Alzheimer's disease [275], stroke [267], cardiac arrest [276] and various other pathologies have found that although biomarkers of CNS injury do not currently form part of regular clinical practice, they hold promise as diagnostic and prognostic markers. Their role in infections of the CNS has generated less research. From the available studies, however, three key biomarkers with potential have emerged, namely S100B, neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP). This chapter will focus primarily on these proteins but also briefly summarise studies on other potential biomarkers.

Limitations of biomarkers

Biomarker analysis is prone to some methodological pitfalls. The technique with which samples are collected has implications for their suitability, for example, hemolysed blood samples may be contamination by biomarkers released from erythrocytes [277]. The collection tube may influence biomarker concentrations and tubes appropriate to the biomarker of interest should be used [278]. Samples should preferably be stored at -70 degrees Celsius as early as possible to prevent antigen degradation, however this varies as a function of biomarker stability. Multiple freeze-thaw cycles and prolonged storage can also lead to biomarker breakdown [279]. Currently the primary method to test for biomarkers is the immunoassay, which demonstrates good sensitivity, is simple and inexpensive to

administer, and for which various kits are commercially available. This technology exploits the high affinity and specificity with which antibodies bind to their antigens ensuring that only the target antigen in a sample will bind even when it is present in very low concentrations and in the presence of many other analytes. Immunoassay plates are coated with antibodies for the biomarker of interest; when test samples are added to the plate the target antigen (biomarker) binds to the antibody and the degree of binding provides a measure of the biomarker present. Since the interaction of antibody and antigen is not associated with a quantifiable physical or chemical change, the binding event is measured by an auxiliary reaction in which one of the immunoreactants is labelled with a substance that can easily be detected by spectrophotometry [280]. Platforms differ based on the antibody and label used to detect the antibody-antigen binding; commonly used options include enzymes (enzyme-linked immunosorbent assays [ELISAs]), radioisotopes (radioimmunoassays [RIA]), fluorophors (immunofluorometric assays), and chemiluminescent compounds (chemiluminescence immunoassay). Electrochemical immunoassays, which are label-free and rely on changes in charge densities or conductivities to detect antibody-antigen binding, are also becoming increasingly available [280-282]. Upper and lower detection limits are variable for different assays and therefore comparison of reference values and pathology-related measurements across testing platforms is challenging, highlighting the need for the establishment of standardised operating and testing procedures. Large-scale multi-centre trials would enable accumulation of sufficient samples for well-powered studies to establish standards, reference ranges and appropriate disease-specific cut-off values. Much biomarker work is still confined to dedicated laboratories or projects; however, the true translation of biomarkers from bench to bedside requires the development of rapid, user-friendly, technologically undemanding tests that can be utilised on demand at a hospital and clinic level [261].

S100B

S100B belongs to the larger S100 family of small acidic proteins approximately 10-12 kDa in size and composed of subunits A and B [283]. These proteins regulate intracellular processes including cell growth, transcription and differentiation. The extracellular concentration of S100 proteins determine their activity; at nanomolar levels they have trophic effects while at micromolar levels they may cause cell damage and apoptosis. In the CNS, subunit B is found in highest concentration [283] and is synthesized by astrocytes, oligodendrocytes and Schwann cells [284] It is involved in cell to cell communication [285], cell growth and intracellular signal transduction as well as the development and maintenance of the CNS. Several S100B assays are available which detect both S100AB and BB, but currently a normal reference range for S100B remains uncertain. Labs conducting S100B assays are therefore required to generate reference ranges for their population and studies in patients should include a representative control group. Brain injury results in leakage of S100B into the CSF and passage into the blood stream through transient disruption of the BBB or via CSF circulation [266].

S100B is metabolized by the kidneys and excreted in the urine. Levels usually normalize within 24 hours after an acute injury; therefore, persistent elevation of S100B may reflect ongoing or secondary cellular injury [286,287]. At elevated levels S100B may be neurotoxic by inducing apoptosis, causing the release of pro-inflammatory cytokines as well as nitric oxide from astroglial cells, and contributing to oxidative stress [258,284,285,288]. Therefore, elevated levels do not only reflect tissue damage but may also exacerbate it [288]. This protein is easily quantified in various biological samples including CSF, blood and urine. It remains stable over a wide range of temperatures (room temperature to -70°C) for up to 48 hours after collection and does not degrade after freezing making it a suitable candidate for daily laboratory measurement and batch analysis in studies [289,290].

S100B is elevated in various CNS pathologies including TBI [270,272], Alzheimer's disease [275], dementia [274,283], stroke [291,292] and subarachnoid haemorrhage [273]. Extracranial sources of S100B include white and brown fat, skin, skeletal muscle, melanocytes and adipocytes [263,293-295]. Clinically, elevated blood levels are reported in melanomas or long bone injuries [263,266].

Reference values

S100B levels differ across various developmental phases from the fetal period through to old age [296]. Stages of fetal development are associated with differential S100B levels in amniotic fluid and cord blood suggesting that elevated S100B within a physiological range may represent its neurotrophic role in brain maturation[297,298]. Age related S100B levels are, therefore, probably due to the role of S100B in the maturation of glial cells, formation of synapses and general brain morphogenesis which occur most frequently during early childhood [299]. The pattern of age-related differences is however difficult to interpret in the existing literature. Portela et al [299] collected serum samples from three age groups of healthy donors; neonates, children (4-16 years) and adults (18-70 years). They found no differences based on sex, but age demonstrated a significant negative correlation with S100B with the highest values found in neonates. After the age of 20 years, the baseline levels seemed to stabilise. Gazzolo et al [300] also found that S100B levels in serum were associated with age in a detailed study of 1004 children between the ages of 0 and 15 years. Highest values were recorded during the first year of life and then again between 7 and 13 years. The authors hypothesize that these epochs correspond to the greatest spurts of growth and maturation; infancy and early adolescence. Different peaks of S100B were also found based on sex, again a possible manifestation of the growth phenomenon which varies between the sexes. Bouvier et al [301] found that serum S100B levels decreased with age. Their results in an exclusively paediatric cohort showed that the highest levels of S100B were recorded before two years. The permeability of the BBB, higher protein turnover in neuronal cells, low renal secretion of S100B as well as dynamic CNS development in children under two years are possible explanations proposed for this finding. However, Spinella et al [288] found a positive correlation between age and

CSF S100B levels in their study on 107 paediatric patients ranging from three days to 17.8 years. Although a significant positive correlation was found between age and S100B levels, the degree of variance attributed to age was only 4%. These results have been mirrored in an adult study conducted by Nygaard et al [302], who found that S100B levels in the CSF increased with age with 43% of the variability attributed to age in men, and 30% in women. Overall S100B levels were also significantly higher in men. This study could not detect S100B in serum samples. Contrary to the above mentioned studies Wiesmann et al [303] selected 200 healthy blood donors which they divided equally in five age bands each of approximately nine years spanning from 18 to 65 years. Their study found no significant age- or sex-related differences.

These seemingly opposing findings between studies may be a function of whether or not children are included in the study cohort as evidence suggests the largest influence of age early in life. Furthermore, the heterogeneity of normal cohorts selected to establish reference ranges is challenging because much of the inter-individual difference cannot be accounted for and sample sizes often are not large enough per age group to adequately compensate for this variability. The selection of so-called 'normal' samples is also subject to interpretation - some studies have chosen individuals with symptoms which could indicate neurological disease and warrant invasive procedures like a lumbar puncture, while others have selected completely healthy individuals. These are important considerations when evaluating the role of S100B or any biomarker in conditions of pathology, defining reference values or matching controls and cases. A summary of reference values for S100B is included in Table5.1 at the end of this chapter.

Studies in CNS infections

Experimental studies

Bertsch et al [292] measured serum S100B levels in a mouse model infected with CNS Candida Albicans. They found that levels were significantly elevated relative to control mice, and peaked within one day post-infection followed by a subsequent decline. The kinetics of S100B appear to reflect the infective process whereby pathogenic entry into the CNS leads to an immediate inflammatory response, cell destruction and BBB breakdown. When the infection is contained, tissue damage is halted and S100B levels return to baseline. In a sheep model Garnier et al found that sheep foetuses exposed to infection intra-uterine developed white matter injury. The degree of injury and mortality were positively associated with S100B levels in both the foetal and maternal circulation suggesting the value of S100B in the early detection of foetal white matter injury [304]. Another experimental model reveals the role of S100B as a marker of hypoxic distress in fetal sheep. Elevated plasma S100B levels were associated with markers of metabolic acidosis, redistribution of blood flow from the peripheries and currently used indicators of perinatal hypoxia [305].

Clinical studies in Adults

Infante et al [283] examined S100B in the CSF of adult patients with various neurological pathologies including meningitis, dementia, hydrocephalus, acute cerebral infarction, multiple sclerosis, motor neuron disease and lymphatic leukemia. S100B levels were significantly elevated in the meningitis, dementia and acute cerebral infarction groups with the highest levels associated with dementia. The authors suggest that S100B in acute limited injury may peak early and clear the system swiftly, in which case accurate assessment is highly dependent on the timing of sampling relative to onset of injury. Continued elevation likely represents ongoing injury or the inflammatory activation of glial cells. Unden et al [306] found higher levels of serum S100B in adults with cerebral infections compared with extra-cerebral infections. Viral encephalitis patients demonstrated the highest levels, and bacterial meningitis patients recorded higher levels than those with viral meningitis which is congruous with the established cellular injury associated with these diseases. However, some of their encephalitis and meningitis patients did not demonstrate elevated values despite radiological and clinical abnormalities, suggesting that S100B may produce some false negative results when used as a marker of brain injury. Considering that S100B is a brain derived protein this may have been influenced by the fact that they sampled blood rather than CSF. Further, in one of these encephalitis patients sampling was done a few days later than in those patients with elevated levels; S100B has been shown to peak earlier in the disease process and so the peak may have been missed [283,307]. The authors also suggest that S100B may demonstrate false positive results: some cases with extra-cranial infection and no evidence of cerebral involvement had elevated S100B. The exact extracranial sources of these elevated S100B levels is not known, however, in these instances S100B levels were mostly only marginally elevated and did not reach levels as high as in encephalitis. In general, CNS infections were the predominant source of elevated S100B.

Clinical studies in Children

Having examined reference values in infants, Spinella et al [288] compared CSF S100B levels between normal children and those with established meningitis and found the latter had significantly higher levels. Hamed et al [258] examined levels of S100B and markers of oxidative stress and antioxidative activity in 40 children with bacterial meningitis. S100B levels were elevated in both serum and CSF samples. They contend that brain injury resulting from bacterial meningitis is evidenced by the increased intrathecal production of S100B and markers of oxidative activity, and that the levels of these markers are related to injury severity. A study of infants with bacterial meningitis [308] found elevated levels of CSF S100B with the highest levels recorded in infants who developed encephalitis in addition to their meningitis. A receiver operating curve indicated that S100B levels above 1.0 ug/L were diagnostic for the early detection of bacterial meningitis-encephalitis with a sensitivity of 91% and a specificity of 82% with an area under the curve of 0.92. S100B also surpassed standard

monitoring techniques in identifying the development of encephalitis [308]. Table 5.2 summarises concentrations reported for S100B in clinical studies.

Neuron-specific enolase

NSE (γ-enolase) is a stable cell-specific isoenzyme of the glycolytic enzyme enolase, a dimer protein made up of α, γ and β sub-units measuring 78 kDa. Although highly localised to neurons and neuroendocrine cells, NSE has also been found in erythrocytes, liver, smooth muscle and lymphocytes; however, this is often of the hybrid (αδ) enolase form and has low concentrations (less than 10 ng/mg) [262,266]. In the brain NSE is concentrated exclusively in the cytoplasm of neurons [262]. NSE demonstrates enzymatic activity and is a promising marker of general neuronal function. Normal serum levels range from 5-15 ng/ml. When neuronal membranes are injured, NSE easily diffuses into the extracellular space and CSF [277]. Consequently, increased levels of NSE in the CSF and serum have been identified in several cerebral pathologies including encephalitis, cerebral infarction, Parkinson's, amyotrophic lateral sclerosis, spinocerebellar degeneration, cervical spondylosis, polyradiculoneuritis and TBI [274,309,310]. NSE is also elevated in small cell lung cancer [266] and paediatric neuroblastoma [262].

Reference values

Van Engelen et al [311] sought to establish reference values for NSE, S100B, and myelin basic protein (MBP). They collected CSF samples in 79 control patients ranging from 1 to 60 years of age. Their findings suggest a significant increase with age which was similar across all three markers, with a median increase of 1% per year. Possible explanations proposed for this finding relate to an increase in cell and myelin loss with age and/or a parallel reduction in CSF bulk flow. Nygaard et al [312] studied CSF and serum NSE levels in normal individuals ranging from 20-90 years of age. In the serum they found no differences based on sex and age, but in CSF there was a positive significant association with age and males had higher values. The authors suggest that the increase in NSE levels with age may be attributed to increased NSE concentration in the face of stable cell turnover, increased cell turnover with age, or an increased NSE half-life due to reduced CSF bulk flow. Sex dependency may be explained by similar mechanisms. In addition this study found no correlation between the NSE levels in the two biological fluids. However, the association with age is not a consistent finding. In a paediatric study Rodriguez-Nunez et al [313] examined NSE levels in the CSF of 37 children aged 1 month to 13 years. Their findings did not suggest an association with age. Casmiro et al [314] studied both CSF and serum in an exclusively adult population of 108 individuals and also found no significant association with age, however their sample was skewed to patients over 60 years old. NSE levels were not associated with sex and no correlation between serum and CSF values was demonstrated. [314]

This in conjunction with findings of Nygaard et al [312] suggests that serum cannot substitute CSF and that normal serum NSE does not necessarily exclude the presence of CNS injury, although this requires validation in a sample of patients with CNS disease. Studies on reference ranges in NSE are subject to the same limitations and cautionary interpretation as for S100B. Reference values for NSE are summarised in Table 5.3.

Studies in CNS infections

Experimental Studies

The authors are unaware of any studies of NSE levels in experimental models of CNS infection. However, a study of NSE in an ischemic rate model is relevant as ischemia is an important determinant of poor outcome in CNS infections. Hardemark et al [315] correlated NSE levels in CSF with the development and size of infarcts in rats by sampling fluid pre- and post-MCA occlusion over several days. NSE levels peaked between 24 and 72 hours after occlusion with a subsequent return to baseline over six days. Infarct size was significantly correlated with NSE levels and the area under the NSE concentration curve. These findings suggest that sequential analysis of CSF NSE reflects the development as well as size of infarcts. The temporal variation of NSE reflects the dynamic nature of the ischemic process which may occur over several days and is subject to inter-individual variation. The timing of sampling as the injury evolves is therefore an important consideration.

Clinical Studies in Adults

Lima et al [277] examined whether NSE levels correlate with severity of neurological impairment. They sampled serum and CSF in 51 adults: 11 with meningitis, 7 with encephalic injuries, 25 with neurocysticercosis and eight controls. They found no age or sex differences. NSE levels were elevated only for the group with encephalic injuries, and only in CSF. It is worth noting that none of the meningitis group was neurologically compromised and so the degree of neurological injury is debatable. Song et al [316] measured NSE in the CSF and serum of adult patients with tuberculous meningitis (TBM) (n=15) and aseptic meningitis (n=28) in comparison with controls. While serum and CSF NSE levels were not significantly different across the three groups, the CSF/serum NSE ratio was significantly higher in the TBM group. This ratio was significantly correlated with the diagnosis of TBM in univariate and multivariate analysis demonstrating its potential as a diagnostic marker. The sample size of this study was small and provides a limited picture of NSE levels in meningitis.

Clinical studies in Children

There is a dearth of literature available in children, with most of the research in TBI [270,309,310,317]. Rodriguez-Nunez et al [318] examined CSF markers of cell hypoxia and NSE to discern the utility of these markers in the differential diagnosis of meningitis. They examined 160 control children and 100

children with bacterial, viral or TB meningitis and found that NSE levels in bacterial meningitis and TBM cases were not significantly higher than controls, did not differ based on sex, and were not different between meningitis groups. Only the viral meningitis group had significantly elevated levels; however, this was attributed to nine cases with mumps etiology in whom NSE levels were highest overall. Two limitations of this study are: first, their TBM group was very small, comprising only 0.9% of the meningitis cases, and second, the CSF samples were collected very early in the disease course (1-16hrs post onset of symptoms). Experimental work in animal models suggested that NSE levels tend to increase beyond 24 hours post-injury [315], therefore sample may have been taken when hypoxia had not been sufficiently prolonged or intense to induce cell death and cause elevations in NSE. In a study of 20 comatose children suffering from acute encephalitis, acute encephalopathy and Reye's Syndrome, Nara et al [319] found that both CSF and serum NSE levels were significantly elevated relative to controls. NSE was associated with neurological compromise and showed higher initial values and subsequent increase in patients with more severe brain damage and poorer outcome. Their findings support the variability of NSE levels at different disease stages and between patients. Contrary to other studies these authors found a good correlation between serum and CSF NSE. NSE concentrations from clinical studies are summarised in Table 5.4.

Combining S100B and NSE

Lins et al [307] performed sequential paired CSF and serum measurements of S100B and NSE in patients with bacterial and viral meningitis. Results from 32 adult patients revealed significantly elevated serum and CSF S100B in patients with bacterial meningitis only. Elevated S100B was associated with lesions on CT or MRI and a higher CSF/serum albumin ratio. In most patients S100B levels decreased after the initial samples, except in a patient with TBM in whom biomarker levels in CSF and serum continued to climb, peaking by day 27. NSE was not significantly elevated in this study. Mokuno et al [274] examined CSF S100B and NSE in patients with a range of neurological pathologies including encephalitis, meningitis, cerebral infarction, Parkinson's disease, sclerosis and others. Levels of NSE and S100B were elevated in several of these conditions, but not always simultaneously suggesting that their presence may reflect the kind of tissue damage: glial versus neuronal. Levels were highest in encephalitis and cerebral infarction and S100B normalised earlier than NSE.

Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) forms an integral part of cytoskeleton of astrocytes. The molecular mass is estimated to be between 50 and 53 kDa, and the soluble fragment released into CSF and the cerebral extracellular fluid is approximately 41 kDa [320-322]. It is involved in modulating the motility of astrocytes and providing structural stability to astrocytic processes, and plays an important

role in maintaining myelin and white matter architecture as well as BBB integrity [320,323]. Astrocytes respond rapidly to injury or inflammation by undergoing hypertrophy, proliferation and various enzymatic changes [324]. Reactive astrogliosis is a prominent feature of cells in the penumbral zone surrounding tissue injury and is characterized by increased concentrations of GFAP [320]. GFAP is also released in the event of cell death [320,321,325] and appears to be specific to central nervous system injury making it a valuable marker of neurological damage and an important component in neuro-biomarker assessment [326].

This marker is frequently investigated to detect gliosis by immunostaining in brain tissue samples [327] or as a cell marker for damage due to infection [328,329]. Various in-house assays to detect GFAP in the CSF and serum have been developed and have shown good sensitivity in detecting GFAP in both biological fluids [322,330,331]. Commercially available assays are now easily available. The exact half-life of GFAP is uncertain; studies in TBI, stroke, intracerebral haemorrhage and subarachnoid haemorrhage demonstrate variability in the release kinetics of GFAP and its temporal profile of elevation ranges from 24 hours to 4 days [321,326,332-335], but few studies have systematically measured GFAP serially. It is suggested that while GFAP clears the bloodstream rapidly, persistent elevation may reflect ongoing secondary injury which contributes to poorer outcome [325,332]. Elevated concentrations of GFAP in TBI have demonstrated high sensitivities and specificities as a marker of injury and predictor of outcome [325,326,336]. Research in stroke patients has found serum GFAP concentrations to be a promising tool in differentiating stroke of ischemic and haemorrhagic origin [335,337]. GFAP is significantly associated with head CT scan abnormalities, infarct volume and perturbations in other physiological variables like raised intracranial pressure and poor cerebral perfusion pressure [332,338]. This neuromarker has also been examined in the diagnosis of brain tumours and assessment of tumour volume [339,340] and has been studied in Alzheimers and dementia [341,342].

Reference ranges

There is little available research defining normative values for age in GFAP. The relationship between age and GFAP concentrations is also contentious; a study examining GFAP in individuals from the paediatric to the adult age ranges found GFAP to be independent of age and elevations in the elderly were attributed to the increased prevalence of Alzheimer's disease and dementia [341]. A study in teenagers and adults found that GFAP was positively related to age and appeared to increase from the age of 50 years, reaching the highest concentrations at 75 years [343]. The same group conducted a study in a patient sample ranging from 1.3-29 years, and recorded significantly lower concentrations in younger children [344]. The increasing trend of GFAP during the life course is attributed to expansion of the fibrillary astrocytes in the maturing brain [344], and it is possible that early neurodegenerative

mechanisms may also influence concentrations in older subjects. GFAP reference ranges are outlines in Table 5.5.

Studies in CNS infections

Research on the diagnostic and prognostic value of GFAP has focussed largely on TBI and stroke; data on measurement of soluble GFAP in the CSF or serum of humans with cerebral infections is sparse and there are no experimental animal models to our knowledge.

Clinical studies in adults

A proteomics study aimed to identify proteins which could be used to differentiate between bacterial and viral meningitis. Their analyses in serum and CSF revealed that CSF GFAP was significantly more elevated in patients with bacterial meningitis, suggesting its value as a marker to discriminate between the two meningitides early, and to direct appropriate treatment [345]. A study on Lyme neuroborreliosis demonstrated that GFAP concentrations were elevated in the CSF of affected individuals relative to control patients, suggesting that the disease process was not only limited to the meninges but affected the parenchyma as well. GFAP responded positively to antibiotic treatment [346]. Further studies have looked at GFAP in combination with other neuromarkers. In a cohort of patients with varicella-zoster virus CNS infections, Grahn et al [347] measured CSF S100B, GFAP and neurofilament protein light chain (NFL), a potential marker of neuronal injury. NFL showed the highest and most prolonged elevation, followed by GFAP whereas S100B levels were normal. This suggests that damage may have involved astrogliosis while the astrocyte cell membrane remained intact, and that persistent or secondary injury may be responsible for ongoing NFL elevation. A review by Bonneh-Barkay et al [348] summarises research into biomarkers for encephalitis in adults; CSF NSE, NFL, GFAP, S100B, a variety of proinflammatory cytokines, and soluble Fas (an apoptotic marker) were identified in herpes encephalitis.

Clinical studies in children

A multi-centre paediatric study combining a panel of biomarkers of neurological injury demonstrated that GFAP was elevated in the CSF of children with infectious or inflammatory cerebral disease and elevations in this neuromarker were attributed to astrogliosis. Contrary to reports on healthy controls, this study found that GFAP was negatively associated with age, and highest GFAP concentrations were observed in patients less than 5 years. This may reflect greater vulnerability of the immature brain to injury [349]. Tsukahara et al [350] examined CSF S100B, GFAP and Tau protein (a microtubule-associated protein found in neuronal axons) in children with acute encephalitis/encephalopathy. CSF biomarker levels were only elevated in patients with poor outcome and S100B had the best predictive accuracy with respect to outcome. GFAP showed poor sensitivity (40%) but was very specific

(100%). Tau protein had a sensitivity of 70% and specificity of 85%. Combining several markers allowed for the best predictive value for outcome. GFAP concentrations recorded from clinical studies are included in Table 5.6.

Other Potential Biomarkers

Advances in genomics, transcriptomics, proteomics and metabolomics offer opportunities for investigating various molecular aspects of the host-pathogen interaction in CNS infections. These include pathogen-specific molecular or metabolic signatures, pathogen-directed alteration to host gene expression patterns, genetic susceptibility to infections, inter-individual genetic variation in response to pathogen exposure, and the association between gene expression profiles and disease outcome. These technologies therefore have the potential to contribute biomarkers which could accurately diagnose the disease as well as the offending pathogen, allow early selection of appropriate treatment, identify patients at greater risk of severe disease and poor outcome, and allow treatment tailored to both host and pathogen characteristics. This field is still in a fledging state, however, especially with respect to CNS infections [351]. In the field of proteomics, Goonetilleke et al [352] studied the CSF protein profile of patients with pneumococcal meningitis using two dimensional gel electrophoresis. Elevated proteins were associated with inflammation, cellular damage and metabolic derangement in non-survivors and could provide insight into novel biomarkers and drug targets. In a proteomics study on the CSF of TBM patients, Kataria et al [353] found 18 differentially expressed proteins. Up-regulated proteins of human origin included: arachidonate 5-lipoxygenase, which contributes to the pathophysiology of TB by weakening the host's immune responses; GFAP, a marker of tissue damage; and anti-thrombin-III which indicates the involvement of coagulation pathways. Down-regulated human proteins included: apolipoprotein E and A-1, which are important for neural growth and repair, immune regulation and their anti-inflammatory properties, and transthyretin, which aids in reducing brain oedema, neuronal death and inflammation. Mycobacterial proteins identified in the CSF could present diagnostic markers for swift detection of the causative agent in patients with meningitis. Metabolomics is a high throughput technology which allows for a broad unbiased analysis of the full complement of metabolites present in biological samples. It is increasingly being used in disease diagnostics, therapeutics and drug development as it provides insight into the combined effect of metabolites from the infecting organism, inflammatory cells and cerebral cellular responses to pathogens and therapeutics[354]. In a study on patients with meningitis and ventriculitis, Coen et al[355] demonstrated that metabolomics of the CSF could rapidly diagnose these infections and showed improvement on routine CSF cell count and chemistry. Subramanian et al[356] developed an expert system to aid in the differential diagnosis of meningitis using a unique combination of clinical features and metabolic fingerprint.

Future work

More in depth studies looking at a broader panel of markers are required. Using a combination of neurological markers may also be useful in tracking injuries of various tissue types and may mitigate the influence of extracranial sources. Combining inflammatory and tissue injury markers has demonstrated improved prognostic power in TBI [271] and may prove beneficial in CNS infections as well. Few of the available studies in CNS infections examined biomarkers in relation to outcome. Several studies have confirmed significant associations between biomarker levels and radiological and clinical outcome in TBI [272,357,358] since these are the current tools available it is important to ascertain the strength of the associations with biomarkers in infectious injury.

Conclusion

Despite the limited number of studies on biomarker levels in CNS infections the literature available suggests that in both adults and children biomarkers of neurological injury have the potential to answer several important questions; 1) when presenting symptoms and history are non-specific, is there CNS involvement, 2) what is the nature of the pathological process, 3) what is the likely causative pathogen, 4) which cells of the CNS are affected, 5) how severe is the injury, and 6) is the disease process responding to intervention? Biomarkers of neurological injury carry promise as markers of diagnosis as well as prognosis and may serve as surrogate end-points for determining the response to novel interventions. Further studies on biomarkers in this field will add to our understanding of their utility. When conducting biomarker studies it is important to consider limitations presented by heterogeneity in the study cohort, the relative value of CSF versus serum and the choice of testing platform.

Table 5.1: S100B Reference Ranges (µg/L)

Authors	Age group	N	Specimen	Mean (SD)	Median	Range
Paediatric cohort						
Bouvier et al (2011)[a]	0-2 years	139	serum	0.21 (0.12)		0.07-0.83
	>2 years	97		0.11 (0.03)		0.07-0.2
Gazzolo (2003)[a, b]	0-1 year	85	serum		0.95	0.44-2.55
	2-7 years	461			0.73	0.44-1.06
	9-11 years				1.65	0.91-1.74
	11-12 years	32			0.45	0.39-0.45
	13-14 years	35			1.23	1.12-2.01
	14-15 years	18			0.78	0.5-0.87
Spinella et al (2004)	<1 months	16	CSF		0.79	0.52-1.32
	1-2 months	29			0.6	0.44-0.84
	2-3 months	11			0.61	0.4-0.81
	3-12 months	20			0.58	0.46-0.73
	1-5 years	10			0.96	0.77-1.33
	5-10 years	8			0.87	0.65-1.07
	10-15 years	7			1.3	0.87-1.8
	15-18 years	6			1.2	0.66-1.8
Mixed cohort						
Portela (2002)	Neonates	19	serum		1.79	1.57-2.44
	4-9 years	16			0.37	0.27-0.41
	10-15 years	8			0.31	0.26-0.37
	16-20 years	10			0.11	0.02-0.2
	21-25 years	13			0.1	0.05-0.15
	26-30 years	9			0.1	0.06-0.15
	30-70 years	54			0.06	0.03-0.1
Van Engelen et al (1992) [c]	1 year		CSF			0.9-2.6 [d]
	20 years					1.1-3.3
	40 years					1.3-4
	60 years					1.6-5
Adult cohort						
Wiesmann (1998)	18-65 years	200	plasma		0.05	0.02-0.1 [e]
Nygaard et al (1997)	20-89 years	110	serum	undetectable		
			CSF	1.9 (0.7) ♂ 1.5 (0.5) ♀		

N = sample size, Range = 25th to 75th percentile unless otherwise stated, SD = standard deviation, CSF= cerebrospinal fluid, ♂ = male, ♀ = female

[a] Additional age related data available in original articles

[b] Sample sizes derived from article where possible

[c] Sample sizes not stated in article

[d] Range reflects 5th – 95th percentiles

[e] 10th-90th percentile

Table 5.2: S100B data from clinical studies (µg/L)

Authors	Age	Pathology	N	Specimen	Mean (SD)	Median	Range	Cases > Controls
Paediatric cohort								
Hamed et al (2009)	< 15 years	Controls	20	serum	0.13 (0.01)		0.11-0.16	
	<15 years	Bacterial meningitis	40	serum	0.22 (0.07)		0.13-0.57	✓
				CSF	0.61 (0.03)		0.55-0.7	
Spinella et al (2004)	0.08-1.5 years	Controls	107	CSF		0.71	0.48-1.07	
		Meningitis	34	CSF		1.1	0.91-1.4	✓
Gazzolo (2004)	38 weeks	Controls	44	CSF		0.16	0.11-0.33	
	38 weeks	Bacterial meningitis	44	CSF		1.34	0.84-1.78	✓
Mixed cohort								
Infante (2003)[a]	1-79 years	Controls	22	CSF	0.88 (0.08)			
		Lymphocytic meningitis	19		2.51 (0.71)			✓
		Bacterial-fungal meningitis	10		5.06 (2.43)			✓
		Dementia	6		2.09 (0.62)			✓
		Acute cerebral infarction	10		1.69 (0.26)			✓
Adult cohort								
Unden et al (2004)[b]	15-84 years	Viral encephalitis	5	serum	0.58			✓
Lins et al (2005)	41 (13) years	Controls	13	serum	0.05 (0.03)			
				CSF	1.22 (0.49)			
		Bacterial meningitis	11	serum	>controls			✓
				CSF	>controls			✓
		Viral meningitis	13	serum	<controls			X
				CSF	<controls			X
Mokuno et al (1983)[a]		Controls	18	CSF	0.33 (0.09)		0.16-0.52	
		Encephalitis	6		1.9 (1.8)		0.34-5.1	✓
		Meningitis	24		0.6 (0.48)		0.06-2.4	✓
		Cerebral infarction	10		1.4 (1.4)		0.12-4.1	✓
		Parkinson's disease	18		0.58 (0.23)		0.21-0.96	✓
		Cervical spondylosis	9		0.51 (0.2)		0.24-0.84	✓
		Acute P (R) N	5		0.83 (0.62)		0.2-1.8	✓
		Chronic P (R) N	6		1.5 (2.3)		0.34-6.6	✓

N = sample size, Range = 25th to 75th percentile, SD = standard deviation,' >' greater than, '<' less than, CSF = cerebrospinal fluid, P(R)N = poly-(radiculo)neuritis, ✓ = cases> controls at $p<0.05$, X = case>controls $p>0.05$

[a] Only pathologies with S100B levels significantly greater than controls included in table, further detail in original articles [b] Several other pathologies included in the study had elevated S100B levels, however, no descriptive statistics are reported in the paper

Table 5.3: NSE Reference Ranges (µg/L)

Authors	Age group	N	Specimen	Mean (SD)	Median	Range
Paediatric cohort						
Rodriguez-Nunez et al (1999)	1 month – 13 years	37	CSF	1.5 (1.01)	1.5	0-4.8
Rodriguez-Nunez et al (2003)	1 month – 13 years	160	CSF	1.5 (1.01)		
Mixed cohort						
Van Engelen et al (1992) [a]	1 year		CSF			2.2-10.2 [b]
	20 years					2.7-12
	40 years					3.1-13.8
	60 years					3.8-16
Adult cohort						
Casmiro et al (2005)	62.7 (16.7) years	108	serum	8.7 (3.9) [c]		
			CSF	17.3 (4.6)		
Nygaard et al (1998)	20-89 years	87	serum	7.1 (3.6)		6.2-8.1 [d]
			CSF	5.1 (1.6) ♂ 4.1 (1.4) ♀		

NSE = neuron specific enolase, N = sample size, Range = 25th to 75th percentile unless otherwise stated, SD = standard deviation, CSF = cerebrospinal fluid, ♂ = male, ♀ = female, [a] Sample sizes not stated in article, [b]Range reflects 5th – 95th percentiles, [c] Based on 98 patients' data, [d] 95% confidence interval

Table 5.4: NSE data from clinical studies (µg/L)

Authors	Age	Pathology	N	Specimen	Mean (SD)	Median	Range	Cases> Controls
Paediatric cohort								
Rodriguez-Nunez et al (2003)	1-168 months	Controls	160	CSF	1.52 (1.01)			
		Viral meningitis	46		2.87 (1.21)			✓
		Bacterial meningitis	45		2.47 (1.59)			X
		Tuberculous meningitis	9		2.25 (0.49)			X
Mixed cohort								
Lima et al (2004)	13-82 years	Controls	8	Serum	4.7 (2.3)	4.9		
				CSF	6.3 (3.6)	6.1		
		Meningitis	11	Serum	5.8 (4.6)	5.4		X
				CSF	4.9 (4.6)	4.4		X
		Neurocysticerosis	25	Serum	6.6 (3.4)	7.1		X
				CSF	3.9 (3.2)	3		X
		Encephalitis	7	Serum	20.9 (19.2)	9.2		✓
				CSF	35.8 (39.2)	29.7		✓
Adult cohort								
Lins et al (2005)	41 (13) years	Controls	13	Serum	5.82 (1.75)			
				CSF	6.78 (2.1)			
		Bacterial meningitis	11	Serum	5.1 (1.3)			X
				CSF	8.38 (3.7)			X
		Viral meningitis	13	Serum	5.73 (3.7)			X
				CSF	8.23 (3.4)			X
Mokuno et al (1983) [a]		Controls	18	CSF	4.5 (1.2)		1.3-7.2	
		Encephalitis	6		11.3 (12.9)		3.4-40	✓
		ALS	10		6 (2.1)		2.5-10	✓
		Cerebral infarction	10		9.9 (6.2)		3.3-25	✓
		Parkinson's disease	18		7 (3.6)		0.8-16	✓
		Cervical spondylosis	9		8.7 (3.4)		4.1-14.4	✓
		Acute P (R) N	5		6.2 (2.3)		4-10	✓
		Chronic P (R) N	6		2.5 (0.9)		1.2-4.2	✓
		Spinocerebellar degeneration	10		7.2 (2.5)		3.2-10.8	✓
Song et al (2012)	Adults	Control	37	Serum	10.42 (6.06)			
				CSF	9.46 (5.76)			
		Tuberculous meningitis	15	Serum	8 (2.17)			X
				CSF	12.35 (3.52)			X
		Aseptic meningitis	28	Serum	9.57 (7.16)			X
				CSF	9.79 (6.1)			X

NSE = neuron specific enolase, N = sample size, Range = 25th to 75th percentile unless otherwise stated, SD = standard deviation,' >' greater than, '<' less than, CSF = cerebrospinal fluid, ✓ = cases> controls at $p<0.05$, X = case>controls $p>0.05$, [a] Only pathologies with S100B levels significantly greater than controls included in table, further detail in original article

Table 5.5: GFAP Reference Ranges (µg/L)

Authors	Age group	N	Specimen	Mean (SD)	Median	Range
Mixed cohort						
Rosengren et al (1992)	1.3-29 years	13	CSF	66.6 (17.2)		
Rosengren et al (1994)	16-77 years	25	CSF			100-1300
Fukuyama et al (2001)	1-25 years	13	CSF	2.96 (1.04)		
	26-55 years	9	CSF	2.80 (1.46)		
	> 65 years	8	CSF	3.99 (1.55)		
Adult cohort						
Rosengren et al (1994)						
Vissers et al (2006)	Not specified	46	Serum			<0.014-0.066
Van Geel et al (2002)	21-70 years	72	Serum		0.15	0.15-0.49
Missler et al (1999)	20-65 years	70	Serum		0.004	0.002-0.049
Dotevall et al (1996)	20-61 years	24	CSF	121 (87)		

N = sample size, Range = minimum-maximum, SD = standard deviation, CSF = cerebrospinal fluid

Table 5.6: GFAP data from clinical studies (µg/L)

Authors	Age	Pathology	N	Specimen	Mean (SD)	Mdn	Range	Cases> Controls
Paediatric cohort								
Shahim et al (2013)	0.4-15.9 years	Infectious and inflammatory CNS disorders	101	CSF			0.5-100	
Tsukahara et al (2013)	0.3-15.8 years	Acute encephalitis/ encephalopathy		CSF	24.53 (2.63)			
Adult cohort								
Jesse et al (2013)	49 (12) years	Bacterial meningitis 1 [a]	7	CSF	176.97 (195.3)			✓
				Serum	0.75 (1.23)			x
		Bacterial meningitis 2[a]	21	CSF	22.53 (32.37)			✓
Dotevall et al (1996)	20 years	Lyme neuroborreliosis	20	CSF	592 (596)			✓
Grahn et al (2013)	15-80	Varicella zoster virus	20	CSF			0.34-2.67	

GFAP = glial fibrillary acidic protein, N = sample size, MDN= median, Range = minimum – maximum, unless otherwise stated, SD = standard deviation,' >' greater than, '<' less than, CSF = cerebrospinal fluid, ✓ = cases> controls at $p<0.05$, X = case>controls $p>0.05$. [a] Data reported from 2 study centres, patients from the second centre only had CSF examined

CHAPTER 6: BIOMARKERS OF INFLAMMATION IN TBM: CYTOKINES AND CHEMOKINES

The prolific host inflammatory response is considered one of the most important determinants of poor outcome in TBM. Cerebral inflammation is responsible for the formation of the thick basal exudate which coats the major vessels of the brain and causes cerebral vasculitis, which obstructs the flow of CSF and precipitates HCP and raised ICP, and which ultimately puts the brain at risk of ischaemia. Understanding the cerebral inflammatory response to TBM, therefore, is key in understanding the pathophysiological consequences of the disease and in developing avenues for effective treatment. This chapter aims to review the current literature on the central nervous system (CNS) immune response to TBM, focussing on the role of cytokines.

CNS INFLAMMATION

Traditionally it was believed that the CNS was an immune naïve domain; however, the CNS is indeed capable of mounting a local immune response manifested by the intrathecal production of inflammatory mediators. Unfortunately CNS inflammation is often ineffective in destroying the invading pathogen [18]. An important difference between the CNS and other bodily tissues is the presence of the blood brain barrier (BBB) which effectively reduces the access of leukocytes and plasma components to the intracranial compartment. Consequently, CNS inflammation is dependent primarily on local production of inflammatory mediators although some systemically produced cytokines may gain access through specific transport systems on the BBB or through a leaky BBB [18]. Once bacteria gain access to the brain, the poor innate local immunity allows their survival and replication. The release of bacterial wall components in the CNS triggers an inflammatory response characterised by the production of cytokines and the recruitment of leukocytes [25-27].

Cytokines

Cytokines are a network of proteins secreted by leukocytes and other non-immune cells which interact to stimulate, generate, and regulate an immune response through pro- and anti-inflammatory activities. These include the attraction of leukocytes and the transmigration of these cells from the circulation into the target tissue through the activation of adhesion molecules on the endothelial surface of blood vessels. Regulation of the immune response is governed by the anti-inflammatory activities of cytokines; the specific nature of the immune process, therefore, is dictated by the temporal course of the immune response and the synergistic interplay between pro- and anti-inflammatory cytokines [359].

Chemokines

Chemokines are a group of cytokines which exhibit chemoattractant qualities that induce leukocyte migration. They are divided into two subfamilies, based on whether their cysteine residues are adjacent to each other (CC) or separated by an intervening amino acid (CXC). The CC subfamily attracts predominantly monocytes/macrophages, but also lymphocytes, basophils, eosinophils, natural killer (NK) cells and dendritic cells. They bind to receptors CCR-1 through to CCR-9, and play a key role in granuloma formation against TB. The CXC subfamily attracts mainly neutrophils and is further divided into the ELR-CXC and the non-ELR-CXC chemokines, depending on the presence of the ELR motif on the first cysteine of their amino acid sequence. Their receptors have been labelled CXCR1 through to 5. Most chemokines are induced by pro-inflammatory cytokines including IL-1, TNF-α and IFN-γ. Release at the site of infection generates a chemoattractant gradient that enables the navigation and homing of effector leukocytes. Chemokines act on the target cell's membrane protein composition to facilitate the chemotaxis of leukocytes from the circulation into inflamed tissues. They also induce or inhibit angiogenesis, hematopoietic precursor cell development, embryogenesis, lymphogenesis and wound repair. Chemokine release initiates the activation of serine or threonine kinases which regulate chemokine activity by phosphorylating chemokine receptors and thereby down-regulating chemokine-mediated leukocyte responses [18,360-362]. In the brain they play an important role in the recruitment of leukocytes from the blood stream into the cerebral site of infection [360,363].

Cytokine release

Microglia and astrocytes are the resident immune cells of the CNS. Microglia act as CNS mononuclear phagocytes participating in the innate immune response. In their resting state microglia exhibit an immunologically quiescent phenotype that lack phagocytic activity. In comparison, active microglia are highly phagocytic and are involved in the induction of neuroinflammation and regulation of T-cells through antigen presentation [364]. Astrocyte foot processes form a key part of the BBB and activated astrocytes consequently play an important role in controlling leukocyte infiltration into the CNS. Astrocytic and microglial release of proinflammatory cytokines initiates and regulates CNS inflammation. These immune mediators include TNF-α, IFN-γ, IL-1β, IL-6, IL-8, IL-12 and nitric oxide (NO) [365] as well as chemoattractant cytokines. Additional sources of cytokine release include ventricular ependyma and meninges [18]. Upon release cytokines induce the rapid although transient expression of cell adhesion molecules on vascular endothelial cells. Leukocytes attracted to the site of inflammation adhere to these endothelial cells and migrate from the circulation into the CSF via diapedesis [162]. Evidence suggests that some cytokines may perform dual roles exhibiting pro- and anti-inflammatory actions depending on the disease course, their concentrations, and their synergistic interaction with other mediators. The consequences of these different distribution patterns

of pro and anti-inflammatory mediators on pathogen clearance and brain integrity is not yet fully understood [365]. Several of these immune molecules may have both beneficial and deleterious effects on the CNS depending on their concentrations, at which point during the disease course they are released and the duration and nature of the inflammatory condition. Chronic activation has been implicated in cytotoxicity [364].

A study of physiological levels of cytokines in the CSF found that the ratio of pro- to anti-inflammatory cytokines was greater in the CSF than in serum. This may reflect a tendency in the CNS towards a pro-inflammatory immunologic pattern; however, several immune mediators also play an important role in embryogenesis and the maintenance of normal CNS function [365]. There is a paucity of data on normal values for cytokines in the CSF. Maier et al [365] examined physiological levels of CSF cytokines in 113 adults. Their results report medians and ranges for IL-6 (6, 1-34 pg/ml), IL-8 (39, 5-90 pg/ml) and IL-10 (0.9, 0-39 pg/ml). As part of a study on paediatric traumatic brain injury, Bell et al [366] established means and standard deviations for 20 healthy control children for IL-6 (20.6 ±5.8 pg/ml) and IL-10 (8.9 ±7.5 pg/ml). An intrinsic challenge in establishing reference values is the variability introduced by differences in testing platform (for example enzyme-linked immunoassay versus Luminex)[367,368], age [369,370], heterogeneity amongst sample individuals [370] and sample size across studies [365].

Cytokine effects on the brain

Although inflammatory mediators are important in mounting a defence against the invading pathogen, the inflammatory process often precipitates substantial damage to the brain in several ways that are summarized below.

BBB permeability. The BBB is integral to maintaining homeostasis in the brain, allowing the influx of required nutrients and the efflux of by products while preventing the movement of cytotoxic molecules into the CNS. This barrier is composed of specialised endothelial cells lining the lumen of cerebral vessels which are functionally and morphologically unique. Among their exclusive properties are intercellular tight junctions, selective transporters and the presence of enzymes which constitute a metabolic barrier [371]. These properties effectively and tightly regulate access to the CNS. Increased permeability of the BBB is considered a hallmark of CNS infection [18] and is thought to be a consequence of tight junction opening, augmented pinocytotic activity and/or the formation of transendothelial channels [371]. This facilitates the leakage of proteins and other molecules into the cerebral space which contribute to CNS inflammation, alterations to the neuronal microenvironment, and vasogenic oedema with increased intracranial pressure due to the protein-induced osmotic gradient. Experimental models of meningitis have implicated TNF-α, IL-6, IL-1β and cytokine-induced NO, and matrix metalloproteases (MMPs) as major contributors to BBB disruption [18,371,372].

MMP-9 has been implicated in BBB breakdown in TBM and the fact that it may remain elevated for up to 60 days after the initiation of treatment suggests that the BBB may be compromised months after the initiation of TB treatment[372]. Chemokines have also been implicated in BBB breakdown by way of chemotaxis-induced leukocyte migration into the brain [363]. Neutrophils migrating into the CSF further contribute to BBB damage by the release of toxic oxygen species and vasoactive lipid autocoids such as platelet activating factor, leukotrienes and prostaglandins [162]. This breakdown of the brain's defences increases its vulnerability to further damage.

Cerebral blood flow (CBF) and metabolism – The inflammatory exudate in the subarachnoid space coats the vessels of the Circle of Willis causing vasculitis, vasospasm and vascular occlusions with resulting ischemia. CBF is further compromised by the loss of cerebral pressure autoregulation which subjects the brain to fluctuations in systemic blood pressure and places it at risk of both hypertension (increased intracranial pressure and cerebral oedema) and hypotension (ischemia) [206]. Uncoupling of metabolic demand and CBF increases this risk. Changes in CBF have been shown to correlate with cytokine concentrations. Elevated CSF IL-1 and IL-6 were associated with increased blood flow velocity in the middle cerebral artery recorded with transcranial Doppler in patients with bacterial meningitis [210]. TNF-α has been implicated in reducing CBF by increasing the vascular reactivity of the capacitance vessels, and by decreasing cerebral oxygen uptake and cellular metabolic demand. It also appears to increase cerebral anaerobic metabolism manifested by an increase in CSF lactate. Several of these changes are mediated by TNF-induced NO activation [209]. IL-1β may increase cerebral blood volume by reducing the apparent diffusion coefficient in the brain and increase BBB permeability [373].

Effect on CNS and vascular cells. Endotoxins reduce metabolism and may alter astrocytic morphology. TNF-α may cause cell injury through the production of reactive oxygen free radicals[207] and may contribute to vascular injury by promoting thrombosis [30], and cytokines may have a direct effect on neurons [18].

The classic hallmarks of CNS inflammation thus include BBB disruption, leukocyte infiltration from the circulation into the brain, cerebral oedema formation, activation of CNS resident immune cells, and release of immune mediators [359,363].

CNS INFLAMMATION IN TBM

Studies on inflammation in TBM have primarily focussed on a particular selection of cytokines. This review highlights the cytokines which have been commonly investigated.

Cytokines

TNF-α

In the inflamed CNS TNF-α is secreted by activated microglia and astrocytes. This pro-inflammatory mediator modifies the BBB, induces adhesion molecule expression on cerebral microvascular endothelial cells and activates infiltrating systemic immune cells as well as CNS glial cells. It also appears to play an important role in initiating meningeal inflammation [374]. At high concentrations TNF-α can cause severe illness, including cachexia, septic shock and death [18,364].

IFN-γ

This is a characteristic cytokine of Th1 immune cells and a potent inducer of TNF-α and NO production. It is involved in endothelial cell activation and induction of class I and II MHC molecules[18,375]. It is also an important cytokine in the formation and maintenance of TB granulomas [376]. Together with TNF-α and vitamin D3, IFN-γ may induce an antimicrobial pathway that results in the inhibition or destruction of *Mtb* in macrophages, although the bacillus may impair the macrophage response to IFN-γ. It may exhibit anti-inflammatory properties by inhibiting pro-inflammatory cytokine production or upregulating the production of their antagonists and thereby helps regulate the immune response [377].

IL-1 β

IL-1β is secreted by activated microglia and astrocytes [18,364]. It activates both resident and infiltrating immune cells thereby stimulating the production of further TNF-α and IL-6, chemokines and adhesion molecules. It also stimulates phagocytic functions and tissue repair. It is implicated in various CNS pathologies, is involved in the initial stages of meningeal inflammation and may have a detrimental effect on the brain [374]. IL-1β may increase BBB permeability by recruiting neutrophils, impair diffusion in the brain and lead to an increased cerebral blood volume [373]. Experimental data indicate that IL-1β stimulates astrocytic production of NO and that this is enhanced by the co-presence of TNF-α and IFN-γ [378]. NO is an important inflammatory mediator as well as a free radical; over-production may lead to tissue damage and further inflammation [379]. IL-1 receptor agonist (IL-1Ra) is an important regulator of immunity released in response to IL-4, IL-10 and IFN-γ. It occupies the IL-1 receptors and inhibits the binding of IL-1β. Large quantities of IL-1Ra are required to suppress IL-1β and it has been suggested that IL-1Ra may limit the aggressiveness of the immune response and the extent of disease except when levels are inadequate in the face of a prolific pro-inflammatory process. The balance of IL-1β and IL-1Ra is, therefore, an important indicator of disease severity [171]. IL-1β is further regulated by the IL-1 decoy receptor which binds IL-1β without producing any signal and IL-1 receptor accessory protein which blocks IL-1β activity [380].

IL-6

IL-6 is secreted mostly by astrocytes and is involved in both acute and post-acute inflammation. It promotes inflammation by stimulating IFN-γ release but also regulates immune responses by suppressing gene expression of pro-inflammatory cytokines, and promotes the proliferation of astrocytes and neuronal survival in the CNS [364,381].

IL-10

IL-10 is an anti-inflammatory cytokine produced by activated monocytes and T-cells that inhibits the production of pro-inflammatory cytokines TNF-α, IL-1α, IL-1β, IL-6 and IL-12 as well as some chemokines. It is, therefore, important in the controlling inflammation and conferring protection on the CNS from the potential harm of pro-inflammatory cytokines like TNF-α [18,382-384].

IL-12

Activated monocytes and dendritic cells are the predominant sources of IL-12, which activates cytolysis, induces IFN-γ synthesis in NK cells and T-cells and bridges innate and adaptive immunity by stimulating the generation of Th1 cells [18]. IL-12 is composed of 2 covalently linked chains, the constitutively expressed p40 and the induced p35 which form the bioactive IL12p70. IL12p70 is difficult to detect in the CSF of patients with CNS infections while the p40 subunit has been detected [364,375].

IL-8 (CXCL-8)

This low molecular weight cytokine is a potent chemoattractant and activator of neutrophils and lymphocytes [25,170,361]. It promotes adherence to the vascular endothelium by enhancing the expression of β_2-integrin, which allows the migration of neutrophils from the vascular space to the site of IL-8 production [25]. IL-8 also eliminates microorganisms by enhancing bactericidal activity through nonoxidative mechanisms.

Interferon-inducible protein -10 (IP-10/CXCL-10)

IP-10 is produced by astrocytes and astroglia in response to TNF-α, IL-1β and IFN-γ[385]. In addition to attracting T lymphocytes, this chemokine also stimulates NK cells and promotes T cell migration [361].

Growth regulated oncogene (GRO)

GRO is secreted by neurons and astrocytes. It interacts with type 2 CXC receptor on neutrophils and is an important chemokine in the attraction of neutrophils to the CNS [386,387]. It is also involved in oligodendrocyte proliferation and improves neurological recovery after demyelination [388].

Monocyte chemoattractant protein -1 (MCP-1/CCL-2)

This is the most potent activator and chemotactic factor for monocyte recruitment and migration and also attracts CD4+ T cells. Elevated levels in the serum may indicate a strong gradient to recruit monocytes to a local site of infection [361,389]. MCP-1 is present in the perivascular space of the BBB and is key in increasing BBB permeability by altering the tight junction complexes on the endothelial cells making up the BBB. It acts through the receptor CCR2 which is present on both endothelial cells and monocytes and it has been suggested that the MCP-1 induced recruitment of monocytes may further contribute to BBB breakdown. Targeting MCP-1/CCR2 may be an avenue to regulate CNS inflammation and reduce BBB breakdown [362]. Elevated levels of this chemokine are also implicated in an increased susceptibility to TBM as it appears to down regulate human macrophage IL-12 production, a cytokine integral to initiating the immune response to TB [390]. TNF-α and IL-1β have been shown to induce MCP-1 [385].

Macrophage inflammatory protein 1-α (MIP-1α /CCL-3)

MIP-1α induces activation and proliferation of T-cells and macrophages, recruits mononuclear cells and promotes Th-1 cell differentiation [387,390,391].

Regulated upon-activation, normal T-cell-expressed and secreted (Rantes/CCL5)

This chemokine is involved in chemotaxis and activation of mononuclear cells, it also induces activation and proliferation of T-cells. In the CNS it is secreted by astrocytes and microglia [390,391] in response to TNF-α and IL-1β[385].

Studies in TBM

Animal models

The earliest work describing the pathogenesis of TBM was done by Rich and McCordock using a guinea pig model of TBM. Infecting the animals hematogenously with TB did not cause direct meningeal inflammation but was associated with the development of a tubercle in the sub-pial tissue adjacent to the meninges, the Rich's focus, from which bacilli were discharged into the CSF resulting in meningitis [20].

Although the guinea pig is a very sensitive model for TB, immunological reagents are not currently available to investigate the immune response and subsequent work has focussed on other animals [392]. In a rabbit model of TBM, animals were infected with a non-virulent strain of mycobacteria that secreted murine TNF-α. Results indicated that the production of TNF-α rendered this non-virulent strain virulent as the TNF-α triggered an inflammatory cascade, stimulated the breakdown of the BBB

and an influx of leukocytes, precipitated cerebrovascular damage and blocked control of *Mtb*. These findings suggest that TNF-α may be a determinant in the pathogenicity of TBM [393]. In subsequent studies with this model these authors studied the therapeutic effects of immune modulators on cytokine levels and outcome, their findings are discussed later in this paper [394,395].

Although mice are not as sensitive to TB infection they have the advantage of generating an innate immune response which bears similarities to that in humans and genetically modified species allow the investigation of specific immune mediators[392]. Mazzolla *et al* [396] investigated the role of natural resistance-associated protein 1 (Nramp1) in murine resistance to TB infection. Nramp1 resistant and Nramp1 susceptible mice were intracranially infected with *Mycobacterium bovis* and the immune response was investigated. Results showed that the mice differed in microbial load and in the kinetics and concentrations of immune mediators MIP-2, IL-12, IL-1β, IFN-γ, IL-6 and TNF-α. This suggests that Nramp1 may alter cytokine expression and plays a role in whether host defences are able to contain the infection. In a murine model by Van Well et al [397] using intracerebral inoculation, cytokine levels were not elevated in brain homogenates relative to controls. MCP-1 and MIP-2 (an animal analogue for IL-8) were, however, elevated for up to 24 weeks post-infection demonstrating that while an acute inflammatory response was not discernible a chronic response had occurred. Be *et al* [398] developed a murine model focussed on hematogenous dissemination to the CNS through intravenous inoculation with *Mtb*. They found that although *Mtb* colonies were recovered from the brain, no inflammatory process was discernible. In comparison, the lung demonstrated significantly higher cytokine levels, leukocyte infiltration as well as gross TB pathology in the tissue. It is postulated that the inflammatory response in the CNS may be delayed by its poor immune baseline and that overt inflammation may have been detected if animals were sacrificed weeks or months later. Using intracerebral inoculation, Lee *et al* [399] compared peripheral and CNS immune responses to TB infection. They found that the CNS uniquely demonstrated the presence of IFN-γ IL-17 double-positive CD4 T-cells and that microglia were the primary source of intracranial TNF-α suggesting that resident CNS cells played an important role in the local immune response. Bolin *et al* [400] developed a swine model for disseminated TB by inoculating pigs through the blood, trachea or lymph nodes. Only a few animals developed meningitis, but none fulminant TBM. All of these animal models employed either intravenous or intrathecal inoculation as it is challenging to develop a model which accurately reflects human pathogenesis of TBM from a primary pulmonary infection. An attempt was made by Pando *et al* [39] whereby mice were intratracheally inoculated with *Mtb* strains isolated from the CSF of patients with TBM. The animals developed disseminated as well as cerebral TB, likely through hematogenous seeding as is presumed to occur in humans. Examination of the brain showed evidence of sub-pial lesions which had ruptured into the surrounding subarachnoid space and produced an inflammatory exudate, and immunohistochemistry was positive for IFN-γ and TNF-α, as well as mycobacterial antigens in astrocytes, microglia, ependymal and meningothelial cells. Mice

infected with strains isolated from pulmonary TB did not result in remarkable cerebral pathology, suggesting that strains of a specific genotype may have greater ability to disseminate from the lungs to the CNS.

Patient studies

Cytokine levels

Elevated CSF levels of TNF-α, IFN-γ, IL-1β, IL-10, IL-6, IL-8, IL-2, MCP-1 and MIP-1α have been reported in adult and paediatric TBM [25,162,170,381,401-404]. TNF-α levels have sometimes been recorded at low levels [372,381,401,404]. The interplay between TNF-α and its receptors may contribute to the biological activity of this cytokine based on its concentration [372]. IL-12p70 was either undetectable in CSF or detected only at low levels [170,381]. This may have been due to the inhibitory presence of IL-10 in the samples tested or may suggest that IL12 has a poor presence in the CSF. Cytokine levels may vary across studies, even when the same testing platform has been used, possibly as a function of the timing of sample collection, the synergistic interplay between pro- and anti-inflammatory cytokines, and variability in the strain of *Mtb* [162]. To exemplify, IL-10 levels in adult CSF were 10-fold lower in a study by Kashyap et al[402] relative to Mastroianni et al [170]; however, the low levels of IL-10 in the former may indicate a developing rather than an established inflammatory cascade in which pro-inflammatory cytokines were still dominant. Little data are available on children. Due to the large variability in cytokine levels within adult cohorts, it is difficult to determine age differences as paediatric values mirror values from some adult studies and diverge from others. In a study examining both adults and children age was not found to have a significant effect on cytokine levels [25]. The ubiquitous finding across all studies is that cytokine levels are elevated in TBM with some decrease in levels after the initiation of treatment. The degree of the attenuating influence of treatment, however, varies between cytokines, and inflammation is continuous despite drug administration. Table 6.1 and 6.2 provide levels of cytokines and chemokines reported in the TBM literature.

Differences between CSF and serum values

Systemic levels of cytokines may not reflect the immune response at the site of disease. Increased serum levels may result from an overproduction of local CNS mediators that leak into the circulation, thereby generating a gradient for the recruitment and migration of leukocytes to the active site [382]. Few studies have examined both CSF and serum cytokine levels simultaneously; in an adult TBM study Thwaites et al [372] showed that CSF far outstripped serum as a sample for the detection of TBM related cytokines. In serum TNF-α was undetectable, but IL-8, IFN-γ and IL-10 had initial elevations followed by an immediate steep decline within the first few days or the first week, and serum values were up to 40 times lower than those found in CSF. In a study by Mastroianni et al [360] IL-8, MIP-1α

and MCP-1 were not detectable in serum. These findings suggest the compartmentalisation of the immune response to the site of infection. However, in a paediatric study Babu et al [403]found that levels of TNF-α and IL-8 were elevated in both CSF and serum relative to controls. As a result of the sparse data and the poor detection of cytokines in serum, the remainder of this article focuses on CSF findings.

Temporal profile

Two experimental *in vitro* studies [383,384] of human monocytes and microglial cells stimulated with lipopolysaccharides demonstrated the acute release of pro-inflammatory markers including TNF-α, IL-1α, IL-1β, IL-6 and IL-8 by 4-8 hours followed by the delayed production of anti-inflammatory cytokine IL-10 at 7-8 hours. These kinetics are mirrored in patient studies; initial pro-inflammatory cytokine levels are highest followed by a subsequent decline over several weeks with cytokines only becoming undetectable months after the commencement of treatment. TNF-α peaks early and usually remains most chronically elevated with levels detectable for up to 16 months post-initiation of treatment [170,401,404]. Persistent elevation regardless of treatment suggests the importance of TNF-α in the chronic on-going inflammatory process in the CNS. Depletion of TNF-α during the chronic phase of pulmonary TB has been associated with granuloma destruction and exacerbation of infection [405]. Extrapolated to the CNS, TNF-α may be involved in the immune reaction to TBM as well as protection against it [170]. IFN-γ, IL-6 and IL-8 appear to have an initial peak followed by persistent elevation in TBM although not for as long as TNF-α [170,372,404]. In a study examining the relationship between intracranial granuloma (IG) formation and IFN-γ, Mansour et al [376] found that admission CSF levels of IFN-γ were not associated with the presence of IG's on computed tomography (CT) brain scans but they were associated at 1 month post-admission in spite of IFN-γ levels being highest at admission. The authors suggested that the on-going inflammatory process rather than the intensity of the initial response may be important for IG formation. IFN-γ, in conjunction with other immune factors, plays a role in this process. Initial levels of anti-inflammatory cytokines like IL-10 may be low if the samples are taken when the inflammatory cascade is still developing. The prolonged elevation of IL-10 in patients may down regulate continued inflammatory activity in TBM [170,372,376,402]. Whether this preserves the CNS or hinders the immune clearance of the TB infection is unclear. IL-8 appears to decrease most rapidly in TB meningitis; since IL-8 is predominantly a neutrophil attractant, this raises questions about differential immune cell attraction and activation in the inflammatory process over time [25,360]. In an experimental study investigating the production of cytokines by microglia infected with different strains of *Mtb*, it was found that the virulence of a strain may be determined by the degree to which it is able to supress the immune response in the brain with more virulent strains inhibiting early release of TNF-α and IL-1β [406]. It is clear, therefore, that many factors relevant to the host as well as the pathogen may contribute to a heterogenenous temporal profile of

cytokines; however, the prolonged elevation of cytokines in the CSF of TBM patients is a common observation.

Cytokine synergism

In vitro studies have shown that microglial cell cultures treated with TNF-α and IL-6 resulted in a dose-dependent production of IL-10, which subsequently reduced the expression of class II MHC and inhibited the production of pro-inflammatory cytokines at a transcriptional level. Furthermore, IL-10 exhibited autoregulatory control of its own mRNA synthesis in monocytes [383,384]. In human studies positive correlations have been found between TNF-α, IL-1β, IFN-γ and IL-10 in adults [170,171] while in a paediatric cohort only IFN-γ and IL-1β were significantly associated and not TNF-α and IL-1β [401]. Hasan et al [382] suggests that a positive correlation between the pro-inflammatory cytokine IFN- γ and the anti-inflammatory cytokine IL-10 represents a balance between pro- and anti-inflammatory mediators which may be lost in cases of severe extrapulmonary TB disease. Akalin et al [171] found that the ratio between IL-1β and IL-1Ra indicates the aggressiveness of the inflammatory process and the severity of disease when skewed towards IL-1β. Similarly, the association between chemoattractants and anti-inflammatory cytokines may also indicate the degree of control exerted over the inflammatory process. Therefore, one possible mechanism for disease progression could be an imbalance of anti-inflammatory cytokines in the face of elevated proinflammatory mediators. In a comparative study across levels of TB disease severity, the balance between and levels of pro- and anti-inflammatory mediators varied [382]. IL-8 and IFN-γ have shown an association with MMP-9 and its inhibitor TIMP-1[372]

Association with disease markers

Cytokines demonstrate mixed associations with CSF markers of disease and data across studies are conflicting. In several studies TNF-α and IL-10 were not associated with CSF protein, glucose or white cell count but showed a correlation with CSF adenosine deaminase (a marker of local immune response) [170,171,401]. The association between TNF-α and CSF lactate is mixed; some studies finding an association [209,372] while others found none[401]. Correlation results for IFN-γ have not shown an association with any CSF markers except lactate and conflicting results have been found for its association with CSF protein [170,401]. IL-1β was significantly correlated with CSF protein and lactate only in a paediatric study [401]. Further evidence [171,381] demonstrates no association between cytokines and CSF white cell counts which supports the theory that resident CNS cells are responsible for generating inflammation in the CNS. Cytokine levels have also remained persistently elevated despite normalisation of these CSF markers. It has also been suggested that the lack of an association between chemokine IL-8 and CSF leukocyte levels may denote that IL-8 has a role primarily in the early recruitment of leukocytes [25,360]. MCP-1 and MIP-1α have been associated with monocyte levels

[360]; however, factors other than cytokines are also important in leukocyte recruitment and maintaining CSF pleocytosis.

Association with severity and outcome

The association between cytokine levels and various markers of injury severity and outcome is also conflicting. The British Medical Research Council grading system [76] for TBM has shown a significant correlation with outcome [58,372] and so remains a widely used approach to quantifying disease severity on admission. Patients in Stage I present with a Glasgow Coma Score (GCS) of 15/15 and no focal neurology, in Stage II with a GCS 11-14 or 15 with focal neurology, and in Stage III with a GCS ≤ 10. Several studies [162,170,381,401,402,404] have found no association between TBM stage and the levels of TNF-α, IL-10, IL-1-β, IL-6 or IL-8. However, in a couple of studies a significant positive correlation was demonstrated between the levels of TNF-α and IFN-γ and TBM stage [403] and for TNF-α and IL-1β between Stage I and II [407], although the potential contribution of duration of illness cannot be excluded as a confounding factor.

The association between cytokines and mortality and morbidity also is poor. TNF-α, IL-1β and IL-10 have shown no association with the development of neurological sequelae [170,171]. TNF-α, IL-6, IL-10, IL-1β and IL-8 do not show a correlation with 3 month clinical outcome and TNF-α, IL-2, IL-6, IL-1β, IL-8, IL-10 and IFN-γ levels have not been associated with mortality [25,171,360,372,381,402,404]. Only in HIV infected (HIV+) patients were reduced levels of IFN-γ associated with death [381]. These results are surprising in light of the prevailing theory that the prolific host immune response is responsible for the poor outcome in TBM and suggest that other factors play a role in the pathophysiology and course of this disease while inflammatory markers serve to indicate an ongoing inflammatory process. The degree to which the host responds to an invading pathogen as manifested in the degree of cytokine production, recruitment of immune cells and inflammatory mediators may be determined by genetic factors. This genetic variability in conjunction with the compartment of inflammation and the progress of disease contribute to variations of severity and outcome in individual patients [18].

A study examining magnetic resonance imaging (MRI) at 60 days found that hydrocephalus correlated positively with IFN-γ, IL-10 and IL-6; tuberculomas correlated positively with IL-6 and IL-10 and infarcts with IL-8 and IL-10 [114]. In a more recent study [162] examining the relationship between CSF cytokines and MRI findings in TBM patients, cytokine levels on admission were not correlated with initial MRI findings (including the presence of exudate, tuberculomas, infarcts and hydrocephalus) or radiological findings seen after 3 months. This study was conducted on a small sample size and levels for individual cytokines demonstrated large variation across this cohort. These factors in combination may have contributed to the lack of correlation, or it is possible that the level of inflammation does not directly reflect the level of tissue damage occurring as a result of ischemic

processes. In the study on IFN-γ and intracranial granuloma formation mentioned above [376], the authors found that it was not the level of initial IFN-γ but rather the prolonged elevation that may have a role to play. Therefore, considering concentrations of cytokines as well as their chronicity may be important in elucidating the relationship between radiological findings and cytokines.

Association with HIV

In a study examining the cytokine profiles of HIV+ patients with TBM, Patel et al [407] found no difference between HIV+ and HIV uninfected (HIV-) individuals for TNF-α, IFN-γ and IL-10. The CD4 counts between these 2 groups, however, were not significantly different and perhaps selection of a more immune compromised HIV cohort may have produced a different result. Results also indicated a poor correlation between CD4 count and cytokine levels suggesting that there may be other sources of cytokine release. The authors also contend that the immune response in the context of HIV may be neither Th1 nor Th2 but rather a non-differentiated response as would be seen early in immune activation. In a larger study by Simmons et al [381] only the levels of IL-10 differed significantly between HIV+ and HIV- TBM patients, with lower levels of IL-10 and a subsequently higher IFN-γ:IL-10 ratio found in the HIV+ cohort. The authors suggest that this finding may indicate a more pro-inflammatory immune response in HIV. It is noteworthy that this appears contrary to the suggestion that the HIV+ immune response to TBM is impaired, as evidenced by less basal enhancement seen on CT scans and less exudate in post-mortem studies[192,193]. Reduced IFN-γ levels were significantly associated with mortality in the HIV+ patients and it is possible that IFN-γ may confer some protective advantage. Further evidence suggests that TNF-α and IL-10 are not associated with HIV status [170]. A recent study on adult paradoxical TBM-IRIS found that a combination of high TNF-α and low IFN-γ in admission CSF significantly predicted the development of paradoxical TBM-IRIS [111].

The effect of immunomodulatory adjunctive chemotherapy

Despite the fact that the inflammatory cascade has been identified as an important source of brain damage in TBM, very few therapeutic studies aimed at modulating the immune response have been conducted. One study in children [101] and a later study in adults [172] showed that adjunctive steroid therapy improved mortality, although the mechanism of action was unclear. An *in vitro* study of microglia [408] found that infected microglia released a range of cytokines including TNF- α, IL-6, IL-1β, MCP-1, Rantes, IP-10 and IL-8. Upon treatment with dexamethasone either pre- or post-TB infection, there was a marked decrease in almost all these inflammatory mediators. An *in vivo* study [394] showed that treatment with thalidomide, a drug with immunomodulatory effects, decreased CSF TNF-α levels and leucocytosis, and that in combination with anti-TB antibiotics it improved survival to 100% from 50% achieved with antibiotics alone .

In a formal study on thalidomide in children, the safety and tolerability phase demonstrated that thalidomide decreased CSF levels of TNF-α, reduced basal enhancement on CT brain scans, and promoted the resolution of established tuberculomas while decreasing the incidence of new ones [103]. These findings, however, were not replicated in the randomised controlled trial which had to be prematurely aborted due to adverse side effects in the thalidomide arm of the trial [104]. Cytokine levels between treatment and placebo groups were not significantly different; however, there was an increase in plasma IL-12 in the thalidomide group, which may signal an enhanced Th1 response. The authors suggest that, in the context of an already exaggerated baseline immune response, this may have led to the worsening of vasculitis and ischemia in these children, contributing to their poor response to treatment. In a large randomised controlled trial in adults, dexamethasone improved mortality and decreased the incidence of hydrocephalus and infarction on MRI, but it did not significantly affect the levels or temporal profile of a large range of inflammatory mediators including IL-6, IL-10, IL-12p70, IL-1β, TNF-α and IL-8, IP-10, MCP-1, RANTES and MIG in CSF or blood[172]. The authors suggest that the benefit of steroids, therefore, may not be through immunosuppression but via mechanisms unrelated to inflammation. Furthermore, the question is raised as to whether immune mediators have as much of a causal role in the poor outcome of TBM disease as current thinking suggests. Several additional studies examining inflammatory mediators in TBM have found that the administration of steroids has not significantly altered cytokine levels or their temporal profile [25,162,170,360,401]. In part this may be due to late presentation in which case much of the inflammatory response has already developed and the delivery of therapeutic molecules to the target site across the BBB may also be limited [18].

There are a few case reports [377,409-411] in immune competent and compromised patients of the use of IFN-γ to treat protracted CNS TB refractory to regular anti-TB drugs and steroids. These reports suggest that IFN-γ aids in disease resolution. In the report by Coulter *et al* [377] gene analysis demonstrated a down-regulation of pro-inflammatory cytokine genes post-IFN-γ administration, supporting the idea that IFN-γ may also have an immunomodulatory role. Lee *et al* [411] suggest that IFN-γ may enhance drug penetration through the BBB and thereby strengthen their activity intracranially. The domain of cytokine therapy in TB is still in evolution and further research is required.

Comparison with bacterial meningitis

Studies suggest that, relative to TBM, concentrations of pro- and anti-inflammatory markers in acute bacterial meningitis are detectable at higher levels, span a much larger range during disease onset, are more easily detected in CSF samples, and decrease rapidly post treatment initiation [171,412,413]. In comparison with TB and viral meningitis, bacterial meningitis exhibits a more aggressive pro-

inflammatory process characterised by high levels of pro-inflammatory cytokines like TNF-α and lower levels of immune regulators like IL-1Ra [171]. The ongoing low level cytokine release in TBM appears indicative of chronic release in an on-going pathological process. A similar trend is seen for chemokines; in a study on CSF IL-8, MCP-1 and MIP-1α in patients with pyogenic and TB meningitis, the authors found that all three chemokines were elevated in meningitis cases on admission; however, pyogenic meningitis demonstrated the highest IL-8 and MCP-1 levels followed by a rapid decrease in the CSF over a 4 week period. In comparison TBM patients had more moderately elevated chemokine levels which remained chronically elevated and were detectable up to 32 weeks post-admission. These findings matched those of 2 paediatric studies of CSF IL-8 levels in purulent, TB and aseptic meningitis [25,414]. The differences in cytokine profiles may reflect differences in disease and the nature of immunity with pyogenic meningitis being characterised by an acute, neutrophil dominated, early resolving pattern relative to the chronic, mononuclear pattern of TBM. The role of IL-8 in pyogenic meningitis may be in the initiation of inflammation while in TBM it participates in the on-going inflammatory process. The mean duration of illness pre-admission was longer in TBM patients [25]; it is possible, therefore, that an early acute rise in chemokine levels may already have been missed in the TBM cohort due to late presentation. Bacterial and TB meningitis further diverge with respect to cytokine associations with outcome; whereas the correlation between cytokines and outcome in TBM is tenuous, several studies have shown that levels of cytokines in bacterial meningitis are indicators of disease severity[415,416] and predictors of outcome [415,417,418].

Cytokines as diagnostic markers

Several studies have examined specific cytokine levels across bacterial, viral, aseptic and TB meningitis. To date no single cytokine or combination of cytokines allows differential diagnosis with acceptably high sensitivity and specificity [18]. The distinction between bacterial and TB meningitis relative to aseptic meningitis is easily made by comparing cytokine levels because aseptic meningitis shows much lower levels. However bacterial meningitis is better distinguished by the swift decline in levels versus the chronic elevation in TBM [25,171,360,414,419]. The unique kinetics may reflect the differential attraction and activation of immune mediators, the inflammatory response and clinical course between these meningitides.

IFN-γ has shown greater sensitivity but less specificity than PCR for *Mtb* in the diagnosis of TBM [420]. IFN-γ levels are affected by anti-TB medication while PCR for *Mtb* is dependent on the bacillary load in the CSF; using both tests in combination has therefore been recommended. Although it was found that below a certain threshold of IFN-γ TBM could be excluded, this test did not differentiate between TBM and other meningitides. Studies have found that IFN-γ release assays (IGRA) are promising for the diagnosis of pulmonary TB, however they are not able to distinguish between latent and active TB. Combining IGRA with multiple cytokines and chemokines has shown that latent versus active TB

demonstrates different cytokine and chemokine correlations with IGRA. This research suggests that the diagnostic accuracy of IFN-γ in TB may be enhanced when combined with a panel of other immune markers. The utility of inflammatory mediators as diagnostic markers is limited by costs and by the fact that they are currently not routinely measured [18] however, research is ongoing and results from extra-pulmonary TB samples including pleural fluid are uncovering the diagnostic potential of cytokines and chemokines [421].

Conclusions

Cytokines are increased in TBM samples, especially in CSF. The inflammatory response in the CNS to TBM is distinct from other meningitides and appears to be acute initially followed by a low level but on-going chronic phase despite treatment. Conflicting data on elevation and cytokine associations between studies may result from variation in the frequency and duration of sampling, the timing of sampling relative to disease onset and treatment commencement, the synergism between cytokines, and the test platform used. Many of these factors are challenging to monitor and control and discrepancies between studies are consequently not likely to be easily resolved. The theory that poor outcome is a function of the prolific host immune response is perhaps too simplistic; poor outcome is not strongly associated with immune mediator levels and although steroids improve mortality they have no significant effect on cytokine levels and may provide benefit through channels other than mediating the inflammatory response. It is clear that other factors are also of importance, particularly the ischemic process which takes place as a result of raised intracranial pressure and cerebral blood vessel damage. Steroids may have limited benefit in TBM because they are unable to mitigate the vasculitis and infarction contributing to poor outcome. Markers of inflammation do not necessarily reflect the degree of cerebral tissue injury and the severity of the disease; therefore, assessing cytokines in combination with other markers of injury may improve the understanding of the pathophysiology, the disease course and outcome of TBM. Additionally, the interaction between host factors (such as nutrition, immune status, concomitant illness, age, genetics and anatomical site of infection) and pathogen factors (like strain, virulence and drug resistance) add to the complex picture of this disease. Treatment remains challenging and novel methods of intervention may benefit from an approach which encompasses these multiple factors.

Table 6.1: Cytokine concentrations in TBM patients (pg/ml)

Authors	Sample	TNF-α	IFN-γ	IL-1β	IL-6	IL-10	IL-12p70
Donald et al (1995) M = 27 months N=30	CSF[1] Week 1 Week 2 Week 3 Week 4	 13 (10-41) 10 (8-17) 10 (8-17) 10 (8-17)	 578 (10-2559) 383 (0-2348) 432 (12-2076) 282 (5-1303)	 24 (12-199) 39 (10-136) 22 (10-177) 15 (10-32)			
Mastroianni et al (1997) M = 32.3 (12-58) years N=15	CSF[1]	94.7 (13.8-236)	56.7 (<5-236)			46 (6.7-249)	<5
Yaramis et al (2001) M = 3.7 years N=37	CSF[3] Week 1 Week 2 Week 4 Week 6 1 year	 134 85.3 53.8 38.1 16.8			 504.9 128.4 26.3 5.2 5.1		
Thwaites et al (2003) >15 years N=21	CSF[4]	66 (108) 0-412	708 (887), 0-3156			2, 0-14	
Simmons et al (2006) Md 38 (15-88) years N=408 HIV- N= 89 HIV+	CSF[2]* HIV- HIV+	Not detectable in all patients	11.59 (3.19) 9.87 (3)		36.97(2.46) 40.45(3.46)	4.35 (2.2) 2.03 (2.77)	Not detectable in all patients
Babu et al (2008) M = 35.2 (6-80) years N=31	CSF Serum	35.4 (2.56) 65.6 (5.18)	77.6 (2.33) 125 (16)				
Kashyap et al (2009) M = 38 (20-72) years N=60	CSF[2] Def_TBM Prob_TBM	 29.22 (16.94) 44.64 (20.29				 4.22 (12.27) 4.22 (12.27)	
Misra (2010) M = 31.4 (10-50) years N=16	CSF[1] Admission 3 Months	 4.78 (1.8-114.64) 0 (0-15)		 0 (0-113.9) 0 (0-5.8)	 1284.65 (9.6-5000) 64.6 (0-3197.5)	 33.75 (0-296.3) 0 (0-270.1)	

CSF = cerebrospinal fluid, IL = interleukin, TNF-α= tumour necrosis factor alpha, IFN-γ = interferon gamma, IL-1ra = interleukin 1 receptor agonist, HIV= human immunodeficiency virus. M= mean, Md = median. N= sample size. [1] median and range, [2] mean (standard deviation [SD]), [3] median, [4] mean (SD), range. * Values converted from log values. Def_TBM = culture confirmed TBM, Prob_TBM = clinically suspected TBM

Table 6.2: Chemokine concentrations in TBM patients (pg/ml)

Authors	Sample	IL-8	MCP-1	MIP-1α
Mastroianni et al (1998) 30 months-58 years N=11	CSF[1]	963(123-1236)	808(235-1375)	26.6(16.3-48)
Yaramis et al (2001) M = 3.7 years N=37	CSF[3] Week 1 Week 2 Week 4 Week 6 1 year	 1931.7 431.6 112.9 68.1 60.2		
Thwaites et al (2003) >15 years N=21	CSF[4]	8297 (9640), 430-41298		
Yilmaz et al (2003) 1 month – 14 years N=5	CSF[2]	4112 (458)		
Simmons et al (2006)* Md = 38 (15-88) years N=408 HIV- N= 89 HIV+	CSF[2] HIV- HIV+	 25.79 (1.79) 23.81 (1.88)		
Misra (2010) M = 31.4 (10-50) years N=16	CSF[1] Admission 3 Months	 500.6 (45.1-5000) 105.35 (0-5000)		

CSF = cerebrospinal fluid, IL = interleukin, MCP-1 = Monocyte chemoattractant protein – 1 alpha, MIP-1α = Macrophage inflammatory protein 1 α, HIV= human immunodeficiency virus. M= mean, Md = median. N= sample size. [1] median and range, [2] mean (standard deviation [SD]), [3] median, [4] mean (SD), range. * Values converted from log values.

SECTION C: STUDY OUTLINE

CHAPTER 7: METHODOLOGY

Working hypotheses and study objectives

This study hypothesised that markers of neurological injury (singly and combined with inflammatory markers) are:
1) elevated in TBM, and
2) elevated concentrations are associated with injury severity and outcome.

The primary objectives of this study were:
1) to examine the profile of neurological and inflammatory biomarkers in acute paediatric TBM in lumbar CSF, ventricular CSF and serum, and
2) to examine the relationship between biomarkers and 6 month clinical and neurodevelopmental outcome (assessed using the Paediatric Cerebral Performance Category Scale [PCPS] and Griffiths Mental Development Scales, extended version [GMDS-ER])

The secondary objectives were:
1) to examine the association between biomarkers and measures of injury severity (clinical examination, radiology and physiological measures of brain ischaemia),
2) to examine the association between biomarker concentrations and patient and clinical characteristics, and
3) to examine novel approaches to detect brain ischaemia in TBM, using near-infrared spectroscopy and brain tissue oxygen monitoring.

Study design

This was a descriptive cross-sectional study that examined biomarkers of neurological injury and inflammation across different sample types in cases and controls, and a cohort study of the associations between biomarker concentrations and outcome and injury severity.

Scientific and ethics approval

Scientific and ethics approval for this study were granted by the local scientific research and human ethics review boards (ethics number 318/2010, Appendix 2). Hospital permission was obtained from the Red Cross War Memorial Children's Hospital Research Committee.

Study funding

Funding for this project was generously provided by the Clinical Infectious Diseases and Research Initiative (CIDRI) funded by the Wellcome Trust.

CHAPTER 8: PATIENT SELECTION

TBM Cases

TBM cases were selected from children treated for definite or probable TBM with hydrocephalus at Red Cross War Memorial Children's Hospital (RXH) between October 2010 and August 2013. The diagnosis of patients included in this study was evaluated according to criteria outlined in a recent consensus statement on diagnostic criteria for TBM [69]. Definite cases were defined as patients in whom TB was cultured from the CSF or acid-fast bacilli were visualised in the CSF. The diagnosis of probable TBM was made based on a combination of clinical, bacteriological and radiological criteria as outlined in the Case Definitions chart (Appendix 1). Patients with other concomitant neural pathology or systemic infection were excluded. Inclusion and exclusion criteria are outline in Table 8.1.

Table 8.1: Study inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Diagnosed with definite or probable TBM	Other neural pathology
Diagnosed with hydrocephalus	Systemic infection other than TB
	Anti-TB treatment ≥ 3 days before study enrolment

Controls

Two groups of controls were selected: Group A served as controls for biomarker concentration comparison. Group B served as controls for developmental outcome scores according to the GMDS-ER.

Group A

This control group consisted of 2 patient categories:

Category 1: This group of patients served as controls to establish normative biomarker concentrations and comprised children presenting to the Neurosurgery Unit at RXH for elective spinal surgery for a fatty filum – a congenital disorder characterized by an abnormal amount of fat in the filum terminale (non-neural structure) of the spinal cord. This is not a progressive disease, is not associated with ongoing acute neurological injury or inflammation and the operation is primarily prophylactic; therefore biomarker levels were not anticipated to be elevated.

Category 2: To control for possible extra-cranial TB sources of S100B and NSE a second control group of children with pulmonary TB (pTB) was enrolled to validate the specificity of biomarkers for

neurological TB injury. As both these groups of patients were selected as convenience controls it was not possible to age-match them with TBM cases.

Group B

Studies on the development of children from low socio-economic status (SES) backgrounds in Cape Town demonstrated that low income, poor nutrition, family instability, and low levels of maternal education and childhood stimulation are significantly associated with poor baseline development, particularly after the age of 1 year [422,423]. By virtue of it being a state institution most children admitted to RXH stem from these impoverished backgrounds and it is likely that pre-morbidly these patients would perform poorly on a developmental test developed in a first world setting like the United Kingdom. Therefore, in an attempt to isolate the association between development and TBM specifically, a control group of children from similar social circumstances was included. Children from a similar SES, language and culture group as TBM cases coming from seemingly stable homes and without a recent history of illness or developmental delay were sourced from siblings, family, friends and neighbours of TBM cases. Controls and cases were age-matched as best as possible.

Sample size calculation

Estimation of the number of *cases* was powered by the sample size required for outcome analysis. Studies which have compared neuromarker concentrations between *good and poor outcome* groups in TBI demonstrate meaningful differences [272,332,336,424]. It is reasonable to hypothesise that biomarker levels will be higher in unfavourable versus favourable outcome; therefore, we estimated the sample size using a one-sided test with an alpha level of 0.05. The smallest reported standardised mean difference noted in biomarker studies in paediatric TBI was for S100B and therefore sample size calculation was based on these data. The standardised mean difference between favourable and unfavourable outcome groups was 0.82, which is considered a large effect size (mean [standard deviation] unfavourable = 1.6[1.6] and favourable = 0.6[0.4]). This suggested a required sample size of approximately 42 patients for an adequately powered study (80%). One limitation of this sample estimation was that multiple comparisons were not accounted for. However, considering budget constraints, the time for patient accrual, and the fact that this was a pilot study aimed at collecting preliminary data, this sample size was selected.

There is little research on cytokines in children with TBM, and none on markers of neurological injury such as S100B, NSE and GFAP. Research on neuro- and inflammatory markers in paediatric head injury has shown effect sizes ranging from 1.1-9.6 [272,425] and inflammatory markers in TBM have shown effect sizes of 17-24 [403] when comparing *cases with controls*. These effect sizes and the

very low biomarker levels and little variability seen in controls suggested that a small sample size would be sufficient to demonstrate a significant difference in biomarker levels due to TBM. Therefore, 11fatty filum controls undergoing elective spinal surgery and 9 controls with pTB were included.

Subject Recruitment

Patients meeting the inclusion criteria were identified from referrals or admissions to the casualty, paediatric and neurosurgical wards of RXH. On admission, parents were approached for consent after the study had been fully explained in their preferred language - English, Afrikaans or Xhosa with the assistance of a translator where needed.

Patient treatment

Antimicrobial medication and steroids

All patients were treated with the standard 4 drug regimen of rifampicin, isoniazid, pyrazinamide and ethambutol for 2 months followed by 4 months continuation of rifampicin and isoniazid at weight-dependent doses. Prednisone was given adjunctively for the first 3 weeks as per standard of care. Anti-retroviral treatment (ART) was administered in HIV+ patients; the timing of ART initiation was determined by the infectious diseases consultants.

Management of Hydrocephalus (HCP)

All patients had an admission CT brain scan once TBM was suspected and patients with radiological evidence of HCP were referred to the neurosurgery unit. Tuberculous HCP is an unusual form of HCP which may be communicating or non-communicating. Successful medical treatment of the former has been demonstrated, obviating the need for surgery [126]. As outlined in Chapter 1 there are variable protocols reported for treating tuberculous HCP, each with advantages and disadvantages. Our protocol is based on the work of Schoeman et al [126]. Establishing the communicating nature of HCP involved performing an AEG according to described procedure [119]; briefly 5-10 ml of air was injected into the lumbar subarachnoid space (SAS) after which the patient was sat in an upright position for 30 minutes to allow the air to move up the spinal canal into the cranium. Air in the ventricular system suggests CHCP (positive test) and air in the basal cisterns or over the convexity of the brain but not in the ventricular system suggests NCHCP (negative test). Occasionally, no air is visible within the cranium, which may be due to a technical problem or impaired passage of air in the spinal canal due

to spinal exudates and arachnoiditis. If results were inconclusive on the first attempt, the AEG was repeated to exclude the possibility of technical errors. If necessary, an EVD was placed first to ensure safe continuation of the procedure in patients who may have had NCHCP. The procedure is safe and was performed either by the paediatrician or neurosurgeon treating the patient, but a neurosurgeon was always informed and able to take the child to theatre for an EVD or shunt immediately should non-communication have been diagnosed. Patients who were considered clinically too unstable or had a very depressed LOC on admission immediately received an EVD as a temporizing measure to control raised ICP. Thereafter, HCP in these children was investigated using an AEG and /or column test. Baseline skull X-rays were performed in all children after EVD insertion to exclude the presence of air in the ventricles as a result of surgery and the drain was clamped while the child sat upright after the AEG was performed. Column tests have been described [120] and briefly involved clamping the EVD distally to the drainage system and connecting a manometer to the proximal port. An LP was performed with the patient lying in the lateral position with a manometer connected to the LP needle. The lumbar and ventricular opening pressure (OP) readings were recorded before and after the release of CSF. CHCP was suggested by similar initial values and an equal change after CSF release. When readings did not correlate NCHCP was assumed. Column test results were considered in combination with AEG results were possible. NCHCP was treated with a VPS or ETV [120,426]. Patients with CHCP or who had undergone an ETV were medically treated with acetazolamide and Lasix in conjunction with serial LPs [126]. The OP was recorded during serial LPs as a temporal measure of ICP. Cessation of serial LPs occurred once the ICP had normalised and remained low over a minimum of 2 LPs performed at least 1 week apart. In general, medical treatment continued for 3 weeks or until the OP settled. A VPS was inserted if medical therapy failed to control HCP (defined by resolution of hydrocephalus on follow up brain imaging and normalised OP on LP). The protocol for patient management is outlined in Appendix 3.

Hyponatremia

Hyponatremia was slowly corrected, mostly using fluid resuscitation with normotonic or hypertonic fluids. The distinction between CSW and SIADH is not easily made [112] and we were guided in large part by patient volume status and laboratory urine results. Perturbations in any other organ systems were treated accordingly. Details about the patient monitoring are provided in Chapter 12.

SECTION D: STUDY RESULTS

All analyses in this project were conducted with the assistance of a qualified statistician using the statistical software programs STATA (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP) and R (*R Development Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.*). Data are presented as median (range or interquartile range) due to the non-parametric distribution. Statistical tools used for the various analyses are outlined in the relevant methods sections. The significance level for all analyses in this project was set at the 0.05 level.

All figures and tables are numbered according to the chapter number and their order in the chapter.

CHAPTER 9 SUMMARY
Patient and admission clinical characteristics

This chapter aimed to provide a profile of the study patients in terms of their clinical admission and demographic characteristics, CSF diagnostic and chemistry results, treatment and outcome. A brief summary of TBM patients who were ineligible for study inclusion was also included.

Data were collected from patient records and laboratory databases at RXH. Clinical outcome was assessed at routine 3 and 6 month outpatient hospital follow up and included mortality at 6 months, dichotomised clinical outcome (good or poor) and morbidity in survivors according to the Paediatric Cerebral Performance Category Scale. Neurodevelopmental outcome was assessed at 6 months using the Griffiths Mental Scales extended version (GMDS-ER).

The main results were as follows:

1. From October 2010 to August 2013 RXH admitted 74 children with probable or possible TBM. HCP was diagnosed in 71.6% of these patients. HIV co-infection was recorded in 8% of patients, and the mortality rate was 13.5%.
2. The 44 patients included in this study were young (≤5 years, n=37, 84.1%) and presented with the typically reported clinical signs and symptoms of TBM. Only 2 patients (5%) were co-infected with HIV.
3. Lumbar and ventricular CSF chemistry results were significantly different in terms of protein, glucose, chloride and lymphocytes.
4. The culture positivity yield was 53.8%, which is an improvement on historical rates and those reported in the literature, and is possibly due to the larger volumes of CSF sent for culture. No cases of drug resistance were reported in the study cohort, although 2 cases of drug resistance occurred in the patients not eligible for study inclusion
5. CHCP was more common than NCHCP (n=3, 8%). Medical treatment was successful in 57% of patients with CHCP. NCHCP was treated with a VPS or ETV.
6. The mortality rate by 6 months was 16% (n=7). Four of these patients died within the first 10 days after admission. Almost half (n=21) of the full cohort made a full clinical recovery; 11 patients (25%) suffered mild-moderate disability, 2 patients were severely disabled (4.5%) and 3 patients (6.8%) were considered vegetative.
7. TBM cases demonstrated neurodevelopmental deficits in all developmental domains assessed and the impact of TBM on development was multidimensional; the impact of socio-economic circumstances and prolonged hospitalisation were important contributory factors.

CHAPTER 9: PATIENT AND ADMISSION CLINICAL CHARACTERISTICS

Methods

Admission clinical and demographic data

General demographic and clinical data for TBM cases were prospectively collected, including gender, age, sex, immunization history, TB contacts, previous medical history and underlying disease including HIV. Clinical data included presenting symptoms, duration of symptoms before admission, clinical and neurological signs. TBM severity was determined using the refined British Medical Research Council criteria [81] on admission and after 1 week: Stage I: GCS 15 without focal neurology, Stage IIa: GCS 15 with neurological deficit/ GCS13-14 with/without neurological deficit, Stage IIb: GCS 10-12 with/without focal neurological deficit, Stage III: GCS < 10 with/without neurological deficits.

In-hospital data

Clinical data collected during hospital admission included neurosurgical interventions (EVDs, VPSs and ETVs), lumbar punctures (OP), Mantoux results (where performed), and medication.

Laboratory data from CSF included glucose, protein, chloride, white cell count and differential, TB culture, Ziehl-Neelsen staining for AFBs, TB PCR and drug sensitivity as well as culture for other bacteria. TB culture results from tracheal aspirate, gastric wash and sputum were documented. Haematological data included blood sodium levels and HIV reactivity.

Radiology findings: All imaging was reviewed by experienced paediatric radiologists, including chest x-rays (CXR), computed tomography (CT) brain scans, magnetic resonance imaging (MRI) of the brain and spine and magnetic resonance angiography (MRA). Further details are provided in Chapter 11.

Outcome data

Mortality was recorded as deaths by 6 months.

Clinical outcome

Outcome was assessed at 6 months after initiation of treatment. For survivors, clinical outcome was assessed at 3 and 6 months post-diagnosis by a senior neurosurgeon who was blinded to the biomarker results. A second senior neurosurgeon scored the follow-up notes according to the Paediatric Cerebral Performance Category Scale (PCPS - Appendix 4) which has been validated in hospitalised paediatric patients for the assessment of physical and cognitive disability [427]. Outcome scores were examined as follows: for the cohort overall scores were dichotomised at 6 months as 1-3 (good outcome) and 4-6 (poor outcome which included death). For survivors, scores were categorised into 3 groups; 1 (normal outcome), 2-3 (mild-moderate disability) and 4-5 (severe disability).

Neurodevelopmental outcome

At 6 months developmental outcome was assessed using the GMDS-ER (Appendix 5) which has been validated for a South African paediatric cohort using all three major languages, English, Afrikaans and Xhosa [428], and which has been used in a number of studies of development after TBM [106,145]. This test battery is the internationally accepted gold-standard assessment of development in children aged 0-8 years old and comprises 6 sub-scales:

1. Locomotor – tests physical development in young children including the ability to sit, walk, run, jump, balance and master stairs.
2. Personal-social - assesses personal and social development including the ability to respond socially to interactions, independence in eating, dressing and bathroom activities, and knowledge of personal details, such as birthday and address.
3. Hearing and Language – examines the growth and development of language through tasks which test vocabulary, sophistication of speech and language expression as well as comprehension.
4. Eye and hand coordination – assesses the handwork and visual ability of the child with tasks involving building blocks, drawing and manipulation of objects.
5. Performance - tests skill in manipulation, speed of working, precision, pattern recognition through form boards, model construction and pattern-making.
6. Practical reasoning – focuses on numerical reasoning and simple problem solving with tasks involving counting, memory and reasoning.

Scores are assigned for the number of items passed in each sub-scale. The raw scores for each subscale as well as the general quotient (GQ - mean of all the raw scores) are then compared against

established British norms for age providing an estimate of the age-equivalence of the patient's performance.

The Baby Scales version of the test battery is designed for children aged 0-2 years; it assesses only sub-scales 1-5 and is therefore scored according to a separate system of normative values. TBM cases were scored according to the age appropriate scoring system and those patients who crossed between the Baby Scale and the 3-8 year version had to be scored on the 3-8 year system while compensating for the additional Scale 6. To allow for unified reporting across the 2 versions of the test battery, analysis focussed only on age equivalents as these are comparable across the Baby Scale and the 3-8 years, sub-quotients are not reported. Children older than 8 years did not undergo developmental assessment.

TBM cases underwent GMDS-ER testing as part of their routine 6 month outpatients follow up. The candidate was trained in administering the test battery and personally tested all English and Afrikaans speaking children (cases and controls). Xhosa-speaking children were assessed with the assistance of a Xhosa-speaking colleague trained and experienced in conducting GMDS-ER assessments in Xhosa. Mothers were requested to be present when possible and maternal level of education was ascertained. All patients were assessed for hearing difficulties either at RXH or at BCH prior to the developmental assessment. The assessor was blinded to biomarker levels.

Controls

Group A (biomarker controls): Category 1: Data collected from fatty filum control patients included age, sex and previous medical history.
Category 2: Data collected from pTB controls included age, sex, previous medical history and sputum TB investigation results.
These data were extracted from patient medical records and the NHLS database.

Group B (Neurodevelopment controls): Data collected included age, sex, previous medical history and maternal level of education.

TBM patients not eligible for study enrolment

The study had strict inclusion and exclusion criteria, the most important of which were the presence of hydrocephalus and the timing of treatment initiation. To place the findings of this selected study cohort in the general context of TBM at RXH, some basic demographic, radiological and laboratory data were also collected for patients who were treated for suspected TBM at RXH during the duration

of the study but were not eligible for study inclusion. These patients were evaluated according to the consensus diagnostic criteria and patients meeting the definite, probable or possible TBM diagnoses were included.

General demographic and clinical data collected from hospital records included gender, age, sex, immunization history, TB contacts and underlying disease including HIV (when available). Clinical data included admission GCS and MRC stage on admission, and outcome data when recorded. From the NHLS database CSF diagnostic (culture, staining for AFBs, PCR and drug sensitivity) and chemistry results for the initial LP (glucose, chloride, protein, cell count) were also recorded as well as the results of TB investigations from other specimens. Imaging findings on admission CT brain and CXR were obtained from the RXH radiology database. Outcome data collected from patient folders only included in-hospital mortality as most of these patients are discharged to secondary hospitals and clinics and are not followed-up at RXH.

Analysis

CSF chemistry

To ascertain if there was a rostro-caudal gradient in CSF chemistry time-linked lumbar and ventricular CSF chemistry results taken on admission were compared using the Wilcoxon signed rank test. In patients who did not have both lumbar and ventricular CSF taken initially, the first available combination of the two sample types was used for analysis.

Association with clinical outcome

The association between patient and admission characteristics and outcome (mortality, 6 month clinical outcome and survivor morbidity at 6 months) was analysed using the Chi-square or Fisher's exact tests. The association between CSF chemistry and 6 month outcome was investigated using Mann-Whitney's U.

Neurodevelopmental outcome

First, TBM case and control results were compared to the British norms in terms of age-equivalents; the distribution of scores above and below the age-equivalence was calculated. Next, cases were compared to controls using a general estimating equation (GEE) which incorporated subscale (locomotor, personal-social, language, eye-hand coordination, performance, reasoning, GQ) and group (case/control). As the term 'neurodevelopmental delay' has a very specific definition which is

not fully applicable to this patient cohort, the term 'neurodevelopmental deficit' was adopted to indicate that TBM cases were developmentally behind relative to the controls. The median raw score for each sub-scale and the GQ were calculated for the control group. This was done in age bands of 0-24, 25-48, 49-96 months to accommodate for age-related function. The raw scores per sub-scale and for the GQ of the TBM cases were compared to these medians; if the raw score fell below the control median the TBM case was defined as having neurodevelopmental deficit on that sub-scale or the GQ. The median was selected as the threshold for comparison because the distribution of scores below the control median most closely matched the distribution of scores below the age-equivalents of the British norms. A flow diagram of this method is outlined in Figure 9.1.

The relationship between clinical and neurodevelopmental outcomes were analysed to ascertain whether the two outcome measures were aligned. As the GMDS-ER testing was limited only to children who were physically and cognitively able to participate in testing, clinical outcome for this analysis was dichotomised as normal (PCPS 1) or mild-moderate disability (PCPS 2-3). Each patient's neurodevelopmental score (deficit or no deficit) for the GQ and each sub-scale were examined in association with their clinical score using the Chi-square or Fisher's exact test.

Figure 9.1: Flow chart for defining neurodevelopmental deficit in cases

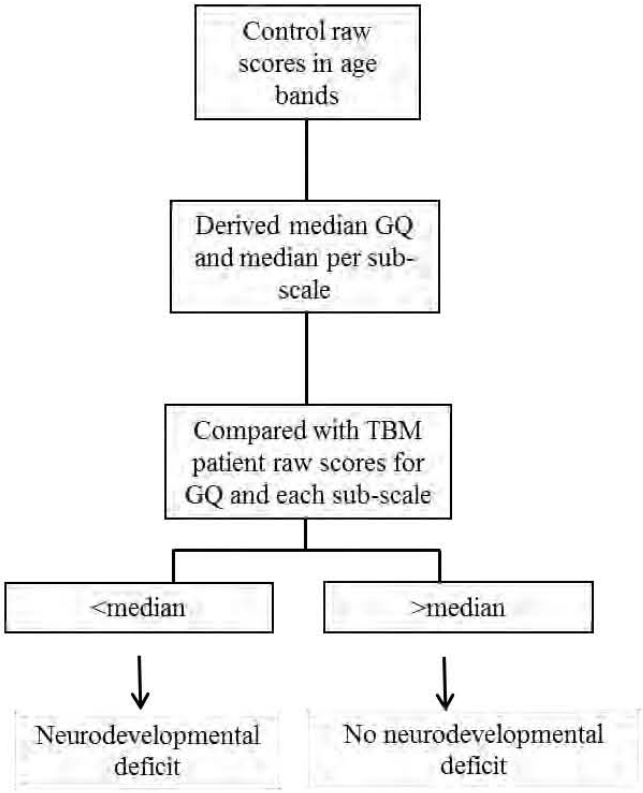


Figure 9.1 illustrates how TBM patients were classified as having a neurodevelopmental deficit. Median for each sub-scale and the GQ were derived from control median raw scores. TBM scores were compared to these medians in determining neurological deficit

CHAPTER 9: PATIENT AND ADMISSION CLINICAL CHARACTERISTICS

Results

Seventy-four patients who met the consensus criteria for definite, probable or possible TBM were treated at RXH from 01 October 2010 to 29 August 2013. Among these patients HCP was diagnosed in 53 patients (71.6%); 44 (83%) of the HCP patients were enrolled in this study and 9 (17%) were excluded. Reasons for exclusion included having been started on TB treatment >72 hours before admission to RXH (n=3), HCP had not been diagnosed on admission when treatment was started (n=1), initial misdiagnosis as viral or bacterial meningitis (n=2), HCP was treated before the diagnosis of TBM was made (n=1), spinal TB had been treated before the diagnosis of TBM was made (n=1), and HCP was due to obstruction caused by multiple posterior fossa tuberculomas which accompanied TBM (n=1) – a flow diagram of patient selection is provided in Figure 9.2.

Figure 9.2: Flow diagram of patient selection

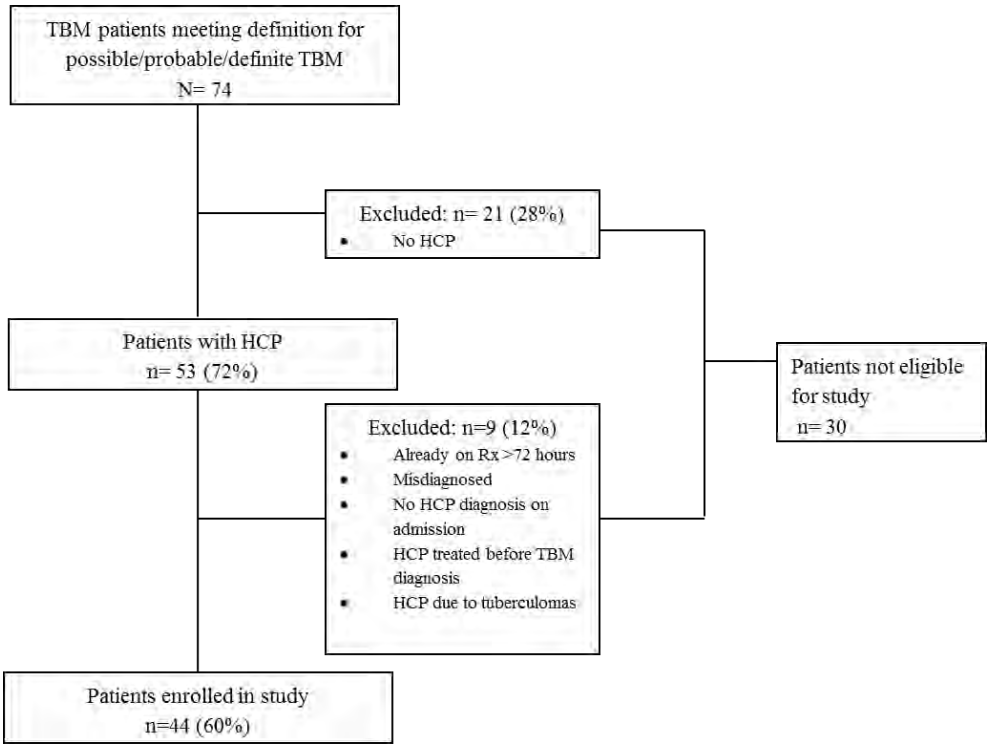


Figure 9.2 demonstrates the process by which patients eligible for inclusion in the study were identified from among all the admissions for TBM at RXH

HCP = hydrocephalus, Rx = treatment

Complete TBM cohort

The median age for the 74 patients admitted to RXH was 3.9 (0.25– 13) years and 42 (56.8%) were boys. Definite TBM was confirmed on CSF culture in 31 (42%) patients and 2 had drug resistance. Most patients presented with MRC Stage II TBM (n=41, 55.4%), the HIV positivity rate was 8% (n=6) and the mortality rate was 13.5% (n=10). Details on patients ineligible for study enrolment are included in Appendix 6.

TBM Study population

Patient Admission Characteristics

TBM Cases

The median age for cases was 3.3 years, most children were < 5 years old (68.2%) and boys were more common (63.6%). Ninety percent of patients presented with Stage II or III TBM. By 1 week after admission clinical staging had improved in 21 (47.7%) patients and worsened in 20.5% (n=9). None of these patients had previous documented TB or had taken TB medication. A positive TB contact was reported in less than half the patients; known contacts included parents, care givers and close family members or friends. The median duration of symptoms was 7.5 days and the median number of admissions to health care facilities or practitioners before presenting to RXH was 2 per patient (maximum 5). Symptoms of pulmonary TB (pTB) including persistent cough and night sweats were not commonly reported, but weight loss and failure to thrive were noted in half the cohort. The most commonly recorded signs and symptoms were an altered level of consciousness (n=40, 90.1%), meningism (n=34, 77.2%), fever (n=30, 68.2%) and loss of appetite (n=27, 61.4%). Focal neurology included unilaterally non-reactive pupils (n=6, 13.6%), cranial nerve palsies (n=10, 22.7%), paresis (n=12, 27.3%) and aphasia (n=8, 18.2%). Papilloedema was infrequently reported, however the fundi were not examined in all admissions and may be difficult to assess accurately. Photophobia was recorded in 4 patients. Over 80% (n=26) of patients had been vaccinated against TB and almost 70% (n=20) who were tested demonstrated a positive TST. HIV status was tested in 43 patients (97%) and was positive in 2 children (5%). Where maternal HIV history was obtained (n=31), 8 patients (25.8%) were recorded as having been exposed to HIV; 6 patients (75%) had not sero-converted. Demographic and admission characteristics and their association with outcome are outlined in Table 9.1.

Controls

The median age was 2.75 (0.75-8) years for fatty filum controls (n=11) and 3.67 (1.25 – 11.83) years for pTB controls (n=9). Both groups were divided evenly between boys and girls. All pTB controls were treated according to the standard medical regimen; only 2 patients (22.2%) were sputum smear or culture positive- the expected rates of smear or culture positivity are < 20% in children with pTB.

Table 9.1: Admission demographic and clinical characteristics

Characteristic	Value		Outcome association	
Demographic characteristics				
Age	3.3 (0.3 – 13.1) years		*p=0.96*	
0-2 years	19 (43.2)			
3- 5 years	18 (40.9)			
> 5 years	7 (15.9)			
Gender	Male 28 (63.6)		*p=0.1*	
	Female 16 (36.4)			
Admission characteristics				
MRC Staging	Admission	Week 1	*p=0.01**	*p<0.001**
1	4 (9)	18 (40.9)		
2a	17 (38.6)	8 (18.2)		
2b	14 (31.8)	5 (11.4)		
3	9 (20.5)	11 (25)		
Dead	0	2 (4.5)		
Symptom duration	7.5 (1 – 90) days		*p=0.05**	
Weight loss/failure to thrive	22 (50)		*p=0.74*	
Night sweats	4 (9)		*p=0.56*	
Cough > 2 weeks	8 (18.2)		*p=0.08*	
Focal neurology [a]	21 (47.7)		*p=0.32*	
Altered level of consciousness	40 (90.1)		*p=0.56*	
Lethargy and sleepiness	36 (81.8)		*p=0.66*	
Meningism	34 (77.2)		*p=0.1*	
Fever	30 (68.2)		*p=0.52*	
Loss of appetite	27 (61.4)		*p=0.49*	
Bulging fontanelle (n=9) [b]	5 (55.6)			
Vomiting	24 (54.5)		*p=0.1*	
Seizures	14 (31.2)		*p=0.11*	
Headache (n=37) [c]	15 (40.1)		*p=0.02**	
Papilloedema	9 (20.1)		*p=0.68*	
Irritability	11 (25)		*p=0.02**	
Recent TB contact	21 (47.7)			
Vaccination (n=33) [d]	26 (78.8)			
TST (n=29)	20 (69)			
HIV infection (n=43)	2 (4.7)		*p=0.02**	
HIV exposure (n=31)	7 (22.6)		*p=0.48*	
Diagnostics				
CSF TB culture (n= 39)			*p=0.56*	
positive	21 (53.8)			
negative	18 (46.2)			

Values reported as median and range or number (percentage), Outcome is dichotomised (good and poor) at 6 months. * = statistically significant result

[a] Focal neurology includes pupillary response, paresis, cranial nerve palsies and aphasia, [b] For children with open fontanelles, [c] Pre-verbal children under the age of 1.5 years excluded, [d] Parent or guardian not present or Road to Health Card missing,

Laboratory results

Diagnostics

Forty-one patients had CSF sent for TB diagnostic investigation; for 2 patients CSF TB investigations were not requested by the treating clinician and in one patient the only CSF sample obtained was insufficient for TB culture - these patients fulfilled the remaining diagnostic criteria for probable **T**BM. An additional 2 patients had CSF sent for culture more than 7 days after the initiation of treatment. Definite TBM was confirmed in 22 of the 39 remaining patients (56.4%); 21 had positive CSF TB cultures and/or visible AFBs and in 1 patient only AFBs were visualised. All culture positive patients were positive on PCR and none had drug resistant TB. Although two thirds of these cultures were confirmed on the initial CSF sample, culture positive results were also obtained from subsequent samples up until the 5th sample sent. Of the initial samples sent for culture, 31 were lumbar CSF and 13 were ventricular CSF (some patients had both lumbar and ventricular CSF sent); 16 (51.6%) of the lumbar and 8 (61.2%) of the ventricular CSF samples tested culture positive. One patient had disseminated TB confirmed on ultrasound and abdominal imaging, 3 patients had positive TB culture on tracheal aspirate, 1 on gastric wash and 1 on sputum. No bacteria other than *Mtb* were cultured on CSF during the sample collection phase of this study.

CSF chemistry and white cell count

Admission CSF chemistry and cell counts are summarised in Table 9.2. Eight patients (21%) had normal admission lumbar CSF glucose; however, 2 among them developed abnormally low glucose in subsequent samples. Only 2 patients (5.3%) had normal admission lumbar CSF protein values and a total white cell count (WCC) < 10/cu mm; however, these were all elevated by the second sample. Three patients (8.3%) had initially normal lumbar CSF chloride values, these decreased below the reference range in subsequent samples. Lymphocytic predominance >50% was seen in the majority of lumbar (84%) and ventricular CSF (90%) samples. CSF/blood glucose ratios could not be calculated as blood glucose tests are not routinely done at RXH. Analysis with outcome revealed that elevated polymorphonuclear cells in the lumbar CSF trended towards an association with better outcome at 6 months ($p=0.06$). Lumbar CSF glucose and chloride were significantly lower than ventricular CSF ($p<0.001$ and $p=0.04$ respectively), and lumbar CSF protein and white cells were higher ($p<0.001$). The average CSF/serum albumin ratio was elevated in lumbar and ventricular CSF samples for TBM cases relative to controls ($p<0.001$). Lumbar ratios were elevated above the 95th percentile of control values (6.1) in 31 patients (97%) and ventricular ratios in 18 patients (85.7%). Ratios were significantly higher in lumbar than ventricular CSF ($p=0.002$). Elevated CSF/serum albumin ratios were not associated with outcome ($p=0.12$).

Table 9.2: Admission CSF chemistry and cell counts

CSF chemistry and white cell count	Lumbar N= 38	Ventricular N = 20	Difference lumbar vs ventricular	Association with outcome
Glucose (mmol/l)	1.6 (0.3-4.8)	3.25 (1.2-4.3)	*p<0.001**	*p=0.4*
Normal (2.3-3.9)	*8 (21)*	*18 (90)*		
Abnormal (< 2.3)	*30 (78.9)*	*2 (10)*		
Chloride (mmol/l) [a]	106.5 (93.0-131.0)	110 (94.0-126.0)	*p=0.04**	*p=0.78*
Normal (120-130)	*3 (8.3)*	*4 (20)*		
Abnormal (< 120)	*33 (91.7)*	*16 (80)*		
Protein g/l	1.98 (0.34-40.88)	0.52 (0.15-1.47)	*p<0.001**	*p=0.2*
Normal (0.2-0.8/ 0.15-0.45)	*2 (5.3)*	*9 (45)*		
Abnormal (> 0.45/ >0.8) [b]	*36 (94.7)*	*11 (55)*		
Polymorphonuclear Cells (/cu mm)	18.5 (0-280.0)	2.5 (0-74.0)	*p=0.01**	*p=0.06**
Lymphocytes (/cu mm)	144 (6.0-715.0)	19.5 (0-85.0)	*p<0.001**	*p=0.25*
Total white cell count	169 (0 – 901.0)	22.5 (0 -139.0)		
Normal (< 10)	*2 (5.3)*	*4 (20)*		
Abnormal (> 10)	*36 (94.7)*	*16 (80)*		
Lymphocyte predominance				
>50%	*32 (84)*	*18 (90)*		
>90%	*14 (36.8)*	*12 (60)*		
CSF/serum albumin ratio				
	29.68 (0.3-588.6)	10.3 (2.5-288.5)	*p=0.002**	*p=0.12*
Normal	1 (3.1)	5 (21.7)		
Abnormal	31 (97)	18 (85.7)		

Data presented as median (range), *number (%)*, association with outcome analysed with lumbar CSF, [a]admission chloride values were only available for 36 patients, [b] Protein references ranges are age dependent: >1 year and <1 year respectively, * = statistically significant

Hyponatremia

Serum hyponatremia was present on admission in 38 patients (86.4%). The average sodium value was 128 ±6 mmol/L (range 117-145). Sodium levels were slowly normalised using hypertonic saline.

Patient management

Medical management

All patients were treated with the standard anti-TB regimen and adjunctive steroids. Patients with CHCP and those who had an ETV were treated with diuretics for 3 weeks. Only 2 patients received ART.

HCP management

AEGs and columns tests were performed in 43 patients, the 44th patient was initially thought to have bacterial meningitis and underwent an immediate shunt procedure without an AEG; TBM was subsequently diagnosed. CHCP was diagnosed by a positive AEG and/or column test in 34 patients (79.1%) and NCHCP by a negative test in 3 patients (7%). In 6 patients AEGs were attempted but a result could not be obtained; 4 patients failed LP (LP needle was in the subarachnoid space but no CSF could be collected - likely due to the presence of spinal arachnoid exudate) and the AEGs could not be definitively performed, and 2 patients with an EVD in situ died before a clear result on AEG could be obtained. The median opening pressure on LP was 24 cmH_2O (1-51 cmH_2O).

Patients with CHCP (n= 34) – Twenty-eight of these patients were treated medically. Sixteen among them (57.1 %) successfully completed medical treatment; ICP was controlled by 3 weeks and did not deteriorate. Twelve patients failed medical treatment; for 7 patients this was evident during their hospital stay (acute failure), and 5 patients had late deterioration after discharge to a rehabilitation or secondary level care centre (delayed failure). For the group with acute failure raised ICP had not normalised by 3 weeks (n=4), deteriorated acutely and necessitated a VPS (n=1) or deteriorated after normalising (n=2). Average time to VPS was 3.1 ± 1.2 weeks. Patients with delayed failure experienced ICP deterioration a median of 10 weeks post admission at which time they were shunted. Four patients with CHCP were considered too ill on admission to undergo a trial of medical treatment and were shunted soon after admission as the imperative was to definitively control ICP early. One patient failed LP and had to be shunted, and in 1 patient the results of the AEG were misinterpreted as NCHCP leading to a VPS when medical treatment would have been appropriate. A flow diagram of HCP management is outlined in Figure 9.3.

Patients with NCHCP (n= 3) – VPSs were inserted in 2 patients, the third patient had an ETV 4 days post-admission but re-presented after 7 weeks with raised ICP. She was subsequently shunted.

Uncertain – AEG result unclear or no AEG performed (n= 7) – five of these cases (71%) were treated with a VPS, one patient had an EVD and died before a shunt could be inserted, one patient had an ETV with no subsequent deterioration in ICP.

Surgical procedures- EVDs were used as a temporising measure to treat high ICP in 31 patients (70.5%) and a total of 25 patients (56.8%) had a VPS inserted. Shunt complications included malfunction (n=3), over-drainage (n=1), infection (n=1) and wound dehiscence (n=1 patient with a poor nutritional status). Surgical procedures are detailed in Table 9.3.

Figure 9.3: HCP Management

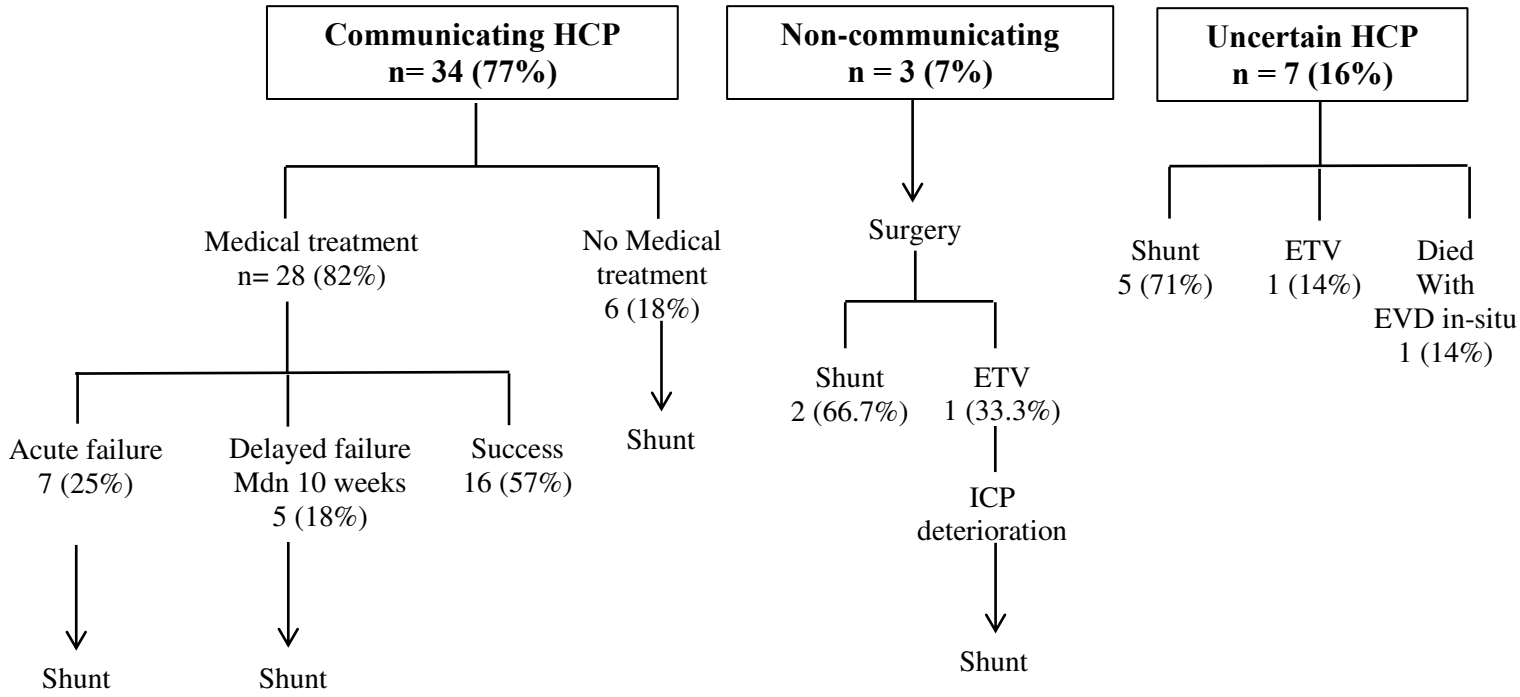


Figure 9.2 demonstrates how HCP was managed in patients with CHCP, NCHCP and in whom the nature of HCP was uncertain. Percentages for CHCP, NCHP and uncertain HCP were calculated based on the total cohort number of 44 patients – when patients with uncertain HCP are excluded, CHCP was diagnosed in 92% of patients and NCHCP in 8%. Mdn=median

Table 9.3: Surgical HCP management

Neurosurgical Intervention		Value
Initial EVD		
	CHCP	21 (61.8)
	NCHCP	3 (100)
	Uncertain	6 (100)
	Total [a]	31 (70.5)
Shunt		
	CHCP (n=34)	18 (52.9)
	NCHCP (n=3)	3 (100)
	Uncertain (n=7)	4 (57.1)
	Total (n=44)	25 (56.8)
ETV [b]		2 (4.5)

This table details the frequency of surgical procedures for treatment of HCP in patients with CHCP, NCHCP and in whom the nature of HCP was uncertain. [a] This total includes all patients who had an EVD– the 31st patient was initially treated for bacterial meningitis and did not have an AEG to establishing the nature of HCP, [b] One patient had NCHCP and the other had an uncertain AEG result

Patient Clinical Outcome

Mortality - Overall mortality by 6 months was 16% (n=7). Four of these patients died within the first 10 days after admission (median 4 days), 2 patients died after 7 weeks and 1 patient died by 6 months. All deaths were the direct result of TBM. Six patients (13.6%) continued to improve after 3 months and 1 patient who was vegetative at 3 months had died by 6 months.

Six month dichotomised clinical outcome - According to the PCPS by 6 months good outcomes (PCPS 1-3: normal –mild/moderate disability) were recorded in 32 patients (72.7%) and 12 patients (27.2%) suffered a poor outcome (PCPS 4-6: severe disability-died). Almost half of the full cohort (n=21) made a full clinical recovery with no neurological morbidity; 16 patients (36.6%) suffered some form of disability including one or more of the following: motor weakness (n=7), motor deficit (n=4), visual deficit (n=4), hearing deficit (n=2), memory deficit (n=1), cranial nerve palsy (n=1), and 3 patients (6.8%) were considered vegetative.

Six month survivor morbidity - Amongst the 37 survivors at 6 months, 21 patients (56.8%) had no neurological fall-out, 11 (29.7%) had mild-moderate disability, and 5 (13.5%) patients were severely disabled or vegetative.

There was no loss to follow-up. Outcome data categorised by MRC stage are presented in Table 9.4.

Associations with mortality

MRC staging at admission trended towards significance in association with mortality as more deaths occurred in severe categories IIb and III ($p = 0.06$). MRC staging at 1 week was similarly predictive of mortality ($p<0.001$). Mortality was significantly higher among females ($p=0.05$).

Associations with 6 month dichotomised outcome

Admission or patient characteristics which were significantly predictive of poor outcome (PCPS 4-6) at 6 months included: MRC staging on admission ($p=0.01$) and at week 1 ($p<0.001$) with poorer outcomes occurring with severe stages IIb and III and a shorter duration of symptoms ($p=0.05$- measured as a continuous variable). A higher incidence of headaches and irritability were reported amongst patients who survived. A full list of variables assessed with outcome is provided in Table 9.1. Age was not significantly associated with 6 month outcome and the 5 infant patients all survived; 1 had a normal outcome, 3 demonstrated mild disability (motor weakness) and 1 had moderate disability (motor weakness and cranial nerve VI palsy). They did not differ from the cohort as a whole on any clinical or admission variables.

Associations with morbidity

Morbidity at 3 and 6 months did not differ in their relationship with outcome; consequently results at 6 months are reported. Only MRC staging at week 1 was a significant predictor of morbidity (*p<0.001*); poorer outcomes were seen in children with Stages IIb and III and children who presented in Stage I all made good recoveries. For survivors, children less than 2.5 years tended to be at greater risk of disability (*p=0.06*).

Table 9.4: Clinical outcome at 6 months

		Admission			Total
Stage	**I**	**IIa**	**IIb**	**III**	**N=44**
Full recovery (PCPS 1)	3 (6.8)	12 (27.3)	6 (13.6)	0	21 (47.7)
Mild disability (PCPS 2)	1 (2.3)	2 (4.5)	3 (6.8)	2 (4.5)	8 (18.2)
Moderate disability (PCPS 3)	0	1 (2.3)	1 (2.3)	1 (2.3)	3 (6.8)
Severe disability (PCPS 4)	0	0	2 (4.5)	0	2 (4.5)
Vegetative (PCPS 5)	0	1 (2.3)	0	2(4.5)	3 (6.8)
Died (PCPS 6)	0	1 (2.3)	2 (4.5)	4 (9.1)	7 (15.9)
		Week 1			**Total**
Stage	**I**	**IIa**	**IIb**	**III**	**N=42**
Full recovery (PCPS 1)	15 (35.7)	5 (11.9)	1 (2.4)	0	21 (50)
Mild disability (PCPS 2)	3 (7.1)	2 (4.8)	1 (2.4)	2 (4.8)	8 (19)
Moderate disability (PCPS 3)	0	1 (2.4)	2 (4.8)	0	3 (7.1)
Severe disability (PCPS 4)	0	0	0	2 (4.8)	2 (4.8)
Vegetative (PCPS 5)				3 (7.1)	3 (7.1)
Died (PCPS 6)	0	0	1 (2.4)	4 (9.5)	5 (11.9)

This table presents clinical outcome data according to MRC Staging on admission and at week 1. Data are presented as number (percent). Data for Week 1 are presented out of 42 patients, 2 patients had already died by week 1

Neurodevelopmental outcome

TBM cases - Twenty-six TBM cases (69% boys) between the ages of 12 and 76 months (median 37 months) underwent neurodevelopmental assessment using the GMDS-ER. Patients who exceeded the age limit of the test (> 8 years, n=5), with severe clinical disability (n=5) and who had died (n=7) could not be evaluated. One patient was excluded due to documented baseline neurodevelopmental delay from alcohol and cigarette exposure in-utero. Auditory assessments were normal in all children and one patient suffered from visual deficits requiring special schooling for the visual impairment. The majority of mothers (90%) had not completed high school, 5% had completed high school and 5% were pursuing tertiary education.

Control Group - This group comprised 25 children with a median age of 41 months (10 – 81 months), 15 were boys (60%) and 10 were girls (40%). None of these children had a previous history of illness or TB and none had established developmental delay. Half of the mothers had completed high school, 36% had not and 16% had tertiary education.

In comparison to established norms

The raw scores for each subscale and the overall general quotient (GQ) for the TBM cases and controls were compared to British norms. The *control group* performed at an age appropriate level for the locomotor and personal-social subscales but 40% of children performed below their age equivalent on the eye-hand coordination, performance and reasoning subscales. The GQ scores fell within the first standard deviation of the equivalent GQ for age. Most of the *TBM cases* performed at a level below their age equivalent on all subscales and scored a median of 3 standard deviations below the normative GQ for age as detailed in Table 9.5.

Table 9.5: Control and Case scores in relation to British normative age equivalents

	Normal Controls	TBM Cases
Locomotor		
< age equiv	2 (8%)	24 (92.3%)
≥ age equiv	23 (92%)	2 (7.7%)
Personal-Social		
< age equiv	2 (8%)	20 (76.9%)
≥ age equiv	23 (92%)	6 (23.1%)
Language		
< age equiv	19 (76%)	24 (92.3%)
≥ age equiv	6 (24%)	2 (7.7%)
Eye-Hand Coordination		
< age equiv	17 (68%)	26 (100%)
≥ age equiv	8 (32%)	0 (0)
Performance		
< age equiv	14 (56%)	25 (96.2%)
≥ age equiv	11 (44%)	1 (3.8%)
Reasoning[a]		
< age equiv	13 (76.5%)	14 (100%)
≥ age equiv	4 (23.5%)	0 (0)
GQ		
< age equiv	12 (48%)	26 (100%)
≥ age equiv	13 (52%)	0 (0)

Data reported as number (percent), [a] only children > 2 years could be assessed on this sub-scale

Cases versus controls

A GEE to investigate the differences between TBM cases and controls revealed that TBM cases were significantly less likely to perform at an age-appropriate level than controls across all sub-scales (OR 0.7, CI: 0.03-0.14, *p<0.001*). The number of TBM patients who scored below the median control value on each sub-scale and the GQ was as follows: 22 (84.6%) on the Locomotor sub-scale, 23 (88.5%) on Personal-Social, 18 (69.2%) on Language, 19 (73.1%) on Eye-Hand co-ordination, 20 (76.9%) on Performance, 11 (78.6% - only 14 patients were old enough to be tested on this scale, >2 years) on the Reasoning sub-scale and 21 (80.8%) on the GQ.

No association was demonstrated between the clinical and neurodevelopmental scores for the sub-scales or the GQ. Clinical and neurodevelopmental outcomes are detailed in Table 9.6.

Table 9.6: Neurodevelopmental and clinical outcome scores

Neurodevelopmental sub-scale	Clinical outcome		
	Normal	**Mild-moderate disability**	**Total**
Locomotor			
Developmental deficit	9 (34.6)	2 (7.7)	11 (42.3)
No deficit	7 (26.9)	8 (30.7)	15 (57.7)
Personal Social			
Developmental deficit	10 (38.5)	2 (7.7)	12 (46.2)
No deficit	6 (23.1)	8 (30.7)	14 (53.8)
Language			
Developmental deficit	9 (34.6)	3 (11.5)	12 (46.2)
No deficit	7 (26.9)	7 (26.9)	14 (53.8)
Eye hand co-ordination			
Developmental deficit	10 (38.5)	2 (7.7)	12 (46.2)
No deficit	6 (23.1)	8 (30.7)	14 (53.8)
Performance			
Developmental deficit	10 (38.5)	2 (7.7)	12 (46.2)
No deficit	6 (23.1)	8 (30.7)	14 (53.8)
Reasoning[a]			
Developmental deficit	4 (15.3)	1 (3.8)	5 (35.7)
No deficit	7 (26.9)	2 (7.7)	9 (64.2)
GQ			
Developmental deficit	8 (30.7)	5 (19.2)	13 (50)
No deficit	8 (30.7)	5 (19.2)	13 (50)

This table displays the frequency of neurodevelopmental deficits in categories of clinical outcome [a] Only 14 children were old enough (>2 years) to be assessed on the Reasoning sub-scale

HIV co-infection

Only 2 patients were co-infected with HIV, both of whom had been diagnosed before admission to RXH for TBM. Patient 1 was a 4 year old male with definite TBM who presented in Stage IIb and progressed to Stage III after 1 week. He had defaulted on ART and had an admission CD4 count of 34 cells/μL. CSF investigations revealed low glucose, high protein and a predominance of lymphocytes. His CXR was suggestive of TB. His admission CTB demonstrated moderate diffuse basal enhancement and right basal ganglia infarcts had developed by his 3 week MRI. He was treated with standard medical TB and CHCP treatment and started on ART after 1 week. His ICP had normalised

by the time he was discharged with a GCS of 15. He was readmitted after 6 weeks for severe HCP which was treated with a VPS. Two weeks later he represented with extensive basal meningeal enhancement and a new streptococcus pneumonia meningitis, a very uncommon pathogen in shunt infections. He was treated with antibiotics and his shunt was revised. At this point his condition deteriorated and on discharge 4 weeks later he had a GCS of 12 and suffered from visual and speech deficits. His shunt was revised once again due to blockage one month after insertion. He was severely disabled at 3 and 6 months (PCPS 4).

Patient 2 was a 12 year female with probable TBM who presented in Stage IIa with a history of having defaulted on ART and a CD4 count of 28 cells/μL on admission. Her OP on LP was 17cmH_20, she was diagnosed with CHCP on AEG and started on medical treatment for TB and HCP as well as ART from day 1. Her CXR was normal and CSF chemistry demonstrated low glucose and high protein. CSF lymphocytes and neutrophils were equal initially but lymphocytes were predominant by the second CSF sample on day 3. Her admission CTB showed moderate HCP with mild focal enhancement. Later on the day of admission her GCS deteriorated from 13 to 7 and she was immediately taken to theatre for an EVD. Post-operatively her GCS remained 5T and her pupils were fixed and dilated. Over the next 5 days her scans showed evolving global infarction and she passed away on the 9^{th} day likely as a result of progressive cerebral ischemia and infarction.

This sample size of 2 HIV+ patients was too small to conduct analysis with outcome.

CHAPTER 9: PATIENT AND ADMISSION CLINICAL CHARACTERISTICS

Discussion

From October 2010 to August 2013 RXH admitted approximately 24 patients with possible, probable or definite TBM per year, comprising mostly young children < 5 years old presenting with moderate TBM according to the MRC staging criteria. HCP was diagnosed in 71.6% of these TBM patients, HIV co-infection was recorded in 8% and the mortality rate was 13.5%. The RXH patient profile mirrored that of paediatric patients admitted over the last 2 decades to our neighbouring institution in the Western Cape, Tygerberg Hospital (TBH) who reported similar outcomes (mortality 12.9%) [22,45]. The HIV co-infection rate in patients from RXH was higher than recorded by the TBH group (8% vs 3.8%) and this likely reflects the increasing national prevalence of HIV over the last 20 years [148].

TBM study population

This study enrolled a select group of patients with HCP who were younger and presented with more severe stage TBM than the group of patients ineligible for enrolment. The study cohort reflected the general patient profile reported by TBH in terms of the socio-economic group, age (majority < 5 years), delayed presentation and predominance of moderate-severe stage TBM on admission [45]. Delayed presentation is likely due to the limited access to effective early health care, poverty and poor education which are so ubiquitous to the population which TBH and RXH serve. Most patients presented with the typically reported symptoms of a low level of consciousness, meningism, fever and loss of appetite or failure to thrive [45,48,49]. Although commonly reported, these symptoms are not specific to TBM and there may be no previous or family TB history to support the presumptive TBM diagnosis. The delay in presentation and frequent health care seeking at medical facilities prior to RXH admission highlight the continued difficulty of diagnosis.

Almost 50% of patients demonstrated an improvement in their MRC staging after 1 week in hospital. This is likely as a consequence of treating HCP and hyponatremia which improve the GCS and focal neurology and reduce seizure activity due to ICP control and the correction of electrolyte imbalances. This suggests that the MRC staging on admission is influenced by factors other than the parenchymal injury associated with TBM. Therefore, new tools are required to specifically detect and assess the severity of intracranial injury so as to facilitate prompt and appropriate management and the prudent direction of limited resources to patients in greatest need. The rate of HIV was low in this cohort and testing occurred in almost all patients allowing the opportunity for timeous ART initiation. The

Western Cape has one of the lowest rates of paediatric HIV infection and the reduction of mother-to-child HIV transmission has been a national goal for over a decade [429], although challenges to achieving wide coverage of preventing mother-to-child transmission are still being addressed.

Laboratory results

The CSF TB culture positivity yield of 53% for the study population was a marked improvement on historical RXH rates (≈30%, unpublished data from a retrospective study conducted in anticipation of this project) and rates for the cohort of patients not eligible for study inclusion (33.3%). This was also higher than the 11.7% culture yield reported over 20 years from TBH [45]. This may be attributed to the greater number of samples and the larger volumes of CSF sent for microbiology. There is evidence to suggest that larger volumes of CSF increase the culture yield [430], consequently, this prospective study aimed to increase the volumes of CSF sent for culture and clinicians were encouraged to take up to 10 ml of CSF when performing AEGs or during therapeutic LPs. However, culture results took up to 44 days to be reported; presumptive diagnosis continues to be the basis for treatment initiation but sensitive, specific and rapid diagnostic tests are urgently required. Evidence of TB from biological samples other than CSF was rare (11.4%). It is encouraging to observe the very low rate of drug resistance recorded, although this may be an underestimate due to the limited rate of culture positivity. The higher rate of drug resistance reported from BCH [94] discussed in the Chapter 2 is a skewed reflection from the more severe spectrum of patients as that institution specifically admits patients with complicated or drug resistant TB, but serves as an important reminder of the importance of treatment compliance and the risk of resistance present in the community.

Laboratory findings demonstrated typically low glucose, high protein and lymphocytic predominance due to the adaptive immune response to TB. In the few samples in which this was not initially the case, most of the subsequent samples revealed these patterns; this highlights the value of repeated CSF testing in presumptive TBM diagnosis. Lumbar CSF protein and lymphocyte counts were higher than in ventricular CSF which in turn exhibited higher levels of glucose and chloride. The rostro-caudal gradient of higher protein in the lumbar than ventricular CSF is observed under both normal and pathological conditions. In the healthy brain the successive addition of serum proteins as the CSF flows from the ventricles to the thecal sac is responsible for the observed gradient [431]. In the context of cerebral infection lumbar CSF protein concentrations may be further augmented as more blood-derived proteins influx into the CSF along its course from the ventricles to the lumbar space due to BBB injury (indicated by an elevated CSF/serum albumin ratio) and reduced flow rate (due to inflammatory exudate obstructing CSF flow) [431]. Additionally, in TBM most of the inflammatory process occurs in the SAS and this would precipitate higher protein and lymphocytes in the lumbar CSF. It has also been contended that the lumbar meninges may be more permeable to the diffusion of serum proteins, and that immunoglobulins produced by the local inflammatory response add

cumulatively to the protein concentration as the CSF flows caudally [432-434]. Glucose enters the CSF through the choroid plexus in the lateral ventricles and through carrier mediated transport into the extracellular fluid of the brain and spine. Decreased lumbar glucose may be attributed to cumulative bacterial glucose consumption along the rostro-caudal axis and impaired lumbar meningeal glucose transport due to inflammation [431-433]. This study's findings suggest that lumbar CSF content cannot be inferred from ventricular CSF and vice versa. The typically anticipated low glucose, high protein and high lymphocytes in TBM apply to lumbar CSF and it is important to be cognisant of this when examining ventricular CSF in the absence of lumbar CSF so as to avoid missing the TBM diagnosis.

Patient management

NCHCP was diagnosed in only 8% of patients in whom an AEG result could be obtained; a much lower rate than reported in the literature (14-25%) [45,64,101]. However, uncertainty about the nature of HCP existed in 16% of patients which could have reduced the reported frequency of both CHCP and NCHCP. Spinal imaging demonstrated that spinal arachnoiditis was present in 72% of patients (Chapter 11); spinal adhesions and exudate may create a barrier to the flow of CSF or the movement of air up the spinal canal resulting in unsuccessful AEGs; indeed more severe spinal arachnoiditis was associated with failure to LP ($p=0.01$). As spinal disease has not been described in paediatric TBM, the frequency of spinal arachnoiditis may not have been appreciated and failed AEGs due to spinal disease may have been incorrectly categorised as NCHCP. Additionally it is our institutional practice to investigate HCP fully with AEGs and column tests before making the diagnosis of NCHCP so as to avoid unnecessary shunts where possible. In spite of this approach, over half of this cohort received a VPS. This is more frequent than studies published from TBH (30-40%), the only other institution which, to our knowledge, routinely investigates the communicating nature of HCP [22,45,64,101,126]. This may be attributed firstly, to the high rate of failed medical treatment observed; 53% versus 25% reported from TBH [22,45]. The definition of failed medical treatment is however variable. At our institution the definition is quite strict - the ICP had to be normal on at least 2 consecutive LPs conducted 1 week apart with an absence of delayed ICP deterioration in conjunction with CT follow-up confirming HCP resolution before medical treatment was considered successful. Secondly, this may be attributable to institutional concern about the risks of chronic uncontrolled raised ICP on an already ischaemic brain which has been demonstrated by continuous ICP and brain oxygen monitoring in TBM patients (discussed in Chapter 12). Shunt complications were low, documented in 6 patients (24%) over the last 3 years, and in 2 of these patients the VPS was complicated by HIV and poor nutrition. Despite the high rate of shunting this is an improvement on complication rates of 32-60% reported over 6-12 month follow-up in the literature [22,127]. Due to the challenges inherent in performing ETVs and the low rate of NCHCP these were performed infrequently.

Clinical outcome

Although the overall mortality rate at RXH was similar to TBH, the mortality for the study cohort was slightly higher than reported for TBH (16% vs 13%). However, this patient cohort represented a very particular selection of patients with HCP who represent the more severe spectrum of TBM disease. Indeed the study group was composed of the more severely infected patients treated at RXH as demonstrated by more severe presentation, greater CSF perturbations and higher mortality relative to patient ineligible for study inclusion (16% vs 10%).

It is a reassuring finding that more than 70% of this cohort demonstrated good outcomes at 6 months; almost half made a complete recovery and disabilities were mostly mild. This reflects positively on the acute in-hospital care. However, 80 % of the patients who underwent neurodevelopmental follow-up performed poorly; this demonstrates that although these children may appear grossly normal, they are left with deficits which will present them with very real challenges as they develop, most importantly the difficulty of completing their education successfully. Most of these children stem from impoverished homes which have limited finances and child caring options. Their communities generally have a baseline deficit of schools which can provide adequate support and stimulating environments to nurture development, especially for children requiring special attention. Adding disability, whether it be motor, cognitive, speech or sensory to already disadvantaged circumstances makes for real challenges in the short and long term. The 12 patients who suffered poor outcome (death or severe disability) presented with moderate - severe stage TBM and demonstrated clinical deterioration within the first week, whereas all patients who presented with mild TBM made a good recovery. These findings highlight the importance of early diagnosis before the window of opportunity to halt disease progression has closed, and demonstrate the acute progression of TBM disease in children who suffer poor outcomes. It is tempting to suggest that an earlier presentation would improve outcomes; however, the signs and symptoms of TBM are non-specific and diagnosis and treatment initiation may still be delayed.

It is noteworthy that 2 patients made unexpectedly good recoveries. One young boy (2.8 years) presented with Stage 4 TBM; his cerebral imaging showed cerebral oedema and moderate HCP which was treated with a VPS. He suffered a prolonged depressed LOC (GCS<8), had multiple daily seizures for more than a month after admission and was not considered for resuscitation. By 6 months he had made a remarkable recovery; he was mobile, interactive and functioning well (PCPS 2) although his performance on the Griffiths demonstrated neurodevelopmental deficit. A second young boy (4.1 years) presented with severe TBM and HIV co-infection, demonstrated infarction in his right basal ganglia on his admission scan and experienced a difficult hospital course including multiple admissions, concomitant streptococcus pneumonia meningitis and multiple shunt revisions. At 6 months he was unable to sit unsupported, to stand or feed himself and did not interact with his

environment (PCPS 4), he was too disabled to be assessed with the Griffiths. After 1.5 years, however, he had made a marked recovery and presented to the outpatients department as a happy, mobile, interactive and functioning child. He was then functionally able to engage in the Griffiths test although his performance was weak. These two cases demonstrate that recovery may be possible in some children in whom the early clinical picture would suggest otherwise.

Associations with outcome

The associations between admission characteristics and clinical outcome were weak; this may in part be a function of the low mortality seen in this group. It was an unexpected finding that a shorter duration of symptoms was associated with poor outcome at 6 months. This may reflect the aggressive nature of the disease in the patients who died as opposed to a low-level chronic disease process playing out over weeks in children who survived. This is also manifest in the acute deterioration within the first week after hospitalisation in patients who died or suffered severe disability. Alternatively, the duration of symptoms may have been poorly reported - the non-specific nature of TBM symptoms may have masked their relevance to parents, and 2 patient's mothers had recently died and histories taken from relatives were sparse. Children under the age of 2.5 years were at a higher risk of a poor outcome, however, this association did not hold for infants as none of the infants in this study died and all made good recoveries by 6 months. A greater number of deaths were seen amongst female patients. Males and females did not differ significantly on any admission or clinical variables and with such a small number of deaths (n=7) it is possible that this may be a chance finding.

Among the laboratory CSF findings only higher polymorphonuclear cell counts appeared to be associated with outcome. There are data to suggest that neutrophils contribute to the early defence against *Mtb* and that the recruitment of these white cells to the site of disease improves outcome [435]. It is possible therefore, that patients who had higher neutrophil counts were admitted at an earlier stage of TBM disease, and were consequently more likely to have a good outcome.

Neurodevelopmental outcome

Assessments with the GMDS-ER demonstrated that TBM cases performed poorly in comparison to peers drawn from similar backgrounds. This would suggest that, having attempted to take the influence of socio-economic status and culture into account, TBM was associated with compromised development. Infarcts are significantly associated with poor development after TBM, particularly in locomotor performance [145]. While there is certainly an organic component to developmental deficits, the impact of TBM extends to the outpatient period as well. Many of these patients were hospitalised at BCH for 6-9 months. Although the doctors and support staff at that institution provide the best care their limited resources allow, these children are exposed to a number of factors that may further

compromise their development. Firstly, normal procedure dictates that children are dressed, fed and washed by the nursing staff. Consequently, these children do not develop independence in these tasks, and this is reflected in their poor test scores on the personal-social subscale with up to 70% of TBM patients demonstrating deficits in this area. Secondly, BCH staff comprise English, Afrikaans and Xhosa speaking individuals who often speak a mixture of languages to the children. This may have contributed to their poor language scores (90% performing below controls) as communication takes place in languages other than the mother-tongue and the language foundation of these young children remains weak. Thirdly, these children are not often exposed to environments external to the hospital ward and playground. Consequently, their locomotor development was limited by their environment. For example, BCH does not have any stairs and poor stair climbing was ubiquitous among TBM cases at BCH, likely due to the lack of exposure rather than motor impairment. Fourthly, teaching at BCH is limited by human and material resource constraints. Certain skills like drawing, writing and recognising body parts were poor in all BCH children and this may be attributed, at least in part, to their limited exposure to tasks that build these skills or knowledge. Fifthly, these children live predominantly at BCH during their hospitalisation and may only see their parents on occasional weekends. Parents often live far from the hospital and need to work and care for other children limiting their opportunities to visit and fetch their children from BCH. The impact of maternal or family separation and the absence of maternal input may have a very real impact on the wellbeing and development of these children.

As part of this study GMDS-ER assessments were also conducted with the non-TBM children admitted to BCH in an attempt to control for the impact of 6 month hospitalisation at BCH on development. However, apart from complicated TB, poor social circumstances are one of the admission criteria to BCH and 4 out of the 8 children assessed came from difficult home environments. It is well established in the literature that poor social circumstances can significantly negatively impact a child's development [423,436] rendering 50% of this potential control cohort developmentally compromised at baseline. Additionally 2 children had established speech delay. Due to the developmental disadvantages intrinsic to this group they were not considered an optimal control group and recruitment for GMSD-ER assessment ceased.

The 10 children who were not admitted to BCH were either admitted to other institutions with similar limitations (n=2) or discharged home (n=8). Children cared for at home were subject to the financial, schooling and care-giver limitations inherent to their poor SES. Several mothers struggled to care for their children as a result of commitments to work or other children and crèche was financially inaccessible for several families. The impact of TBM on development is therefore multidimensional comprising the organic injury, as well as the environmental, social and educational limitations imposed by their disease and prolonged treatment in both the short and long term.

The absence of an association between neurodevelopmental outcome and clinical outcome may be due to differences inherent in the nature of the outcome being assessed by the 2 tools. Clinical outcome assessment was based on the overall neurological picture of the child thereby assessing outcome on a very general level with a focus on neurological deficits discernable on physical examination. The GMDS-ER assessed outcome in specific functional categories which covered motor and sensory performance as well as cognitive and social performance. Furthermore, the GMDS-ER assessed not only whether an activity could be performed but also the quality with which the activity was performed; therefore, it detected both gross and subtle deficits over a broad range of function, many of which may not be apparent on physical examination. Additionally, clinical outcome is determined largely by pathology, whereas neurodevelopmental outcome is influenced by pathology in combination with social, educational and psychological factors.

HIV co-infection

The small number of HIV+ patients did not allow formal analysis across the infected and un-infected patients groups; however, these 2 patients demonstrated admission characteristics and CSF findings similar to those of HIV- patients. Both children fell into the poor outcome group (1 died and the other had severe disabilities after 6 months); however, they had both defaulted on ART a number of months before their TBM admission and with the small sample size it is not possible to conclude whether appropriately treated HIV co-infection would also expose children to a greater risk of poor outcome. The patient who survived demonstrated worsening basal enhancement 10 weeks after ART initiation (drug-sensitivity had been confirmed). This may represent a TBM-IRIS response or represent ongoing TBM disease as seen in one of the HIV- patients who re-presented with worsening tuberculomas and enhancement 7 weeks after anti-TB treatment initiation. Although TB culture and drug-sensitivity could not be confirmed on the latter patient, the very low overall rate of resistance at RXH would suggest that his deterioration was not likely due to drug resistance. HIV exposure was not associated with outcome.

Limitations

Data on admission and demographic characteristics were drawn from patient folders and from interviews with parents when possible. The data were therefore subject to variable qualities of history taking and the accuracy or fullness of parental reporting may have been limited by language and the subjectivity with which parents experienced and recalled their child's illness. A number of these patients presented to RXH without a parent or guardian present and the accompanying information from the referral hospital, often sparse, had to be used in lieu of a proper history. Other children were brought in by family members, other than the mother, who had limited knowledge of the child's previous medical history. The frequency of admission characteristics may therefore have been underestimated. TSTs are usually performed on admission to the medical wards; when patients were

admitted directly to the neurosurgical ward or ICU or transferred there soon after admission these tests often were not performed as they did not form part of the routine ward procedures. Placing outcome of this HCP study group in the context of overall TBM outcomes at RXH was curtailed by the limited follow-up for non-HCP patients as these patients are generally not assessed in the Outpatients Departments of RXH. An accurate assessment of RXH's TBM outcomes would require a prospective study which tracks children as they are discharged to secondary and primary level services.

For the neurodevelopmental outcome it was not possible to exactly match cases and controls for age and gender, and several of the control children's mothers were more educated. However, the average age of the children with mothers who had tertiary education was 13.5 months. Molteno *et al's* study in Cape Town found that child development was similar across all SES groups till approximately 12 months of age; only after infancy did the association between the deceleration in development and maternal education/SES become more apparent [422]. Therefore, some of the discrepancy between maternal levels of education may have been mitigated in this group. It remains possible, however, that the control group performed superiorly due to better home stimulation and that this may have falsely augmented developmental deficits associated with TBM. Establishing an appropriate control group is challenging, and although the GMDS-ER has been used for several local TBM studies they have focussed on within group comparisons and none have included comparisons to a control group [106,145]. Future projects will continue to build an appropriate control group against which to compare the GMDS-ER outcomes of patients admitted to RXH or similar institutions.

CHAPTER 10 SUMMARY

Biomarkers of neurological injury and inflammation

In this chapter, analysis aimed to establish if neuromarkers S100B, NSE and GFAP were elevated in TBM, associated with patient clinical characteristics and prognostic of clinical and neurodevelopmental outcome. A panel of inflammatory markers was also analysed.

Biomarkers were analysed in serum, lumbar CSF and ventricular CSF on admission and for the next 3 weeks using ELISA for neuromarkers and Luminex multibead array assay for inflammatory markers. Biomarker concentrations in patients were compared against control patients and their temporal profile was examined. Various neuromarker indices were analysed in association with outcome (clinical and neurodevelopmental) including initial and highest concentrations as well as the trend over time. PCA combining neuro- and inflammatory markers over time was examined in association with outcome.

The main findings were as follows:

1. Neuro- and inflammatory markers were elevated in the lumbar and ventricular CSF of TBM cases relative to controls on admission and for up to 3 weeks, however, serum concentrations were not. This suggests that CSF S100B, NSE and GFAP are indicators of cerebral injury in TBM and that the immune response in TBM is compartmentalised to the brain.
2. Neuromarker concentrations were higher in the ventricular than lumbar CSF, likely reflecting the largely cerebral origin of neuromarkers. Inflammatory concentrations were higher in lumbar than ventricular CSF, probably reflecting the predominantly subarachnoid location of much of the inflammatory response.
3. Neuromarkers were poorly associated with patient and admission clinical characteristics suggesting that they provide additional information about brain injury that is not reflected in the history and clinical examination.
4. Elevated neuromarker concentrations within the first week and overall were prognostic of poor outcome. The strongest predictor of outcome was an increasing trend in neuromarker concentrations over time.
5. PCA combining all 3 neuromarkers over time demonstrated that concentrations continued to increase in patients who died, while combined inflammatory concentrations decreased. This suggests that it is not the ongoing immune response which is responsible for the progressive brain injury associated with TBM, but rather secondary pathological processes that are initiated by the inflammatory process.

6. Associations between inflammatory markers and outcome were poor and combining neuro- and inflammatory markers did not improve the association with outcome.

CHAPTER 10: BIOMARKER ANALYSIS

Methods

Sample collection

Samples for biomarker examination included lumbar CSF, ventricular CSF and serum. A volume of 3 ml of CSF and 3 ml of blood were collected (where possible) for study purposes. CSF was collected in sterile tubes and blood in SSTII tubes for serum collection (BD Vacutainer, Plymouth, UK). All CSF and serum samples were collected at the time of clinically indicated lumbar punctures, neurosurgery or phlebotomy.

TBM Cases

Admission samples: Lumbar CSF samples were collected from routine diagnostic LPs. In patients who were too unstable for an LP an EVD was placed, from which ventricular CSF was collected at insertion. In some cases a lumbar CSF specimen was also collected at the same time (immediately pre-EVD insertion). Blood samples were collected at the time of LP or in the operating theatre if blood had not already been drawn for clinical purposes. If blood had already been drawn, a study sample was taken with the next planned phlebotomy. Where possible, samples were taken before the commencement of anti-TB treatment and steroids. Samples from patients started on treatment for more than 3 days were excluded.

Serial samples: Additional CSF and blood samples were collected once per week during weeks 1-3. In patients with CHCP lumbar CSF was collected at the time of clinically planned serial LPs. Ventricular samples for these patients were taken during planned neurosurgical procedures including EVD insertion and removal or shunt insertion if these patients failed medical treatment. For patients with NCHCP ventricular CSF was collected during EVD insertion and removal, shunt insertion and/or ETV. Any lumbar CSF samples for these patients were collected during AEGs or column tests to establish that the HCP was non-communicating. Blood samples were time-linked with CSF samples when possible or taken as part of clinically indicated blood draws.

Controls

For the fatty filum controls blood was drawn after the patient was anaesthetised at the time of checking haemoglobin/arterial blood gas and before the operation began to minimise the risk of possible extracranial sources of neurological biomarkers released in response to surgical procedures to

skin and bone. Lumbar CSF samples were taken before the dura was opened as part of the operative procedure. For pTB controls samples were taken at the time of clinically indicated phlebotomy. All samples were collected before treatment initiation or within 48 hours thereof.

Sample processing

Within the constraints of a busy 24 hour clinical service, sample processing occurred immediately after sample collection. When this was not possible, samples were refrigerated at 5°C for < 5 hours or frozen at -20 to -70°C overnight. Frozen blood samples were not used due to the potential for contamination from intracellular proteins released from blood cells. In the laboratory samples were centrifuged at 4000 rotations per minute for 5 minutes (1780 relative centrifugal force), the supernatants were preserved and the cells discarded. Samples were examined for serum hemolysis and CSF xanthochromia and affected samples were excluded from NSE analysis due to the contribution of NSE of erythrocyte origin. Samples were aliquotted into smaller volumes for ELISA and Luminex testing and frozen at -70°C for batch analysis. Multiple freeze thaw cycles were avoided. Serum albumin testing was run immediately in the in-house National Health Laboratory Service (NHLS) laboratory; CSF albumin testing was batched for analysis at the NHLS laboratory at Groote Schuur Hospital.

Sample analysis

Neuromarkers

Commercially available enzyme-linked immunoassays (ELISA) were used to analyse S100B (Merck Millipore, Billerica, USA), NSE (DRG Diagnostics, Marburg, Germany) and GFAP (Merck Millipore, Billerica, USA). Batch analysis was performed thrice during the 3 years of patient enrolment based on the accumulation rate of samples. Testing procedures and ELISA kits were standard across the 3 analysis episodes. The full complement of each patient's samples (serum and CSF) were analysed on the same plate; samples were not analysed in duplicate. All assays were carried out according to manufacturer instructions. Briefly, the ELISA testing procedure was carried out using 96 well plates coated with monocolonal antibodies directed at the target analyte. Patient samples were incubated in the coated wells with an enzyme conjugate made up of anti-analyte antibodies conjugated with horseradish peroxidase. This facilitated binding of the enzyme to captured target analyte molecules. Free enzyme conjugate was washed away and the quantification of immobilized antibody-enzyme conjugate was measured spectrophotometrically at an absorbence of 450nm. The amount of bound peroxidase was proportional to the concentration of the target analyte in patient samples and analyte concentrations were derived by interpolation using a standard curve

generated from reference standards of known concentration. Testing kits for S100B and GFAP were validated for both serum and CSF. The NSE assay was validated for serum; however, since no NSE assays validated for CSF are available in South Africa, both sample types were analysed on this test kit. Normal reference ranges were suggested by the manufacturers for NSE only (0-12 ng/ml).

Inflammatory markers

The panel of cytokines and chemokines examined included IL-1β, IL-1Ra, IL-6, IL-10, Il-12p40, TNF-α, IFN-γ, IL-8, GRO, MCP-1, IP-10, MIP -1α, VEGF and RANTES. These were analysed on the Bio-Plex platform (Bio-Rad Laboratories, Hercules, CA, USA), using customised 14-plex MilliplexTM kits (Millipore, St Charles, MO, USA), according to the manufacturer's instructions. This technique uses fluorescent labels to uniquely colour-code beads which are each coated in a specific capture antibody. When patient samples are added to the bead-containing wells individual analytes are captured by the beads specific to that analyte. Steptavidin PE is added and acts as the reporter molecule to complete the interaction on the surface of each bead. The beads pass rapidly through a laser which excites the dyes coating them while a second laser excites the fluorescent label PE on the reporter molecule. This enables the identification of individual beads and quantification of the fluorescent signal of their reporter molecules from which analyte concentrations may be derived using a standard curve of known concentrations. The luminex plates were validated for serum and CSF in the Clinical Infectious Diseases and Research Initiative (CIDRI) laboratory.

Data preparation

Biomarker values that fell below the lowest limit of detection (LLOD) of the testing kit were assigned the value 0.01. Neuromarker values that exceeded the highest limit of detection (HLOD) and were extrapolated from the standard curve were accepted if the standard curve was linear, variability around the curve was very low (R^2 = 0.98-0.99) and quality control values fell within the expected ranges. For cytokine concentrations which were so elevated that the luminex software did not extrapolate a value, the highest value measured for that analyte by that testing kit was assigned + 1. Unfortunately, limited samples did not allow repeat experiments with diluted samples.

Biomarker data were reviewed in the clinical context of each patient. Samples taken within 24-48 hours after procedures like EVDs, ETVs, CSF shunts and AEGs were identified and excluded to avoid the contribution of invasive procedures to inflammatory and injury marker concentrations. Samples that were not immediately refrigerated or not processed and frozen within 12 hours of collection were excluded as analyte degradation may have occurred.

Data Analysis

Neuromarker data were analysed according to *marker type* (S100B, NSE, GFAP [neuromarkers] or inflammatory markers) and *sample type* (lumbar CSF, ventricular CSF and serum) over time. The primary focus of this analysis was to describe the profile of neuromarkers and their association with clinical and neurodevelopmental outcome as potential indicators of brain tissue injury in TBM. Inflammatory markers were introduced in the outcome analyses as complements to the neuromarkers to ascertain whether adding inflammatory markers to the neurological markers enhanced prognostic power.

Analysis was, to some extent, curtailed by the large number of tests inherent in analyzing up to 17 analytes in 3 sample types over multiple time points in a small sample size; this was particularly relevant to the 14 inflammatory markers. Univariate and multivariate analysis were used for neuromarkers where possible, but multivariate analysis is limited as the number of variables which can be analysed is restricted by the sample size and the level of significance requires adjustment for multiple testing. Principal component analysis (PCA) is a data reduction technique which overcomes the limiting factors of multivariate analysis by allowing complex data sets with multiple data points over multiple variables and time points to be analysed simultaneously within a small sample size [437]. It allows the major sources of variation in a multidimensional data set to be identified and classified according to overarching principal components (PC). It is an unbiased approach as it makes no assumptions as to which biomarkers are of importance. It does not, therefore, explain the biology of the relationships between variables but rather how biomarkers contribute to variability between patients and over time. Consequently, PCA was adopted to analyse the full data set, or large parts thereof. PCA involves transforming the data set from having multiple variables to having fewer variables, or PCs, which are combinations of the original variables that explain as much of the variability in the data as possible. The covariance among the original variables is reduced into a set of PCs which are not correlated with each other but which are common to all variables by calculating a weighted average for each data point for each biomarker type included in the analysis. The first principal component (PC1) represents as much of the variation as possible, the second PC represents as much of the remaining variation and so forth. The quality of the PCA fit is determined by the amount of variability the analysis accounts for in the smallest number of PCs. Therefore, a strong PCA would account for the majority of variability in the data set with the first 2 PCs. PC scores are represented as Z-scores which can be plotted on a 2-dimensional plane to represent the sample points. The plot can be composed of multiple axes which represent the original variables and each point is interpreted in terms of its distance relative to the axes [438]. Further explanation of PCA is provided in Appendix 7.

Neuromarker and inflammatory marker profiles

Descriptive analysis

Descriptive statistics including median and interquartile range, and minimum and maximum for the 17 analytes of interest in the 3 sample types of interest (lumbar CSF, ventricular CSF and serum) were calculated.

Neuro- and inflammatory marker elevation

Neuro- and inflammatory marker concentrations of TBM cases were compared to those of controls using Mann Whitney's U with a Holm p-value adjustment to control for multiple testing. Elevated concentrations were defined as being > 95th percentile of control values.Ventricular CSF could not be sampled from the fatty filum control patients. In absence of cerebral injury or infection it is unlikely that biomarkers of neurological injury or inflammation would be elevated in any CSF compartment and ventricular biomarker concentrations should theoretically be similar to lumbar CSF. Consequently, ventricular and lumbar CSF drawn from TBM cases were compared to fatty filum lumbar CSF. TBM serum values were compared to the serum values of both the fatty filum and pTB control groups. The serum concentrations of the two control groups were also compared to each other. PCA bi-plots were generated to depict the distribution of data for both cases and controls in the 17 biomarkers and 3 sample types.

Temporal profile

Temporal profiles were evaluated graphically using box-and-whisker plots. Neuromarker concentrations were compared against control values at each time point. This temporal comparison could not be conducted for the inflammatory markers due to multiple testing with 14 analytes; therefore, only admission samples were compared with controls.

Inter-biomarker associations

Concentrations of the 17 neuro- and inflammatory markers at admission were correlated using Spearman's R. Cluster analysis was used to examine the similarities among the biomarkers by calculating the distances between a patient's data points. The smallest differences between biomarkers indicated the greatest similarity between them and these biomarkers were clustered together. The distances between cluster groups were calculated and the clusters exhibiting the smallest difference were connected. This was represented in a cluster dendrogram.

Additional correlations amongst the neuromarkers included overall median concentration for the sampling period and median concentration at each time point during the sampling period for the 3 sample types. To examine the interaction between neuromarker type (S100B, NSE, GFAP), sample type (lumbar CSF, ventricular CSF and blood) and time a GEE model was constructed which accommodated for intra-individual variability. Additionally time-linked lumbar and ventricular CSF neuromarker concentrations were compared to further explore the differential between the 2 CSF compartments; the presence of spinal arachnoiditis (discussed further in Chapter 11) was included in this analysis using a GEE.

PCA plots were constructed to depict the distribution of admission lumbar CSF, ventricular CSF and serum values on the same graph for all the neuromarkers, and on a separate graph for all the inflammatory markers.

Association with admission and patient characteristics

The association between initial neuromarker concentrations (admission or week 1 concentration when no admission concentration was available), and patient/admission characteristics (Chapter 9) was evaluated using the Chi-squared or Fisher's exact test. The last recorded S100B, NSE and GFAP concentrations in lumbar and ventricular CSF were analysed in association with failed medical treatment and ICP deterioration to ascertain whether neuromarker concentrations may predict these events.

Clinical outcome analysis

Outcome variables analysed included mortality at 6 months, dichotomized clinical outcome at 6 months (poor outcome [PCSP 4-6: severe disability- death] vs good outcome [PCPS1-3: normal – mild/moderate disability]), and survivor morbidity at 3 and 6 months (normal [PCPS 1], mild-moderate disability [PCPS2-3], severe disability [PCPS 4-5]).

Univariate analysis

The relationship between neuromarker concentrations and outcome was examined using Mann-Whitney's U (mortality and 6 month outcome), the Kruskal-Wallis for survivor morbidity categorized across 3 levels, and Chi-square for categorical data. Neuromarker indices investigated in association with outcome included the initial concentration (admission or week 1 concentration when no admission concentration was available), highest in week 1 (highest from among admission and week 1 values), and highest overall (across the sampling period). As a measure of the trend over time, the absolute change (Δ) in the neuromarker concentration was calculated (change from initial to 2nd week

sample – the last available sample after admission was used for patients who were not sampled for as long as 2 weeks, or the week 3 sample was used in patients who did not have a sample at week 2). This last index could only be calculated for lumbar CSF as ventricular samples were too few.

Outcome analysis with inflammatory markers was limited to avoid multiple testing with 14 analytes; however, the predictive value of individual admission cytokine concentrations could be of relevance in clinical practice, where available testing may be limited to a few key analytes. Therefore, univariate outcome analysis for each cytokine was conducted and the p-values were not adjusted for multiple testing as part of hypothesis testing to observe whether any cytokines demonstrated potential as prognostic markers in TBM.

Principal Component Analysis (PCA)

PCA was used to derive Z-scores combining all 3 neuromarkers or all 14 inflammatory markers for each patient, these z-scores combining multiple analytes were used as a statistical approximation of a single index for neurological injury which incorporates the contributions of multiple biomarkers and a single index for inflammatory markers. These were calculated for initial concentrations of lumbar CSF (Z-Neuro Initial LP, Z-Inflam Initial LP) and ventricular CSF (Z-Neuro Initial V, Z-Inflam Initial V), and for the absolute change (Z-NeuroΔ, Z-InflamΔ). The association between these variables and outcome was analysed using Mann-Whitney's U. PCA plots for mortality were constructed for Z-Neuro Initial LP and Z-Inflam Initial LP and Z-NeuroΔ and Z-InflamΔ to ascertain whether the combination of neuro- and inflammatory markers demonstrated an association with outcome. Variables which were not significantly associated with outcome were not carried forward into further outcome analyses. Statistical tools to assess the association between the temporal profile of neuro- or inflammatory markers and outcome are limited by a single outcome variable in the face of multiple biomarker data points. Consequently, PCA was used to derive 3-dimensional plots for the combined neuromarkers over time and the combined inflammatory markers over time using the Z-scores at each time point; the temporal profiles of patients who died were highlighted.

Receiver operating characteristic (ROC) analysis

ROCs were constructed to identify the thresholds of highest S100B, NSE and GFAP as well as ΔS100B, ΔNSE, ΔGFAP and Z-NeuroΔ which best predicted mortality and 6 month clinical outcome with the optimal combination of sensitivity and specificity. The efficacy of these variables in predicting poor outcome is indicated by the area under the curve (AUC), where the perfect curve would have an area of 1.0.

Multivariate analysis

Logistic regression models for mortality and morbidity were constructed for ΔS100B, ΔNSE, ΔGFAP and Z-NeuroΔ since these neuromarker indices demonstrated the strongest association with outcome on univariate analysis. Covariates were added to these models to determine whether their inclusion altered the relationship between the neuromarkers and outcome. Covariates were drawn from patient admission characteristics (Chapter 9) and radiology variables (Chapter 11) which were significantly associated with clinical outcome or biomarker concentrations on univariate analyses.

Developmental outcome analysis

The association between developmental outcomes and neuromarker concentrations was analysed by examining the association between the developmental score (deficit/no deficit) for the GQ and the 6 sub-scales and neuromarker indices including: highest S100B, NSE and GFAP (lumbar and ventricular), ΔS100B, ΔNSE, ΔGFAP as well as Z-NeuroΔ. These analyses were conducted using the Chi-square or Fisher's exact test.

CHAPTER 10: BIOMARKER ANALYSIS

Results

A total of 301 samples from 44 TBM cases were collected for analysis: 131 lumbar CSF, 108 serum and 46 ventricular CSF samples. The average number of samples collected per patient was 7.3, with a minimum of 2 and a maximum of 15. Seventeen samples were excluded: 7 lumbar CSF samples (5 were post-EVD/shunt/AEG, 2 were not frozen within 12 hours), 6 serum samples (4 post-EVD/shunt/AEG, 2 were not frozen within 12 hours) and 4 ventricular CSF samples (3 post-EVD/shunt/AEG, 1 was not frozen within 12 hours). For NSE analysis a further 15 hemolysed serum samples were excluded. For Luminex analysis an additional lumbar and serum sample were excluded as the plate reader was unable to detect any analyte beads in those samples, likely due to a technical error. Sample collection continued for up to 5 weeks depending on the HCP management of each patient.

For the fatty filum controls, 11 lumbar CSF and 11 serum samples were collected, of which 1 pair was excluded from NSE analysis due to hemolysis. Nine serum samples were collected from TB controls, of which 2 hemolysed samples were excluded from NSE testing.

Assay results

All quality control values in the ELISA and Luminex assays fell within the expected ranges. All standard curves generated in the ELISA results had R^2 values between 0.98 and 0.99. Details of the LLOD for each test kit are outlined in Table 10.1. For GFAP, serum samples accounted for most of the results <LLOD.

Table 10.1: ELISA Results: Lowest limits of detection

Analyte Limit of detection	S100B LLOD	NSE LLOD	GFAP LLOD
Plate 1	0.02	6.56	1.5
Plate 2	0.02	6.53	1.5
Plate 3	0.02	4.67	1.3
Plate 4	0.01	4.09	0.4
Plate 5	0.04	2.48	1.6
Plate 6	-	-	1.6
% samples < LLOD	9.78	8.95	35.97

This table demonstrates the lower limit of detection (LLOD) for each of the ELISA plates used to assay neuromarkers S100B, NSE and GFAP, as well as the percentage of the total samples which fell below these limits. GFAP required 6 plates, as the plate is set up to run fewer samples per plate than the S100B and NSE kits

Table 10.2: Luminex results: Lowest limits of detection

Analyte	Plate 1		Plate 2		Plate 3		Plate 4		Plate 5	
	LLOD	**%**	**LLOD**	**%**	**LLOD**	**%**	**LLOD**	**%**	**LLOD**	**%**
GRO	15.9	*2.6*	17.1	*2.6*	16	*12.8*	16.4	*2.6*	16	*12.7*
IL-Ra	2.5	*20.5*	12.1	*5.1*	8	*30.8*	16	*6.4*	16	*26.8*
IL-12p40	2.8	*26.9*	2.4	*6.4*	16	*34.6*	3.19	*11.5*	16	*19.7*
IP-10	3.1	*0*	3.8	*0*	3.2	*25.6*	16	*0*	3.2	*38*
MCP-1	3.1	*0*	3	*0*	16	*0*	3.2	*0*	3.2	*1.4*
MIP-1α	4.1	*16.7*	4.1	*23*	3.2	*34.6*	3.2	*10.3*	3.2	*15.5*
TNF-α	3.1	*1.3*	3.1	*0*	3.2	*1.3*	3.2	*0*	3.2	*5.6*
VEGF	80.1	*3.8*	84.3	*8.9*	80	*12.8*	80	*25*	80	*29.6*
IFN-γ	3.1	*5.1*	3.1	*11.5*	16	*8.9*	3.2	*1.3*	3.2	*9.9*
IL-10	3.1	*3.8*	3.5	*2.6*	3.2	*10.3*	3.2	*0*	3.2	*9.9*
IL-1β	2.9	*57.7*	3.1	*38.5*	3.2	*41*	3.2	*41*	3.2	*29.6*
IL-6	3.1	*7.7*	3.1	*10.3*	3.2	*0*	3.2	*11.5*	3.2	*19.7*
IL-8	3.3	*0*	3.2	*0*	3.2	*1.3*	3.2	*0*	3.2	*1.4*
RANTES	16.6	*51.3*	2.5	*50*	3.2	*55.1*	3.2	*52.5*	3.2	*52.1*

This table details the lower limit of detection (LLOD) of the inflammatory cytokines assayed using Luminex technology, as well as the percentage of samples which fell below those limits for each plate used, indicated by "%"

Descriptive statistics

Tables 10.3 and 10.4 outline the descriptive statistics for all biomarkers for the 2 control groups and the TBM cases for the duration of sampling (patients treated for longer than 3 weeks had samples taken till treatment ended).

Table 10.3: Descriptive statistics for neuromarkers in all sample types (µg/L)

Marker		S100B			NSE			GFAP		
Sample		**LP**	**V**	**S**	**LP**	**V**	**S**	**LP**	**V**	**S**
Admission		n=36	n=23	n=36	n=36	n=23	n=30	n=36	n=23	n=36
	Median	1.37	3.24	0.08	17.62	29.07	16.74	8.92	93.31	0.01
	Min	0.01	0.91	0.01	0.01	0.01	0.01	0.01	2.73	0.01
	Max	8.57	9.82	0.59	89.49	104.86	73.75	181.09	269.96	20.25
	p25	0.7	1.34	0.02	8.48	20.53	11.36	3.25	33.86	0.02
	p75	3.07	5.30	0.14	38.12	48.89	27.08	24.16	172.37	1.77
Week 1		n=29	n=9	n=26	n=29	n=9	n=21	n=29	n=9	n=26
	Median	1.37	5.37	0.11	24.67	39.61	16.09	6.06	168.64	0.01
	Min	0.16	1.07	0.01	0.01	17.38	0.01	0.01	8.23	0.01
	Max	9.13	16.25	1.00	94.97	154.86	42.60	298.66	344.40	16.29
	p25	0.57	1.13	0.02	14.32	34.61	8.8	2.1	13.37	0.01
	p75	3.46	9.12	0.23	46.42	91.97	24.21	21.84	331.87	0.01
Week 2		n=31	n=6	n=24	n=31	n=6	n=23	n=31	n=6	n=24
	Median	0.77	1.05	0.04	14.44	19.42	12.86	3.38	66.30	0.01
	Min	0.16	0.29	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Max	10.53	2.53	1.06	78.92	80.64	104.67	164.43	270.50	15.88
	p25	0.4	0.62	0.03	8.21	10.82	7.99	0.03	17.09	0.01
	p75	1.84	2.47	0.22	28.8	46.01	19.88	9.38	100.01	2.72
Week 3		n=21	n=5	n=14	n=21	n=5	n=13	n=21	n=5	n=14
	Median	0.48	1.10	0.06	10.52	29.20	12.74	3.26	196.12	0.89
	Min	0.01	0.12	0.01	0.01	0.01	5.82	0.01	18.55	0.01
	Max	16.9	11.86	0.44	101.92	44.64	50.08	257.8	281.20	20.79
	p25	0.22	0.86	0.03	8.75	13.86	7.66	0.67	62.29	0.01
	p75	1.01	3.81	0.11	20.08	34.52	18.62	10.27	271.99	2.21
Week 4		n=11	n=2 [a]	n=7	n=11	n=2 [a]	n=5	n=11	n=2 [a]	n=7
	Median	0.62	4.26	0.07	7.54	55.40	16.28	3.46	18.98	0.01
	Min	0.01	0.90	0.03	0.01	35.47	0.01	0.01	15.74	0.01
	Max	1.41	7.63	0.15	44.34	75.33	32.70	30.64	22.23	31.72
	p25	0.03	0.90	0.05	6.51	35.47	14.07	1.62	15.74	0.01
	p75	0.83	7.63	0.12	18.34	75.33	22.45	9.4	22.23	1.4
Week 5		n=2	n=0	n=0	n=2	n=0	n=0	n=2	n=0	n=0
	Median	0.1			3.78			8.12		
	Min	0.1			0.01			0.67		
	Max	0.1			7.54			15.57		
	p25	0.1			0.01			0.67		
	p75	0.1			7.54			15.57		

Controls	**Fatty filum**		**pTB**	**Fatty filum**		**pTB**	**Fatty filum**		**pTB**
	LP[1]	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**
	n=11	n=11	n=9	n=10	n=10	n=7	n=11	n=11	n=9
Median	0.36	0.05	0.1	6.88	13.4	28.12	0.03	0.76	1.17
Min	0.01	0.03	0.01	2.87	8.2	10.42	0	0.01	0.01
Max	0.44	0.15	0.5	12.46	26.23	50.82	1.19	5.90	5.90
p25	0.21	0.04	0.07	4.51	10.9	13.39	0	0.24	0.01
p75	0.39	0.1	0.11	10.42	20.9	29.34	0.62	2.51	1.79
p5	0.01	0.03	0.01	2.87	8.2	10.42	0.01	0.01	0.01
p95	0.44	0.15	0.5	12.46	26.23	50.82	1.19	5.9	5.79

LP = lumbar CSF, V = ventricular CSF, S = serum, p= percentile, min = minimum, max = maximum, [a] increase in week 4 reflects data of 2 patients- 1 failed medical treatment and required a VPS (responsible for max value)

Table 10.4: Descriptive statistics for inflammatory markers in all sample types (pg/mL)

Marker		TNF-α			IFN-γ			IL-6		
Sample		**LP**	**V**	**S**	**LP**	**V**	**S**	**LP**	**V**	**S**
Admission		n=36	n=23	n=36	n=36	n=23	n=30	n=36	n=23	n=36
	Median	266.32	109	15.46	1095.67	684.79	9.66	1700.91	873.54	7.61
	Min	76.97	20.63	0.01	140.99	118.89	0.01	218.79	38.35	49.95
	Max	1604.31	1010.17	50.88	8541.02	3888.62	48.9	18448.02	13643.62	10.20
	p25	180.14	75.49	8.42	619.97	307.39	4.12	1101.84	415.62	1.96
	p75	493.68	171.6	19.11	2809.42	1080.8	19.58	4863.87	2272.35	12.35
Week 1		n=29	n=9	n=26	n=29	n=9	n=26	n=29	n=9	n=26
	Median	160.16	39.89	13.48	278.44	239.02	6.07	595.86	377.59	4.73
	Min	34.63	12.09	3.36	48.44	57.14	0.01	0.01	43.08	0.01
	Max	1329.57	712.6	52.55	10275.4	1055.49	75.84	10464.67	5174.25	392.83
	p25	83.49	32.65	8.86	149.23	112.76	0.01	328.33	58.56	0.01
	p75	295.86	74.18	17.94	793.26	259.64	13.05	2066.4	791.99	12.43
Week 2		n=30	n=6	n=23	n=30	n=6	n=23	n=30	n=6	n=23
	Median	95.15	67.55	11.45	244.51	271.32	4.43	373.81	343.71	0.01
	Min	25.48	15.8	3.99	0.01	25.92	0.01	21.68	18.89	0.01
	Max	402.75	141.27	25.31	2197.81	787.56	44.78	5175.25	1210.65	46.97
	p25	48.62	50.58	7.19	80.71	108.88	0.01	91.83	170.51	0.01
	p75	138.29	137.06	14.94	465.49	507.03	13.57	604.53	500.6	5.73
Week 3		n=21	n=5	n=14	n=21	n=5	n=14	n=21	n=5	n=14
	Median	56.84	90.7	11.7	126.68	93.92	0.01	351.6	244.88	2.78
	Min	8.49	17.74	4.12	0.01	31.33	0.01	5.65	5.22	0.01
	Max	216.71	118.5	50.26	757.17	315.17	268.25	1586.25	21146.37	67.69
	p25	26.16	29.96	5.52	66.08	67.74	0.01	120.81	59.89	0.01
	p75	84	110.26	16.64	154.25	184.12	7.04	482.87	561.8	4.74
Week 4		n=11	n=2	n=7	n=11	n=2	n=7	n=11	n=2	n=7
	Median	56.05	63.76	13.8	69.71	62.31	0.01	216.2	412.64	0.01
	Min	20.5	58.08	5.35	21.1	45.61	0.01	12.87	23.32	0.01
	Max	299.88	69.44	39.14	715.18	79.01	116.18	1117.09	801.96	35.62
	p25	34.94	58.08	6.48	49.34	45.61	0.01	62.02	23.32	0.01
	p75	129.88	69.44	29.52	209.65	79.01	9.15	678.55	801.96	6.8
Week 5		n=2	n=0	n=0	n=2	n=0	n=0	n=2	n=0	n=0
	Median	83.63			199.71			745.76		
	Min	52.6			84.3			110.24		
	Max	114.65			315.11			1381.28		
	p25	52.6			84.3			110.24		
	p75	114.65			315.11			1381.28		
Controls		**Fatty filum**		**pTB**	**Fatty filum**		**pTB**	**Fatty filum**		**pTB**
		LP[1]	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**
		n=11	n=11	n=9	n=10	n=10	n=7	n=11	n=11	n=9
	Median	0.01	12.09	27.4	0.01	0.01	13.1	0.01	0.01	34.39
	Min	0.01	7.7	11.84	0.01	0.01	7.97	0.01	0.01	3.82
	Max	0.01	27.82	135.02	0.01	261.3	196.87	0.01	47.63	458.64
	p25	0.01	9.75	23.16	0.01	0.01	11.42	0.01	0.01	7.21
	p75	0.01	20.84	33.06	0.01	6.24	20.9	0.01	0.01	40.48
	p5	0.01	7.7	11.84	0.01	0.01	7.97	0.01	0.01	3.82
	p95	0.01	27.82	135.02	0.01	261.3	196.87	0.01	47.63	458.64

LP = lumbar CSF, V = ventricular CSF, S = serum, p= percentile, min = minimum, max = maximum, LP and serum samples in week 2 have 1 less sample relative to neuromarkers as this pair of samples was excluded due to a technical error during Luminex analysis

Marker		IL-12p40			IL-1β			IL-Ra		
Sample		**LP**	**V**	**S**	**LP**	**V**	**S**	**LP**	**V**	**S**
Admission		n=36	n=23	n=36	n=36	n=23	n=36	n=36	n=23	n=36
	Median	43.34	4.85	10.69	6.7	0.01	0.01	456.2	72.65	33.31
	Min	0.01	0.01	0.01	0.01	0.01	0.01	19.02	0.01	0.01
	Max	279.46	63.99	126.18	58.76	27.14	16.37	1572.11	699.77	223.29
	p25	20.25	0.01	0.01	0.01	0.01	0.01	204.17	28.42	0.01
	p75	62.8	32.03	45.2	15.04	6.89	3.31	810.35	195.84	84.48
Week 1		n=29	n=9	n=26	n=29	n=9	n=26	n=29	n=9	n=26
	Median	44.96	2.39	17.29	0.01	0.01	0.01	182.06	62.91	31.18
	Min	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Max	330.96	114.41	114.21	99.39	50.68	13.62	2074.47	252.14	619.99
	p25	25.2	0.01	0.01	0.01	0.01	0.01	47.26	0.01	0.01
	p75	79.95	12.54	38.13	3.7	8.86	1.01	736.48	88.53	68.73
Week 2		n=30	n=6	n=23	n=30	n=6	n=23	n=30	n=6	n=23
	Median	50.99	23.7	21.47	0.01	0.01	0.01	71.99	46.68	19.02
	Min	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Max	356.42	133.38	92.16	12.31	18	14.6	2060.31	154.38	184.79
	p25	30.99	16.74	0.01	0.01	0.01	0.01	2.53	0.01	0.01
	p75	100.02	35.15	44.04	4.1	13.79	1.52	210.34	89.94	69.48
Week 3		n=21	n=5	n=14	n=21	n=5	n=14	n=21	n=5	n=14
	Median	41.37	64.48	13.99	0.01	0.01	0.01	2.53	31.57	38.74
	Min	0.01	8.08	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Max	217.54	146.87	236.27	8.47	5.58	50.42	293.77	1492.95	384.11
	p25	4.87	12.89	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	p75	65.95	87.23	23.44	0.01	3.8	0.01	59.28	51.87	52.69
Week 4		n=11	n=2	n=7	n=11	n=2	n=7	n=11	n=2	n=7
	Median	30.99	65.28	0.01	0.01	0.01	0.01	0.01	1.27	37.8
	Min	0.01	45.53	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Max	273.4	85.02	176.57	3.7	0.01	33.5	618.6	2.53	261.35
	p25	25.2	45.53	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	p75	59.27	85.02	52.47	3.41	0.01	0.01	130.97	2.53	66.3
Week 5		n=2	n=0	n=0	n=2	n=0	n=0	n=2	n=0	n=0
	Median	64.37			0.01			22.09		
	Min	52.47			0.01			0.01		
	Max	75.62			0.01			44.17		
	p25	52.47			0.01			0.01		
	p75	75.62			0.01			44.17		

Controls		**Fatty filum**		**pTB**	**Fatty filum**		**pTB**	**Fatty filum**		**pTB**
		LP[1]	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**
		n=11	n=11	n=9	n=10	n=10	n=7	n=11	n=11	n=9
	Median	0.01	29.25	25.69	0.01	0.01	0.01	0.01	16.53	69.36
	Min	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Max	0.01	99.76	74.31	0.01	6.89	9.95	0.01	80.09	279.49
	p25	0.01	5.74	6.51	0.01	0.01	0.01	0.01	0.01	23.65
	p75	0.01	41.58	35.11	0.01	0.01	0.01	0.01	30.74	72.85
	p5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	p95	0.01	99.76	74.31	0.01	6.89	9.95	0.01	80.09	279.49

LP = lumbar CSF, V = ventricular CSF, S = serum, p= percentile, min = minimum, max = maximum

Marker		IL-8			MCP-1			IP-10		
Sample		**LP**	**V**	**S**	**LP**	**V**	**S**	**LP**	**V**	**S**
Admission		n=36	n=23	n=36	n=36	n=23	n=36	n=36	n=23	n=36
	Median	1981.46	490.57	20.14	1947.4	4597.47	143.21	35505.4	24924.98	550.97
	Min	487.48	23.41	4.31	507.8	1627.85	20.22	5919.14	970.83	165.43
	Max	6539.84	1370.34	280.93	6257.75	15327.69	684.11	77800.54	77800.54	3654.24
	p25	1122.93	177.8	16.68	1510.62	2678.13	78.21	25618.52	7422.5	395.26
	p75	3396.45	806.4	36.2	2954.85	9227.79	225.95	69388.13	74447.62	1080.1
Week 1		n=29	n=9	n=26	n=29	n=9	n=26	n=29	n=9	n=26
	Median	1115.03	706.78	26.27	1280.36	2581.72	207.33	25618.52	15672.62	463.92
	Min	197.78	35.16	5.73	421.86	500.45	34.4	1959.68	488.21	146.81
	Max	7766.9	3399.34	236.15	6799.66	9936.64	792.72	78500.87	74531.67	4861.21
	p25	467.65	94.41	17.43	906.29	2238.52	164.24	8734.63	1217.97	226.1
	p75	2307.95	906.85	40.62	1702.15	3655.25	348.78	58255.83	69676.58	704.59
Week 2		n=30	n=6	n=23	n=30	n=6	n=23	n=30	n=6	n=23
	Median	804.1	323.85	21.77	1466.31	4045.46	225.1	25357.92	12102.38	324
	Min	126.07	48.82	0.01	467.46	1894.41	45.62	1847.29	2804.47	162.49
	Max	2424.95	749.45	969.47	9046.45	11696.31	740.67	76541.2	74447.62	1582.56
	p25	407.35	182.55	13.82	892.7	2395.89	143.34	8481.32	4147.57	194.98
	p75	1291.6	608.44	40.12	2295.7	6962.93	326.09	63333.72	35471.74	507.72
Week 3		n=21	n=5	n=14	n=21	n=5	n=14	n=21	n=5	n=14
	Median	651.52	244.96	17.42	1866.22	1821.05	263.32	14252.21	27490.36	312.1
	Min	37.35	57.55	6.72	245.94	336.85	71.19	445.64	396.94	113.99
	Max	3329.57	805.35	108.11	5474.77	4258.12	878.79	74447.62	74447.62	3768.51
	p25	301.26	187.91	13.91	1006.69	1796.2	159.45	3284.14	20746.36	205.44
	p75	1355.66	354.89	28.05	2455.82	2537.43	791.14	25618.52	69382.67	969.06
Week 4		n=11	n=2	n=7	n=11	n=2	n=7	n=11	n=2	n=7
	Median	635.77	498.95	25.91	1506.78	1816.67	331.02	14017.15	35227.77	532.95
	Min	133.58	399.7	10.84	648.07	1784.44	95.63	980.38	7950.87	84.56
	Max	4602.41	598.2	69.42	2938.7	1848.89	866.52	74447.62	62504.67	1002.57
	p25	253.15	399.7	22.19	1016.79	1784.44	232.79	2476.6	7950.87	358.18
	p75	1099.52	598.2	39.96	2539.85	1848.89	428.2	51893.2	62504.67	782.34
Week 5		n=2	n=0	n=0	n=2	n=0	n=0	n=2	n=0	n=0
	Median	1237.83			3270.23			37109.9		
	Min	366.18			1741.39			12431.57		
	Max	2109.48			4799.07			61788.22		
	p25	366.18			1741.39			12431.57		
	p75	2109.48			4799.07			61788.22		

Controls		**Fatty filum**		**pTB**	**Fatty filum**		**pTB**	**Fatty filum**		**pTB**
		LP[1]	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**
		n=11	n=11	n=9	n=10	n=10	n=7	n=11	n=11	n=9
	Median	6.58	9.93	15.37	651.38	246.54	418.17	43.42	272.16	1931.02
	Min	3.26	3.47	7.41	412.88	198.02	160.26	7.76	91.43	807.44
	Max	30.11	42.59	300.62	1475.21	610.11	1543.51	364.18	1012.02	19318.78
	p25	5.07	6.28	12.52	533.75	216.59	339.71	14.44	186.21	996.4
	p75	13.65	18.75	30.44	1175.25	365.65	1084.61	106.29	470.15	2188.88
	p5	3.26	3.47	7.41	412.88	198.02	160.26	7.76	91.43	807.44
	p95	30.11	42.59	300.62	1475.21	610.11	1543.51	364.18	1012.02	19318.78

LP = lumbar CSF, V = ventricular CSF, S = serum, p= percentile, min = minimum, max = maximum

Marker		MIP-1α			GRO			IL-10		
Sample		**LP**	**V**	**S**	**LP**	**V**	**S**	**LP**	**V**	**S**
Admission		n=36	n=23	n=36	n=36	n=23	n=36	n=36	n=23	n=36
	Median	57.79	35.2	3.26	1431.18	166.77	2165.63	420.32	97.22	8.54
	Min	26.48	9.54	0.01	250.17	0.01	873	107.18	41.61	0.01
	Max	256.37	113.17	1694.16	3207.74	819.52	4696.52	1209.5	536.41	78.76
	p25	41.39	24.38	0.01	1010.29	43.9	1793	252.45	61.66	1.78
	p75	78.92	46.58	17.34	1852.98	358.33	2779.23	653.04	255.17	17.86
Week 1		n=29	n=9	n=26	n=29	n=9	n=26	n=29	n=9	n=26
	Median	48.46	31.27	2.49	819.18	109.88	2155.46	140.29	50.24	14.04
	Min	0.01	10.08	0.01	130.51	0.01	127.94	46.45	11.47	0.01
	Max	91.08	140.46	1516.58	4067.42	3269.84	3651.44	873.74	603.21	85.51
	p25	21.5	24.62	0.01	429.08	0.01	1274.39	108.71	29.09	5.47
	p75	62.71	64.35	19.59	1589.94	415.05	2859.11	234.29	153.16	27.43
Week 2		n=30	n=6	n=23	n=30	n=6	n=23	n=30	n=6	n=23
	Median	37.4	37.94	9.54	696.18	179.1	2385.49	156.3	55.89	16.69
	Min	0.01	14.01	0.01	62.24	0.01	1030.95	9.73	34.79	0.01
	Max	92	49.28	864.09	2194.12	502.87	4252.57	439.5	605.45	132.23
	p25	23.3	30.09	0.01	320.24	38.72	1909.51	84.18	35.62	4.92
	p75	53.81	45.13	20.3	1267.34	290.01	3237.48	251.34	518.97	36.53
Week 3		n=21	n=5	n=14	n=21	n=5	n=14	n=21	n=5	n=14
	Median	32.61	22.18	2.44	508.08	107.09	2530.25	156.41	108.99	5.87
	Min	11.87	15.49	0.01	0.01	0.01	956.73	22.22	27.07	0.01
	Max	68.5	66.05	82.85	2898.23	180.76	5093.25	291.39	267.55	159.74
	p25	22.59	21.07	0.01	348.11	73.41	1834.52	95.38	64.57	0.01
	p75	36.28	24.27	19	1351.99	160.21	2984.26	215.3	154.01	25.74
Week 4		n=11	n=2	n=7	n=11	n=2	n=7	n=11	n=2	n=7
	Median	23.06	20.84	8.81	931.36	834.46	2609.84	216.77	163.4	16.88
	Min	8.49	15.19	0.01	77.43	297.65	1334.27	74.58	109.84	8.41
	Max	65.32	26.48	86.67	2425.63	1371.35	3242.75	275.68	216.96	68.74
	p25	17.5	15.19	0.01	277.25	297.56	1597.04	125.43	109.84	12.38
	p75	46.58	26.48	30.89	1785.63	1371.35	2948.99	254.48	216.96	38.48
Week 5		n=2	n=0	n=0	n=2	n=0	n=0	n=2	n=0	n=0
	Median	26.48			1376.51			191.21		
	Min	15.86			228.54			76.78		
	Max	37.09			2524.48			305.63		
	p25	15.86			228.54			76.78		
	p75	37.06			2524.48			305.63		
Controls		**Fatty filum**		**pTB**	**Fatty filum**		**pTB**	**Fatty filum**		**pTB**
		LP[1]	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**	**LP[1]**	**S[1]**	**S[2]**
		n=11	n=11	n=9	n=10	n=10	n=7	n=11	n=11	n=9
	Median	0.01	6.63	0.01	0.01	2273.49	2414.41	0.01	8.01	19.31
	Min	0.01	0.01	0.01	0.01	328.69	1254.55	0.01	0.01	4.08
	Max	10.61	63.47	78.87	0.01	3272.34	3062.44	0.01	31.98	44.67
	p25	0.01	0.01	0.01	0.01	1149.74	1538.76	0.01	3.4	7.58
	p75	7.41	13.14	15.49	0.01	2560.79	2816.48	0.01	18.93	32.26
	p5	0.01	0.01	0.01	0.01	328.69	1254.55	0.01	0.01	4.08
	p95	10.61	63.47	78.87	0.01	3272.34	3062.44	0.01	31.98	44.67

LP = lumbar CSF, V = ventricular CSF, S = serum, p= percentile, min = minimum, max = maximum

Marker		VEGF			RANTES		
Sample		**LP**	**V**	**S**	**LP**	**V**	**S**
Admission		n=36	n=23	n=36	n=36	n=23	n=36
	Median	145.33	136.15	220.27	0.01	0.01	4375.69
	Min	0.01	0.01	0.01	0.01	0.01	1356.4
	Max	836.7	424.78	1442.94	1022.21	0.01	13288.76
	p25	108	0.01	152.5	0.01	0.01	3680.88
	p75	269.13	220.27	382.75	0.01	0.01	7155.71
Week 1		n=29	n=9	n=26	n=29	n=9	n=26
	Median	141.88	67.17	236.19	0.01	0.01	4970.54
	Min	0.01	0.01	0.01	0.01	0.01	0.01
	Max	558.81	376.55	729.24	412.04	0.01	12984.16
	p25	97.28	0.01	130.44	0.01	0.01	4164.56
	p75	194.76	166.22	358.31	0.01	0.01	8418.42
Week 2		n=30	n=6	n=23	n=30	n=6	n=23
	Median	139.7	142.49	247.01	0.01	0.01	5026.29
	Min	0.01	0.01	0.01	0.01	0.01	862.17
	Max	504.41	399.71	973.74	314.6	0.01	11374.73
	p25	0.01	56.6	162.47	0.01	0.01	3800.18
	p75	206.25	309	383.57	0.01	0.01	8559.62
Week 3		n=21	n=5	n=14	n=21	n=5	n=14
	Median	0.01[a]	238.68	180.6	0.01	0.01	4851.89
	Min	0.01	97.28	0.01	0.01	0.01	1615.88
	Max	558.81	327.99	819.39	0.01	0.01	11787.36
	p25	0.01	221.98	150.43	0.01	0.01	4037.31
	p75	184.11	271.79	265.75	0.01	0.01	7747.56
Week 4		n=11	n=2	n=7	n=11	n=2	n=7
	Median	90.24	115.2	166.22	0.01	0.01	4403.37
	Min	0.01	56.6	0.01	0.01	0.01	2381.16
	Max	399.71	173.8	970.41	0.01	0.01	13632.33
	p25	0.01	56.6	135.06	0.01	0.01	3670.28
	p75	127.84	173.8	534.92	0.01	0.01	9796.18
Week 5		n=2	n=0	n=0	n=2	n=0	n=0
	Median	267.93			0.01		
	Min	136.15			0.01		
	Max	399.71			0.01		
	p25	136.15			0.01		
	p75	399.71			0.01		

Controls		**Fatty filum**		**pTB**	**Fatty filum**		**pTB**
		LP[1] n=11	**S[1]** n=11	**S[2]** n=9	**LP[1]** n=10	**S[1]** n=10	**S[2]** n=7
	Median	0.01	327.99	383.57	0.01	7270.56	9843.51
	Min	0.01	0.01	0.01	0.01	3511.32	4984.65
	Max	0.01	816.74	1950.31	0.01	12311.36	12977.91
	p25	0.01	90.24	220.27	0.01	4008.91	7407.29
	p75	0.01	546.56	681.05	0.01	9864.55	11179.14
	p5	0.01	0.01	0.01	0.01	3511.32	4984.65
	p95	0.01	816.74	1950.31	0.01	12311.36	12977.91

LP = lumbar CSF, V = ventricular CSF, S = serum, p= percentile, min = minimum, max = maximum, [a] The LLOD for VEGF was 80, therefore values <80 were assigned 0.01; week 3 concentrations were predominantly <80 and were assigned 0.01, therefore the median appears much lower than the week 2 and week 4 medians

Cases versus Controls

Neuro-markers

S100B **-** In TBM cases, S100B concentrations in lumbar and ventricular CSF samples were elevated compared to controls from admission to week 2 ($p<0.05$). Serum values were not significantly different from fatty filum or pTB controls.

NSE - NSE lumbar CSF values were elevated for the first 2 weeks and ventricular CSF until week 1 ($p<0.05$). Serum NSE concentrations were not elevated.

GFAP - Lumbar CSF concentrations were elevated until week 4 and ventricular CSF until week 3 ($p<0.05$); serum concentrations were not significantly elevated at any time point.

Inflammatory markers

Admission lumbar and ventricular CSF cytokine concentrations were significantly higher in TBM cases relative to control CSF. Only RANTES did not differ between the 2 groups. Serum concentrations for IP-10, IFN-γ, IL-6 and IL-8 were significantly higher than the fatty filum controls, and MCP-1 concentrations were significantly lower; the remaining cytokines did not differ statistically. In comparison to pTB controls, IP-10, MCP-1, TNF-α and RANTES were lower in case serum, and no differences were demonstrated across the other cytokines.

The number of case samples elevated above the 95^{th} percentile of control values is tabulated for the 17 markers in Table 10.5.

PCA plots demonstrate that concentrations of all 17 biomarkers were greater in TBM cases compared to controls in lumbar (Figure 10.1 and 10.4) and ventricular CSF (Figure 10.2 and 10.5), but were not different in serum samples (Figure 10.3 and 10.6).

Fatty filum controls vs pTB controls

There were no significant differences in serum concentrations of the 3 neuromarkers between the fatty filum and pTB controls. Several of the inflammatory markers were significantly higher in the pTB control group, including GRO, IL-Ra, IP-10, TNF-α, IFN-γ IL-10, IL-6, IL-8 and RANTES.

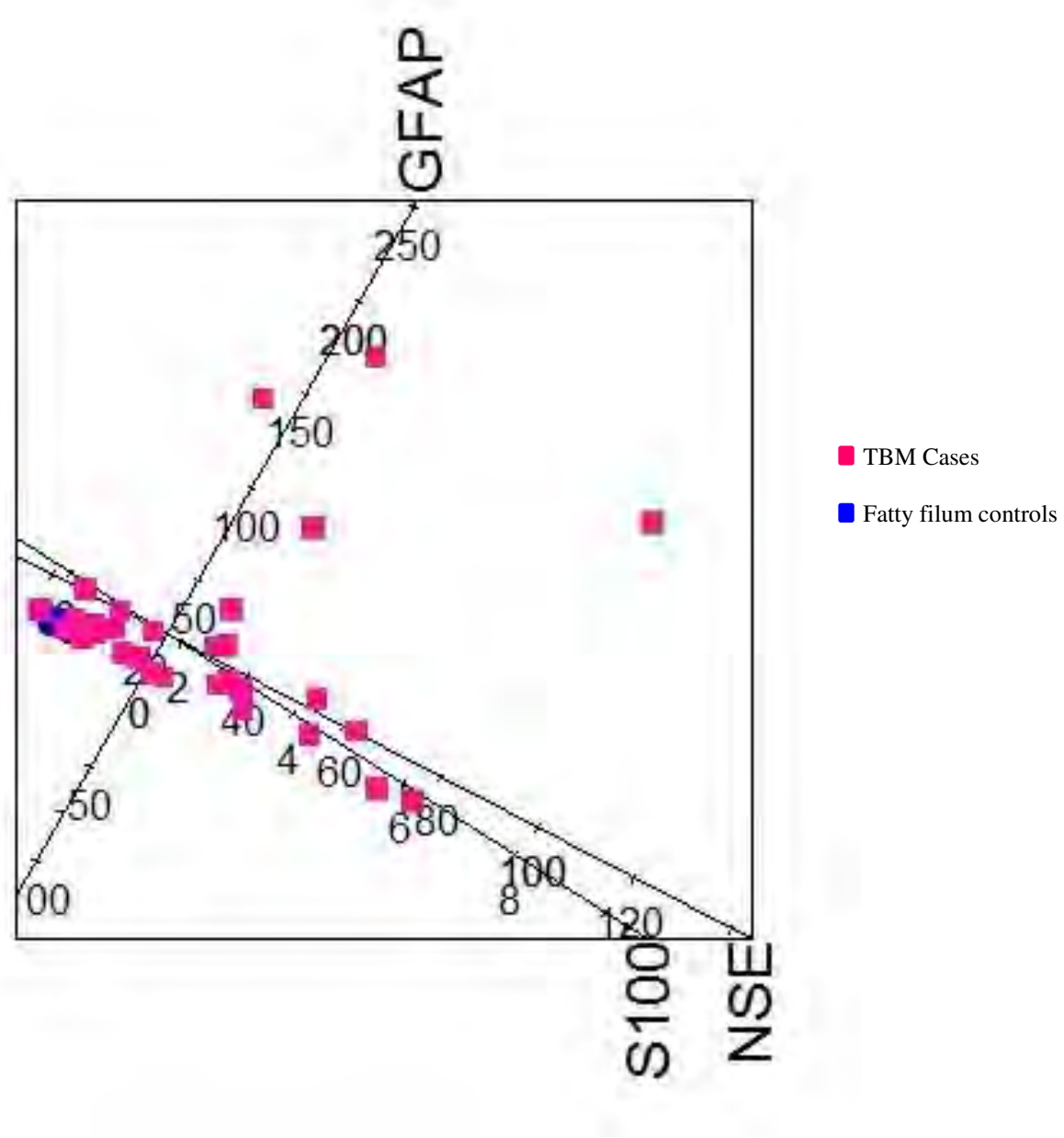
Table 10.5: Percentage of elevated case samples in each sample type

	Sample Type	TBM LP vs Fatty filum LP	TBM V vs Fatty filum LP	TBM S vs Fatty filum S	TBM S vs pTB S
Biomarker		**% > p95**	**% > p95**	**% > p95**	**% > p95**
S100B	Admission	86.11	100	22.22	2.78
	Highest	89.7	96.9	40.5	9.5
NSE	Admission	66.67	91.3	26.67	6.67
	Highest	92.3	90.6	32.5	5
GFAP	Admission	91.67	100	2.78	2.78
	Highest	92.3	100	9.5	9.5
TNF-α	Admission	99.23	100	10.28	0
IFN-γ	Admission	99.23	100	2.78	2.78
IL-6	Admission	99.23	100	2.80	0
IL-12p40	Admission	99.23	100	3.74	6.54
IL-1β	Admission	99.23	100	9.35	7.48
IL-Ra	Admission	99.23	100	23.36	1.87
IP-10	Admission	99.23	100	19.63	0
MIP-1α	Admission	95.38	95.65	8.41	8.41
IL-8	Admission	99.23	95.65	19.63	2.78
MCP-1	Admission	50.77	93.48	8.41	0
GRO	Admission	99.23	100	12.15	20.56
IL-10	Admission	99.23	100	19.63	14.02
VEGF	Admission	99.23	100	6.54	0
RANTES	Admission	99.23	100	2.80	2.80

This table details the percentage of TBM samples (admission and highest overall) which were elevated above the 95th percentile of the CSF and serum concentrations of the control groups – TBM concentrations in lumbar CSF are compared to fatty filum lumbar CSF, TBM case ventricular CSF are compared to fatty filum lumbar CSF, and TBM case serum are compared to serum of fatty filum and pTb control groups. LP= lumbar CSF, V = ventricular CSF, S = serum. % > p95 represents the percentage of case concentrations which fell above the 95th percentile of control values. Admission LP samples (n=36), V (n=23), S (n=36); Highest LP samples (n=39), V (n=32), S (n=42 for S100B and GFAP and n=40 for NSE-hemolysed samples excluded)

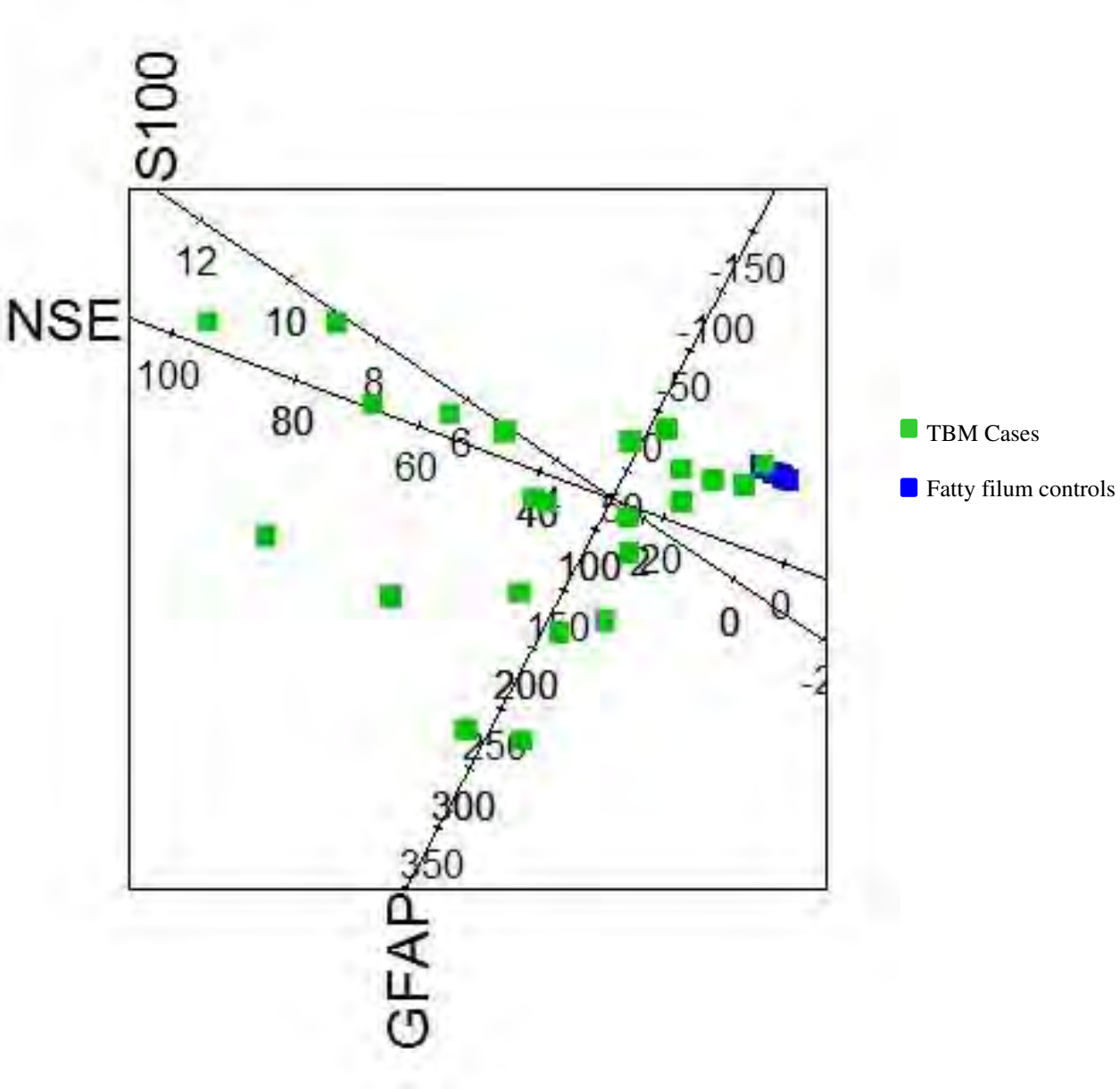
Neuromarkers: PCA plots - cases versus controls

Figure 10.1: Initial lumbar CSF



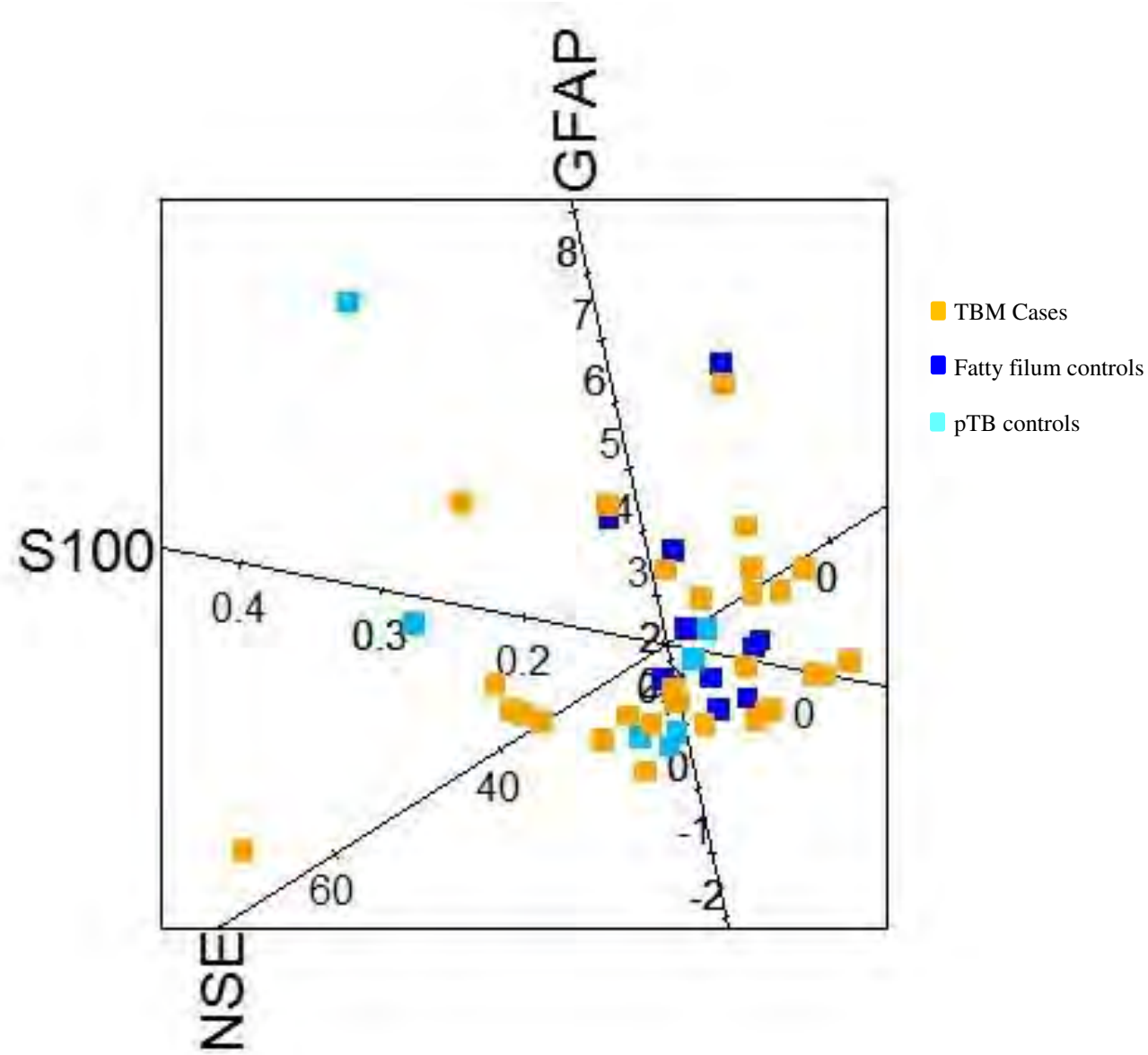
This plot demonstrates lumbar S100B, NSE and GFAP concentrations simultaneously. The weighted average of initial S100B, NSE and GFAP concentrations for each patient were used to calculate a z-score per patient which reflects their concentrations in all 3 neuromarkers simultaneously. Pink markers represent the z-scores of individual patients and the concentration of S100B, NSE and GFAP for an individual patient is indicated by their relative distance from the 3 axes. Blue markers indicate fatty filum controls and demonstrate that control patients had low concentrations on all 3 neuromarkers. TBM cases had higher concentrations relative to controls on all 3 neuromarkers.

Figure 10.2: Initial ventricular CSF



This plot demonstrates admission ventricular S100B, NSE and GFAP concentrations simultaneously using z-scores as described for Figure 10.1 above. Green markers represent the z-scores of TBM patients, and blue markers indicate fatty filum controls - controls had low concentrations on all 3 neuromarkers. TBM cases had higher concentrations relative to controls on all 3 neuromarkers.

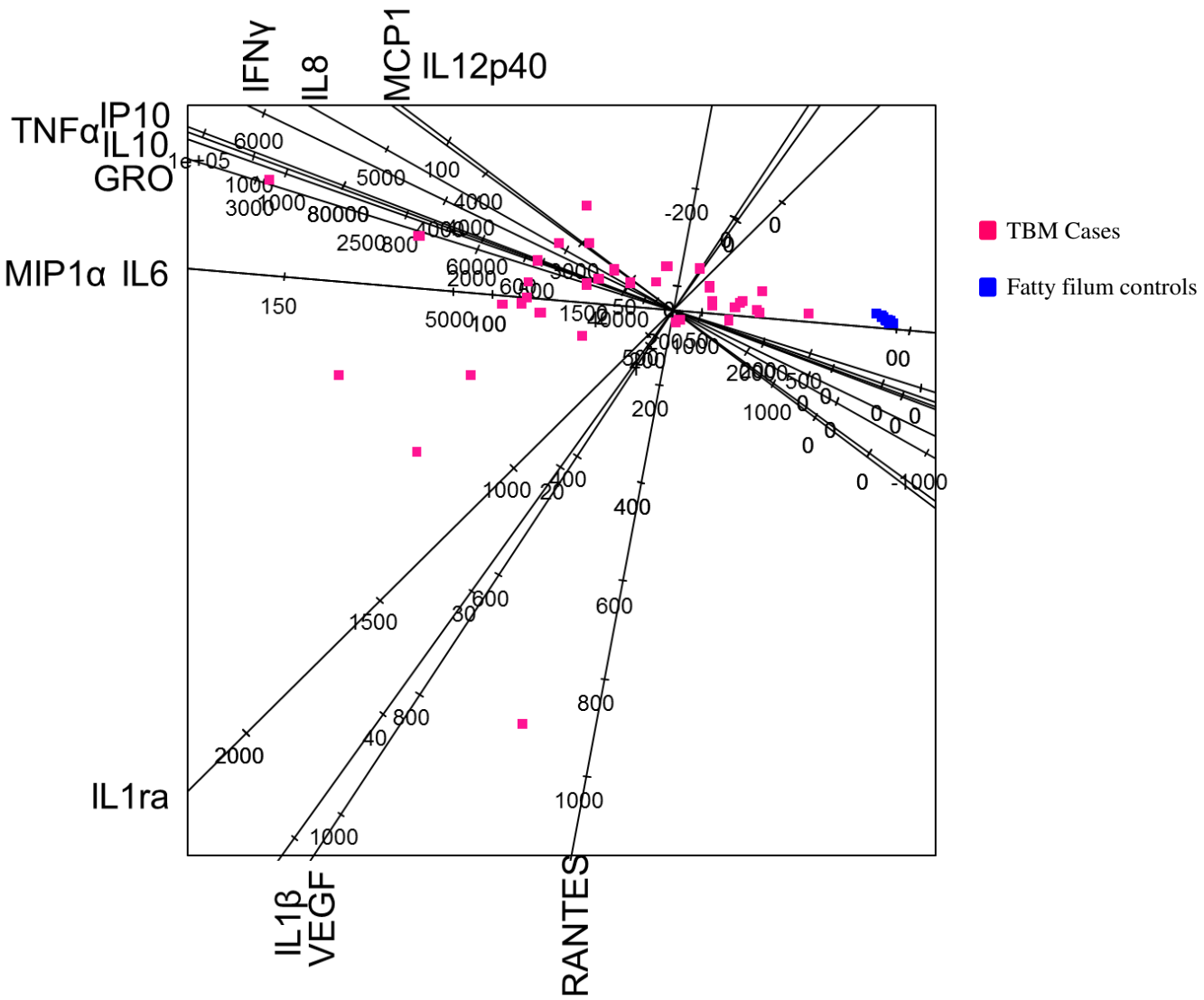
Figure 10.3: Initial serum



This plot demonstrates admission serum S100B, NSE and GFAP concentrations simultaneously using z-scores as described for Figure 10.1 above. Orange markers represent the z-scores of TBM patients, dark blue markers indicate fatty filum controls, and light blue markers indicate pTB controls - control and TBM case concentrations on all 3 neuromarkers overlapped, this provides a graphical depiction of the finding that TBM serum neuromarker concentrations were not significantly different from control concentrations

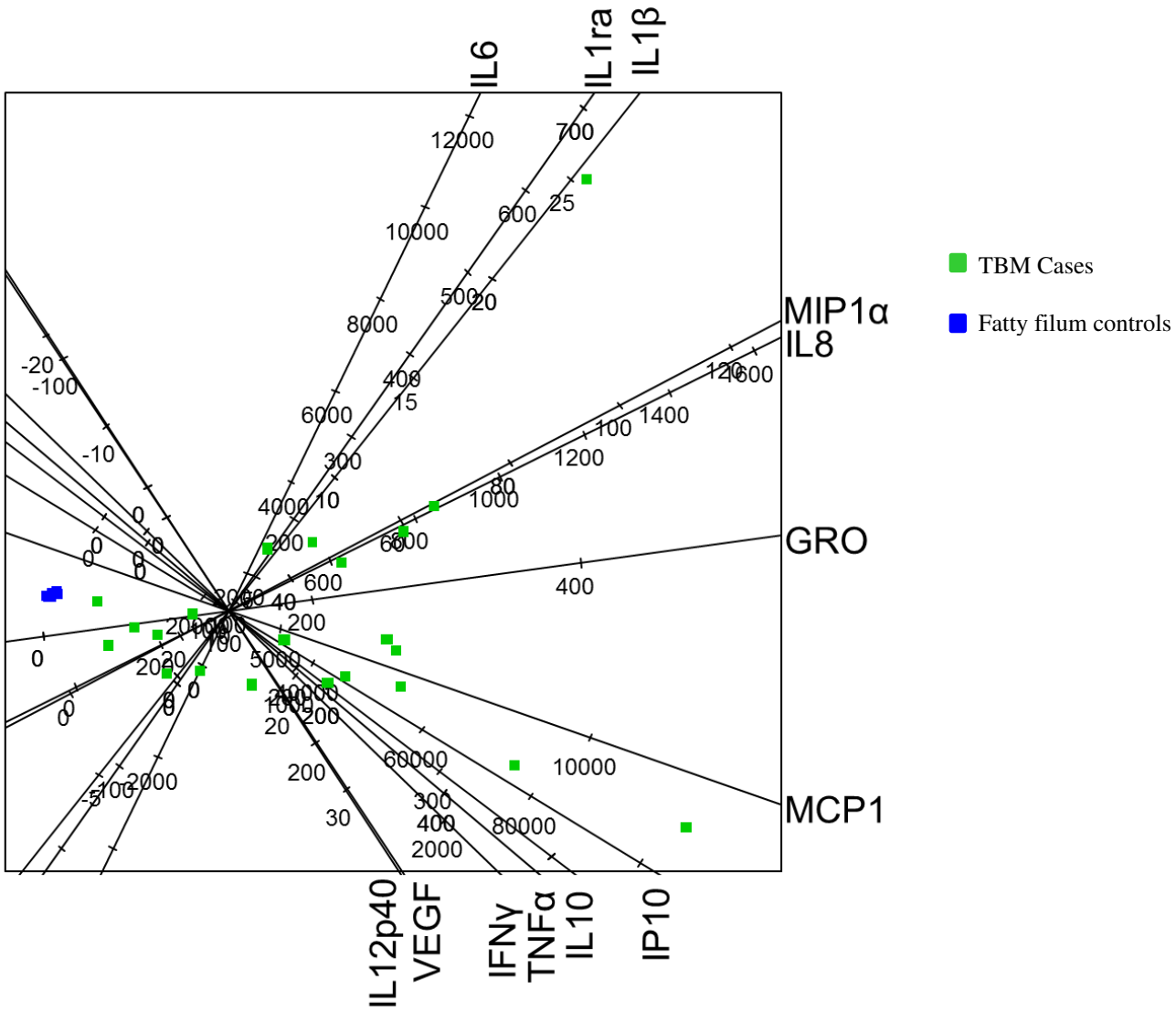
Inflammatory markers: PCA Plots - cases versus controls

Figure 10.4: Initial lumbar CSF



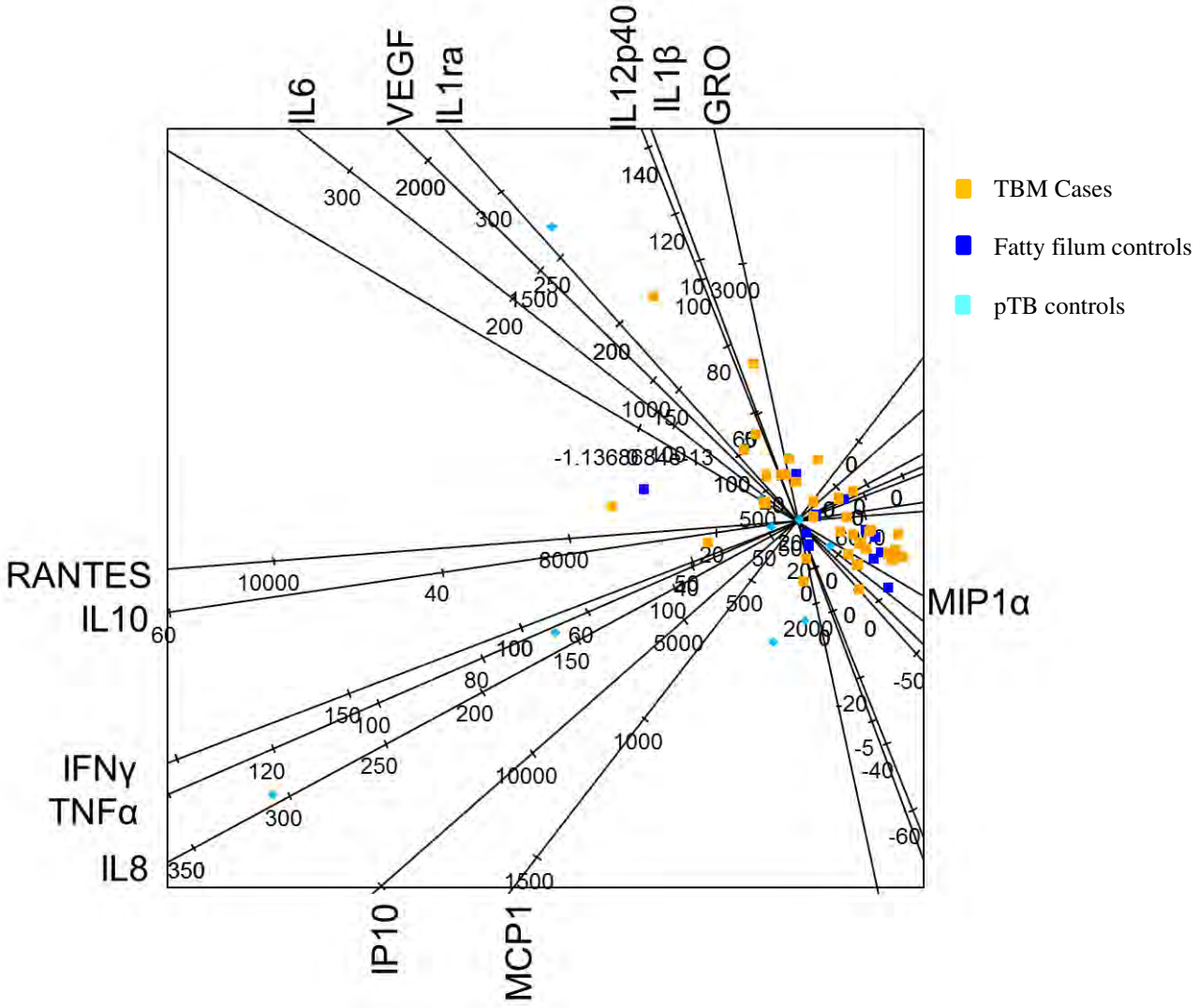
This plot demonstrates admission lumbar inflammatory marker concentrations simultaneously using z-scores as described for Figure 10.1 above. Pink markers represent the z-scores of TBM patients, and blue markers indicate fatty filum controls - controls had low concentrations on all inflammatory markers, TBM cases had higher concentrations relative to controls on all markers

Figure 10.5: Initial ventricular CSF



This plot demonstrates admission ventricular inflammatory marker concentrations simultaneously using z-scores as described for Figure 10.1 above. Green markers represent the z-scores of TBM patients, and blue markers indicate fatty filum controls - controls had low concentrations on all inflammatory markers, TBM cases had higher concentrations relative to controls on all markers

Figure 10.6: Initial serum



This plot demonstrates admission serum inflammatory marker concentrations simultaneously using z-scores as described for Figure 10.1 above. Orange markers represent the z-scores of TBM patients, dark blue markers indicate fatty filum controls, and light blue markers indicate pTB controls – TBM and control serum concentrations overlapped suggesting that they were not different across the groups.

Temporal profile

Graphical representations of the neuro- and inflammatory marker temporal profiles are shown in Figures 10.7-10.10; boxes demonstrate median and inter-quartile range, and whiskers denote the 5th and 95th percentile. In order to maximize the visual demonstration of biomarker temporal profiles, outliers (values >2 times the interquartile range above the median) and ventricular samples from week 4 (n=2 - elevated concentrations belonged to a patient who had failed medical treatment and required a VPS) were excluded from the box-plots. Box-plots with outliers are included in Appendix 8.

Neuromarkers

S100B **-** The median concentration of *lumbar CSF* S100B peaked in week 1, although median values between admission and week 1 samples were almost equal. Concentrations decreased thereafter and had normalized by week 3. *Ventricular CSF* concentrations peaked in week 1 and then declined. *Serum S100B* concentrations were highest on admission followed by a general downward trajectory.

NSE – Median *lumbar* and *ventricular CSF* concentrations peaked in week 1 followed by a gradual downward trajectory thereafter, and normalized by week 3. Median *serum NSE* was highest on admission and in week 1. The increase in ventricular concentrations in week 4 is from the same 2 patients described for S100B; these values were also not significantly different from controls.

GFAP – Median lumbar CSF concentrations peaked at admission, and thereafter decreased. The increase in median concentrations in week 4 is attributable to the 2 patients described above and these samples were not significantly different from controls. Serum GFAP concentrations remained low throughout sampling.

Inflammatory markers

Overall, lumbar CSF inflammatory marker concentrations were highest on admission, followed by a decline thereafter, although they remained higher than controls until week 4. Only GRO levels increased in week 4. Similarly, ventricular CSF markers declined over time. Elevations in IP-10, IL-8, GRO, IL-10, IL-12p40, VEGF and TNF-α in week 4 are attributable to the same 2 patients described for the neuromarkers above.

Box-and-whisker plots over time: Neuromarkers

Figure 10.7: S100B

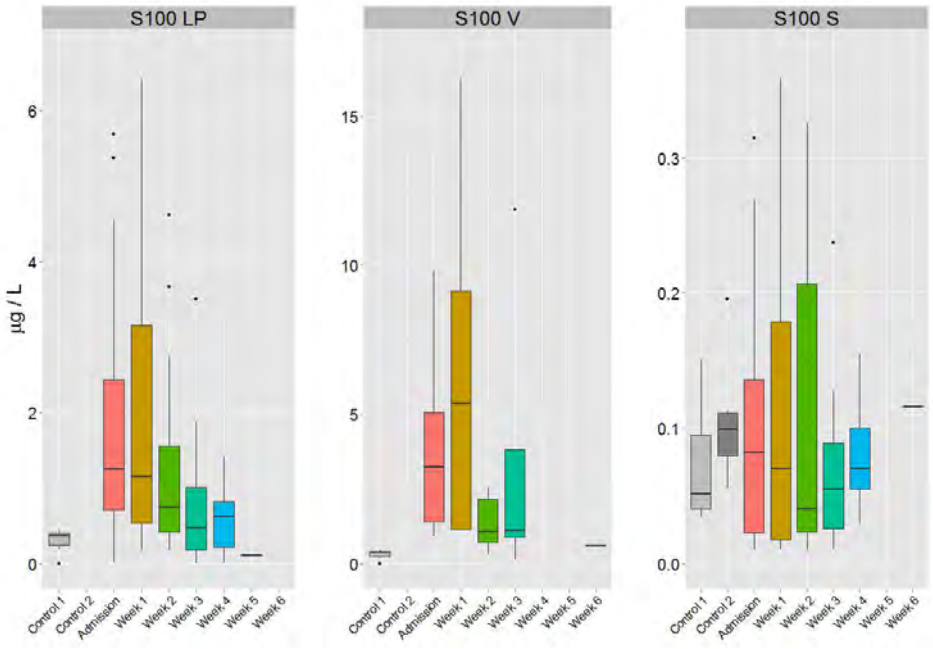
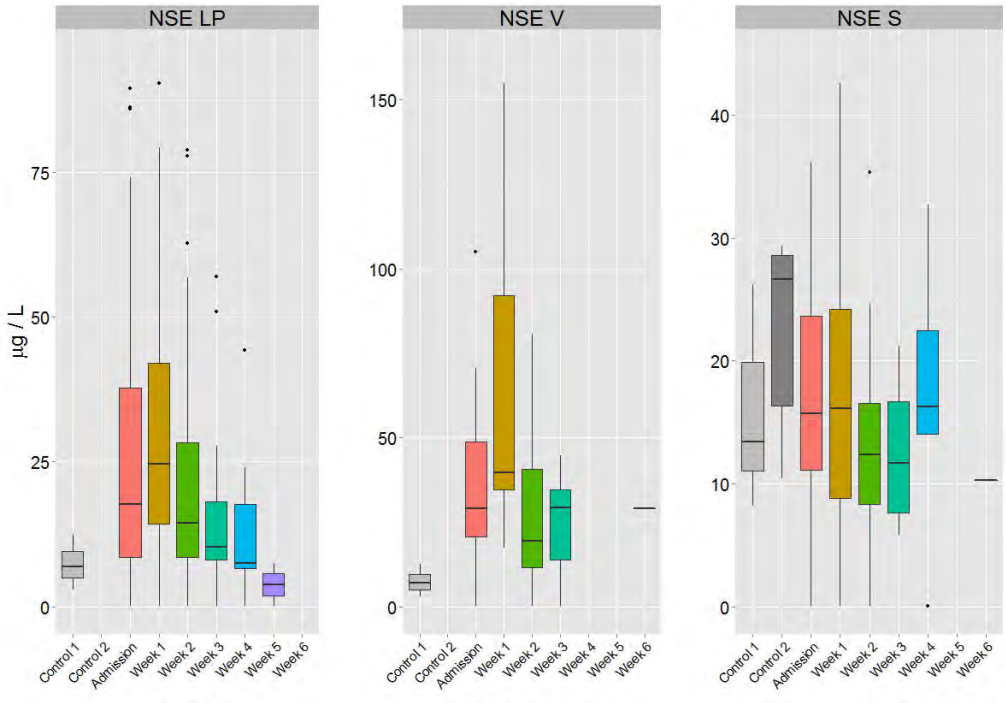
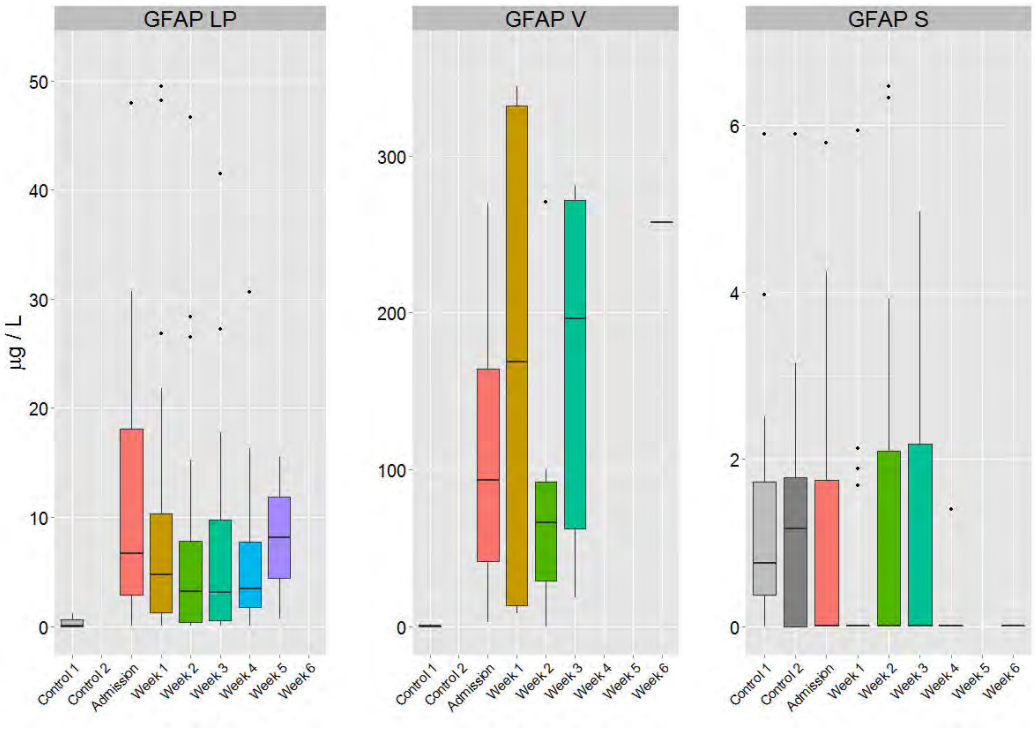


Figure 10.8: NSE



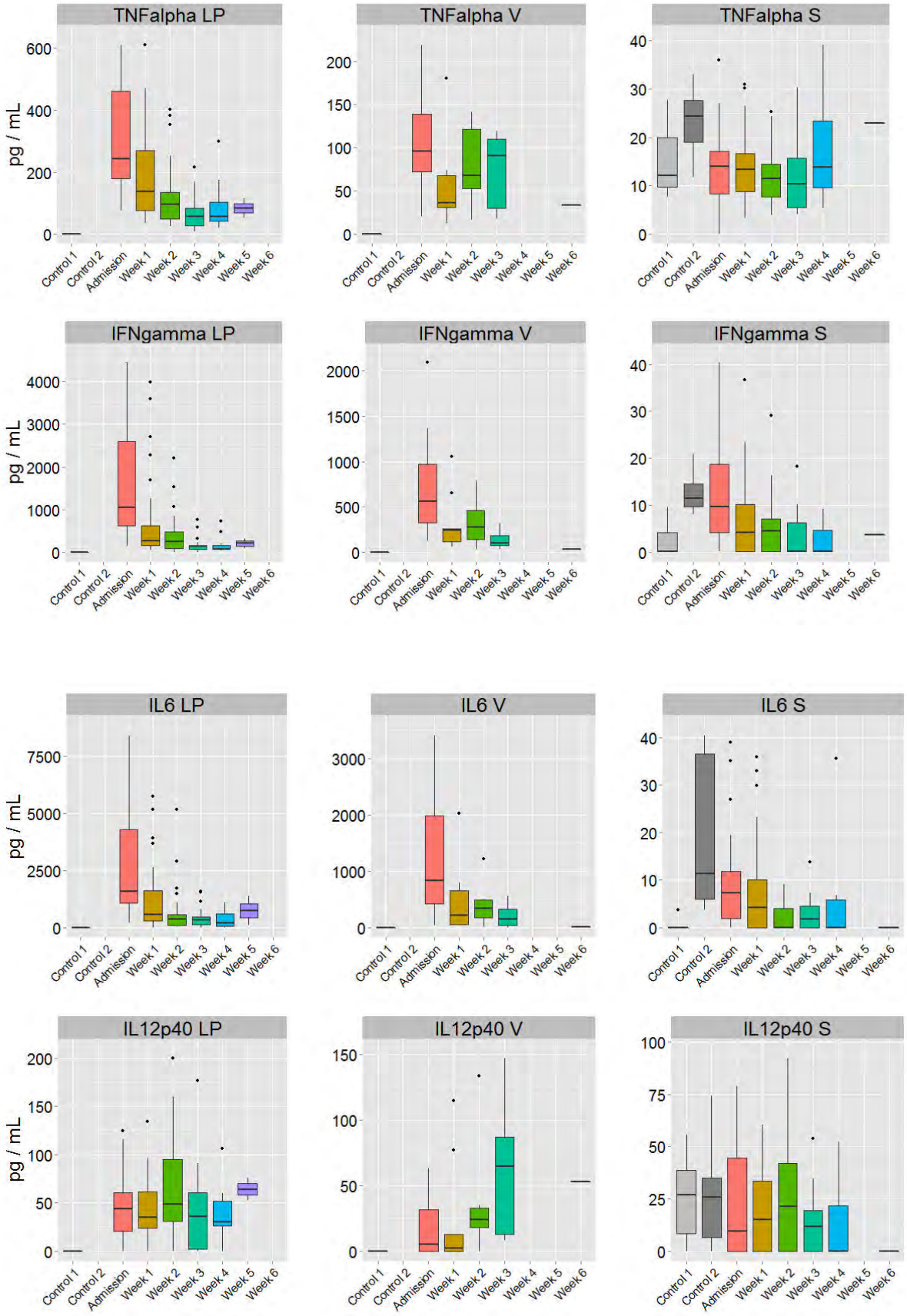
LP = lumbar CSF, V = ventricular CSF, S = serum. Boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile, fatty filum control CSF values are represented in light grey on the lumbar and ventricular plots, and fatty filum control serum values (light grey) and pTB control serum values (dark grey) are represented on the serum plots. TBM case data are presented in colour. Only 1 patient had samples at week 6 (ventricular CSF and serum)

Figure 10.9: GFAP

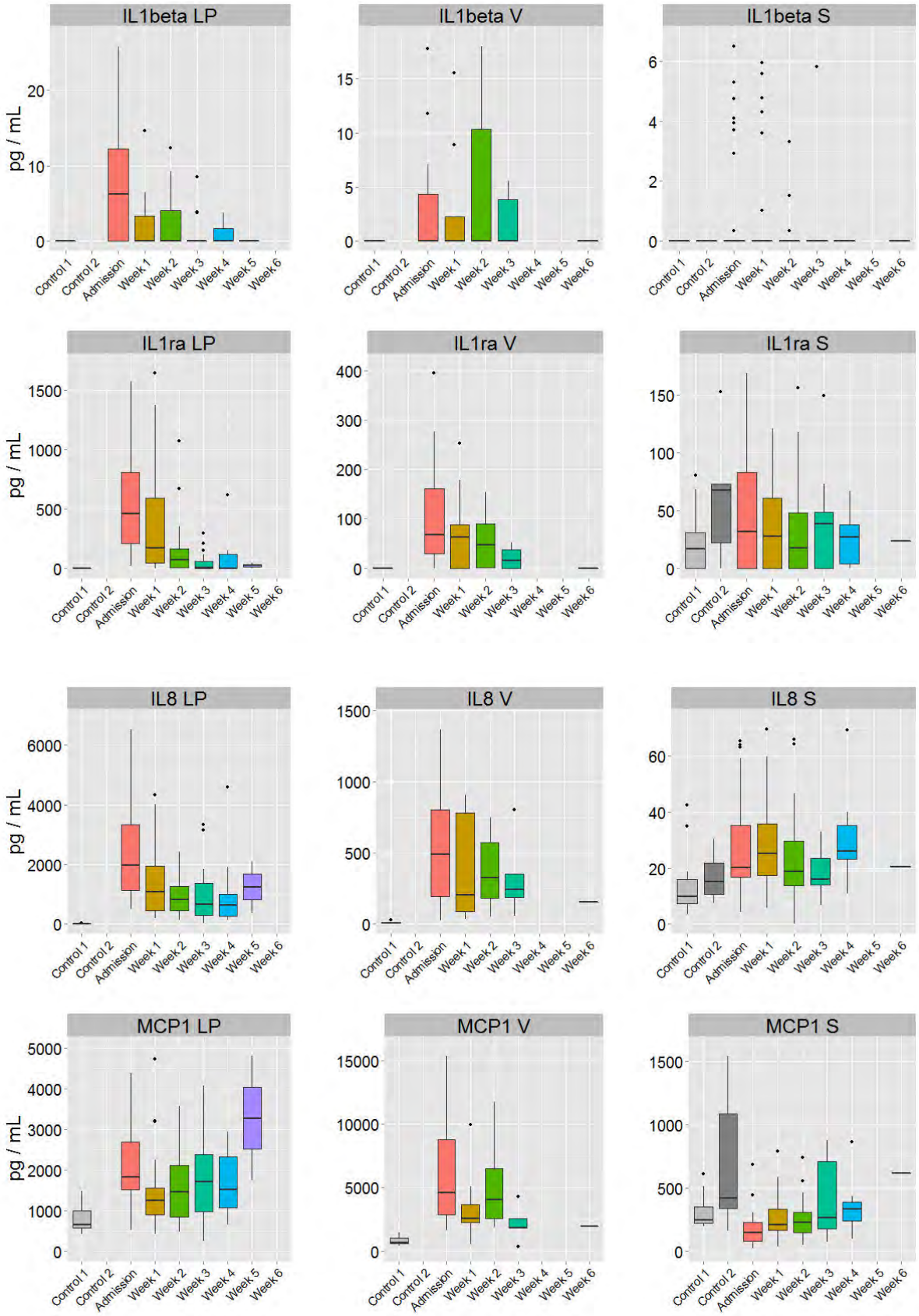


LP = lumbar CSF, V = ventricular CSF, S = serum. Boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile, fatty filum control CSF values are represented in light grey on the lumbar and ventricular plots, and fatty filum control serum values (light grey) and pTB control serum values (dark grey) are represented on the serum plots. TBM case data are presented in colour. Only 1 patient had samples at week 6 (ventricular CSF and serum).

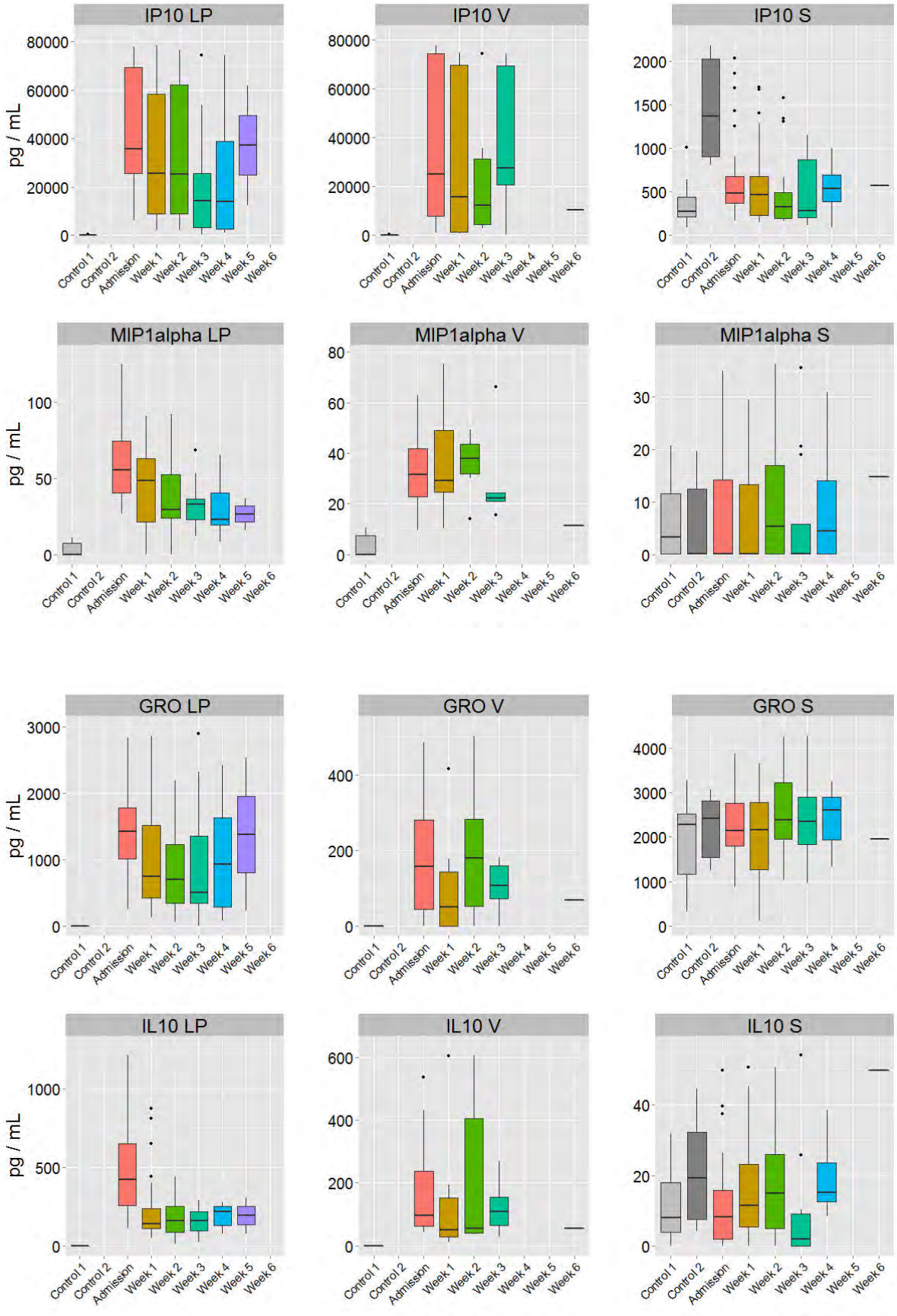
Inflammatory Markers



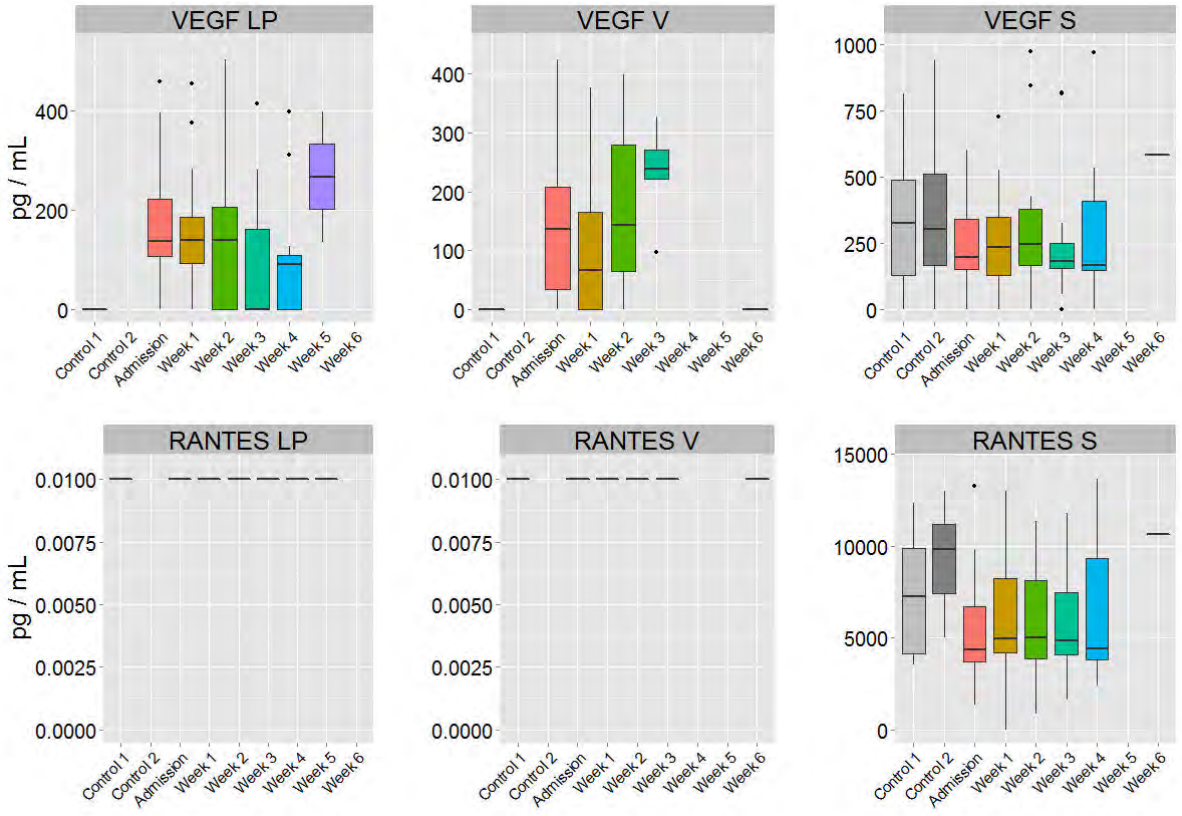
LP = lumbar CSF, V = ventricular CSF, S = serum. Boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile, Fatty filum control CSF values are represented in light grey on the lumbar and ventricular plots, and fatty filum control serum values (light grey) and pTB control serum values (dark grey) are represented on the serum plots. TBM case data are presented in colour. Only 1 patient had samples at week 6 (ventricular CSF and serum).



LP = lumbar CSF, V = ventricular CSF, S = serum. Boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile, Fatty filum control CSF values are represented in light grey on the lumbar and ventricular plots, and fatty filum control serum values (light grey) and pTB control serum values (dark grey) are represented on the serum plots. TBM case data are presented in colour. Only 1 patient had samples at week 6 (ventricular CSF and serum).



LP = lumbar CSF, V = ventricular CSF, S = serum. Boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile, Fatty filum control CSF values are represented in light grey on the lumbar and ventricular plots, and fatty filum control serum values (light grey) and pTB control serum values (dark grey) are represented on the serum plots. TBM case data are presented in colour. Only 1 patient had samples at week 6 (ventricular CSF and serum).



LP = lumbar CSF, V = ventricular CSF, S = serum. Boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile, Fatty filum control CSF values are represented in light grey on the lumbar and ventricular plots, and fatty filum control serum values (light grey) and pTB control serum values (dark grey) are represented on the serum plots. TBM case data are presented in colour. Only 1 patient had samples at week 6 (ventricular CSF and serum).

Inter-biomarker associations

Initial and overall median neuromarker concentrations per patient were positively correlated in S100B, NSE and GFAP for lumbar and ventricular CSF (Table 10.6, Figure 10.11). Overall median serum values per patient for NSE and S100B were positively associated ($r = 0.65$, $p < 0.001$). Correlations of the median S100B, NSE and GFAP concentrations at each time point over sample collection revealed that S100B and NSE were positively associated in both lumbar and ventricular CSF ($r = 0.88$, $p=0.01$ and $r =0.8$, $p = 0.04$ respectively). GFAP was not associated with S100B ($p=0.3$) or NSE ($p=0.6$).

Table 10.6: Correlation matrices for S100B, NSE and GFAP

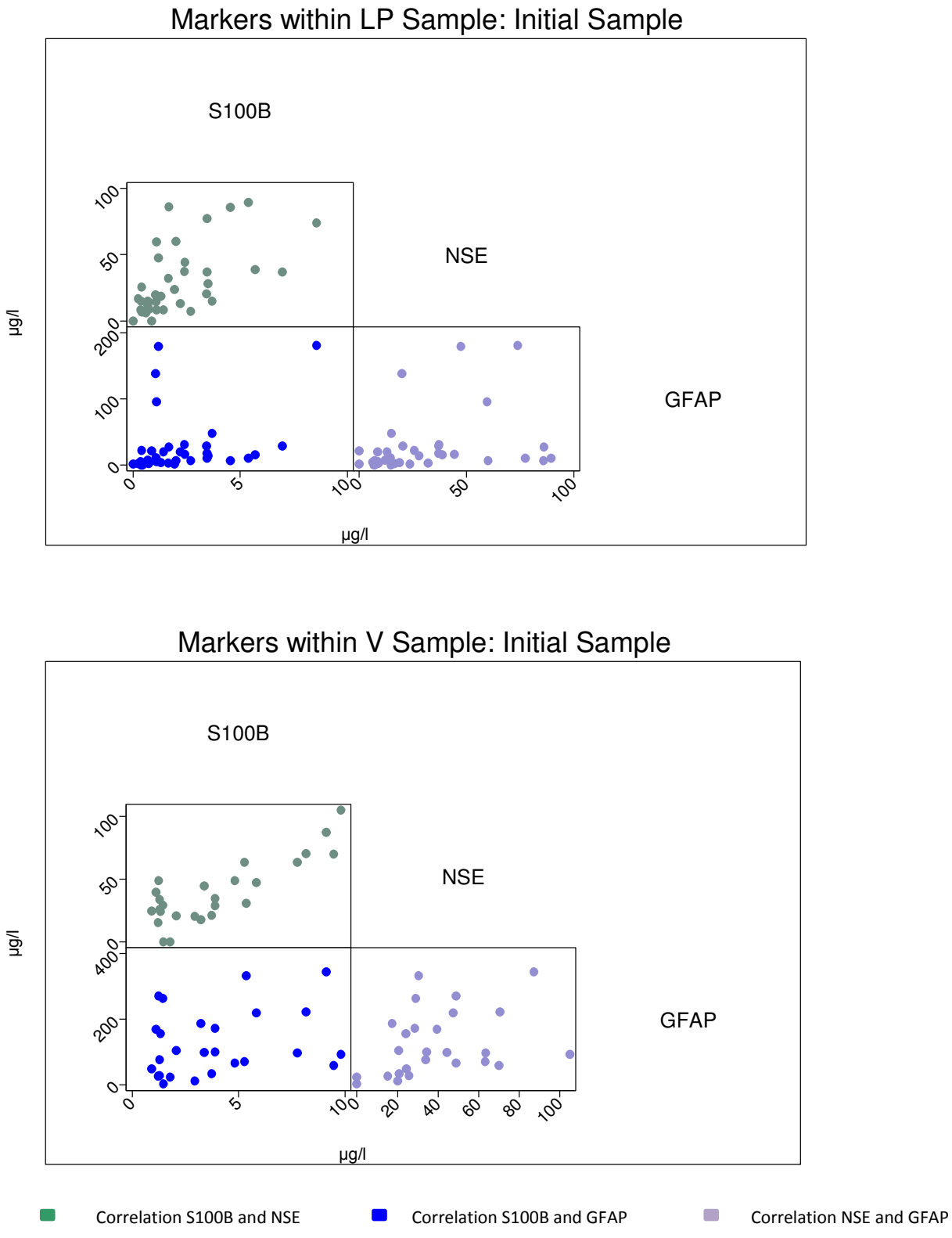
Lumbar CSF	**Initial S100B**	**Initial NSE**		**Median S100B**	**Median NSE**
Initial NSE	r=0.65* *(p<0.001)*		**Median NSE**	r=0.6* *(p<0.001)*	
Initial GFAP	r=0.52* *(p<0.001)*	r=0.47* *(p<0.05)*	**Median GFAP**	r=0.6* *(p<0.001)*	r=0.5* *(p<0.001)*

Ventricular CSF	**Initial S100B**	**Initial NSE**		**Median S100B**	**Median NSE**
Initial NSE	r=0.64* *(p<0.001)*		**Median NSE**	r=0.45* *(p=0.01)*	
Initial GFAP	r=0.25 *(p=0.2)*	r=0.45* *(p=0.05)*	**Median GFAP**	r=0.45* *(p=0.01)*	r=0.32 *(p=0.07)*

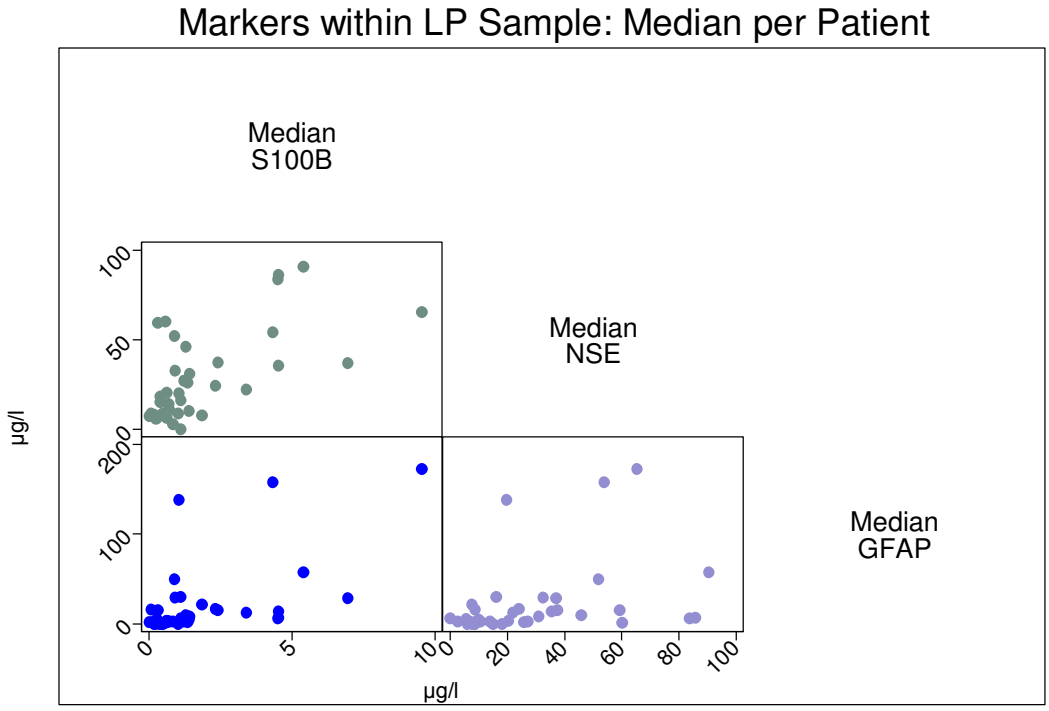
Serum	**Initial S100B**	**Initial NSE**		**Median S100B**	**Median NSE**
Initial NSE	r=0.6* *(p<0.001)*		**Median NSE**	*r=0.65** *(p<0.001)*	
Initial GFAP	r=0.15 *(p=0.37)*	r=0.07 *(p=0.69)*	**Median GFAP**	r=0.12 *(p=0.45)*	r=0.07 *(p=0.65)*

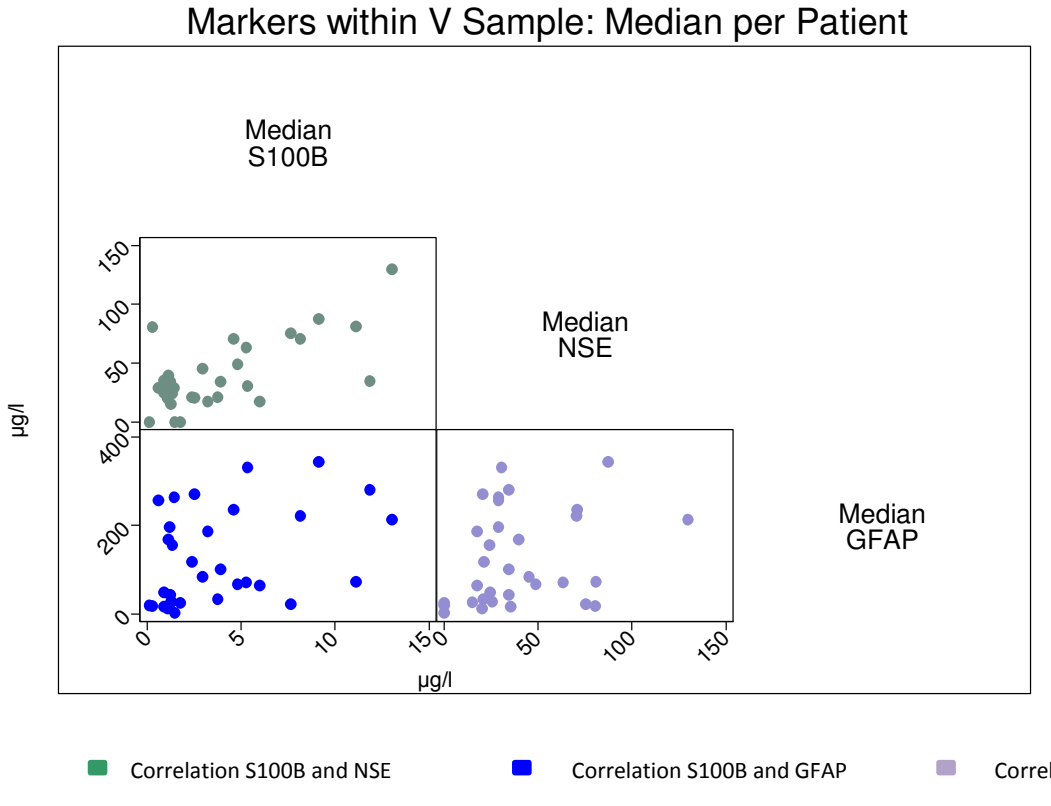
This table depicts results for correlations between initial as well as overall median per patient concentrations for S100B, NSE and GFAP in lumbar CSF, ventricular CSF and serum, * statistically significant

Figure 10.11: Pairs plots for correlations between CSF sample type for neuromarkers



Pairs plots demonstrate scatterplots for the correlation between S100B, NSE and GFAP for the initial lumbar CSF (LP) and ventricular CSF (V) samples





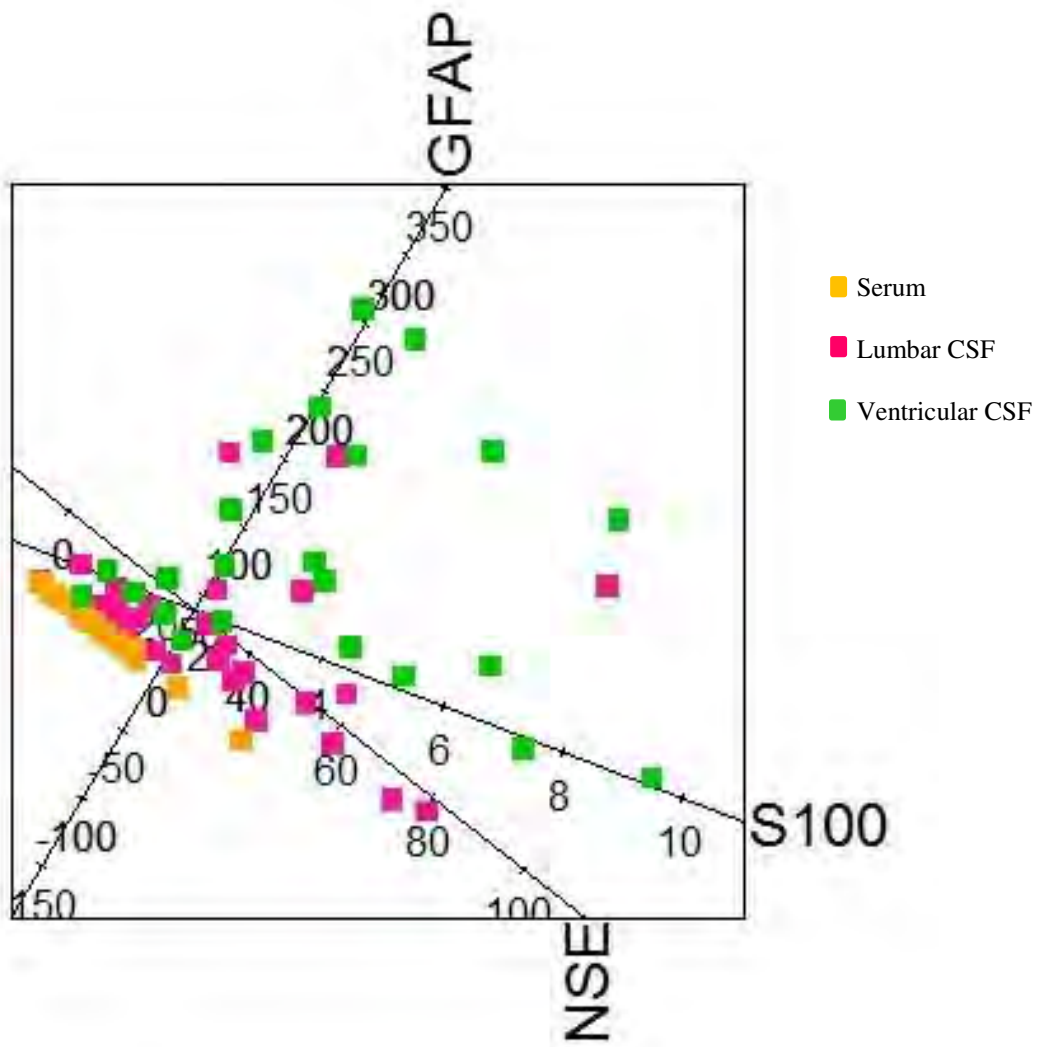
Correlation S100B and NSE Correlation S100B and GFAP Correlation NSE and GFAP

Pairs plots demonstrate scatterplots for the correlation between S100B, NSE and GFAP for the the overall median per patient in lumbar CSF (LP) and ventricular CSF (V) samples

The GEE model demonstrated ventricular CSF concentrations were greater than lumbar CSF concentrations, which was greater than serum concentrations across all time points for S100B, NSE and GFAP. At any time point, ventricular CSF S100B was estimated to be 2.2 μg/L greater than lumbar CSF S100B, ventricular NSE 13.55 μg/L greater than lumbar NSE, and ventricular GFAP 91.18 μg/L greater than lumbar GFAP. Serum S100B was estimated to be 1.59 μg/L lower than lumbar CSF S100B, serum NSE 12.83 μg/L lower than lumbar NSE and serum GFAP 21.59 μg/L lower than lumbar GFAP. Since spinal arachnoiditis and adhesions potentially may influence the rostro-caudal gradient of biomarker concentrations, spinal arachnoiditis was added to the model. In patients with spinal arachnoiditis ventricular S100B remained higher than lumbar S100B but to a lesser degree than seen in patients without arachnoiditis, NSE concentrations were no longer significantly different across the ventricular and lumbar CSF, and ventricular GFAP remained higher than lumbar GFAP irrespective of arachnoiditis. The coefficients and confidence intervals (CI) for the GEE are presented in Appendix 9.

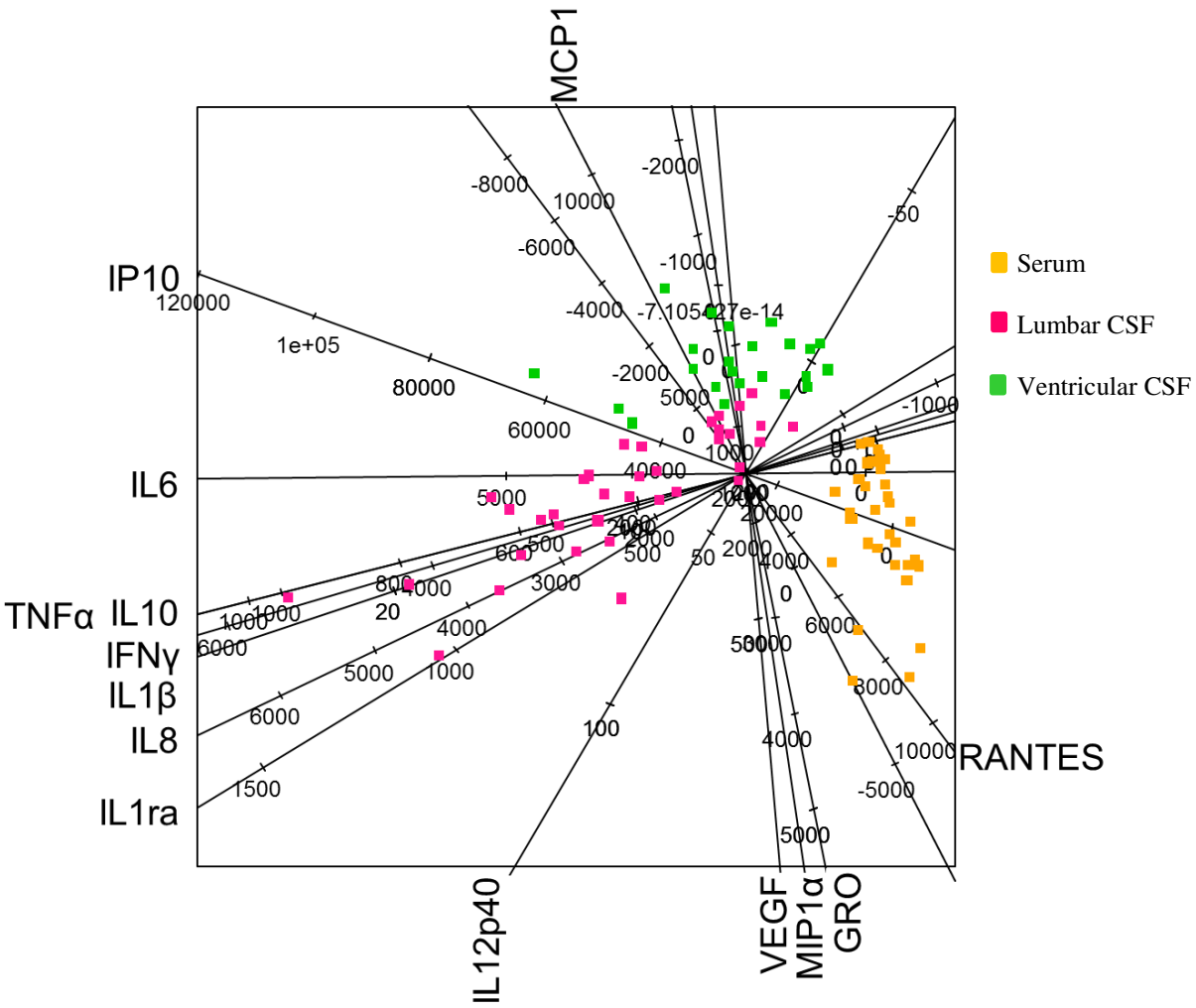
PCA plots visually demonstrate the GEE findings that ventricular CSF concentrations were more elevated than lumbar CSF, which were more elevated than serum across all neuromarkers, as indicated by their position higher along the S100B, NSE and GFAP axes (Figure 10.12). For inflammatory markers overall lumbar CSF concentrations were greater than ventricular CSF, which were greater than serum. MCP-1 and IP-10 demonstrated higher values in ventricular CSF; and GRO, RANTES and VEGF were more elevated in the serum than CSF samples. Similarly, serum inflammatory marker concentrations were lowest (Figure 10.13).

Figure 10.12: PCA Plot: Neuromarkers in all sample types



This plot demonstrates admission lumbar CSF, ventricular CSF, and serum S100B, NSE and GFAP concentrations simultaneously using z-scores as described for Figures 10.1-10.3 above. Green markers represent ventricular z-scores, pink markers represent lumbar z-scores and orange markers represent serum z-scores. This plot illustrates that for all 3 neuromarkers ventricular CSF concentrations were greater than lumbar CSF which were greater than serum concentrations

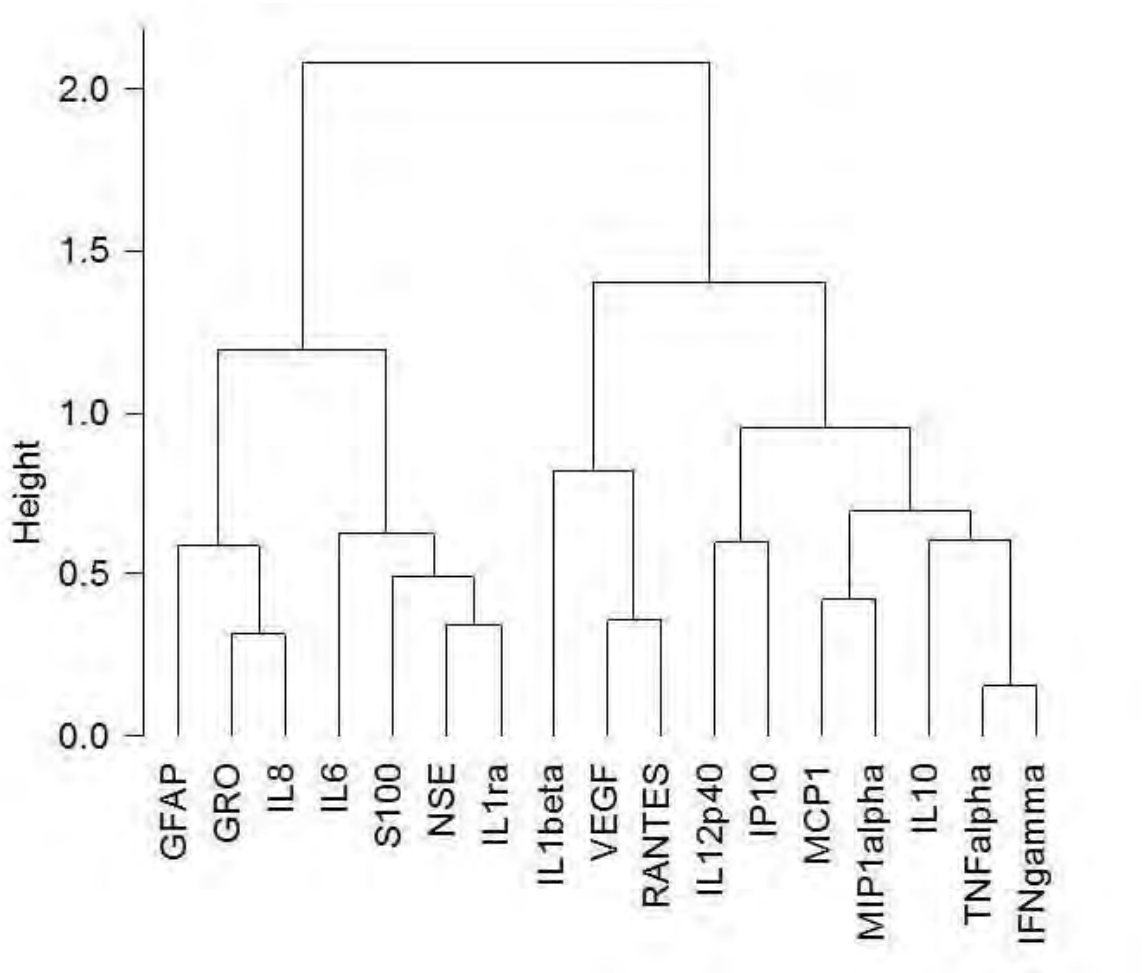
Figure 10.13: PCA plot: Inflammatory markers in all sample types



This plot demonstrates admission lumbar CSF, ventricular CSF, and serum inflammatory marker concentrations simultaneously using z-scores as described for Figures 10.4-10.6 above. Green markers represent ventricular z-scores, pink markers represent lumbar z-scores and orange markers represent serum z-scores. This plot illustrates that lumbar and ventricular CSF concentrations exceeded serum concentrations on all 14 inflammatory markers, and lumbar CSF concentrations were greater than ventricular CSF in most inflammatory markers.

Correlations between neuro- and inflammatory markers and within inflammatory markers are tabulated in Appendix 10. S100B was correlated with IL-Ra, IL-6 and VEGF; NSE was correlated with IL-Ra, TNF-α, VEGF and IL-10; only GRO was correlated with GFAP. Significant correlations were observed between numerous cytokines. Cluster analysis demonstrated that neuromarkers clustered with cytokines GRO, IL-8, IL-6 and IL-Ra.

Figure 10.14: Cluster dendrogram: Neuromarkers and inflammatory markers



The dendrogram represents the results of the cluster analysis – biomarkers which demonstrated the smallest distance between patient data points were clustered together, and clusters exhibiting the smallest difference between each other were connected

Association with patient and admission characteristics

Lumbar NSE trended towards being higher in children < 2 years (*p=0.07*). Lumbar GFAP was significantly more elevated in TB culture positive patients, but this was not replicated in the ventricular CSF. Initial lumbar and ventricular S100B were not associated with admission or patient characteristics.

Association with clinical outcome

Univariate and PCA analysis

Neuromarkers

S100B **-** Absolute Δ in lumbar S100B was significantly predictive of mortality and 6 month dichotomized PCPS outcome (*p<0.001* and *p=0.02* respectively), demonstrating that in patients who died or who had a poor functional outcome, S100B tended to increase. The highest concentration recorded in week 1 and overall predicted clinical outcome at 6 months (*p=0.04* and *p=0.03* respectively). All indices of ventricular S100B was associated with mortality and 6 month outcome as detailed in Table 10.7.

NSE **-** Initial lumbar NSE was a significant predictor of morbidity in survivors at 3 and 6 months (*p=0.02* and *p=0.03* respectively). Highest NSE overall was predictive of mortality and survivor disability and highest in week 1 was associated with 6 month clinical outcome. Absolute Δ was predictive of both mortality (*p=0.01*) and clinical 6 month outcome (*p=0.02*). Initial and highest ventricular NSE were strong predictors of mortality and clinical 6 month outcome.

GFAP **-** Highest lumbar GFAP overall and in week 1 and the absolute Δ were associated with mortality (*p<0.05*). Only highest overall trended towards predicting survivor morbidity at 3 and 6 months. Ventricular GFAP was not a predictor of mortality or survivor morbidity.

*Neuromarkers combined***-** Z-Neuro Initial LP was not predictive of mortality (*p=0.3*) or clinical outcome (*p=0.2*), however, Z-Neuro Initial V was predictive of both (*p=0.03* and *p=0.04* respectively), suggesting that combined initial S100B, NSE and GFAP lumbar neuromarker concentrations were not prognostic; whereas combined concentrations in ventricular CSF were. Z-Neuro Δ significantly predicted mortality (*p<0.001*) and outcome at 6 months (*p<0.01*), indicating that an increasing trend in neuromarkers was predictive of poor outcome.

In summary, results for neuromarkers, singly or in combination, suggest that elevated neuromarker concentrations (as represented by their highest recorded concentrations) and an increasing trend over time were prognostic of poor outcome. Outcome associations for neuromarker indices are summarized in Table 10.7 and p-values in Appendix 11.

Table 10.7: Summary of neuromarker univariate outcome analysis

	Neuromarker Index	Mortality	Outcome	Morbidity	
				3 month	6 months
S100B					
Lumbar	Initial	x	x	✓	x
Ventricular		✓	✓	*p=0.08*	x
Lumbar	Highest in week 1	x	✓	✓	x
Ventricular		✓	✓	*p=0.08*	x
Lumbar	Highest overall	x	✓	✓	x
Ventricular		✓	*p=0.06*	x	x
Lumbar	Absolute Δ	✓	✓	x	x
NSE					
Lumbar	Initial	x	*p=0.07*	✓	✓
Ventricular		✓	✓	x	x
Lumbar	Highest in week 1	x	*p=0.08*	✓	✓
Ventricular		✓	✓	x	x
Lumbar	Highest overall	✓	✓	✓	✓
Ventricular		✓	✓	x	x
Lumbar	Absolute Δ	✓	✓	x	x
GFAP					
Lumbar	Initial	x	x	*p=0.06*	x
Ventricular		x	x	x	x
Lumbar	Highest in week 1	*p=0.07*	x	*p=0.07*	x
Ventricular		*p=0.08*	x	x	x
Lumbar	Highest overall	✓	*p = 0.07*	✓	*p = 0.06*
Ventricular		x	x	x	x
Lumbar	Absolute Δ	✓	*p=0.08*	x	x

This table represents results of univariate analysis of various neuromarker indices with mortality, clinical outcome at 6 months, and morbidity in survivors at 3 and 6 months. Initial concentrations represent admission or week 1 concentration when no admission concentration was available (n= 39 lumbar CSF and 26 ventricular CSF samples), highest overall represents the highest concentration recorded during the duration of sampling, and absolute Δ represents the change in concentration from admission to week 2 (calculated as week 2-admission), ✓ = significant at $p<0.05$, x = not significant, indices trending towards significance are indicated by p-values. A full list of p-values is provided in Appendix 10

Inflammatory markers

Univariate analysis demonstrated that none of the admission CSF cytokines were predictive of mortality or 6 month clinical outcome, except ventricular IL-6 was more elevated in patients with poor clinical outcomes ($p=0.05$).

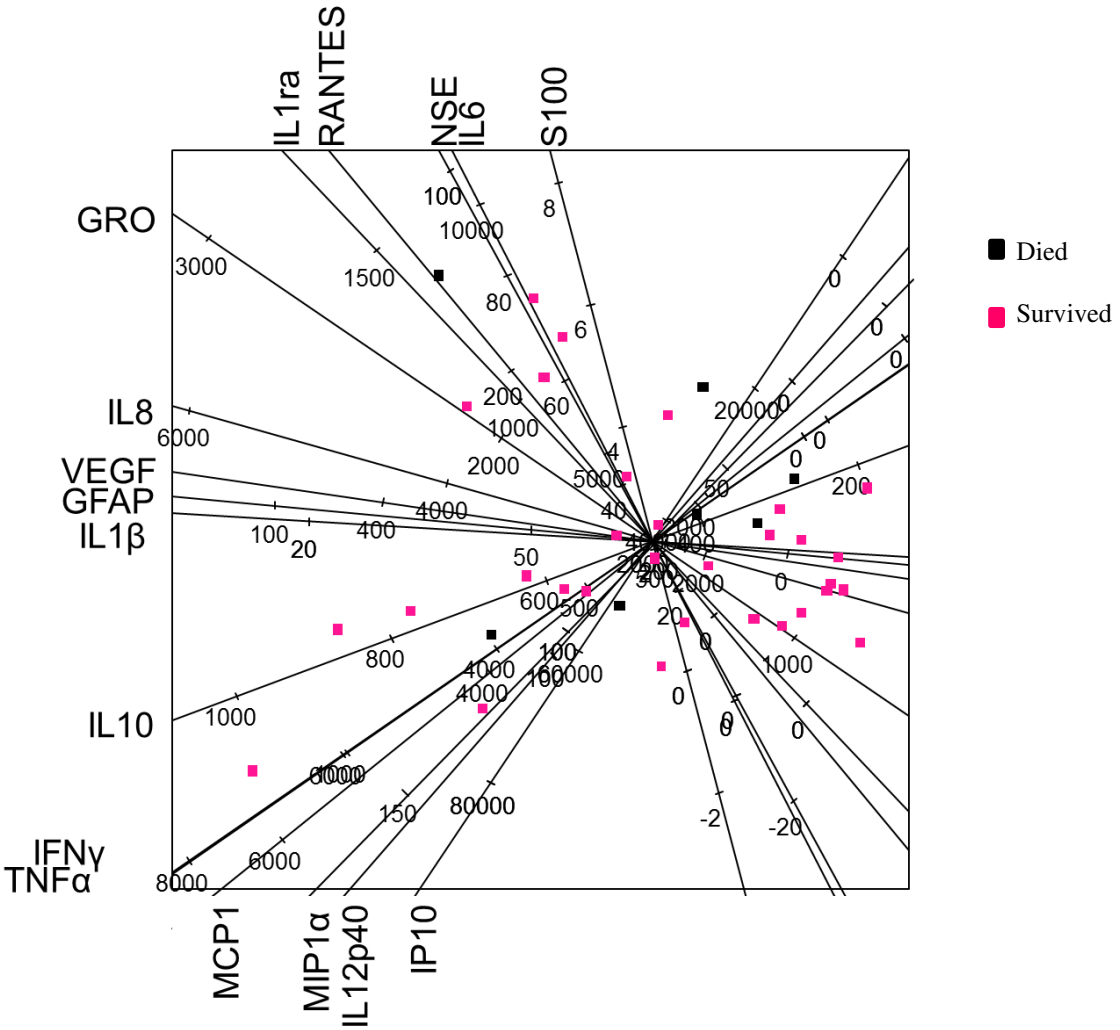
Inflammatory markers combined **-** Z-Inflam Initial LP was not significantly associated with mortality ($p=0.93$) or 6 month outcome ($p=0.95$), neither was Z-Inflam Initial V ($p=0.16$ and $p=0.12$ respectively), or Z-Inflam Δ ($p=0.8$ and $p=0.6$ respectively).

Therefore, the initial concentrations (singly or combined) and the change over time in inflammatory markers (combined) were not predictive of outcome.

Combined neuro- and inflammatory markers

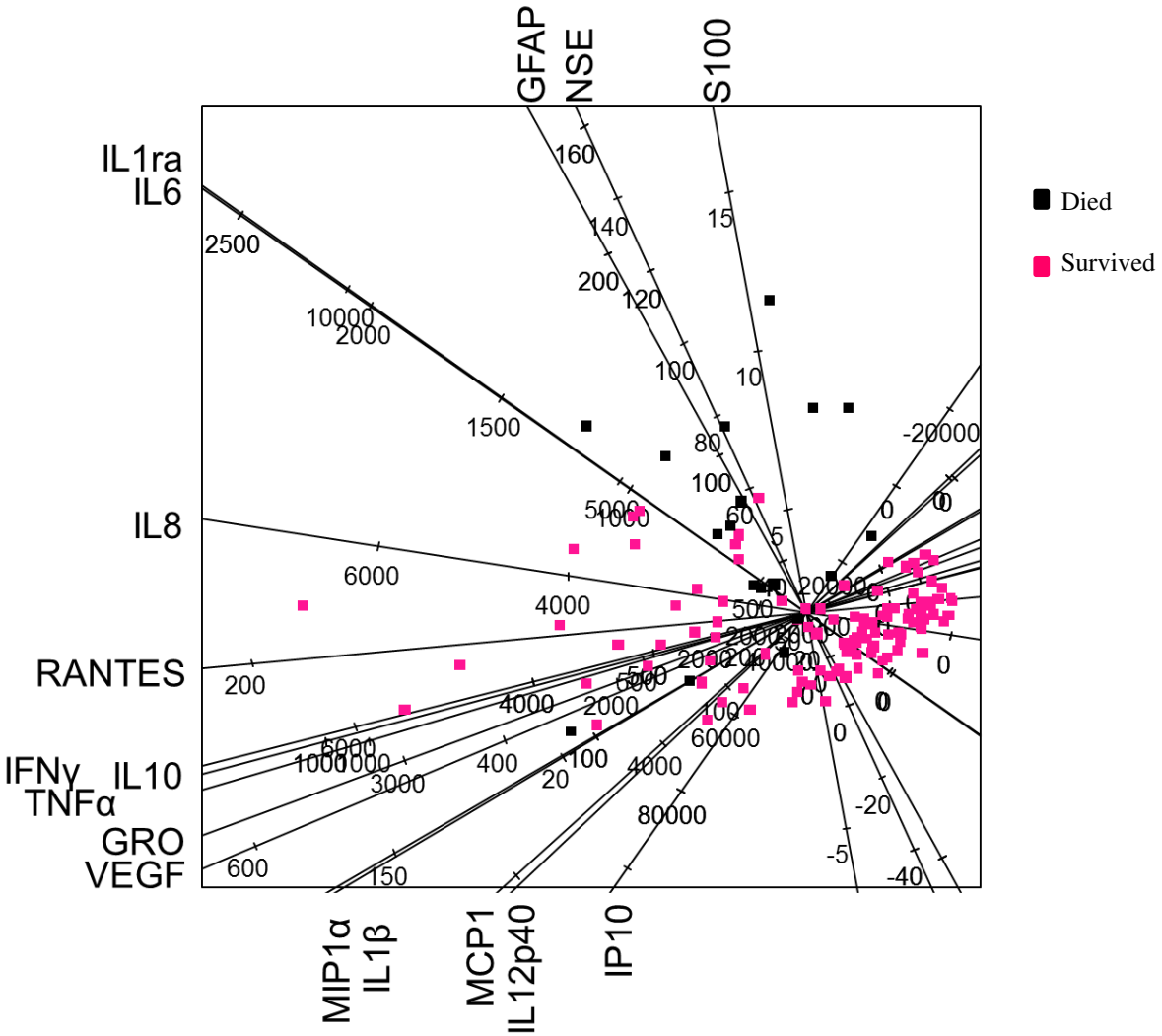
Results from PCA combining Z-scores for neuro- and inflammatory markers were weak. PCA for combined initial neuro- and inflammatory marker concentrations (Z-Neuro Initial LP and Z-Inflam Initial LP) demonstrated that PC1 accounted for 36% variability and PC1+PC2 accounted for only 48% variability. Analysis of the trend in combined neuro- and inflammatory marker concentrations over time (Z-Neuro Δ and Z-Inflam Δ) revealed that PC1 accounted for 32% variability and PC1+PC2 accounted for 47%. This suggests that the combined neuro- and inflammatory data demonstrate a high degree of variability which cannot be explained by only 2 PC's. When the combined data were plotted, patterns associated with mortality were not discernable. In comparison to the PCA results for neuromarkers alone (described above), these results suggest that combining neuro- and inflammatory markers creates more variability in the data and does not add prognostic power. The PCA plots for combined neuro- and inflammatory data are presented in Figures 10.15 and 10.16.

Figure 10.15: PCA Plot – Combined neuro- and inflammatory marker data on admission



This PCA plot demonstrates the combination of neuro- and inflammatory marker concentrations on admission using z-scores. Patients who died are indicated in black, and patients who survived in pink. The distribution of the data suggests there was no discernable pattern predictive of death using combined neuro- and inflammatory markers

Figure 10.16: PCA – Combined neuro- and inflammatory marker data for the trend over time



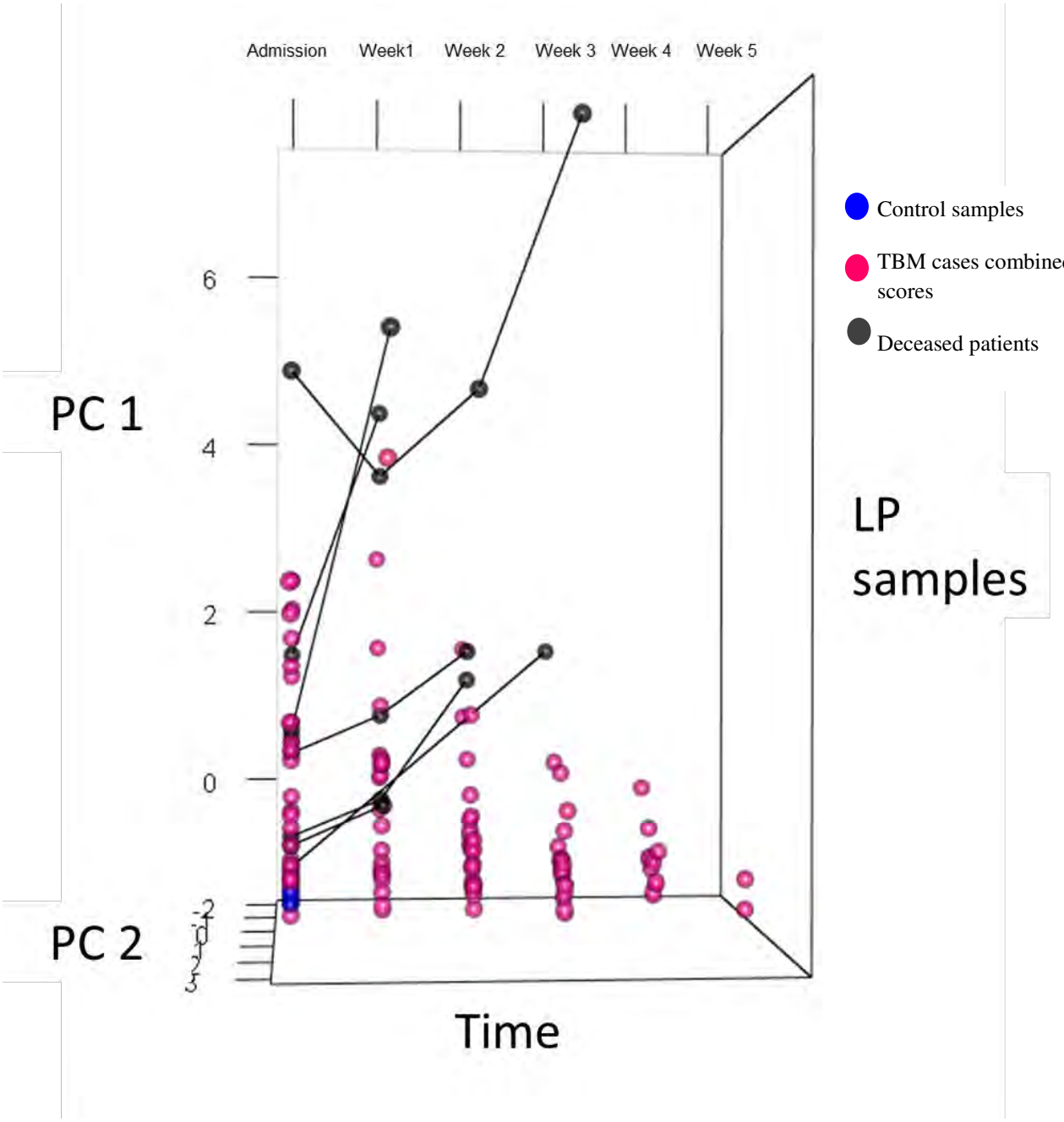
This PCA plot demonstrates the combination of the trend in neuro- and inflammatory marker concentrations over time using z-scores. Patients who died are indicated in black, and patients who survived in pink. The distribution of the data suggests there was no discernable pattern predictive of death using neuro- and inflammatory markers combined

Temporal profile and mortality

PCA plots of the temporal profile of combined neuromarkers illustrated that neuromarker concentrations in the lumbar CSF decreased over time, but that an increasing trend was observed in patients who died. The analysis showed that for the neuromarkers in lumbar CSF, PC1 accounted for 67.58% of the variability in the data, and PC2 accounted for 19.03%. Cumulatively the 2 principle components explained 86.61% of variability in the data over time. In ventricular CSF PC1 and PC2 explained 92.49% of the variability over time (PC= 71.75%, PC2= 20.74%). This demonstrates a good quality PCA fit for the neuromarkers. S100B contributed most to this variability. The PCA plot for combined lumbar CSF neuromarkers is depicted in Figure 10.17.

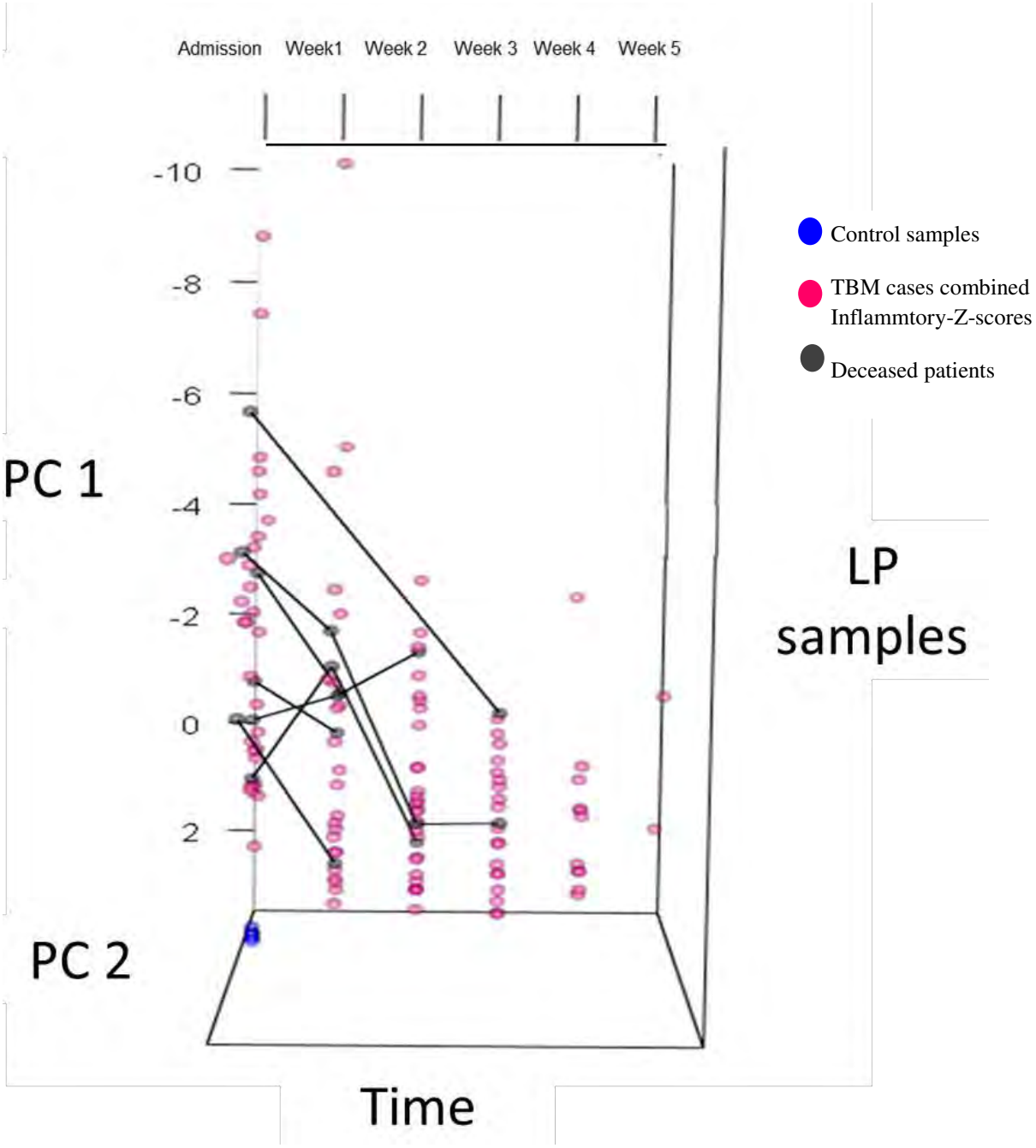
The increasing temporal neuromarker profile in patients who died was not replicated in the PCA plots of the combined inflammatory markers over time; patients who died followed a similar downward trajectory to those who survived, as illustrated in Figure 10.18. For the combined inflammatory analysis over time only 52.52% of the variability was described by the first 2 PCs in lumbar CSF (PC1=43.96%, PC2 =8.58%) and 52.58% in ventricular CSF (PC = 39.04%, PC2= 13.54%).

Figure 10.17: PCA plot: Temporal profile of combined neuromarkers in lumbar CSF



This 3-dimensional PCA plot depicts the z-scores for combined neuromarkers in lumbar CSF over the duration of sampling (admission – week 5). Each pink mark represents a patient's z-score at that time point, and the blue marks represent the fatty filum control patients for whom only 1 sample was collected. The axes represent principle component 1 (PC1), principle component 2 (PC2) and time. This plot demonstrates that patients who died (indicated by black marks) had increasing neuromarker trends, whereas survivors demonstrated a decline in concentrations over time.

Figure 10.18: PCA plot: Temporal profile of combined inflammatory markers in lumbar CSF



This 3-dimensional PCA plot depicts the z-scores for combined inflammatory markers in lumbar CSF over the duration of sampling (admission – week 5). Each pink mark represents a patient's z-score at that time point, and the blue marks represent the control patients for whom only 1 sample was collected. The axes represent principle component 1 (PC1), principle component 2 (PC2) and time. This plot demonstrates that, overall, patients who died (indicated by black marks) had decreasing neuromarker trends, similar to survivors

ROC analysis

Neuromarkers and mortality – ROC's for mortality demonstrated that concentrations of S100B >2.75 µg/L predicted death with a sensitivity of 71.43% and specificity of 70.97%, (area under the curve [AUC] 0.69, CI:0.4-0.97); NSE > 56.84 µg/L with a sensitivity of 77.42% and specificity of 78.95% (AUC 0.81, CI: 0.63-0.99); and GFAP elevations >27.25 µg/L with a sensitivity of 85.71% and specificity of 73.68% (AUC 0.84, CI: 0.71-0.98). ROC analysis using the change in neuromarker concentrations from admission to week 2 demonstrated that ΔS100B > 0.06 µg/L predicted death with a sensitivity of 100% and specificity of 93.1% (AUC 0.97, CI: 0.92-1); ΔNSE > 2.07 µg/L with a sensitivity of 85.71% and a specificity of 65.52% (AUC 0.81, CI: 0.56-1); ΔGFAP > 12.54 µg/L with a sensitivity of 85.7% and specificity of 96.5% (AUC 0.87, CI: 0.64-1); and Z-Neuro Δ > 0.48 with a sensitivity of 85.71% and a specificity of 93.33% (AUC 0.93, CI: 0.8-1). Curves are shown in Appendix 12.

Neuromarkers and clinical outcome - Analysis for 6 month dichotomized clinical outcome showed that S100B concentrations >2 µg/L predicted poor outcome with a sensitivity of 83.33% and specificity of 61.54% (AUC 0.72, CI:0.53-0.9); NSE > 38.86 µg/L with a sensitivity of 75% and specificity of 65.38% (AUC 0.77, CI: 0.62-0.92); and GFAP > 27.25 µg/L with a sensitivity of 50% and specificity of 65.38% (AUC 0.69, CI: 0.51-0.86). ROC analysis using the change in neuromarker concentrations from admission to week 2 demonstrated that ΔS100B >0.06 µg/L predicted poor outcome with a sensitivity of 66.67% and specificity of 95.83% (AUC 0.73, CI:0.51-0.96); ΔNSE >8.67 µg/L with a sensitivity of 58.33% and a specificity of 91.67% (AUC 0.75, CI: 0.54-0.95); ΔGFAP > 12.54 µg/L with a sensitivity of 85.7% and specificity of 96.5% (AUC 0.68, CI:0.48-0.88), and Z-Neuro Δ >0.01 with a sensitivity of 66.67% and a specificity of 84% (AUC 0.79, CI: 0.62-0.97).

ROCs were not constructed for inflammatory markers due to the poor association with outcome.

Multivariate analysis

Z-Neuro Δ, S100B, NSE and GFAP all demonstrated an independent association with outcome on multivariate analysis. The strongest logistic regression model for predicting mortality and 6 month outcome included Z-Neuro Δ. The odds of death were 22 times greater with a 1 unit increase in Z-Neuro Δ (CI: 1.57-324.66, *p=0.02*). Covariates added to this model included MRC staging, symptom duration, age, gender, HIV status, fever, seizures, culture positivity and radiology characteristics HCP, HCP severity and tuberculomas. Infarcts could not be added to the models as all patients who died or suffered a poor outcome had infarcts, and no patients without infarcts died or had a poor outcome; therefore infarcts perfectly predicted outcome. Only seizures altered the model, its inclusion

strengthened the predictive power of Z-Neuro Δ and the odds of death increased to 26 times per unit change (CI: 1.8 – 383.05, *p=0.02*). The next best model was for Δ S100B which was associated with 9 times greater odds of death per unit increase in the absolute change in S100B (CI: 1.9-42.1, *p=0.005*), this relationship with mortality was independent of admission, clinical or radiology covariates. Patients with a 1 unit increase in ΔNSE or ΔGFAP had equal odds of death or survival (CI: 1-1.1, *p=0.02* and CI: 1-1.2, *p=0.01* respectively). Significant covariates in the relationship between ΔNSE and death included HCP severity as patients with more severe HCP had greater odds of death, and tuberculomas, with the odds of death decreasing with tuberculoma incidence (CI: 1.01-1.27, *p=0.02*). The addition of seizures increased the prognostic strength of the model for ΔGFAP (CI: 1.02-1.21, *p= 0.01*) and the odds of death decreased in association with tuberculomas (CI: 1.01-1.2, *p=0.02*).

These models were also predictive of dichotomized outcome at 6 months; the odds of a poor outcome were 4 times greater per unit increase in Z-Neuro Δ (CI: 1.31-13.04, *p=0.02*) and 2 times greater per unit increase in Δ S100B (CI: 0.96-3.65, *p=0.06*). Patients with a 1 unit increase in ΔNSE (CI: 1.0-1.11) or ΔGFAP (CI: 0.1-1.09, *p=0.06*) had equal odds of a poor or good outcome. These relationships were independent of clinical, admission and radiology characteristics.

Developmental outcome analysis

Twenty-six children were included in this analysis; 16 (61.5%) of these children made a full clinical recovery and 10 children (62.5%) suffered from mild-moderate disability. More than half of these patients were delayed relative to controls on all the sub-scales and the GQ (Chapter 9). Biomarker concentrations were not significantly associated with developmental outcome for highest S100B, NSE or GFAP or their trend over time (ΔS100B, ΔNSE, ΔGFAP, Z-Neuro Δ).

CHAPTER 10: BIOMARKER ANALYSIS

Discussion

This study examined markers of neurological injury and inflammation in 44 paediatric patients with TBM. The main findings were as follows: Analysis using ELISAs demonstrated that neuromarkers S100B, NSE and GFAP were elevated in the CSF of TBM patients relative to controls. Across all time points and all neuromarkers, ventricular CSF concentrations were highest, followed by lumbar CSF, and then serum. Neuromarker association with outcome was examined in several ways; results indicated that elevated ventricular CSF S100B and NSE concentrations were strong predictors of mortality and 6 month clinical outcome. Highest lumbar S100B, NSE and GFAP were predictive of poor outcome but the most prognostic index of all neuromarkers was an increasing trend in concentrations. ROC analysis demonstrated that an increase of > 0.06 μg/L in S100B, of >2.07 μg/L in NSE and >12.54 μg/L in GFAP were predictive of mortality with high sensitivities and specificities; S100B was the most sensitive and specific. A combined score of the change in all 3 neuromarkers over time using PCA demonstrated an increasing temporal profile of neuromarker concentrations in patients who died, whereas the temporal profile decreased in patients who survived. Combining neuromarkers with inflammatory markers did not improve their association with outcome.

Inflammatory markers, analysed with Luminex technology, were also elevated in the lumbar and ventricular CSF of TBM patients, however, this was not the case for the serum concentrations of most cytokines. Concentrations were highest on admission and declined thereafter. Associations between inflammatory markers and outcome were poor and the temporal profile of combined inflammatory markers demonstrated a downward trajectory for all patients, including those who died.

Biomarker elevations in TBM cases

Neuromarkers S100B, NSE and GFAP were significantly increased in lumbar and ventricular CSF relative to controls and demonstrated prolonged elevation for up to 3 weeks after hospital admission. This suggests that these markers may be sensitive indicators of ongoing cerebral injury in TBM. As such, they have potential to be used as surrogate markers of injury severity and outcome in clinical and research work and may help reduce sample sizes in preliminary studies.

Serum concentrations were not elevated for any of the neuromarkers relative to the 2 control groups, which suggests that either serum S100B, NSE and GFAP concentrations are not sensitive to brain injury due to TBM, or that our fatty filum control group was not suitable for comparison. It is not clear through which channels brain-derived proteins like S100B, NSE and GFAP enter the peripheral circulation. It is contended that they diffuse passively along their concentration gradient across the blood-brain or blood-CSF barrier [431,439]. Factors which determine the degree to which brain-derived

proteins may be detected in the blood include its intrathecal concentration, the change in its concentration in response to pathology, its molecular weight, and its half-life in the blood. It is suggested that brain-derived proteins diffuse into the blood regardless of BBB disruption [431,439], but that diffusion into the blood may be augmented in the presence of BBB break-down [335]. The serum concentration is further influenced by the dilution of CSF due to the continuous production of CSF and by the flow of approximately 500 mL of CSF into 5 L of blood per day [431]. Consequently, serum concentrations reflect only a fraction of CSF levels and only transiently.

S100B is detectable in the blood early after injury [258,306,307], however, the half-life is estimated to be as short as 30 minutes, and consequently, elevated serum levels may decline swiftly. [440]. NSE is known to be plentiful in the brain and increases rapidly in response to neuronal membrane damage. At 78 kDa it is the largest of the 3 neuromarkers but it is detected well in the serum and appears early after TBI and stroke [309,310,317]. The half-life is approximately 24 hours, suggesting that the timing of sampling is imperative to detection and quantification of this protein. Similarly, GFAP concentrations are elevated in response to cell injury as well as astrogliosis, and serum concentrations are considered promising indicators of stroke and TBI[321,325,332,337,338].The exact half-life of GFAP is uncertain; however, serum concentrations after ischemic stroke may normalise within 6-48 hours after injury [337].

The timing of sampling relative to the onset of injury is clearly an important factor in determining whether biomarker concentrations will be detected in serum and to what degree. The temporal nature of injury in TBM is not clear; due to the chronic nature of the disease the onset of injury cannot be identified, as in the case of traumatic or vascular head injury, and may not be a single discrete event. Disease processes may build up to a peak in tissue injury or injury may follow a fluctuating pattern influenced by multiple dynamic variables like raised ICP, CPP, brain oxygenation and treatment. Sampling in this study was determined by the timing of clinically indicated procedures. It is possible, therefore, that the frequency of sampling may have allowed episodes of ischemia and peaks in serum concentrations to be missed.

Regarding the suitability of the control group, serum S100B and GFAP concentrations were low in the 2 control groups and the similarity of the fatty filum and pTB control groups suggests they were reasonable controls. Serum NSE concentrations for the fatty filum and pTB controls exceeded their CSF concentrations and were equal to or greater than TBM case serum concentrations. The serum control concentrations for this study were also substantially higher than reported normal values and the normal ranges suggested by the kit manufacturer (Tables 5.3). Although all visibly hemolysed samples were excluded, it remains possible that NSE of erythrocyte origin may still have contributed to the serum concentrations due to hemolysis occurring at phlebotomy, during storage or at sample processing. In particular, more of these children had blood drawn when placing an intravenous cannula, which may have caused more hemolysis due to the blood being drawn with negative

pressure. This suggests that serum NSE concentrations need to be interpreted with caution and that sample collection and processing need to be conducted with meticulous care. The sensitivity of NSE to hemolysis is well known, and means of accommodating for the role of hemolysis in NSE serum concentrations have been suggested [441] but are currently too laborious to be used routinely. This susceptibility to the effects of hemolysis may limit the clinical use of NSE as a serum biomarker for cerebral injury in TBM. Overall, these data suggest that S100B, NSE and GFAP are strong markers of TBM-induced injury in the CSF, but are not useful in serum. A serum marker would be useful but not critical because in these patients CSF sampling is routine.

The inflammatory process generated by the CNS in response to TBM was reflected by the elevated concentrations of cytokines in lumbar and ventricular CSF, and the differential between CSF and serum concentrations. Concentrations in CSF were much greater than in serum, which suggests that the inflammatory response is compartmentalised to the site of disease, and that serum is not ideal to measure immunological markers in TBM patients. The pattern of serum cytokines is also worth considering: it has been suggested that elevated serum cytokines in TBM may originate from the CSF and create a gradient to recruit leukocytes to the CNS [382]. The higher serum concentration of GRO and RANTES relative to CSF may be as a result of their role in leukocyte recruitment as both cytokines are involved in chemotaxis. The pTB controls demonstrated the highest concentration of serum cytokines, likely reflective of the active pTB disease process. Cytokine elevations in the serum of fatty filum controls may have been influenced by subtle systemic factors or by the anaesthetic. The low concentrations in their CSF supports the notion that this condition is not associated with inflammation in the CNS.

CSF S100B, NSE and GFAP concentrations in TBM cases were higher than reported for other cerebral infections. Serum values, however, were less impressive and only NSE values were elevated relative to reported pathological and normative values. Overall, CSF inflammatory marker concentrations for this study group were considerably higher and serum values lower than reported in other paediatric and adult TBM studies (Tables 6.1 and 6.2). These differences may be due to the different testing platforms used as well as variability in the timing of sampling and disease course.

Biomarker Temporal profile

S100B and GFAP values were highest initially and NSE peaked in the week 1 sample after admission; thereafter all neuromarkers decreased, likely in response to treatment, however, increasing trends were seen in patients who died early. Inflammatory markers also demonstrated an early downward trajectory over time, suggesting that the inflammatory response was subsiding; however, concentrations remained elevated above normal for a prolonged period. The concentrations of cytokines are dependent on the timing of their release relative to the initiation of the inflammatory

response and, therefore, the timing of sampling is important in assessing the degree of elevation and the temporal profile. As most patients presented late with moderate-severe MRC stage TBM, it is likely that the initial stages of the immune response and the peaks in cytokine concentrations were missed. This may explain the low concentrations of IL-1β which is usually involved in the initial stages of meningeal inflammation, although technical shortcomings of the assay cannot be excluded. Additionally, as discussed above for serum biomarker concentrations, sampling may not have been frequent enough to capture the response of cytokines to the dynamic nature of cerebral injury and inflammation, particularly in the first few days after the initiation of treatment. In one patient who failed medical treatment and required a VPS in week 4, the late increase in neurological and inflammatory markers may suggest that chronically raised ICP is accompanied by neurological injury and ongoing inflammation. Although overall there was no relationship between late elevations in neuro- and inflammatory markers and failed medical treatment, analysis was limited by a small sample size (n=12) and likely heterogeneity in the patient's degree of raised ICP.

Relationship between biomarkers

CSF S100B, NSE and GFAP concentrations correlated with each other, suggesting all 3 markers indicate brain tissue injury occurring in paediatric TBM patients. Several cytokines demonstrated correlations, but these are challenging to interpret. The synergistic or antagonistic relationships between cytokines may depend on the timing of their release relative to injury. It is challenging, therefore, to discern the functional significance of correlations between markers when the timing of injury is uncertain. However, cluster analysis demonstrated grouping of functionally related cytokines: pro-inflammatory cytokines (TNF-α and IFN-γ, IL-12p40 and IP-10), chemoattractants (MCP-1 and MIP-1α) and antagonistic anti- and pro-inflammatory cytokines (IL-10 and TNF-α). VEGF, RANTES and IL-1β likely clustered because their concentrations were low in CSF; VEGF and RANTES appear to be more elevated in the serum and IL-1β concentrations were low possibly because the early phase of meningeal inflammation had passed. A possible mechanism behind the clustering of neuromarkers with GRO and IL-8 may involve the neutrophil attractant function of these chemokines. During the early response to disease high levels of neutrophils are protective against infection. However, it has been found that during active TB disease the degree of neutrophilia is associated with the severity of disease and that the recruitment of neutrophils to damaged tissue may augment the damage through the release of cytotoxic substances[435]. Although this research was conducted in the context of pTB, it is possible that a similar mechanism may apply in the brain whereby recruitment of neutrophils during the active stage of TBM disease leads to increased tissue damage manifest by higher neuromarker concentrations. The peak of the inflammatory cascade may have been missed by the time sampling began, however, neurological tissue injury was still ongoing. This might explain why neuromarkers clustered with IL6 and IL-1ra, which function as immune regulators, rather than pro-inflammatory cytokines like TNF-α and IFN-γ.

Examination of the temporal profile of S100B and NSE and their correlation over time suggests a similar profile of neuronal and astroglial injury characterised by increasing concentrations from admission through week 1. Lumbar CSF GFAP concentrations began to decrease immediately after admission; this may account for the poor temporal correlation with lumbar S100B and NSE and suggests that injury to the astroglial cytoskeleton may precede astroglial and neuronal cell destruction. Alternatively, there is evidence to suggest that GFAP may be more sensitive than S100B and NSE to injury due to the mechanical effect of dilating ventricles on the parenchyma and periventricular white matter [442,443]. A paediatric study on acute HCP found that CSF concentrations of GFAP were higher than S100B or NSE [443]. Histopathology studies have shown that astrogliosis occurs in the periventricular white matter in response to HCP; GFAP is a marker of astrogliosis and is a sensitive marker of periventricular white matter injury [443-445]. Neuronal or astrocyte damage in response to HCP may be more subtle, leading to lower NSE and S100B concentrations [443]. This damage is transient if the HCP is effectively treated [443]. Therefore, the decline in lumbar GFAP after peaking at admission may be attributed to the immediate treatment of HCP and raised ICP in these children. Furthermore, ventricular CSF GFAP demonstrated a more labile pattern and larger fluctuations in concentration relative to ventricular S100B and NSE. Considering that ventricular samples were collected from patients who required ventricular access for poorly controlled ICP this could reflect GFAP's sensitivity to fluctuations in ICP.

The highest CSF neuromarker concentrations were observed in the ventricular CSF for all biomarkers at all time points. This would be consistent with their cerebral origin and a rostro-caudal gradient along the brain-spine axis [439,446]. Lumbar neuromarker concentrations are less elevated, likely due to degradation by proteinases in the CSF over time [266]. In patients with spinal arachnoid lesions, however, the differential between ventricular and lumbar concentrations may be less as the diseased spine may contribute neuromarkers to the spinal SAS. Cytokine concentrations were greater in lumbar than ventricular CSF, likely due to the fact that most of the inflammatory process is occurring in the subarachnoid space.

Association with patient admission characteristics

Only NSE approached an association with age and none of the neuromarkers were associated with gender. This reflects the mixed age and gender-related findings in the literature and the absence of an association with age in this study may be due to the fact that this was an exclusively paediatric cohort whereas age-dependent studies have included adults (discussed in Chapter 5). Additionally, within this paediatric cohort the patient numbers in individual age categories were too small to assess significant differences. The fact that NSE was higher in younger children may be reflective of the relative vulnerability of developing neurons to damage. The relationship between biomarker concentrations and HIV co-infection could not be meaningfully studied as the sample size of HIV+

children was too small (n=2). Neuromarker concentrations on admission were not associated with MRC staging which is the current means of assessing injury severity. This potentially highlights one of the limitations of clinical assessment, i.e. there is little certainty whether the decreased level of consciousness is due to cerebral tissue injury, raised ICP, seizures, or hyponatremia. The overall improvement in staging from admission to week 1 was likely due to the treatment of raised ICP, fluid resuscitation and correction of electrolyte disturbances; therefore, the MRC staging may be affected by potentially reversible pathophysiology in addition to actual brain tissue injury. The absence of strong associations between neuromarkers and clinical characteristics suggests that neuromarkers provide information about brain injury that may not be reflected in the clinical picture of the patient as gathered from history and clinical examination.

GFAP concentrations were significantly higher in culture positive patients in lumbar CSF only. It is unclear why this may be the case; perhaps a higher bacterial load was associated with a more vigorous reaction and consequently more astrocyte cytoskelton damage. However, it is expected that this would be replicated in the ventricular CSF and in the other neuromarkers. GFAP appears to be sensitive to HCP and it is possible that children with more severe HCP may have had greater volumes of CSF drained and sent for culture. Larger CSF volumes are known to increase the culture positivity rate and therefore it is possible that culture positivity is merely a proxy for HCP in relation to GFAP. This would however apply to ventricular CSF too as larger CSF volumes were often drained during the insertion of EVDs relative to LPs. It may be that the ventricular sample size was too small (46 versus 131 lumbar samples) to allow statistical identification of a relationship with culture positivity. Alternatively, it may be a chance finding.

Association with clinical outcome

NSE was the most prognostic neuromarker on admission as elevated concentrations significantly predicted disability at 6 months. Admission S100B and GFAP were not significantly associated with outcome; however, the highest concentration of lumbar CSF GFAP, S100B and NSE in week 1 appeared to demonstrate and association with poor outcome. This was likely due to the evolution of the disease even after the initiation of treatment and patients who died demonstrated a continued rise in neuromarkers. Potentially, this reflects progressive disease during the first week of treatment initiation that may yet be reversible, i.e. the degree of injury severity on admission suggests the likely outcome, but it is the evolution of the disease after admission that is the stronger determinant. The highest concentrations overall in both lumbar and ventricular CSF were predictive of mortality and morbidity, except highest lumbar CSF S100B was not associated with mortality.

An increasing trend in neuromarker concentrations was a strong prognostic marker of poor outcome on univariate analysis and logistic regression. This was best demonstrated with combined neuromarker Z-scores suggesting that TBM involves complex brain injury of more than one cell type

that evolves over several weeks despite the initiation of anti-TB and steroid treatment. Combined neuromarker Z-scores are of interest as they provide a broader picture of what is likely heterogenous brain injury by combining injury markers from various cell types. Assessing several neuromarkers simultaneously may arguable provide greater insight into injury than a single neuromarker which is limited to a single injury type. However, in the resource and time-limited clinical context cerebral injury assessment and prognostication may rely on only a single marker. S100B concentrations were mostly low; from this relatively low baseline, an increase of as little as 0.06 μg/L predicted death with a very high degree of sensitivity and specificity. Change in NSE over time was less sensitive and specific likely because a larger range of NSE concentrations was observed across outcome groups. This was similar for GFAP, likely because this neuromarker is elevated in patients with transient damage, due to raised ICP, as well as irreversible damage. Seizures were significant covariates in the relationship with mortality for the trend in GFAP and the combined neuromarker z-score and increased the odds of death. This may reflect greater severity of injury or seizures may result from raised ICP of which GFAP appears to be a more sensitive marker than NSE and S100B. However, severe admission HCP was a significant covariate strengthening the relationship between NSE trend and mortality; this may reflect the fact that NSE is less sensitive to HCP-induced damage except under circumstances of severe HCP when neuronal damage may be more pronounced. Both severe HCP and increasing NSE likely represent the more injured brain at higher risk of poor outcome. For the trend in NSE and the trend in GFAP the odds of death or survival were equal; however, in patients with tuberculomas the risk of death was lower; this may suggest that the rise of neuromarkers with tuberculoma injury is less significant in predicting outcome than a similar magnitude rise in neuromarkers due to infarction. Alternatively, it may be a chance finding.

Ventricular CSF was a stronger predictor of mortality and 6 month outcome for both S100B, NSE and the combined neuro-Z score, possibly because the rostro-caudal gradient of neuromarkers reduces the predictive power of lumbar CSF samples. GFAP ventricular concentrations were not predictive of death and showed elevated concentrations in patients with both good and poor outcomes, again possibly because of GFAP's sensitivity to transient effects. Ventricular GFAP, therefore, may be more sensitive to injury than specific to the nature of that injury.

Cytokines behave synergistically or antagonistically depending on the timing of their release relative to the onset of injury and the associated cytokine mileu [437]. Consequently, considerable variability exists across cytokine data sets especially when the timing of injury cannot be identified or standardised across the patient cohort. This was demonstrated by the PCA for combined inflammatory biomarkers which only accounted for half the variability of the data set with the first 2 PCs. To determine the functional consequences of the cytokine profile and the association with outcome is, therefore, challenging. Univariate analysis with CSF revealed significant outcome associations with IL-6. The mechanisms for this relationship are uncertain. However, IL-6 concentrations may have

been elevated in patients with a poor outcome as a function of the advanced temporal course of inflammation in these patients. In the post-acute phase IL-6 modulates the immune response and since patients with severe disease may be at a later stage in the inflammatory process than patients with good outcome this may explain the association with elevated IL-6. Combined initial inflammatory biomarker concentrations did not predict outcome, but the peak of inflammation may have been missed. It is possible that the chronicity of cytokine elevation rather than the initial degree of elevation may be more relevant; the long term profile cannot be commented on because sampling in this study did not extend beyond the 4th week after admission for most patients However, all cytokine concentrations decreased markedly from admission to week 4, although even at week 4 many cytokines remained above the normal range.

The increasing trend of combined neuromarkers and decreasing trend of combined inflammatory markers in patients who died suggests that it may be less an ongoing immune response which is responsible for the progressive brain injury associated with TBM, and more secondary pathological processes that are initiated by the inflammatory process that then continue to cause and perpetuate tissue injury via secondary mechanisms. These may include several biochemical, metabolic and vascular consequences of the inflammation. The best recognized consequence is the thick exudate which obstructs CSF flow precipitating raised ICP and causes vascular damage, thereby placing the brain at risk of cerebral ischemia. Other mechanisms may include the loss of pressure autoregulation due to cerebral inflammation and vessel damage which makes the brain vulnerable to fluctuations in CBF; the uncoupling of metabolism and CBF which places the brain at risk of ischemia as well as hyperaemia and raised ICP; cerebral metabolic derangement which may lead to cell death, cytotoxic oedema and further raised ICP; the accumulation of oxygen free radicals associated with elevated cytokine concentrations which may damage brain tissue and contribute to cytotoxic oedema; and BBB destruction by inflammatory mediators predisposing the brain to vasogenic oedema, raised ICP and consequent ischemia. Tissue infarction increases the risk of cerebral electrophysiological disturbances in the form of clinical and subclinical seizures and possibly spreading depolarisation and their detrimental consequences on the developing brain.

Association with developmental outcome

Neuromarker concentrations did not reflect neurodevelopmental outcome scores. This may be due to several reasons. Firstly, only patients who had a relatively good outcome (normal, mild-moderate disability) were able to undergo neurodevelopmental follow-up. Therefore, the more severe spectrum of neuromarker concentrations would not have been represented in this analysis. Secondly, the neurodevelopmental assessment tests broad constructs which require global cerebral function. Differences in neuromarker concentrations within a limited range, and often indicative of focal injury in this patient sub-set, may have been too subtle to be reflected in the performance of these children.

Thirdly, neuromarker concentrations were measured during the acute phase of injury whereas neurodevelopmental outcome was assessed after 6 months. The temporal resolution of the association between the neuromarkers and neurodevelopmental outcome is severely compromised by the scope for healing and the plasticity of the young brain to overcome damage in the intervening 6 months. Fourthly, as discussed in Chapter 9, neurodevelopmental compromise after TBM is not only a function of organic injury, but also of the social, environmental and educational limitations inherent in long-term hospitalisation and poor home caring environments after discharge from hospital. Therefore, while neuromarkers may provide a valuable quantitative measure of brain tissue injury, they may not reflect the qualitative aspects of brain injury or predict the long-term functional outcomes of children who survive TBM.

Limitations

Samples were processed as soon after collection as possible and degraded samples were not included in the analysis. Yet, it is possible that those samples which were not immediately processed and frozen may have been exposed to cell breakdown and sample contamination. However, the robustness of biomarkers to the often unpredictable dynamics of the hospital environment is an important indicator of their appropriateness for wide-spread clinical use. In general S100B, NSE and GFAP concentrations appeared robust in the CSF but serum was very sensitive to cell breakdown and hemolysis and samples not processed swiftly had to be discarded.

Some variability in the LLOD existed across Luminex plates; while some degree of human error is expected in the laboratory environment, this may have been influenced by variability in the Luminex plate reader as 2 plates were read on a different reader. IL-1β concentrations were low in CSF and serum. While this may have reflected physiology it is possible that this cytokine was poorly detected with Luminex testing. Re-running the samples with an ELISA would have clarified whether the low concentrations were real or artefactual. Additionally, for those cytokines which frequently exceeded the HLOD, retesting with diluted samples on ELISA plates would have been beneficial. Unfortunately samples and funds were limited; however, these are preliminary data and future testing protocols will be informed by these results.

The sample size was too small and the age range too large to adequately compare cases with age-matched control patients; however, the role of age in normative biomarker values remains contentious and there is no clear indication of how age influences normal ranges. Raised serum neuromarker and cytokine concentrations in fatty filum controls may be reflective of the general anaesthesia these children were under when the samples were taken. Their CSF neuromarker values were similar to the normal ranges reported in the literature and so they likely represent a reasonable control group. We considered several other possibilities as control patients, but options were limited by the invasive procedures required to obtain CSF. Most children in whom these invasive procedures are warranted

have some form of cerebral pathology or a potential confounder. Patients suspected of meningitis in whom the CSF was normal were considered; however, the signs and symptoms which warranted the investigative LP, such as pyrexia and headache, may be associated with some perturbation in normal physiology. Patients presenting for shunt replacements not associated with an infective process were also considered; however, there is evidence that chronic HCP, even under conditions of normal ICP, is associated with brain tissue injury [442] and the inflammatory response generated by foreign material (the shunt) in the CNS cannot be excluded. We also considered patients who present with seizures and in whom a LP was done revealing normal CSF results; however, seizures likely increase biomarkers. Ideally ventricular CSF from TBM cases should have been compared to ventricular CSF of control patients. However, for reasons mentioned above it would be very unlikely to obtain access to ventricular CSF in healthy patients and ventricular CSF concentrations were so elevated relative to control lumbar CSF as well as case lumbar CSF that it is unlikely they would not have exceeded normal ventricular CSF values.

More frequent sampling at shorter time intervals during the acute phase (admission-week 2) may have provided greater insight into the dynamic nature of disease evolution. Interpreting the temporal profile of biomarkers in TBM is tricky because the timing and pattern of injury is unclear; it is possible that injury does not all occur at a single time point but may rather undergo fluctuations dependent on various dynamic factors like ICP, CBF, systemic blood pressure and saturation, treatment and so forth. Currently there are no tools to identify these patterns in real-time; frequent biomarker sampling could contribute some insight; however, frequent access to the CSF is not ethically possible. More regular blood sampling is also not ethically permissible in young children in whom the blood volume is already low and normal clinical investigations take preference. The fact that CSF neuromarker concentrations remained elevated over time does, however, provide valuable information about ongoing injury. Similarly, the temporal resolution of cytokine sampling was limited by infrequent sampling as well as delayed patient presentation. Insight into the functional role of cytokines was consequently limited. However, the primary aim of this study was to evaluate novel neuromarkers in TBM and describe their profile and association with outcome. Inflammatory markers were analysed complementary to the neuromarkers.

The complexity of the data set significantly limited the statistical tools which could be applied in examining the association with outcome. PCA was a means of combining the multiple biomarkers over time; yet, it does not provide insight into the biology of the disease process being measured and only identifies patterns across the data. However, this study was directed at collecting pilot data and building hypotheses for future research on neuromarkers in TBM.

Cytokines did not demonstrate a strong association with injury severity or outcome. It may be that cytokine quantification is not the ideal means by which to examine the immune response in TBM, and

that more unbiased techniques like transcriptomics which take into account the genetic determinants of the immune response and the heterogeneity amongst individuals would generate a better understanding of how the immune response is associated with outcome. Additionally, this study did not consider the virulence of the *Mtb* pathogen and its ability to suppress the immune response. Examining discrete aspects of the disease process may be somewhat artificial as it is the baseline characteristics of the host, the baseline characteristics of the pathogen and the interaction between them which determine the response pattern to disease.

Conclusion

Neuromarkers are elevated in the CSF of patients with TBM and provide additional insight into existing and ongoing cerebral injury. Elevated admission NSE concentrations may be of value as an early prognostic indicator. Repeat testing within the first week of admission can provide important prognostic information as patients at risk of a poor outcome demonstrate increasing neuromarker concentrations, this is most marked in S100B for which small increases may predict a poor outcome. Elevated GFAP concentrations may reflect transient damage due to HCP as well as permanent ischemic damage and should, therefore, be interpreted in the context of clinical and radiological signs of raised ICP. Individual neuromarkers reflect brain injury of different types and combining several neuromarkers may reflect heterogeneous brain injury; however, this may not be feasible. The most sensitive sample for neuromarker testing is ventricular CSF and lumbar CSF samples should be interpreted with the awareness that they do not reflect the full extent of brain tissue injury; treatment or injury thresholds should ideally be established separately for the 2 CSF compartments. The sensitivity of CSF neuromarkers to injury over time may render them valuable surrogate markers for testing novel interventions. Serum does not appear to be a sensitive sample for neuromarker measurement. The immune response to TBM is compartmentalised to the site of disease in the CNS and inflammatory cytokines are elevated in the CSF but only partly in the serum. The peak of inflammation likely occurs early in the infective process and appears to decrease fairly rapidly after treatment is initiated. Measuring inflammatory markers may not provide prognostic insights, but further examination of the secondary injury processes initiated by the inflammatory process would be of great value.

CHAPTER 11 SUMMARY

Radiology features of TBM

This chapter aimed to describe the radiological findings of this cohort of children with TBM and hydrocephalus. It included novel descriptions of spinal pathology on MRI and vascular pathology on MRA, and examined their associations with neuro- and inflammatory markers as well as outcome.

Data were gathered from admission CT brain scans, and MRIs (brain and spine) and MRAs at 3 weeks. Radiology features were examined in association with clinical variables, neuro- and inflammatory markers (initial and highest), and clinical and neurodevelopmental outcome.

The main findings were as follows:

1. Most patients presented with moderate and severe HCP (n=33, 75%) and almost all had evidence of diffuse enhancement (95.5%). Infarcts and tuberculomas were uncommon on the admission head CT.
2. MRI scans after 3 weeks demonstrated infarcts in 64% of patients, mostly in the MCA territory (92%). Multiple or large infarcts involving both hemispheres were prognostic of a poor clinical outcome.
3. Tuberculomas were visualised in 59% of patients (n=23) by 3 week MRI and delayed/paradoxical development after the acute phase was observed in 10% (n=4) of patients a median of 78 days after treatment initiation.
4. Attenuation and occlusion of the major cerebral vessels (particularly the MCA) occurred in 55.2% of patients (n=16). Infarcts and MRA abnormalities were not statistically associated, suggesting either that some cerebrovascular pathology, such as vasospasm, occurred early and resolved before 3 week imaging, or that there are other factors which cause infarcts. Additionally, all infarcts were documented, including small lacunar infarcts, the vascular origin of which may be too small to see on MRA.
5. Intradural spinal pathology occurred in more than 75% of patients (n=25) and was mostly asymptomatic. It involved arachnoiditis of the cord and nerve roots, exudates, and spinal tuberculomas. All patients who failed LP had moderate-severe spinal arachnoiditis which was associated with increased lumbar CSF protein; this has important clinical implications for the diagnosis of TBM and the establishment of CHCP or NCHCP on AEG.
6. Elevated concentrations of S100B and GFAP were associated with the severity of HCP on admissions suggesting that these neuromarkers may be sensitive to damage induced by raised ICP and/or distended ventricles.

7. GFAP concentrations were elevated in patients with infarcts and tuberculomas and were positively associated with their size, suggesting that GFAP is sensitive to brain damage from various injurious processes and across the spectrum of severity. S100B and NSE were elevated in patients with multiple or large infarcts and likely reflect severe tissue injury.
8. Inflammatory markers were poorly associated with radiology features

CHAPTER 11: THE RADIOLOGICAL FEATURES OF THE BRAIN AND SPINAL CORD IN PAEDIATRIC TBM

Methods

Radiology plays an essential role in TBM: firstly, it contributes to the diagnosis; secondly, it assists with long-term management to guide the treatment of ICP and other surgical complications; thirdly, it provides information on disease resolution or deterioration; fourthly, it aids the assessment of injury severity and prediction of outcome; and finally, it adds valuable insight into the pathophysiology of TBM.

Aim

The objectives of this part of the study were to prospectively describe the following in a cohort of children with TBM and HCP:

1. Radiological features on admission and after 3 weeks
2. The involvement of the spinal cord and nerves
3. Vascular pathology as seen on MRA
4. The relationship between biomarkers (neurological and inflammatory) and radiology features on admission and follow-up imaging

Study Imaging protocol

As part of routine care all patients received a contrast-enhanced CT brain scan on admission when TBM with raised ICP or HCP was suspected. Further CT scans were requested when indicated for deteriorating clinical status or to inform surgical management. All patients were scheduled for an MRI of the brain and spine at approximately 3-4 weeks post-admission, as the Radiology Department's schedule allowed, or earlier if clinically required. MRI scans are not standard of care for patients with TBM at RXH due to resource constraints and the need for a general anaesthetic in younger children. This project was able to fund one MRI per study patient. The decision to conduct MRI scanning at 3 weeks rather than admission was taken to maximise the detection of evolving pathology. Follow up CT scans after discharge were performed where clinically indicated to follow-up HCP and the progress of intracranial or spinal pathology. All data were analysed as part of this study. Results for chest X-ray (CXRs) were also collected and analysed.

Imaging modalities

CT Scans

CT brain scans were performed using a 64 slice Phillips Brilliance scanner. Admission CT scans were performed pre- and post- administration of intravenous non-ionic contrast (Ultravist- 1ml/kg). Follow up scans to assess ventricular size were not routinely done with contrast unless required for tuberculoma investigation.

MRI Scans

The Philips Achieva 1.5 Tesla MRI scanner was used for MR imaging. Patients who were less than 20 kg and stable were sedated orally using Chloral Hydrate. All children for whom sedation was not considered safe had a general anaesthetic. Children (generally > 6 years) who would cooperate with the MRI procedure without requiring sedation or an anaesthetic did not receive any.

Brain imaging included: Axial T2-weighted, axial FLAIR, and 3 DWI (acquired sagitally and reformatted in 3 planes), MRA diffusion and axial T1 post-contrast (Magnevist –gadopentetic acid).

Spinal imaging included: Sagittal T1 and T2, sagittal T1 post-contrast, and axial T2 and T1 post-contrast imaging through the lesion if pathology was detected.

Data collected

Scans were reviewed by three senior paediatric radiologists and a senior paediatric neurosurgeon all experienced in evaluating CT and MRI scans in paediatric TBM. Each radiological feature was assigned to one primary reviewer who examined that feature across all scans included for analysis. Thereafter the scans were all reviewed for a second time by second reviewer from amongst the team to ensure uniformity. Results were compared to the original radiology and neurosurgery reports. Where discrepancies in reporting were noted, scans were re-evaluated by the team until consensus was reached. Due to the paucity of formal standardised criteria for characterising features of TBM, scans were evaluated according to the following criteria set by the reviewers.

<u>Brain imaging</u>

The following features were recorded from brain imaging on CT and MRI

HCP: *mild*- visible temporal horns, rounding of the third ventricle (V3)
moderate – all ventricles dilated, no transependymal fluid shift
severe – dilated ventricles, fluid shift and loss of sulcal markings

Basal enhancement: *focal* – localised enhancement

diffuse–enhancement not limited to a focal region

Pre-contrast hyperdensity: obliterated hyperdense basal cisterns or Sylvian fissures on non-contrasted CT scan

Infarcts: Acute/sub-acute – acute infarcts demonstrated restricted diffusivity on DWI evaluated in conjunction with the actual diffusion coefficient (ADC) map – infarcts positive on DWI were estimated to be < 10 days old, sub-acute infarcts were between 10-14 days old, and established infarcts which were not positive on DWI were >14 days old

Size –small (lacunar), medium (multiple or large infarcts) and global (encompassing all vascular territories)

Location - categorised accordingly to the number of vascular territories as well as anatomical locations – vascular territories were assigned as follows:

Vessel	L hemisphere	R hemisphere	Total
MCA	1	1	2
ACA	1	1	2
PCA	1	1	2
Vertebrobasilar			1
			7

Global infarction included infarcts in all 7 major vascular territories

Tuberculomas and TB abscess: number, anatomical location as well as unilateral or bilateral location

Spinal imaging (MRI with contrast)

Arachnoiditis: visualised as enhancement of the cord or roots; defined as nodules, exudate and clumping of nerve roots; presence of plaques (extra or intra-dural collections of exudate). Spinal arachnoiditis was defined as mild (thin coating of exudate on the cord or nerve roots), moderate (clumping of nerve roots, presence of intra- or extra-dural exudate plaques) or severe (extensive exudate on neural structures and in the subarachnoid space).

Tuberculoma or TB abscess: surface or intramedullary.

MR Angiography

Attenuation and occlusion of the ICA, MCA, ACA, PCA and posterior communicating artery (Pcomm), unilaterally or bilaterally, were recorded.

CXR

Features suggestive of TB on CXR included adenopathy, airway compression, bronchogenic pathology, airspace disease and effusion. The presence of a miliary pattern on CXR was also noted.

Analysis

Statistical tests for categorical data included the Chi-square or Fisher's exact tests for binary variables and Kruskal-Wallis for variables with >2 levels. This analysis aimed to explore whether relationships exist between radiology and clinical/biomarker characteristics rather than determining prognostic markers per se. Therefore, univariate analysis was conducted as part of hypothesis generation and p-values were not adjusted for multiple testing.

Associations between radiological features

The relationships between radiological features recorded on admission and overall were examined.

Association with admission, patient and laboratory characteristics

Relationships between the following were examined:

- Various radiology features: HCP, admission enhancement, infarcts, tuberculomas, spinal disease, vascular abnormality,

- CXR findings,

- Patient characteristics (age, HIV status, MRC staging on admission, culture result, seizures, symptom duration, failed medical treatment and ICP deterioration),

- Admission CSF chemistry (glucose, protein, chloride, polymorphonuclear cells and lymphocytes measured in lumbar and ventricular CSF as well as the differential between the 2 CSF compartments to accommodate for the rostro-caudal gradient),

- The relationship between opening pressure on LP and the severity of HCP was also investigated.

Association with outcome

Analysis between radiological features and outcome included mortality at 6 months and dichotomized clinical outcome at 6 months (poor outcome [PCSP 4-6: severe disability- death] vs good outcome [PCPS1-3: normal – mild/moderate disability]). Radiology features included admission enhancement (including severity), infarcts (including number of vessels involved, size, unilateral/bilateral location, single/multiple), vascular pathology on MRA, tuberculomas (including single/multiple) and spinal disease (spinal arachnoiditis and severity, and spinal tuberculoma).The association between infarcts

(number of vessels involved, size, unilateral/bilateral location, single/multiple) and neurodevelopmental outcome for the GQ and each sub-scale was assessed.

Association with biomarkers

Admission CT scan features were analysed in association with initial lumbar and ventricular CSF S100B, NSE, GFAP and combined initial neuromarker concentrations (Z-Neuro Initial). Analysis was also conducted with initial lumbar and ventricular CSF inflammatory markers and combined initial inflammatory concentrations (Z-Inflam Initial). Radiology features included HCP communication (CHCP or NCHCP), HCP severity (mild, moderate and severe), admission enhancement and enhancement severity, and the presence of tuberculomas and infarcts.

Radiology features overall (including admission, MRI or final CT scans for patients who died before MRI scanning could be performed) were examined in association with highest overall concentrations of the 3 neuromarkers and the 14 inflammatory markers. Additionally lumbar CSF Δ S100B, Δ NSE, Δ GFAP, and the combined change in neuromarkers (Z-NeuroΔ) and inflammatory markers (Z-InflamΔ) were also examined. Radiology features analysed included severity of HCP, infarcts (including number of vessels involved, size, unilateral/bilateral location, single/multiple), vascular pathology on MRA (including the number of vessels involved), tuberculomas (including single/multiple) and spinal disease (spinal arachnoiditis and severity, spinal tuberculoma).

CHAPTER 11: RADIOLOGICAL FEATURES OF THE BRAIN AND SPINAL CORD IN PAEDIATRIC TBM

Results

Admission CT scans

All 44 TBM cases had admission CT brain scans with and without contrast. HCP was present in all patients and HCP severity was scored as moderate in more than half the patients (n=24, 54.5%). The inter-observer agreement in grading HCP severity was 88.1% (*Kappa, p<0.001*). Enhancement was present on 95.5% of admission scans (n=41) and involved the basal cisterns in almost all cases (n=40, 97.6%). Enhancement was more commonly diffuse (n=39, 95.1%) than focal (n=2, 4.9%). Pre-contrast hyperdensity was visible in two thirds of the scans which demonstrated enhancement post-contrast (n=27, 65.9%) and in none without enhancement. Seven patients (15.9%) presented with infarcts on their admission scan, largely involving the MCA territory. Tuberculomas were only reported in 3 admission CT scans (6.8%). No TB abscesses were observed. Details of admission imaging features are provided in Table 11.1, and details of tuberculoma location in Table 11.5.

MRI Brain Scans

MRI scans were conducted in 39 patients at an average of 26 ±11 days post-admission. Four of the 44 study patients died before an MRI could be performed and 1 patient was not scanned due to complications with transport from the facility to which she was transferred. Persistent ventriculomegaly was reported in 24 patients (61.5%); in 12 patients (30.8%) HCP was treated with an EVD or VPS at the time of the scan, and in 3 patients (7.7%) HCP had resolved. Enhancement was present in all 38 patients who received contrast; one patient awoke before contrast could be administered. The pattern of enhancement was predominantly basal (92.1%) and diffuse (89.5%). Infarcts were reported in 25 patients (64.1%) and were limited to 1 or 2 vascular territories in most (n=18, 72%). The most common site for infarction was the basal ganglia (n=20, 80%, Table 11.2). The MCA was the vessel most affected (n=23, 92%), involving MCA perforators (n=22), branches (n= 6) and the major artery (n=1), singly or in combination. Infarcts occurred in the ACA branch territories in 3 patients with infarction (12%) and the major ACA artery in 2 cases (8% - Table 11.3). Only 8 of these 25 infarcted patients (24.1%) demonstrated infarcts on their admission scans; and by the time of DW imaging almost 90% of infarcts were already more than 2 weeks old, the remaining infarcts were acute or a mixture of acute and established. Luxury perfusion was observed in 4 patients (16%) with large infarcts. Tuberculomas were observed in 23 patients (59%), most of which were multiple (56.5%) and occurred bilaterally (47.8%). Summary imaging details are outlined in Table 11.1-11.5. and Figures 11.1-11.7 depict various MRI features.

Table 11.1: Summary of radiology features overall

Radiology features	Admission CT (n=44)	MRI (n= 39)	Early deaths (n=4)	Overall [a] (n=43)	Association with outcome
HCP	n = 44 (100)	n = 24 (61.5)	Treated [b]		
Severity					*p=0.7*
Mild	11 (25)	18 (75)			
Moderate	24 (54.5)	6 (25)			
Severe	9 (20.5)	2 (8.3)			
Periventricular lucency	32 (72.7)	9 (37.5)			
Enhancement	n = 41 (95.5)	n =38 (100) [c]	No contrast		*p=0.6*
Severity					*p=0.05**
Mild	21 (51.2)	15 (39.5)			
Moderate	19 (46.3)	10 (26.3)			
Severe	1 (2.4)	13 (34.2)			
Focal	2 (4.9)	4 (10.5)			
Diffuse	39 (95.1)	34 (89.5)			
Basal	40 (97.6)	35 (92.1)			
Pre-contrast hyperdensity	27 (65.9)				*p=0.18*
Infarcts	9 (20.5)	25 (64.1)	4 (100)	29 (65.9)	*p=0.01**
No. of vascular territories					*p<0.001**
1	6 (66.7)	12 (48)	0	12 (41.4)	
2	3 (33.3)	6 (24)	0	6 (20.7)	
3	0	4 (16)	0	4 (13.8)	
4	0	1 (4)	0	1 (3.4)	
5	0	1 (4)	0	1 (3.4)	
6	0	0	0	1 (3.4)	
7	0	1 (4)	4 (100)	4 (13.8)	
MCA	7 (77.8)	23(92)	4 (100)		
ACA	1 (11.1)	5 (20)	4 (100)		
PCA	0	3 (12)	4 (100)		
Vertebrobasilar	1 (1.11)	6 (24)	4 (100)		
Unilateral	6 (66.7)	14 (56)		14 (48.3)	
Bilateral	3 (33.3)	11 (44)	4 (100)	15 (51.2)	*p<0.001**
Single	5 (55.6)	9 (36)		11 (37.9)	
Multiple	4 (44.4)	14 (56)	4 (100)	18 (62.1)	*p=0.003**
Infarct size					*p<0.001**
Small	3 (33.3)	10 (40)	0	10 (34.5)	
Moderate	6 (66.7)	15 (60)	0	16 (55.2)	
Large	0	0	4 (100)	3 (10.3)	
DWI infarct evolution		n= 23			*p=0.08*
Acute [d]		1 (4.3)		1 (4.3)	
Old [e]		20 (87)		20 (87)	
Mixed [f]		2 (8.7)		2 (8.7)	
Tuberculoma	3 (6.8)	23 (59)	No contrast		*p=0.04**
Single	1 (33.3)	10 (43.5)			*p=0.98*
Multiple	2 (66.7)	13 (56.5)			

This table summarises the frequency of radiology features reported on admission CT scans, final CT scans of the 4 patients who died before 3 week MRI (Early deaths), and on 3 week MRI. The associations between radiology features and 6 month clinical outcome are indicated. Data are presented as number (percent). [a] Overall represents all 43 patients with follow up imaging during their hospital stay (4 deceased and 39 with MRI), [b] HCP was treated with EVDs or VPS [c] Only 38 patients received contrast, [d] infarcts < 10 days old, [e] infarcts > 14 days old, [f] combination of infarcts < 10 days and >10 days old

Early deaths

Four patients died within 10 days of admission (median 4 days) and could not undergo MR imaging but CT scans (average 2.5 ±0.6 scans per patient) were performed in order to monitor their deterioration and direct clinical management. In order to include them in the analyses, the findings of their final CT scans were recorded. These findings are reported separately from MRI findings in Table 11.1 as features from the 2 imaging modalities are not directly comparable due to differences in sensitivities. The admission scans of these 4 patients demonstrated moderate – severe HCP and all received an EVD or VPS. Mild enhancement and no tuberculomas were reported on their admission scans; follow-up imaging was not done with contrast so the evolution of enhancement and tuberculomas could not be determined. Infarcts were visible in only 2 of these patients on admission: in the right basal ganglia in 1 patient and bilateral basal ganglia and left tempero-parietal region in the other. Global infarction involving all 7 vascular territories was observed in all of these patients by their last scan a median of 4 days (3-11 days) after admission. The remaining 3 patients who died underwent MRI and their data are reported with the overall MRI data.

Table 11.2: Anatomical location of infarcts

	Infarct Admission CT	Infarct MRI	Infarct Early deaths	Infarcts overall on post-admission imaging
Frontal lobe	1	4	4	8
Temporal lobe		3	4	7
Parietal lobe		2	4	5
Occipital lobe		3	4	7
Corpus callosum	1	4	4	7
Basal ganglia	7	20	4	24
Thalamus		3	4	6
Cerebellum	1	2	4	5
Brain stem		5	4	9
Infra-tentorial		7	3	10
Supra-tentorial		39	3	42

Data presented as the number of reports of infarcts in each location, some patients were reported to have multiple lesions in multiple locations, Infarcts overall= combination of MRI findings and findings on the final CT scan of patients who died early

Table 11.3: Vascular detail of infarcts on MRI

Vessel	Unilateral	Bilateral	Total
MCA			**23**
Perforator	12	10	
Branch	5	1	
Major vessel	1		
ACA			**5**
Perforator			
Branch		3	
Major vessel		2	
PCA			**3**
Vertebrobasilar			**6**

Data presented as the number of reports of infarcts involving each cerebral vessel and its branches or perforators, patients frequently had combinations of vessels involved in infarction. Total = total number of patients with infarcts in that vascular territory

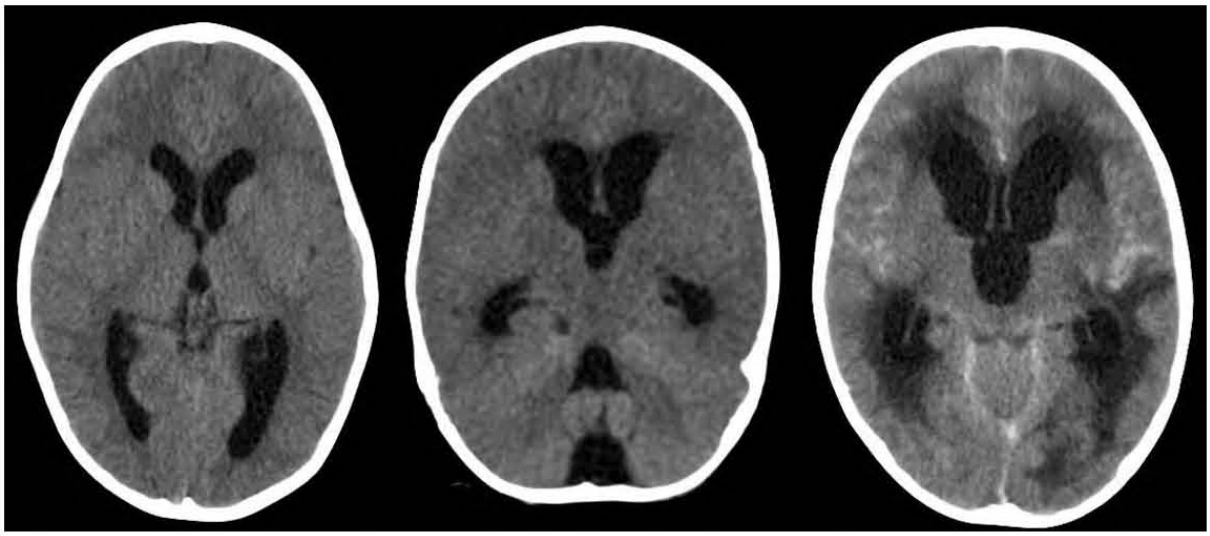
Table 11.4: Infarcts overall by vessel

	Admission CT			Overall post-admission imaging [a]		
	Unilateral	**Bilateral**	**Total**	**Unilateral**	**Bilateral**	**Total**
MCA	5	2	7	13	14	27
ACA		1		3	6	9
PCA				2	5	7
Vertebrobasilar	1			1	9	10

This table presents the frequency of infarcts in the 7 major vascular territories for admission CT scans and for MRI scans and final CT scans of patients who died early

Cerebral radiology features

Figure 11.1: Hydrocephalus (HCP)

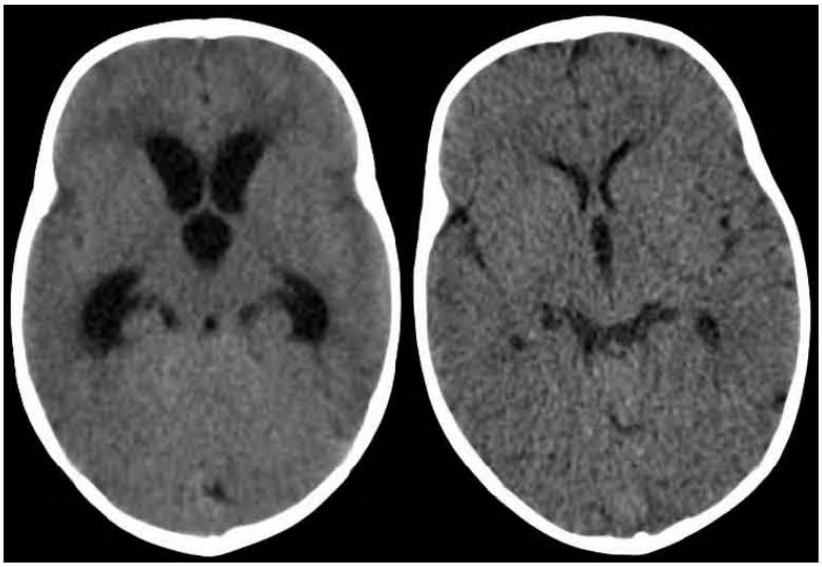


Mild HCP
visible temporal horns, rounding of the third ventricle (V3)

Moderate HCP
all ventricles dilated, no transependymal fluid shift

Severe HCP
Dilated ventricles with periventricular luceny and loss of sulcal markings

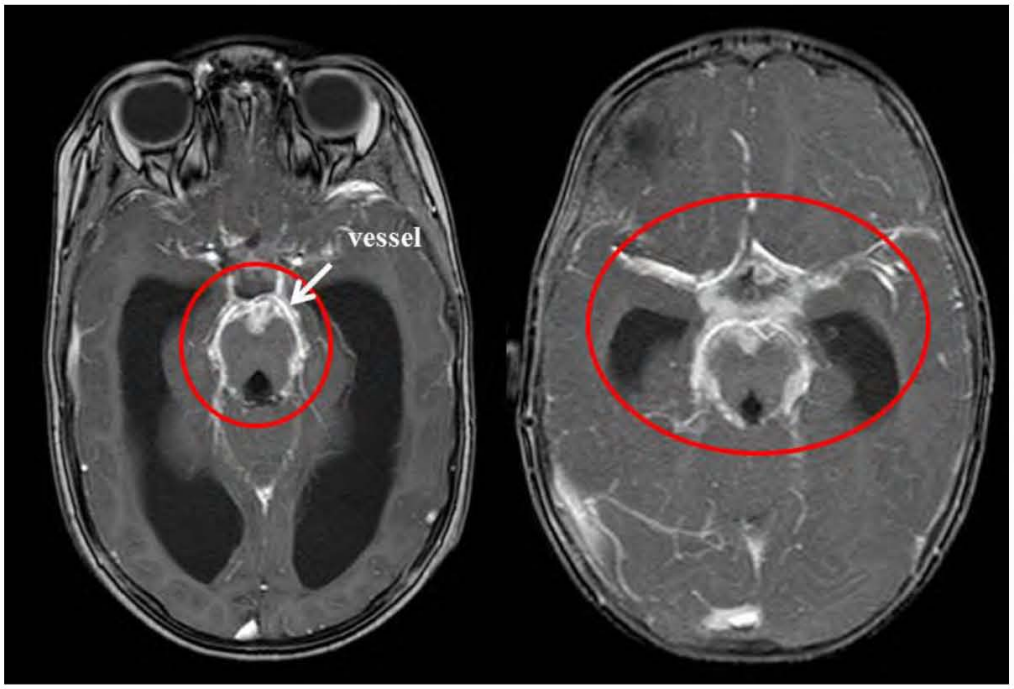
Figure 11.2: HCP resolution



Admission CT scan demonstrating moderate HCP

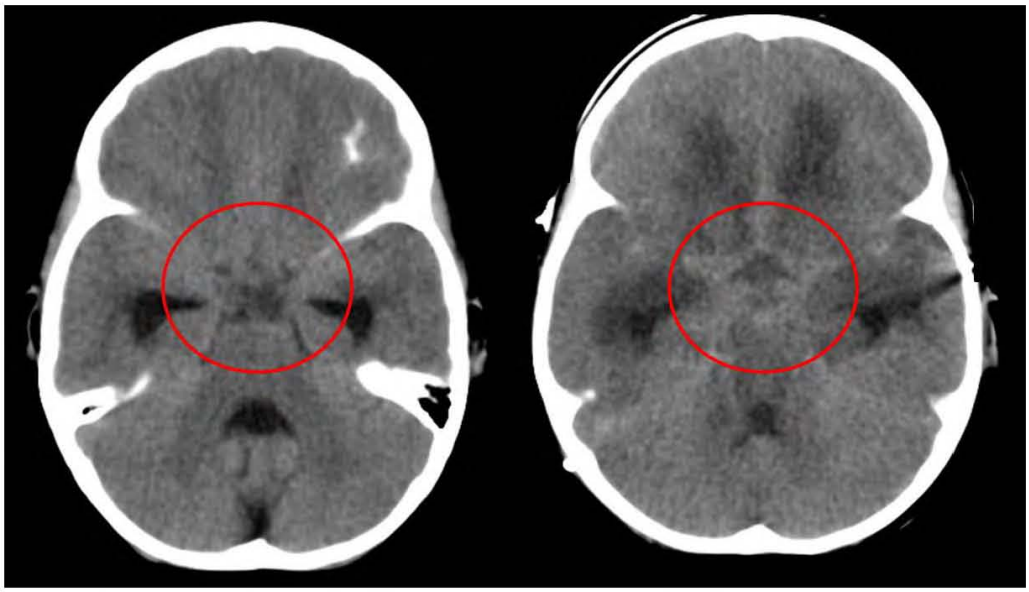
Follow-up CT scan after 35 days demonstrating resolution of HCP on medical treatment

Figure 11.3: Basal meningeal enhancement



Post-contrast scans demonstrating enhancement in the basal cisterns with exudate surrounding the major vessels

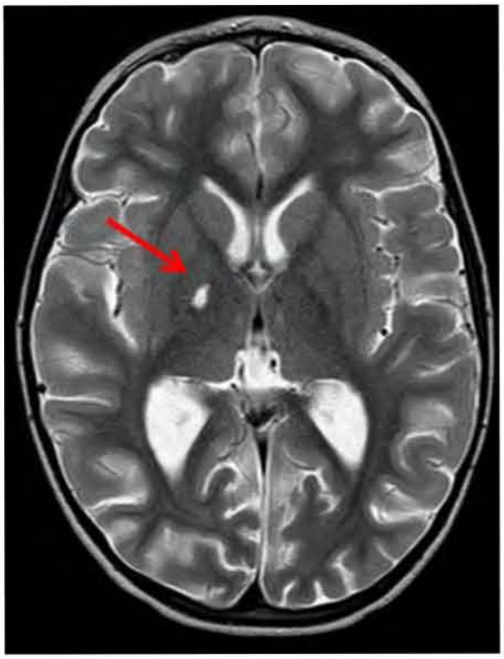
Figure 11.4: Evolution of pre-contrast hyperdensity



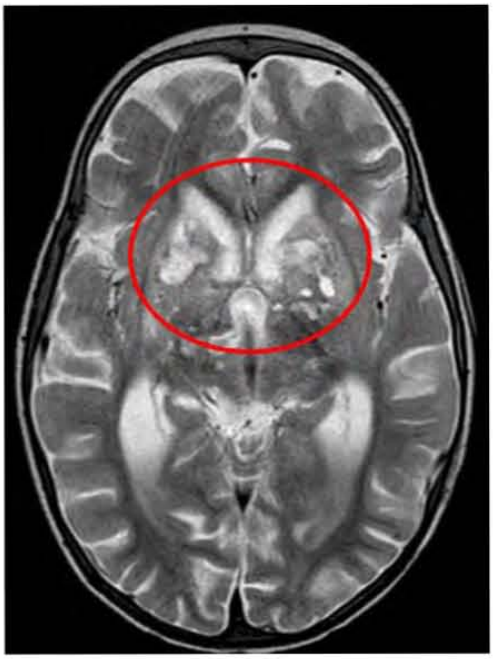
Admission pre-contrast CT scan demonstrating pre-contrast hyperdensity in the basal cistern

CT scan after 11 days in the same patient demonstrating evolution of pre-contrast hyperdensity in a patient on full TB treatment

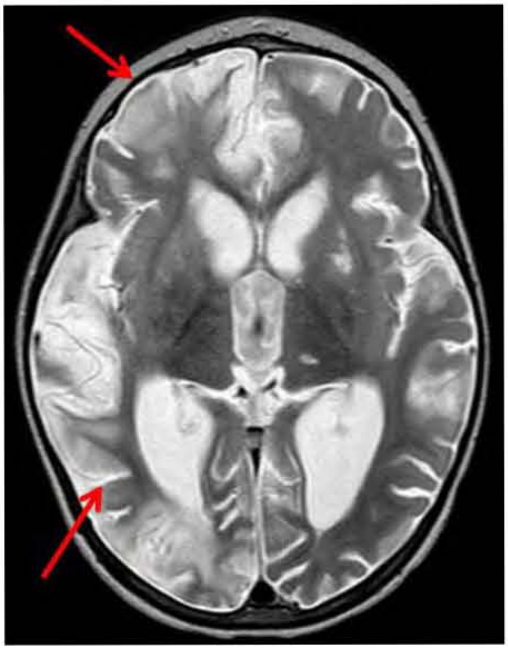
Figure 11.5: Infarcts



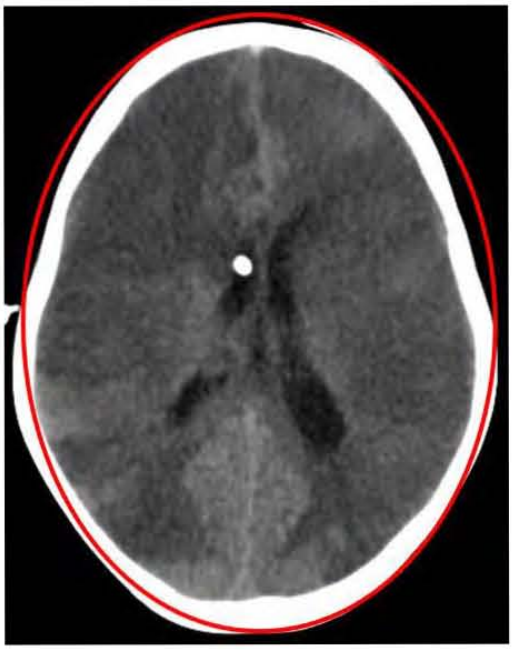
Small lacunar infarct in the right basal ganglia on T2 weighted MRI



Multiple infarcts involving the basal ganglia bilaterally on T2 weighted MRI

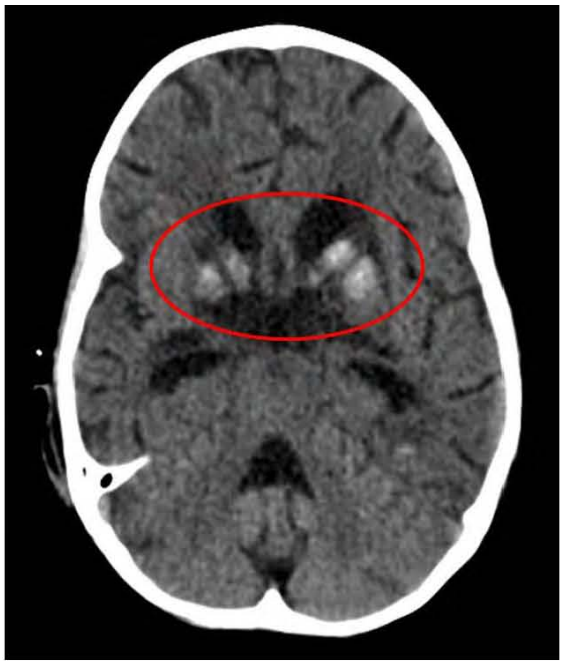


Large vascular territory infarcts on T2 weighted MRI



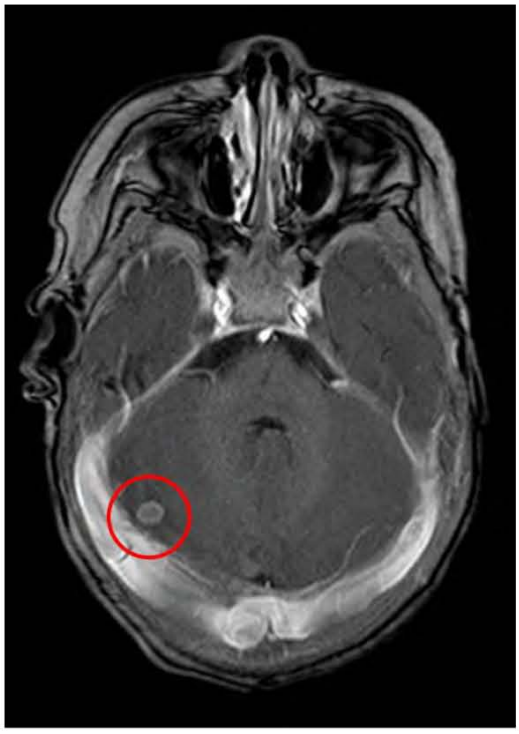
Global infarction on CT scan

Figure 11.6: Luxury perfusion in infarcted areas



Luxury perfusion observed in the infarcted basal ganglia

Figure 11.7: Parenchymal Tuberculoma



Post-contrast MRI demonstrating a tuberculoma in the right cerebellum

Delayed tuberculoma development

Delayed or 'paradoxical' tuberculoma development was defined as the progression of established tuberculomas or the occurrence of new tuberculomas on out-patient follow-up imaging despite treatment. These were observed in 4 of the 37 patients who survived till 6 months (10.8%). These were reported a median of 78 (47-106) days after admission and the initiation of anti-TB treatment. Two of these patients were asymptomatic and the lesions were discovered incidentally on routine 3 month follow-up imaging, and 2 patients presented with signs of raised ICP and progressive HCP. Tuberculomas were mostly bilateral multiple tuberculomas (n=3) and their locations are detailed in Table 11.5. A TB abscess was observed in 1 of these patients, and all 4 scans demonstrated persistent basal enhancement. Images of late onset tuberculomas and evolution of enhancement are provided in Figures 11.8 and 11.9.

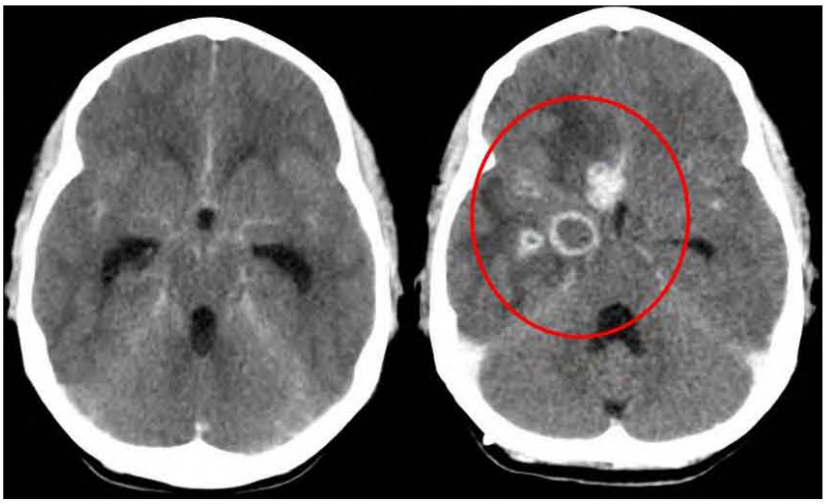
Table 11.5: Tuberculoma location

	Tuberculoma Admission CT	**Tuberculoma MRI**	**Delayed Tuberculomas**
Frontal lobe	1	3	3
Temporal lobe		4	1
Parietal lobe		5	
Occipital lobe		5	
Corpus callosum		1	
Basal ganglia		3	
Thalamus		0	
Cisterns		6	4
Cerebellum	2	11	
Brain stem		3	

Data presented as the number of reports of tuberculomas in each location on admission CT scans, MRI at 3 weeks, and on follow-up imaging

Radiological deterioration

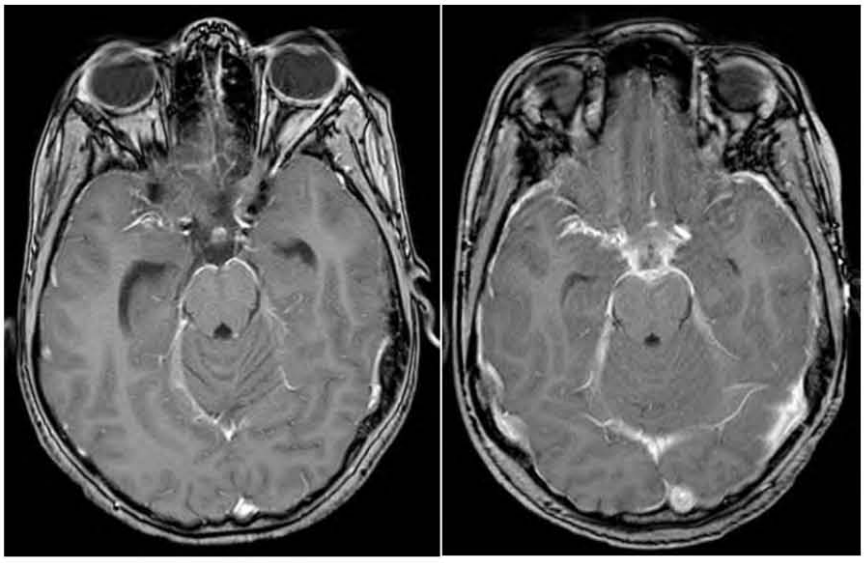
Figure 11.8: Late onset tuberculomas



Admission CT scan demonstrating HCP and enhancement, no tuberculomas

Multiple tuberculomas and a TB abscess observed 4 months after treatment initiation in the same patient with drug-susceptible TB

Figure 11.9: Evolution of enhancement



MRI scan 18 days after treatment initiation demonstrating minor basal enhancement

MRI scan of the same patient145 days after treatment initiation demonstrating progression of diffuse enhancement

MR Angiograms

MRAs were a late addition to the study protocol and were not performed in the first 10 patients. Of the remaining 34 patients, MRAs were performed in 29 patients (74.4%); 3 patients had died and in 2 patients technical complications prevented imaging. MRA was normal in 13 patients (44.8%). Abnormalities in the remaining 16 patients involved predominantly the MCA (93.7%) singly or in combination with pathology in other vessels. The median number of vessels with pathology per patient was 2 (range 1-7). Abnormalities included vessel attenuation, irregular vessel calibre, vessel occlusion and absent vessels. Illustrations of MRA pathology are included in Figure 11.10.

Table 11.6: Frequency of vascular pathology per vessel

ICA		MCA		ACA		PCA		Pcomm	
Unilat	**Bilat**	**Unilat**	**Bilat**	**Unilat**	**Bilat**	**Unilat**	**Bilat**	**Unilat**	**Bilat**
1	1	5	10	3	4	0	0	0	3

Data presented as the number of reports of pathology in each vessel. Unilat = unilateral, Bilat = bilateral

Spinal imaging

Spinal imaging was performed simultaneously with MRI brain scans at an average of 26 ±11 days post-admission. Spinal scans were conducted in 37 patients as 4 patients had died and 3 patients could not be scanned due to technical complications. Images from 4 of these 37 patients were excluded due to movement artefact (n=2), and patients waking before contrast could be administered (n=2). In the remaining 33 patients spinal disease was present in 25 children (75.8%), including spinal arachnoiditis in 24 patients (72%), spinal tuberculomas in 6 patients (18.2%) and intradural extramedullary plaque-like collections of exudate in 3 patients (9%). There was no bony involvement. *Spinal arachnoiditis* involved enhancement of the cord (n=19, 79.2%) and of the nerve roots (n= 23, 95.8%). Exudate filled the thecal sac below the conus and/or caused clumping of the spinal nerve roots in 12 patients (50%). The severity of exudate in the spinal canal was mild in 12 patients (50 %), moderate in 6 patients (25%) and severe in 6 patients (25 %). Among the 6 patients with *spinal tuberculomas*, 5 patients had tuberculomas on the surface of the cord (83.3%) and 1 patient had an intramedullary tuberculoma (16.7%). Most of the patients with spinal disease were asymptomatic (n=23, 92%), 2 patients complained of sensory deficit or pain in the legs. Images of spinal disease are provided in Figure 11.12.

Table 11.7: MR characteristics of the spine accompanying TBM (n=33)

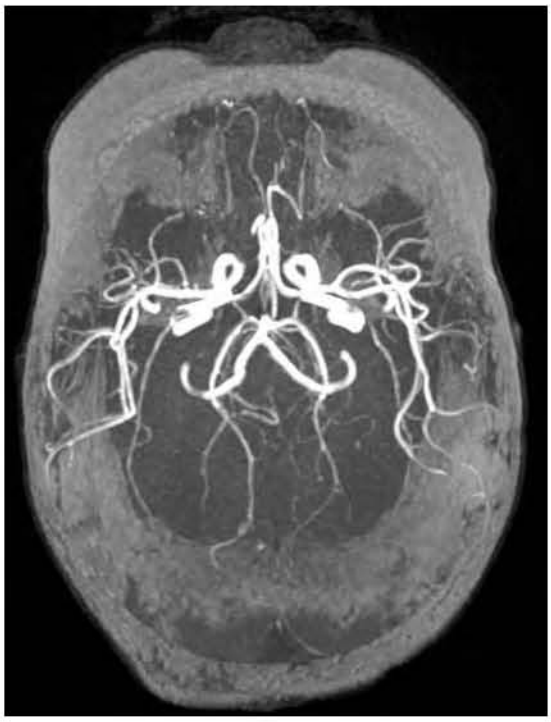
Feature	Value	Association with outcome
Spinal disease overall	25 (75.8)	*p=0.31*
Spinal arachnoiditis	24 (72.7)	*p=0.62*
Severity		*p=0.54*
Mild	12 (50)	
Moderate	6 (25)	
Severe	6 (25)	
Cord arachnoiditis	19 (79.1)	
Nerve root arachnoiditis	23 (95.8)	
Arachnoid nodules	4 (16.7)	
Exudate in the thecal sac	7 (29.2)	
Clumping of nerve roots	10 (41.7)	
Spinal Tuberculoma	6 (18.2)	
Single	3 (50)	
Multiple	3 (50)	

Data are presented as number (percent) of patients reported to have the various forms of spinal pathology, the association with 6 month clinical outcome is reported

Chest imaging

CXRs were performed in 42 patients. Features suggestive of TB were documented in 22 patients (52.4%) and miliary TB in 5 patients (11.9% -none of whom were infants).

Figure 11.10: MRA Abnormalities

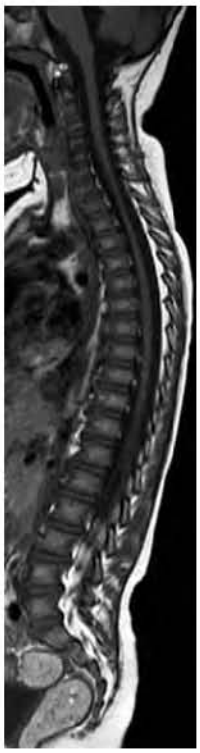


Normal MRA

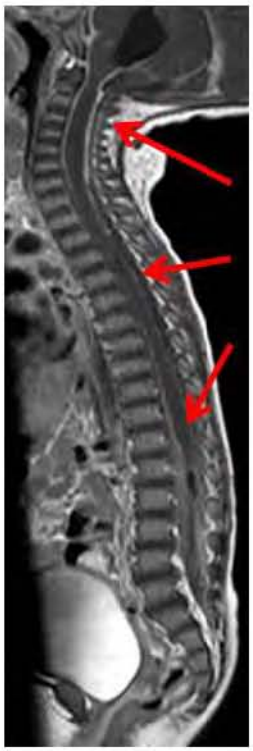
Focal stricture of the right MCA

Absent left ICA[1], attenuated left MCA[2] and bilateral ACA[3]

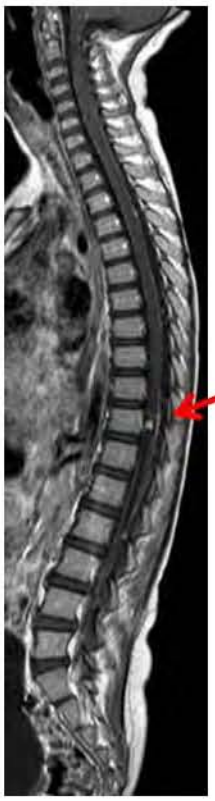
Figure 11.12: Spinal disease



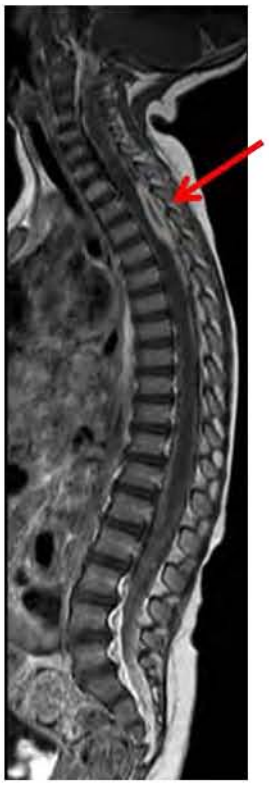
Normal spine



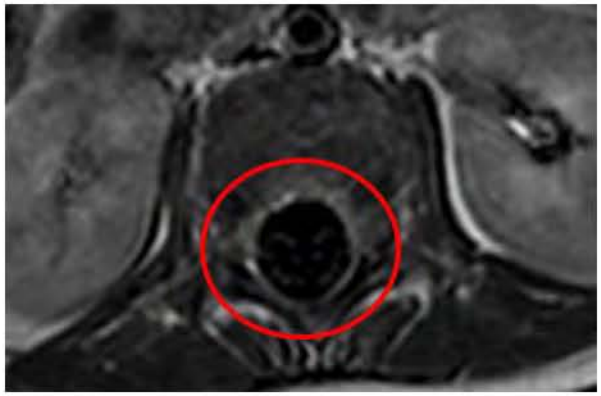
Post-contrast MRI demonstrating spinal enhancement along the length of the spinal cord



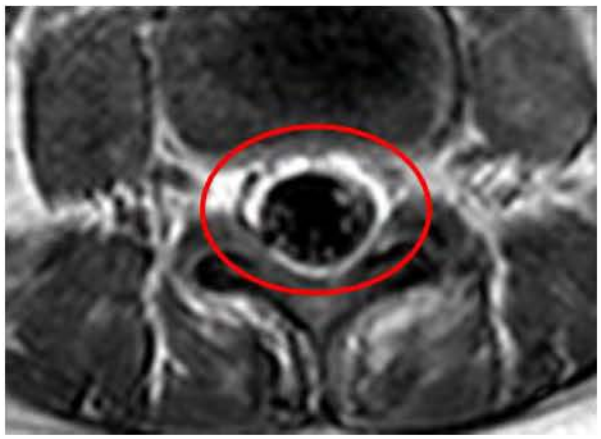
Spinal tuberculoma



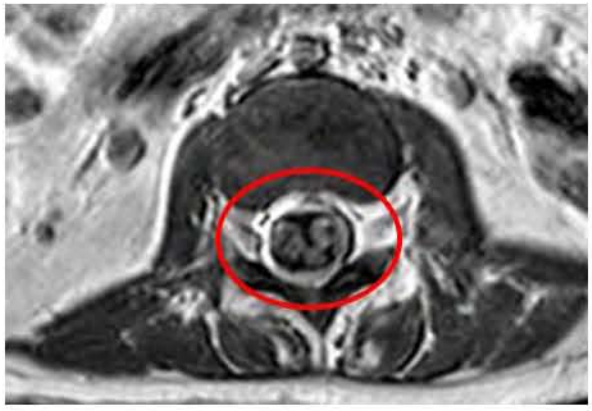
Intradural extramedullary plaque-like exudate collection



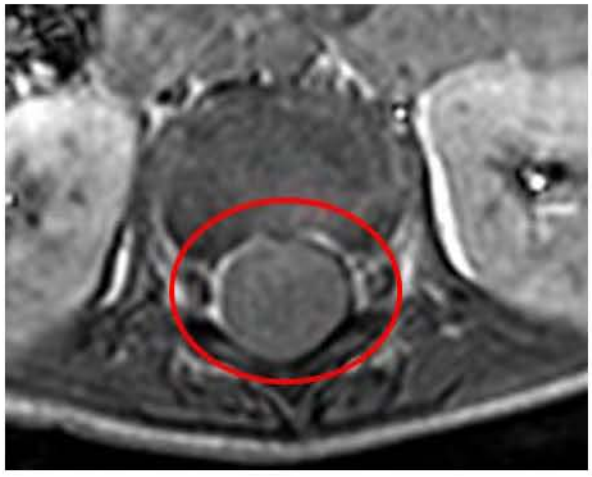
Normal thecal sac



Mild cauda equina arachnoiditis



Moderate cauda equina arachnoiditis



Severe cauda equina arachnoiditis with exudate filling the thecal sac

Analysis results

1. *Associations between radiological features*

No significant relationships were demonstrated between radiology features; only the difference in the incidence of tuberculomas and infarcts approached significance (*p=0.06*) in that infarcts were reported less frequently in patients with tuberculomas, and tuberculomas were reported less frequently in patients with infarcts. Pre-contrast basal hyperdensity was 100% specific in detecting post-contrast enhancement; however, it was only 64% sensitive. Abnormalities on MRA were not associated with infarcts.

2. *Association with admission, patient and laboratory characteristics*

Whether HCP was communicating or non-communicating was not significantly related to any patient or clinical features. The severity of HCP score was not associated with opening pressure (*p=0.48*). Neither HCP severity nor infarcts were related to MRC staging on admission (*p=0.23* and *p=0.43* respectively) or a history of seizures (*p=0.24* and *p=0.31* respectively). Patients with spinal arachnoiditis were 4.3 times more likely to fail LP (*p=0.04*), the severity of spinal arachnoiditis was significantly associated with failed LP (*p=0.002*) and all patients who failed LP had moderate-severe spinal arachnoiditis. Patients with spinal arachnoiditis more commonly experienced a deterioration in ICP after it appeared to have normalised (*p=0.03*), this was not replicated for cerebral enhancement (*p=0.9).* A miliary pattern on CXR was not associated with age; however, having a suggestive CXR was associated with older age (*p=0.03*). Overall radiology features did not differ in patients with HIV co-infection.

Only spinal arachnoiditis and cranial tuberculomas demonstrated significant associations with CSF chemistry. Lumbar CSF protein was higher in patients with more severe spinal arachnoiditis (*p=0.03*). Significant associations with tuberculomas were as follows: lumbar CSF polymorphonuclear cell counts were significantly higher in patients with tuberculomas (*p=0.04*), ventricular CSF lymphocytes were lower (*p=0.03*) and ventricular protein was lower (*p=0.04*). The differential between lumbar and ventricular CSF did not reach significance in the relationship with radiological variables.

3. *Association with outcome*

Mortality - Tuberculomas were more common in patients who survived (*p=0.01*), whereas infarcts trended towards being more common in patients who died (*p=0.08*). In particular, death was significantly associated with multiple (*p=0.03*), bilateral (*p=0.006*), and large infarcts (*p<0.0001*) – all patients who died had global infarcts, some patients who survived had lacunar infarcts. The number of vascular territories involved in infarction was positively associated with death (*p<0.001*); however, vascular pathology on MRA was not (*p=0.41*).

Six month outcome - Mild admission basal enhancement (*p=0.05*) and a higher frequency of tuberculomas (*p=0.04*) were associated with good outcomes. Infarcts were significantly associated with poor outcome (*p=0.01*) and no children who made a full recovery had infarcts while all children with a poor outcome had evidence of infarction. Bilateral (*p<0.001*), multiple (*p=0.003*) and large infarcts (*p<0.001*) involving multiple vascular territories (*p<0.001)* were predictive of poor outcome, but this relationship was not found for vascular pathology on MRA (*p=0.44*). Neither miliary TB (*p=0.41*) nor a suggestive chest x-ray (*p=0.8*) were significantly associated with clinical outcome.

Neurodevelopmental outcome - Infarct presence and severity were not associated with neurodevelopmental deficit on any of the sub-scales or the GQ.

4. *Association with biomarkers*

Admission CT scan - Univariate analysis with admission CT scan features demonstrated that HCP severity was significantly associated with lumbar S100B (*p=0.05*), GFAP (*p=0.01*) and the initial concentration of combined neuromarkers (Z-Neuro Initial, *p=0.01*), where higher concentrations were observed with more severe HCP. Admission lumbar CSF cytokine concentrations were not associated with admission radiology, except for the following: IFN-γ concentrations trended towards being higher in patients with more severe HCP (*p=0.06*); ventricular TNF-α and IFN-γ concentrations were more elevated in patients with mild enhancement (*p=0.05* and *p=0.04* respectively) or no infarcts on their admission scans (*p=0.03* and *p=0.01* respectively).

Table 11.8: Association between neuromarkers and admission radiology

Admission CT	S100B		NSE		GFAP		Z-Neuro	
	LP	V	LP	V	LP	V	LP	V
HCP severity	✓	x	x	x	✓	✓	✓	x
Basal enhancement	x	x	x	x	x	x	x	x
Enhancement severity	x	x	x	x	x	x	x	x
Tuberculoma (n=3)	x	-	x	-	*p=0.06*	-	x	x
Infarcts (n=3)	x	x	x	x	x	x	x	x

This table demonstrates the association between neuromarkers (in the lumbar and ventricular CSF) and radiology features. The relationship between ventricular neuromarkers and tuberculomas could not be investigated as no patients with ventricular samples had tuberculomas. LP = lumbar CSF, V = ventricular CSF, ✓= significant association at p<0.05, x = p>0.05 – actual p-values are detailed in Appendix 13.

Radiology overall –N*euromarkers*

Lumbar CSF - Analysis with radiology features overall (admission and in-hospital follow-up imaging) demonstrated that highest GFAP in lumbar CSF samples was positively associated with the severity of HCP (*p=0.02*) and highest S100B trended towards a positive association (*p=0.06*). However, the highest neuromarker concentrations were not predictive of opening pressure (*p>0.05*). No significant associations were demonstrated with failed medical treatment or ICP deterioration. Highest GFAP was significantly associated with the presence of infarcts (*p=0.05*), whereas highest S100B (*p=0.9*) and NSE (*p=0.8*) were not. The extent of infarction as measured by their size, whether they were unilateral or bilateral, singular or multiple was significantly associated with highest concentrations of GFAP and NSE (p –values in Appendix 14). The number of vessels demonstrating pathology on MRA was positively correlated with highest S100B (*p=0.05*).The overall incidence of tuberculomas did not have a relationship with neuromarker levels, however, highest GFAP was positively associated with multiple and bilateral tuberculomas (*p=0.04* and *p=0.05* respectively). No relationship was observed between highest neuromarker concentrations and spinal disease (*p>0.05*).

Ventricular CSF - Highest ventricular CSF NSE was positively associated with bilateral infarction (*p=0.001*) and highest S100B approached significance in association with infarcts (*p=0.07)*. Highest NSE was positively associated with infarct size (*p=0.003)* and highest GFAP approached significance *(p=0.06*); highest S100B was positively correlated with the number of vessels with pathology on MRA (*p=0.04*).

Biomarker trend - ΔNSE (*p=0.03*), ΔGFAP (*p=0.01*) and Z-NeuroΔ (*p=0.02*) were positively associated with infarcts. Infarct size and number (unilateral or bilateral) were significantly associated with larger ΔNSE, ΔGFAP, Z-NeuroΔ as well as ΔS100B (p-values in Appendix 13). Larger ΔNSE trended towards significance in the relationship with vascular abnormalities recorded on MRA (*p=0.07*). ΔGFAP was negatively associated with multiple bilateral tuberculomas (*p=0.03*). No association was demonstrated between biomarker trend and spinal disease or HCP on admission.

The associations between biomarker concentrations and radiological features are tabulated in Table 11.9 with a full list of p-values is included in Appendix 13.

Table 11.9: Association between neuromarkers and overall radiology

	S100B			NSE			GFAP			Z-NeuroΔ
	Highest		Δ	Highest		Δ	Highest		Δ	
	LP	V		LP	V		LP	V		
HCP severity	*p=0.06*	x	x	x	x	x	✓	x	x	x
Infarcts	x	x	x	x	x	✓	✓	x	✓	✓
Uni/bilat	x	*p=0.07*	✓	✓	✓	*p=0.08*	✓	x	*p=0.06*	*p=0.06*
Single/multi	x	x	*p=0.08*	✓	✓	x	✓	x	x	*p=0.08*
Size	x	x	✓	✓	✓	✓	✓	*p=0.06*	✓	✓
MRA pathology	x	x	x	x	x	*p=0.07*	x	x	x	x
Tuberculoma	x	x	x	x	x	x	x	x	x	x
Uni/bilat	x	x	x	x	x	x	✓	x	✓	x
Single/multi	x	x	x	x	x	x	✓	x	✓	x
Spinal disease	x	x	x	x	x	x	x	x	x	x
Arachnoiditis	x	x	x	x	x	x	x	x	x	x
Tuberculoma	x	x	x	x	x	x	x	x	x	x

LP = lumbar CSF, V = ventricular CSF, Δ = absolute change from week 2-admission, Uni= unilateral, bilat = bilateral, multi = multiple,✓= $p<0.05$, x = $p>0.05$ – p-values detailed in Appendix 12

Inflammatory markers – parenchymal and spinal tuberculomas were positively associated with highest lumbar CSF IL-12p40 (*p=0.04*), IP-10 (*p=0.001*) and MCP-1 (*p=0.02*). Infarcts and infarct severity were positively associated with highest TNF-α, MIP-1α, IL-6 and IL-8 concentrations in lumbar and ventricular CSF. IL-12p40 was negatively associated with vascular abnormalities on MRA and MCP-1 was negatively associated with infarcts. ICP deterioration was associated with the highest concentrations of IFN-γ (*p=0.02)*, TNF-α (*p=0.03)*, MCP-1 (*p=0.05)* and IL-10 (*p=0.02)* in lumbar CSF. Z-InflamΔ was not significantly associated with any of the cranial or spinal radiology features but demonstrated a positive relationship with CXRs suggestive of TB (*p=0.03*). A summary of associations and p-values is included in Table 11.10.

Table 11.10: Association between inflammatory markers and overall radiology

	Parenchymal tuberculoma	Spinal tuberculoma	Infarcts Uni/bilateral	Infarcts Single/multiple
IL-12p40	*p=0.04*	*p=0.02*		
IP-10	*p=0.001*	*p=0.03*		
MCP-1	*p=0.02*	*p=0.05*		
TNF-α			*p=0.05* *p=0.02*	
MIP-1α			*p=0.03* *p<0.001*	*p<0.001*
IL-6			*p=0.05* *p=0.01*	
IL-8			*p=0.02*	

This table presents the significant relationships which emerged from analysis of the association between all inflammatory markers and all radiological features. Non-significant relationships are not shown due to the large number of associations between 14 cytokines (in lumbar and ventricular CSF) and multiple radiological features. Uni= unilateral, p-values in red represent ventricular CSF, p-values in black represent lumbar CSF

CHAPTER 11: THE RADIOLOGICAL FEATURES OF THE BRAIN AND SPINAL CORD IN PAEDIATRIC TBM

Discussion

This cohort of TBM patients demonstrated the typical radiology features suggestive of TBM including HCP, basal leptomeningeal enhancement, infarcts and tuberculomas. Novel descriptions of MRA and spinal pathology for the study cohort provided insight into the pathology of TBM in the cerebral vessels and the extension of disease in the spinal canal.

Radiology findings

Enhancement and exudates: As expected, enhancement involved the basal cisterns and was diffuse in most cases [45,64,114,117,158]. Pre-contrast hyperdensity appeared to be a reliable indicator of moderate-severe enhancement as suggested by other paediatric TBM studies [153], but cases with mild post-contrast enhancement may not be detected, and therefore, contrast-enhanced scans are still optimal as part of the presumptive diagnosis. This is especially important in the early phases of disease when mild enhancement likely represents the initial stages of the inflammatory response (as demonstrated by higher concentrations of ventricular pro-inflammatory cytokines TNF-α and IFN-γ) and the risk of death is low.

Infarction - Infarcts were rarely observed on the admission CT scan and DWI approximately 3 weeks after admission demonstrated that most infarcts were already more than 2 weeks old. This suggests that progression to infarction likely occurs within the first couple of weeks after admission and/or that early CT scans may not reveal the developing infarcts. It is clear in some cases, though, that a pathological process is ongoing even after the initiation of anti-TB treatment and steroids. This appears to be the case even though there is a relatively quick decrease in the inflammatory mediators. This is evident in the progression to infarction, the late development of tuberculomas, and the rise in neuromarkers in some patients. For patients with a fatal aggressive disease process, infarcts progressed rapidly to involve all 7 major vascular territories within the first 10 days, even when no infarcts were visible on the admission scan. Presumably, the consequences of the vascular pathology continue despite the decrease in inflammation. Small lacunar infarcts occurred frequently in patients who made good recoveries; however, multiple or large infarcts involving both hemispheres were prognostic of a poor clinical outcome. Infarcts developed at locations throughout the brain, including the brain stem and cerebellum; however, similar to other studies the MCA territory was most affected [49,64,114,169,178]. This largely involved its perforators into the basal ganglia, and likely reflects the

predilection of the inflammatory exudate to collect in the Sylvian fissure [21]. The predominance of vascular pathology in the MCA vessel seen on MRA mirrored the predominance of infarcts in the MCA territories suggesting that ischemia due to vasculopathy plays an important role in infarct development in TBM. However, the absence of a statistical association between MRA vessel pathology and infarction may be due to several reasons. Firstly, it is possible that vascular pathology, like vasospasm, occurred early within the first few weeks when infarction seems to have taken place, and resolved by the time of MRA. Secondly, this suggests that infarction is not only the result vasculitis, but may also have been the result of further disease processes that lead to brain tissue injury, including the contribution of increased ICP, direct damage from bacterial toxins, damage due to cytokine release of oxygen free radicals, the loss of pressure autoregulation as a result of inflammation, uncoupling of CBF and cerebral metabolism (discussed in Chapter 4).

Tuberculomas Few tuberculomas were identified on admission CT scans. This may be due to the relatively poor sensitivity of CT scans in detecting small lesions; however, lesions may also develop late, despite a drug-sensitive microbe [49,114,137,183]. Tuberculomas were spread throughout the brain, evenly distributed between the supra-tentorial and infra-tentorial compartments, and frequently occurred in the cisterns. Tuberculomas and infarcts were negatively correlated, in that tuberculomas arose more frequently in patients without infarcts, and infarcts were recorded more frequently in children without tuberculomas. Furthermore, tuberculomas were predictive of survival and infarcts were predictive of death. This suggests that the underlying pathophysiological processes in these children may have been different and possibly that processes leading to tuberculoma development may be protective against those leading to infarction. Associations with inflammatory markers revealed that elevations in IL-12p40, IP-10 and MCP-1 were significantly associated with tuberculomas (both parenchymal and spinal) but that MCP-1 was negatively associated with infarcts and IL-12p40 was negatively associated with vascular pathology on MRA. It is tempting to suggest that these cytokines may lead to the recruitment of monocytes and T-cells to the CNS which are able to limit the infection by containing it in a granuloma, but with such a limited sample size and so little information interpretation is speculation at best and further exploration would be necessary. A higher concentration of CSF polymorphonuclear cells were observed in lumbar CSF, however, these were results from admission LPs and the temporal resolution in their association with tuberculomas 3 or more weeks later is questionable. The delayed or 'paradoxical' development of tuberculomas after treatment initiation was observed in 11% of this cohort. This may be the result of a hypersensitivity reaction to bacillary debris released in response to the bactericidal effects of TB medication [24,183] or could represent an immune reconstitution response in immunosuppressed patients, of which this study enrolled very few. Unfortunately, the sample size of patients who developed delayed onset or progressive tuberculomas was too small to meaningful examine their relationship with neuro- and inflammatory markers and no conclusive comment can be made as to whether patterns in these

biomarkers could predict delayed tuberculoma development. The temporal resolution of the relationship would, however, be tenuous as biomarker measurements ceased by week 3 and delayed tuberculomas developed approximately 11 weeks after admission. Long term follow up imaging demonstrated tuberculoma resolution in all patients.

Spinal cord and spinal SAS - To our knowledge, this is the first study to prospectively describe spinal disease associated with TBM in a large, exclusively paediatric, cohort. Spinal arachnoiditis was present in over 70% of these patients and ranged from a mild coating of the cord or roots to an exudate-filled thecal sac. The vast majority of these children had no symptoms or signs pointing to spinal involvement. In many cases the radiological findings were mild; however, several cases demonstrated gross pathology, including extramedullary plaques with compression of the spinal cord and complete or near-complete obliteration of the SAS in the region of the cauda equina. There are several potential clinical implications of these findings: First, the severity of the disease has a bearing on how successful LP and/or AEG procedures are likely to be, and provides a reason for why some of these fail. Obtaining an LP result is an important part of the diagnosis and management of the condition. AEGs are important in our protocol to differentiate between CHCP and NCHCP because none of the non-dynamic tests are reliable in making this distinction [64,112]. A 'negative' result due to arachnoid lesions or exudate preventing the air from travelling up the spinal canal may be misinterpreted as NCHP and lead to an avoidable VPS.

Second, most of these patients were asymptomatic and the spinal cord pathology would not have been detected without a dedicated MR study. In our clinical experience, patients have presented on occasion with clinically significant neurological deterioration after TBM due to spinal cord involvement that was not diagnosed at the time of their TBM treatment. Although most children in this current series who had spinal pathology were asymptomatic, one patient with severe spinal arachnoiditis complained of pain and numbness in his legs. During his hospital stay this was attributed to the neuropathic side effects of ethambutol as his neurological exam was normal; however, these symptoms persisted into his 3 month follow-up. Given his MRI appearance, it is more likely that his symptoms were due to spinal cord and root involvement. Another patient with progressive spinal disease had a sensory deficit in a radicular distribution over the thorax. This study's findings of few spinal-related neurological symptoms may underestimate the true incidence of symptoms as clinical neurological examination was likely suboptimal, especially if patients were young and/or had a depressed level of consciousness, and the lack of mobility was attributed to a generally ill state.

Third, the understanding of the TBM disease process and of the treatment response (for example, cellular count changes and CSF drug levels) is often based on lumbar CSF results, with the presumption that this reflects basal cerebral subarachnoid disease. However, from the findings of the present study, it is suggested that the lumbar CSF results may as much reflect spinal cord and spinal

subarachnoid disease as they do cerebral subarachnoid disease. Additionally, there is a possibility of a relative block to CSF flow, as is well-described with spinal cord tumours in Froin's syndrome [447]. In this case, high protein values may reflect the relative absence of free flowing CSF in the rostrocaudal direction, rather than the severity of cerebral subarachnoid disease. This possibility is demonstrated clearly in the image of severe cauda equina arachnoiditis in Figure 11.12. The contribution of the spinal cord and subarachnoid inflammation and the lack of CSF flow in the cauda equina region may be part of the reason that there is little association between high protein values and outcome demonstrated in Chapter 9. Similarly, whether drug levels determined from lumbar CSF samples are reliable in the face of a large degree of exudate in the thecal sac, can be questioned. Elevated levels of lumbar CSF protein were positively associated with the severity of spinal arachnoiditis, suggesting that very elevated lumbar CSF concentrations of protein may raise the suspicion of spinal arachnoiditis when imaging is not available, as should an inability to obtain CSF from a LP.

Associations with patient and admission variables

The HCP severity score and CSF opening pressures were not significantly associated; this suggests that our interpretation of the degree of HCP is not predictive of the ICP, as was similarly demonstrated by Schoeman et al [64,448]. Factors that may contribute to this discrepancy include non-communication between the ventricular and lumbar CSF space as well as spinal arachnoiditis blocking CSF flow in the spinal canal. More importantly though, it most likely reflects the limitations of our radiological criteria in predicting ICP. This emphasizes the importance of actual ICP measurement in patient management. No association was shown between infarcts on admission and MRC staging; likely because so few children had evidence of infarction on admission CT scans. Although this may be due to the delayed appearance of infarcts and the lack of sensitivity of CT scan to detect early infarction, it is likely that neurological findings are also the result of other phenomena, including raised ICP, cerebral oedema, hyponatremia, seizures, and cranial nerve arachnoiditis. Many of these are potentially reversible, and so likely account for the early improvement in the clinical state in some patients. The severity of HCP was not significantly associated with MRC admission staging, which reflects the poor correlation between radiological criteria and ICP, but also the role of other contributors to raised ICP, including cytotoxic and vasogenic oedema, seizures, hyponatremia and the loss of pressure autoregulation in addition to HCP. Similarly, the absence of an association between seizure history and the radiological features of HCP, infarcts and tuberculomas may also be attributed to the fact that seizures may be brought on by multiple factors singly or in combination. Therefore, signs and symptoms are likely a combination of various processes occurring simultaneously rather than due to one single factor. HCP severity and infarction were not significantly associated with symptom duration. This may be influenced by the subjectivity of parental history reporting (as discussed in Chapter 9), but may also indicate that the temporal disease profile of TBM is

heterogenous across patients; aggressive disease may be associated with severe pathology rapidly, or a protracted disease course may be associated with severe disease due to the build-up of pathology over time. The heterogeneity in responses to TBM may be influenced by genetic vulnerability, and further research into the transcriptome of TBM may elucidate the underlying factors responsible for varied disease manifestations across patients Although miliary TB has been recorded more frequently in younger children suggesting a weaker immune system and a predisposition to TB dissemination, this was not observed in this cohort and may be due to the small sample size of children who had miliary patterns on their CXR. It is uncertain why older children were found to have CXRs more suggestive of TB, especially as the risk of progressing from infection to disease is higher in younger children [449]. However, a normal CXR does not exclude pTB [450] and therefore the prevalence of respiratory disease in younger children may have been underestimated.

Failed medical treatment was not associated with the severity of basal or spinal exudate; however, ICP deterioration occurred more frequently in patients with spinal arachnoiditis. It is possible that this was as a result of opening pressures appearing lower than was actually the case due to the presence of adhesions blocking the flow of CSF in the spinal canal, or the limited CSF release during LP in patients who had severe arachnoiditis. ICP deterioration was significantly associated with elevated pro-inflammatory cytokine levels in the lumbar CSF which may represent an aggressive inflammatory response contributing to or as a result of the exudate and arachnoid lesions. Analysis comparing radiology features between HIV+ and HIV- children could not be meaningfully conducted due to the small sample size of co-infected children.

Association with neuromarkers

The severity of HCP on admission was associated with elevated concentrations of GFAP, and to a lesser extent, S100B. This suggests that these 2 neuromarkers may be elevated in response to injury induced by the pressure of expanding ventricles on the ventricular ependyma and peri-ventricular white matter, often accompanied by opposing pressure from brain oedema as has been suggested in the literature [442,443]. Alternatively, elevated S100B and GFAP and severe HCP could represent a more injured brain, however, this would likely result in increased NSE concentrations as well. Elevated GFAP concentrations differentiated between patients with and without infarcts, and between patients with infarcts of varying severity. Additionally, GFAP was higher in patients with multiple tuberculomas, but the temporal profile of GFAP followed a downward trajectory in these patients. In combination, these findings suggest that GFAP is sensitive to brain damage as a result of various injurious processes and is sensitive to injury across the spectrum of severity. Elevated concentrations of S100B were associated with vascular pathology on MRA and an increasing S100B trend was significantly associated with multiple, large infarcts. NSE was not elevated in patients with small lacunar infarcts but was significantly elevated in those with large infarcts. This suggests that S100B

and NSE may be markers of more severe neurological injury. Neuromarker concentrations were not associated with spinal disease; this may be due to the fact that the brain is the predominant source of these neuromarkers and, therefore, concentrations of S100B, NSE or GFAP released in response to spinal disease may be masked by larger concentrations of cerebral origin.

Elevated concentrations of ventricular CSF IFN-γ and TNF-α were associated with mild enhancement and an absence of infarcts on admission scan. This may denote patients at the early phases of the inflammatory disease process. Patients with CXRs suggestive of TB had increasing temporal combined CSF inflammatory marker profiles. It is possible that concurrent TB infection in a location additional to the brain is associated with an augmented immune response overall, that the immune response may be more prolific in those patients who develop pulmonary disease as well as cerebral disease, or that the temporal link between pulmonary infection and dissemination was shorter in these patients than in patients who may no longer have had signs of disease in their lungs.

Limitations

Due to differences in the sensitivity of CT and MRI in detecting pathology it was not possible to directly compare CT and MRI findings. Comment or analysis on the deterioration or progression of radiology features such as the severity of enhancement or HCP from admission to a MRI in week 3 or 4 was, therefore, not possible. Additionally, the poorer sensitivity of CT relative to MRI may have underestimated the infarcts on admission. However, in resource limited settings where TBM is most likely to occur, MRI scanners are not likely to be standard of care and radiological evaluation most commonly relies on CT scanning.

The timing of the MRI scan may have influenced our findings. MRI is not standard of care at our institution in the early management of TBM, in part because of resource-limitations, but also because this requires deep sedation or general anaesthesia. The timing of MRI scanning at 3 weeks was selected to allow sufficient time for evolving disease to manifest radiologically, and to maximise the detection of pathological consequences. MRAs could not be performed in patients who died early; therefore, the MRA data is skewed to patients who survived. This is true also for the other radiological findings. Arguably, more gross pathology may have been detected in the patients who died early. Data on the condition of cerebral vessels of patients undergoing fatally aggressive disease processes may have added greater insight to the link between infarction and vessel pathology. This is a group about which we require more information to inform possible interventions to interrupt this aggressive course. Similarly, data on spinal pathology was limited to patients who survived and may not represent the most severe form of TBM disease. However, these data still demonstrate the high frequency of spinal and MRA pathology and future studies involving imaging at earlier time intervals after admission may add further insight into the evolution of these disease processes.

Serum and CSF sampling was limited to the early weeks after hospitalisation; however, sampling of patients who returned with delayed tuberculoma development would have added insight into the relationship between these lesions and neuro- and inflammatory markers, and contributed to the understanding of the immunology or tissue injury surrounding this phenomenon of 'paradoxical' tuberculomas.

The p-values of associations reported for radiology and clinical characteristics, outcome and neuromarkers were not corrected for multiple testing which would have substantially reduced the number of significant associations. However, this was an exploratory investigation which aimed largely to build pilot data on the association between biomarkers and radiology and develop hypotheses for future formal testing.

CHAPTER 12 SUMMARY

Monitoring brain oxygenation in TBM

This chapter aimed to examine the potential utility of brain oxygenation monitors in detecting compromised brain oxygenation and its response to interventions.

Data obtained from invasive PbtO2 catheters and non-invasive NIRS monitors were descriptively analysed in terms of their temporal profile and their relationship with ICP, neuromarkers and outcome. The level of agreement between PbtO2 and NIRS data was assessed.

Only a few patients were considered candidates for invasive monitoring with PbtO2, consequently data analysis was limited and is largely descriptive. PbtO2 monitoring was only performed in patients considered to be at high risk for ischaemia based on clinical or radiological findings; this likely introduced bias in patient selection. NIRS was more widely used as it is non-invasive; however, maintaining good sensor-scalp contact in mobile children is challenging and NIRS monitoring could not be performed in all patients; this too would have introduced some bias in patient selection.

The main findings were as follows:

1. PbtO2 and NIRS demonstrated that brain oxygenation is compromised in a sub-set of patients with TBM and episodes of compromise can be detected in real time
2. Brain oxygenation as measured by PbtO2 was very sensitive to fluctuations in ICP, even within the normal range, presumably because of the already narrowed cerebral perfusion pressure in these patients. This suggests that a lower ICP threshold and continuous ICP control may be indicated to maximise perfusion of the injured brain.
3. NIRS values below 50% were significantly associated with poor outcome, suggesting that this may be considered a threshold of compromised brain oxygenation for NIRS monitoring in TBM. However, it is likely that some inter-individual variability exists so the trend in oxygenation and its response to interventions may be as important to consider.
4. Case illustrations demonstrated that PbtO2 and NIRS were sensitive to changes in brain oxygenation in response to interventions aimed at improving brain oxygenation. This suggests that there is potential to treat impaired brain oxygenation in TBM.
5. There was a reasonable level of agreement between PbtO2 and NIRS data and the two monitors demonstrated similar temporal profiles.
6. Neuromarkers were not associated with indices of brain oxygenation as measured by PbtO2 and NIRS. The absence of an association may be due to the small sample, missing early

episodes of oxygenation, the multifactorial nature of outcome determinants, or the fact that episodes of poor oxygenation (in the case of PbtO2) were treated.

CHAPTER 12: MONITORING BRAIN OXYGENATION IN TBM

Methods

Ischemia plays a pivotal role in the pathophysiology and poor outcome of TBM. It is precipitated by various factors, most importantly raised ICP and vascular pathology. However, at present there are no means to reliably diagnose or monitor ischaemia in these patients. Clinical examination does not reveal the aetiology of a depressed level of consciousness and neurological deficits. Repeat brain imaging illustrates the evolving nature of ischemia and brain infarction, but only once the damage is already irreversible. Recently, technological advances have enabled aspects of brain oxygenation and perfusion to be monitored in real-time. All of the available methods have limitations, but may offer some benefit in guiding treatment in patients at risk of ischaemia, although further research is required before monitoring is adopted widely.

As part of this study, data from brain tissue oxygen tension (PbtO2) and near-infrared spectroscopy (NIRS) monitoring in this TBM cohort were gathered. PbtO2 is used routinely at RXH for severe traumatic brain injury, and more recently it has also been used for patients with severe TBM [236]. In general, these monitors are indicated for patients with a severely depressed level of consciousness who are usually ventilated in the ICU. The invasive nature of these monitors limits their use to the most severely ill patients. Often patients at risk of ischaemia initially appear to be mildly affected, but develop infarction later. Consequently, by the time the monitor is inserted, it may be too late to intervene. Therefore, NIRS was investigated as a potential non-invasive monitor to detect compromised brain oxygenation in TBM. NIRS has been examined in several conditions but as yet not in TBM [211,245,246,248,251].

Because the use of PbtO2 monitors is uncommon, we anticipated few patients and so the analysis was planned to be largely descriptive.

Aim

The study aimed to

1. Describe the profile of PbtO2 and its relationship with ICP and outcome in a cohort of paediatric TBM patients with HCP
2. Describe the profile of NIRS and its association with outcome in a cohort of paediatric TBM patients with HCP
3. Compare NIRS and PbtO2 data in patients who had both monitors
4. Illustrate the potential utility of brain oxygenation monitoring in TBM

5. Examine the relationship between PbtO2 and NIRS data and neuromarker concentrations

Invasive monitoring

Monitors

The EVD is considered the gold standard for measuring ICP. It is an inexpensive, accurate and reliable method of monitoring ICP [451]. It is inserted through a burr hole in the skull and guided into the lateral ventricle. PbtO2 catheters (Licox, Integra Neurosciences) were considered for patients with Stage III TBM with a low level of consciousness, particularly if ICU admission and ventilation was required. PbtO2 catheters were inserted via a bolt to ensure correct positioning, except when the skull was deemed too thin in young patients. In these cases the catheter was tunnelled under skin and secured with sutures, no problems with the tunnelled catheters were experienced. The catheter was targeted for frontal white matter 2.5 cm below skin and approximately 2cm from the EVD. Appropriate placement in white matter was confirmed on follow-up CT brain scans. Informed consent was obtained from all parents before insertion of intracranial monitors. Monitors were removed when the patient was considered stable for > 48 hours or when the EVD was replaced with a VPS.

Patient management

The EVD was usually placed to drain at a height of 15 cmH_2O/ 11mmHg above the external auditory meatus. In patients who had low PbtO2, and in very young patients, the height of the EVD was reduced. For monitoring, the EVD was clamped and the pressure transduced to the bedside monitor display. PbtO2 values <20mmHg were considered compromised brain oxygenation [198,212,230]. Low PbtO2 was managed by lowering ICP further, and optimising MAP, systemic oxygenation and ventilation settings. In general, interventions were tested against the PbtO2 response.

Data Collection

Data were collected using a computerised physiologic recording system, ICMPlus® (University of Cambridge, U.K) which samples data at 50 Hz and provides a data point averaged over 10 seconds. Variables recorded included MAP, ICP, PbtO2 and systemic saturation where possible. Data on partial pressure of arterial oxygen (PaO2) were collected from arterial blood gases (ABGs) and hourly ventilator inspired fraction of oxygen (FiO2) from ICU charts.

Non-invasive monitors

Near-infrared spectroscopy (NIRS) sensors (left and right) were placed on the forehead of patients admitted to the ICU as well as group of patients admitted to the medical wards. Since we aimed to collect pilot data on NIRS a quota of NIRS sensors were assigned to the study and consecutive

patients were monitored until the study's quota finished. Data were collected continuously by the monitor at a frequency of 1 data point every 30 seconds.

NIRS monitors were used for research purposes and did not direct patient management. Nasal prong oxygen was encouraged for all TBM children and was used at the discretion of the treating clinician.

Data Analysis

The profile of PbtO2 and relationship with ICP and outcome

Descriptive statistics - PbtO2 catheters were allowed to settle for 2 hours, and data during this time were excluded from analysis to avoid using potentially artefactual data from a stabilising catheter. Data from catheters that were poorly placed were excluded. Descriptive statistics were calculated for PbtO2 as well as for ICP, MAP, CPP (calculated as MAP-ICP), PaO2, inspired fraction of oxygen (FiO2) and systemic saturation (SaO2). Hourly data were used. These physiological variables were only available for patients managed in the ICU where continuous monitoring occurs. The full complement of data was not available for all patients as this was contingent upon the availability of monitors and recording computers. Patients managed in the Neurosurgical High care ward received neurological observations twice daily.

Relationship between PbtO2 and ICP - The relationship between these 2 variables was examined by plotting the temporal profile of PbtO2 and ICP for each patient who had complete data for both variables using hourly data, and a spearman's correlation was performed. Electronic data were examined for case illustrations of the nature of the relationship between ICP and PbtO2 in TBM.

Outcome description - Due to the very small sample size (n=6) statistical analysis with outcome could not be performed; therefore, PbtO2 data for the two outcome groups were examined descriptively and included the lowest PbtO2 recorded for the duration of monitoring, the median PbtO2 ($PbtO2_{24}$) over the first 24 hours of monitoring (or part thereof) and the time spent below PbtO2 thresholds < 5, 10 and 20mmHg. To maximise the representation of PbtO2 less than critical thresholds, high frequency data were interrogated. Clinical outcome at 6 months (poor outcome [PCSP 4-6: severe disability- death] vs good outcome [PCPS 1-3: normal – mild/moderate disability]) was used as the outcome measure.

The profile of NIRS and relationship with outcome

Descriptive statistics and the temporal profile of NIRS data were calculated for the full cohort of patients monitored with NIRS for the left (L) and right (R) sensors. Data is recorded at a frequency of 1 data point per 30 seconds. Descriptive statistics were calculated for the L and R sensors and for the combined average of L and R for the entire recording period. Treatment thresholds for NIRS have not

been established, but normal ranges are thought to be between 60-80% [238,242,243] and animal research has shown NIRS saturation (SO2) <35% to be associated with irreversible neurological deficits [239]. Therefore, as part of exploratory analysis to assess the association between compromised NIRS and outcome, the amount of time spent below various thresholds was calculated. These thresholds included time <40% and <50%, as well as time spent less than the median SO2 for the monitoring period. As monitoring continued for up to 10 days, the median was considered a more representative baseline for the recoding period as a whole than initial recordings at the start of monitoring, and therefore deviation from this median was assessed. Time spent below the specified thresholds was calculated for each patient and examined in association with mortality and clinical outcome at 6 months. NIRS data were also examined for illustrations of its utility as a monitor of ischemia and of the response in brain oxygenation to intervention.

NIRS and PbtO2 monitors

Data collected simultaneously from patients with both PbtO2 and NIRS catheters were combined such that data points were time-linked. PbtO2 is measured in mmHg and normally ranges from 20-40 mmHg; NIRS is measured as percentage saturation and usually ranges between 50 and 100%. To accommodate for the difference in the distribution of the 2 data sets, PbtO2 and NIRS data were converted into standard scores: this was done for the PbtO2 and NIRS data sets separately and involved subtracting each score from the overall cohort mean and dividing by the cohort standard deviation, such that the mean became 0 and the standard deviation became 1. To examine whether NIRS data reflected PbtO2 data the standard scores of the 2 monitors were compared using the Bland-Altman plot which enabled the degree of agreement between PbtO2 and NIRS data to be calculated by plotting the mean difference between PbtO2 and NIRS scores against the mean of both data sets combined [452]. Two lines of agreement were plotted at the level of 2 standard deviations above and below the mean difference value as indicators of the extent to which scores vary about the mean difference and therefore the extent to which PbtO2 and NIRS data differ from each other. Variation within two standard deviations suggests adequate agreement between the two brain oxygenation monitors. The ideal mean difference would be 0. The temporal profiles of the PbtO2 and NIRS data were examined by constructing box-and-whisker plots to ascertain whether the 2 monitors reflected a similar trend in brain oxygenation. Finally, case illustrations were identified which reflected how PbtO2 and NIRS responded to fluctuations in ICP and MAP and in response to interventions.

The association between neuromarkers and brain oxygenation monitors (PbtO2 and NIRS)

The relationships between neuromarkers and data from PbtO2 and NIRS were examined as follows: the admission S100B, NSE and GFAP concentrations were analysed in association with the first 24 hours of PbtO2 and NIRS monitoring; the change (Δ) in S100B, NSE and GFAP were examined in

association with the time spent <5, 10, 20mmHg for PbtO2, and <40%, <50% and less than the median for the monitoring period for NIRS. Spearman's correlation was used for this analysis.

CHAPTER 12: MONITORING BRAIN OXYGENATION IN TBM

Results

The profile of PbtO2 and relationship with ICP and outcome

Descriptives - Six patients were monitored with PbtO2 catheters; 5 were admitted to ICU where continuous physiological monitoring took place, and 1 patient was admitted to the Neurosurgical High Care ward. All 6 patients had an EVD; the transduced EVD signal for ICP could be exported into the monitoring computer for only 3 of the 5 patients in ICU due to technical reasons. Continuous MAP, hourly SaO2 and inspired fraction of oxygen FiO2, and partial pressure of oxygen (PaO2) from routine arterial blood gases were recorded in 5 ICU patients. The median time of PbtO2 monitoring for all patients was 5.5 (4-6) days. A summary of descriptive statistics for these variables calculated from hourly data is provided in Table 12.1.

Table 12.1: Summary of monitored physiological variables for the duration of monitoring

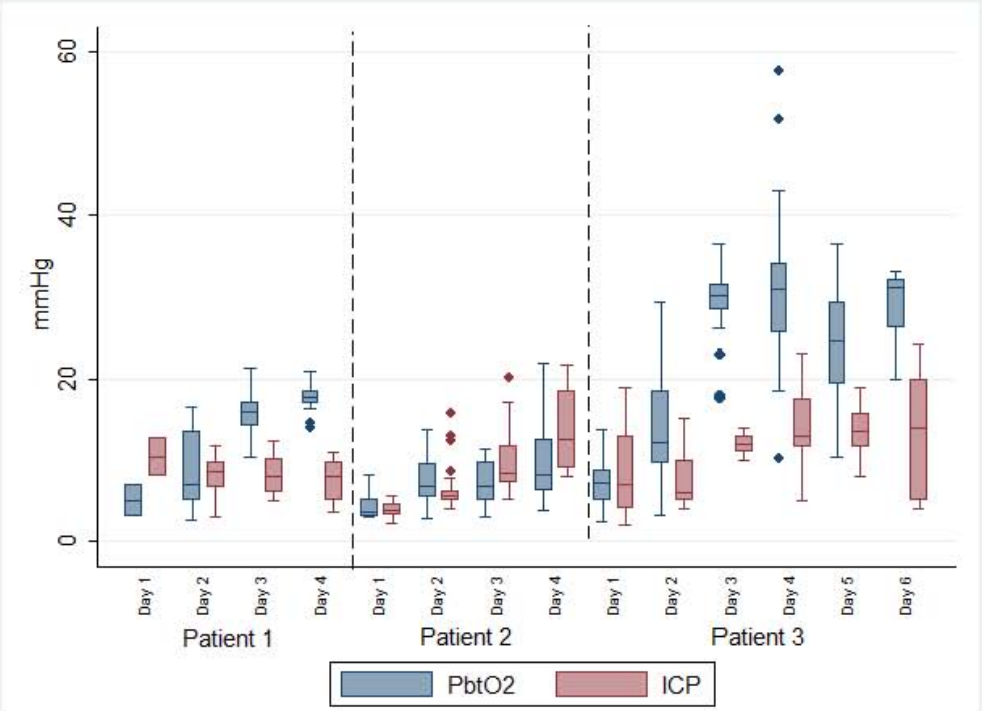
Statistic	PbtO2 mmHg	ICP mmHg	MAP mmHg	CPP mmHg	SaO2 percent	PaO2 kPa	FiO2 percent
Median	20.6	9.15	83.55	71.74	100	23.7	40
Minimum	2.48	2	56.71	48.71	88.66	8.9	21
Maximum	97.69	24	126.2	120.3	100	64	100
P25	12.1	6	74.09	63.73	98.5	15.5	30
P75	30.94	12.64	88.97	76.68	100	30.25	60

This table represents descriptive statistics for physiological variables recorded during PbtO2 monitoring in ICU patients. Statistics have been calculated on hourly data. P25 = 25th percentile, P75 = 75th percentile

Relationship between PbtO2 and ICP – The temporal profile for ICP and PbtO2 for the 3 patients with both monitors are displayed in Figure 12.1. In patient 1 PbtO2 increased and ICP decreased over time; in Patient 2 the ICP increased as did the PbtO2, although to a lesser degree; and in Patient 3 both ICP and PbtO2 increased with a larger increase observed in PbtO2.

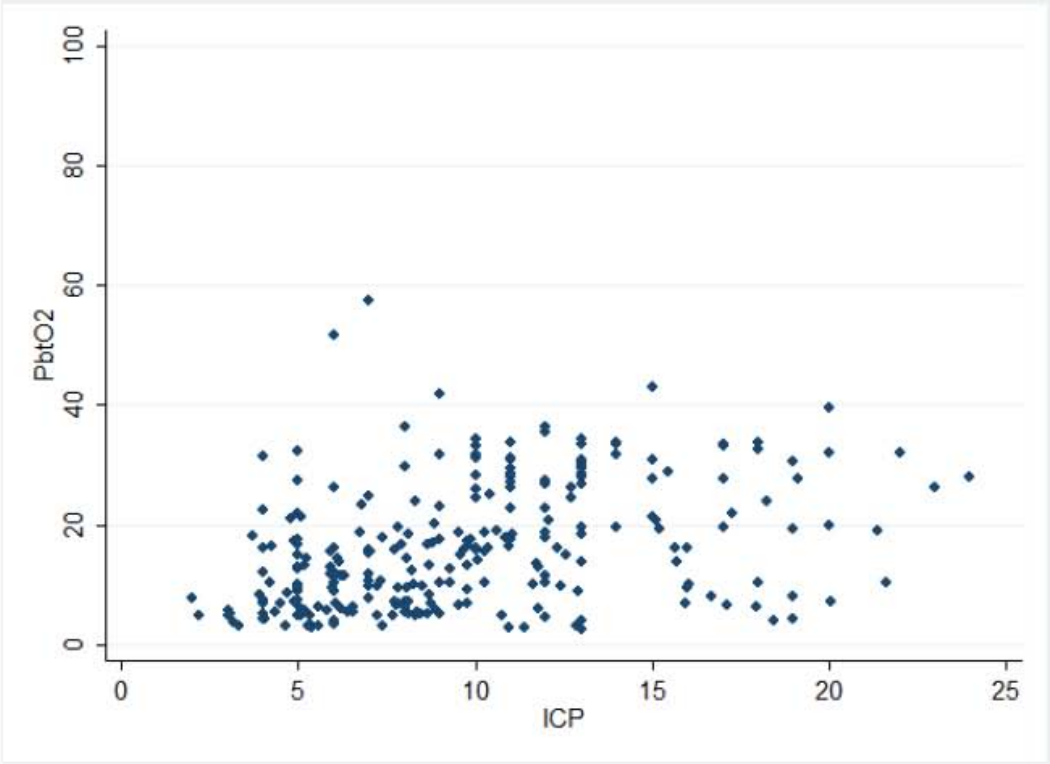
A spearman's correlation between ICP and PbtO2 demonstrated a positive relationship (r=0.44, $p<0.001$) and the scatterplot is included in Figure 12.2.

Figure 12.1: Temporal profile of PbtO2 and ICP



This figure illustrates the temporal profile of 3 patients who had both ICP and PbtO2 monitoring using box-and-whisker plots; boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile. ICP and PbtO2 demonstrated variable relationships across the 3 patients

Figure 12.2: Scatterplot: ICP and PbtO2



The scatterplot demonstrates a positive relationship between ICP and PbtO2 (both measures in mmHg)

Case illustrations for the relationship between ICP and PbtO2 extracted from high frequency data recordings:

Figure 12.3: In this illustration the EVD was clamped in preparation for a VPS and the ICP increased; this was followed by an immediate decline in PbtO2. After the EVD was unclamped and ICP decreased the PbtO2 recovered.

Figure 12.3: PbtO2 in response to increasing ICP

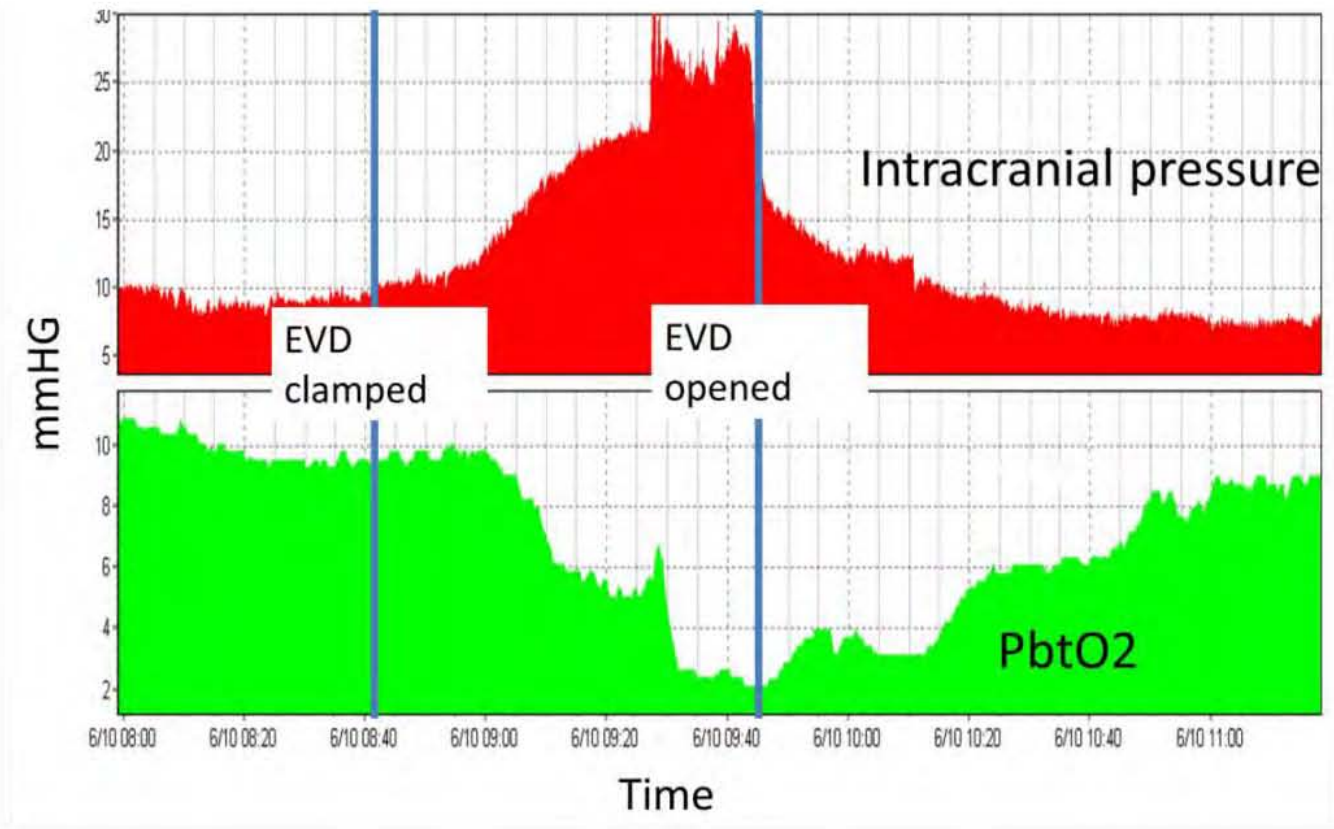
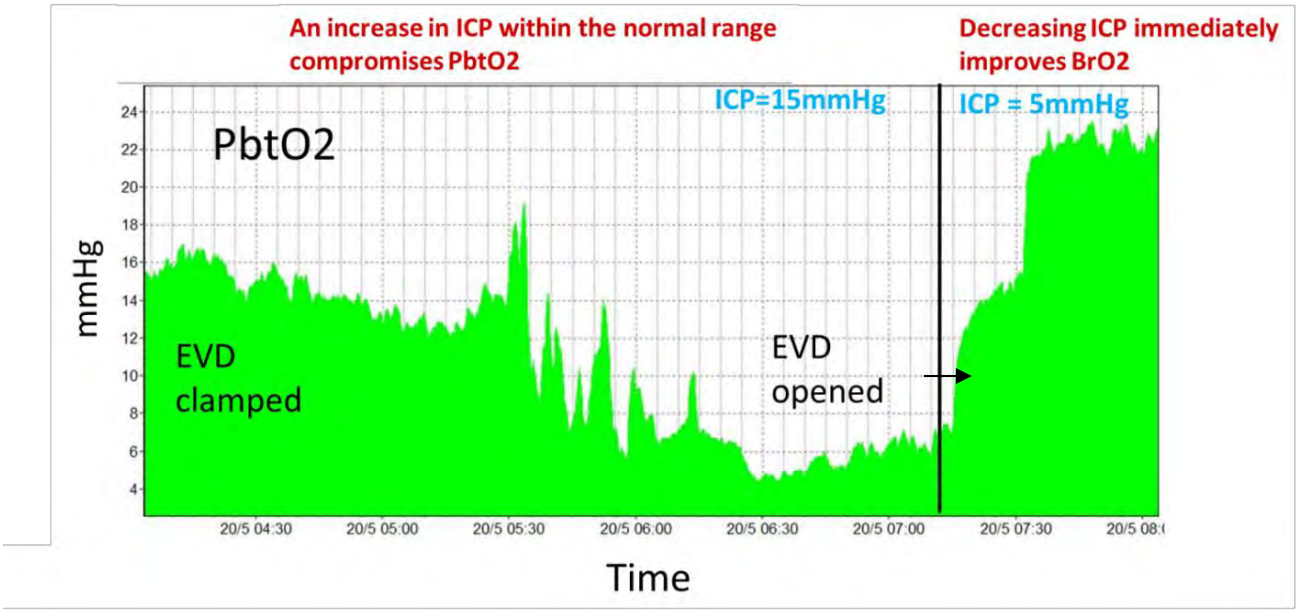


Figure 12.4: The commonly used treatment threshold for ICP in conditions such as traumatic brain injury is 20mmHg/ 27cmH20. Differential thresholds for children are not reliably described and so 20mmHg remains most commonly used [228]. This recording suggests that in patients who already have a compromised baseline perfusion, most likely due to associated vasculitis, fluctuations of ICP even within the normal range may aggravate the poor tissue perfusion, presumably by narrowing an already reduced cerebral perfusion pressure. Unfortunately the electronic ICP trace was not available for this patient.

Figure 12.4: Fluctuations of ICP within the normal range may negatively impact on PbtO2



PbtO2 and outcome – Of the 6 patients who underwent PbtO2 monitoring, 3 had good outcomes (2 patients made a full clinical recovery with a PCPS score of 1, and 1 patient had mild disability with a PCPS score of 2), and 3 patients had poor outcomes (1 patient was severely disabled with a PCPS of 4 and 2 were vegetative with a PCPS score of 5). The poor outcome group had the lowest recorded PbtO2 as well as lower median PbtO2 values within the first 24 hours of monitoring than patients with a good outcome, and spent more time below critical PbtO2 thresholds (<5, <10, <20 mmHg), even though the duration of monitoring was less. These findings are demonstrated in Figure 12.5 and Figure 12.6 and tabulated in Appendix 15. princip

Figure 12.5: Median $PbtO2_{24}$ and the lowest PbtO2 for poor and good outcome groups

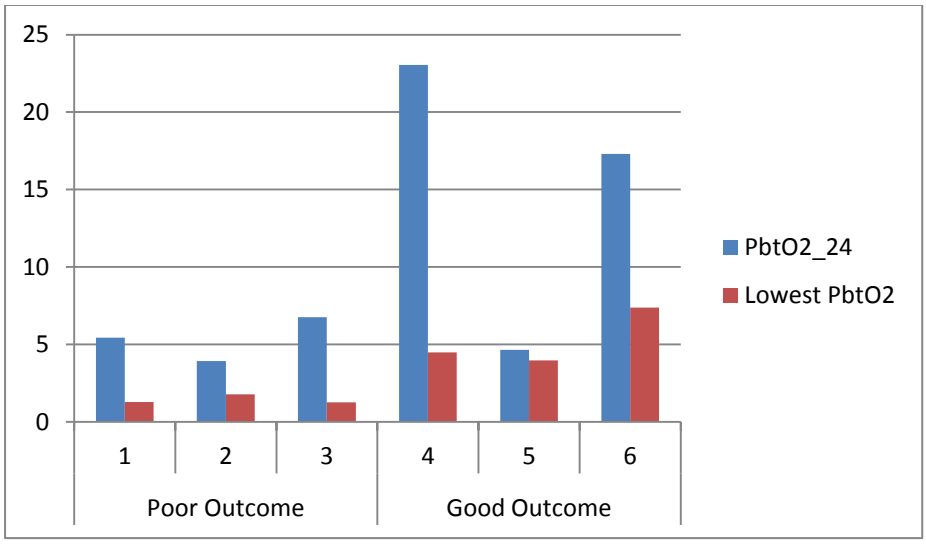


Figure 12.5 demonstrates the median PbtO2 recorded within the first 24 hours (PbtO2_24) and the lowest PbtO2 recorded for the duration of monitoring in 6 patients with poor and good outcomes. PbtO2 represented in mmHg

Figure 12.6: Time spent below critical thresholds of PbtO2

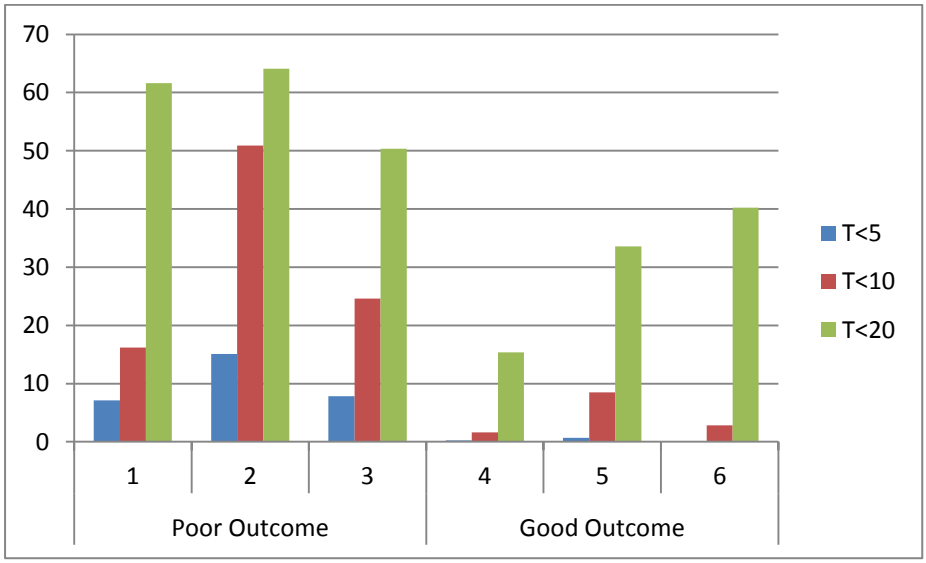


Figure 12.6 demonstrates the number of hours spent with PbtO2 less than critical thresholds (5, 10, 20 mmHg) for 6 patients with poor and good outcomes

The profile of NIRS and relationship with outcome

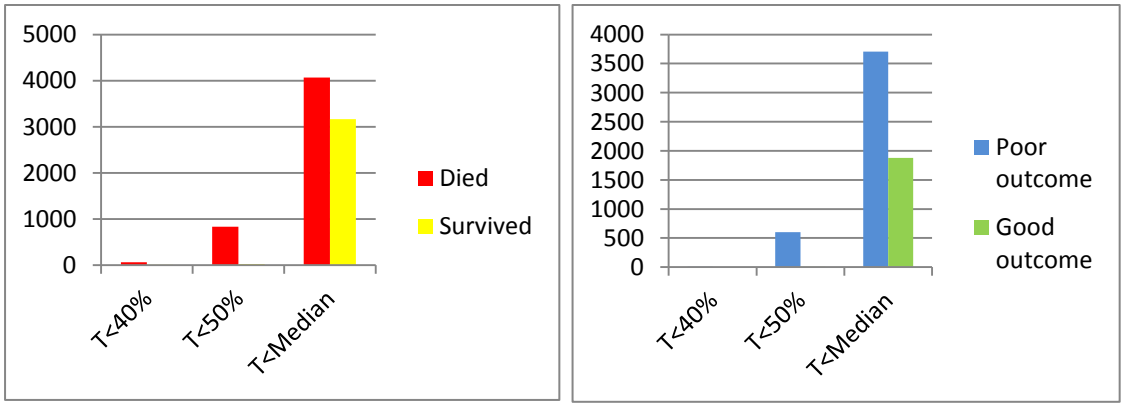
Twenty-one patients were monitored with NIRS, the median number of days of monitoring was 7 (3-11). The median SO2 was 67% (58-84) for the left sensor, 67% (58-90) for the right sensor, and 66% (57.5-86) for the combined L and R. The L and R sensors were not found to be statistically different overall and therefore outcome analysis was conducted on data for the 2 sides combined. The time spent <50% was significantly associated with death and poor outcome (*p=0.02* and *p=0.002* respectively), and time spent less than the overall median was significantly greater in patients who had a poor clinical outcome than in patients with a good outcome (*p=0.01*). Time spent <40% was not significantly associated with outcome, likely because of the small number of data points falling below 40%. These associations with outcome are detailed in Table 12.2.

Table 12.2: NIRS association with outcome

NIRS threshold	Deceased N=5	Survived N=16		Poor outcome N=10	Good outcome N=11	
<40%			*p=0.19*			*p=0.1*
Median	62.5	0.25		4	0	
P25	0	0		0	0	
P75	508	4.5		62.5	1	
< 50%			*p=0.02*			*p=0.002*
Median	832.5	23.25		602.5	8.5	
P25	745.5	3.5		44.5	1	
P75	1171	141.5		1171	21	
Median overall			*p=0.12*			*p=0.01*
Median	4066	3162		3970.75	2850	
P25	3774.5	2501.5		3703	1878.5	
P75	5291.5	3875.5		4121.5	3300.5	

Data are presented as the number of minutes spent below NIRS thresholds

Figure 12.7: Time spent below critical thresholds of NIRS for mortality and clinical outcome



Case illustrations of NIRS recordings in TBM patients:

Figure 12.8 depicts the last 12 hours in a patient who died by day 4 post admission. The NIRS trace demonstrated a marked decline from SO2 >60% to below 40% and did not recover.

Figure 12.8: NIRS recording showing declining brain oxygenation in the hours before death in one patient

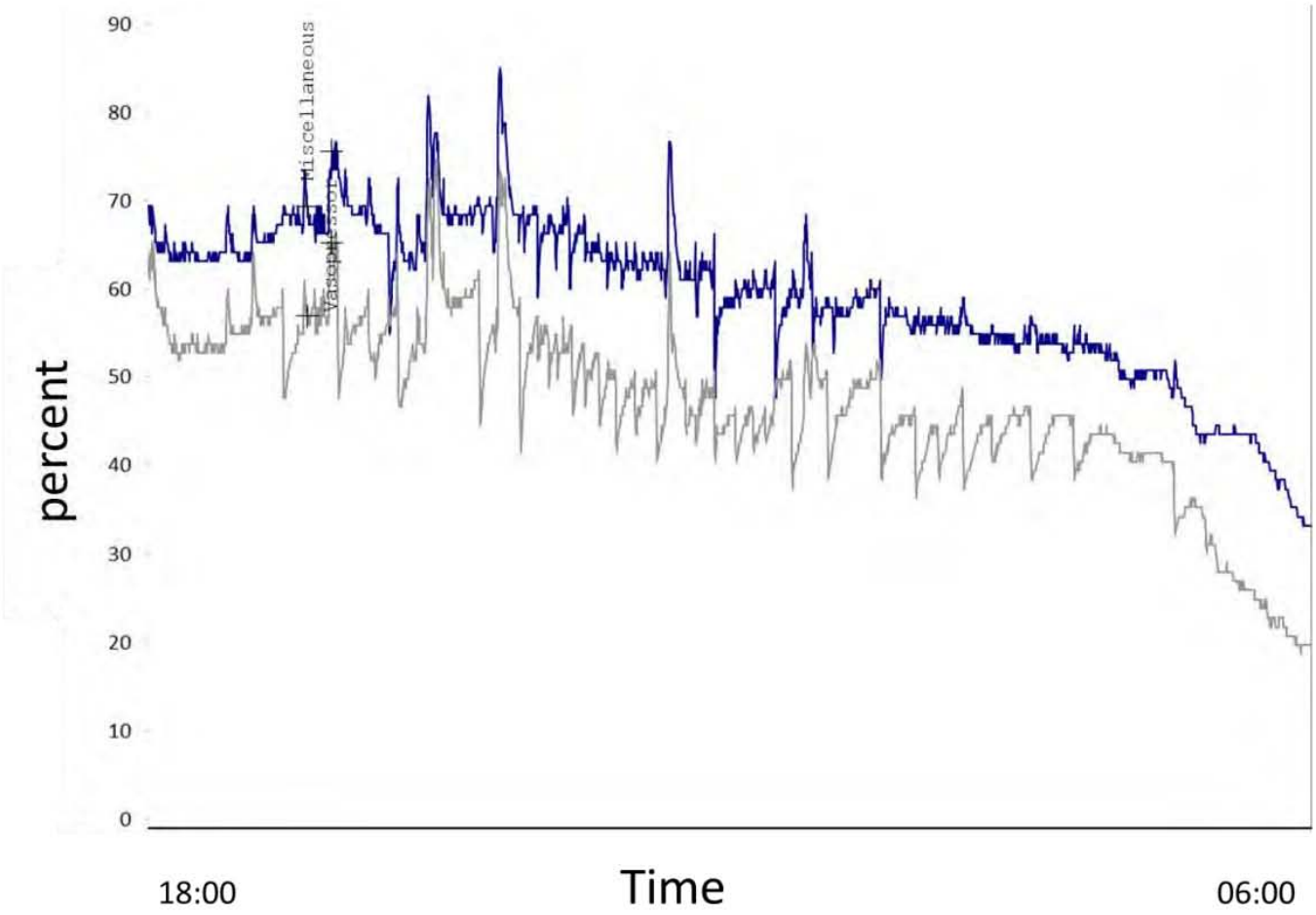


Figure 12.9 demonstrates an increase in the baseline of NIRS after an LP was performed in a patient with CHCP, and likely represents the improvement in CBF following the reduction in ICP. The patient underwent no other interventions during this time apart from the LP.

Figure 12.9: NIRS improves after a lumbar puncture to treat raised ICP

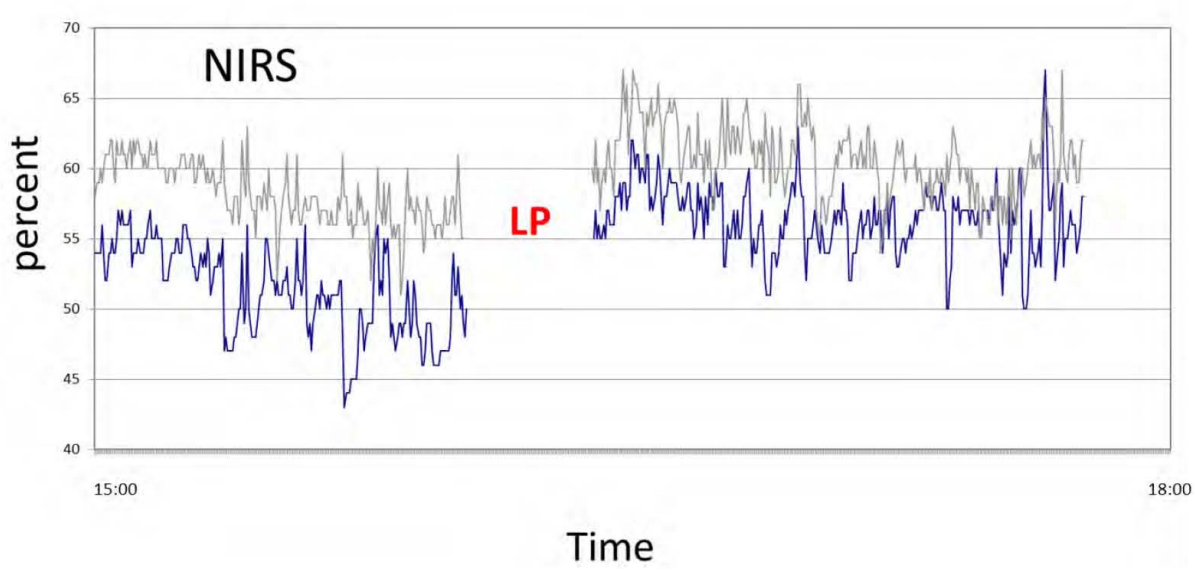


Figure 12.10 illustrates the improvement in the NIRS trace in a patient during the early hours of resuscitation during which MAP, haemoglobin and fluid status were optimised and electrolyte disturbances were corrected, suggesting an improvement in CBF and brain oxygenation.

Figure 12.10: NIRS improves with resuscitation

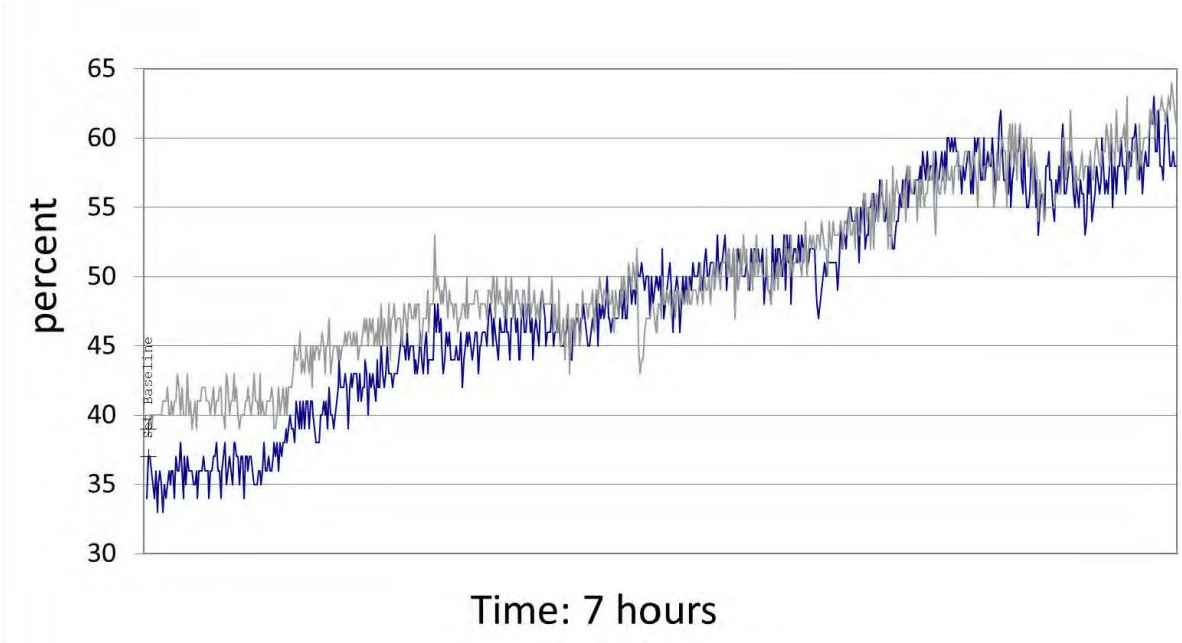
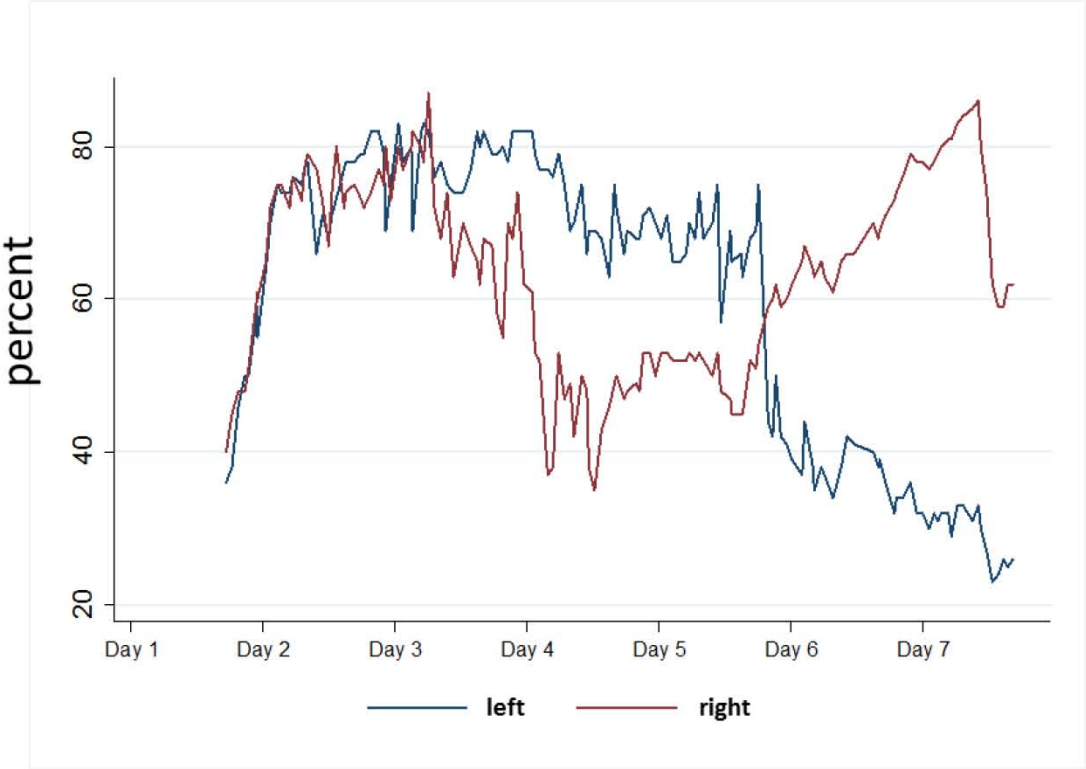
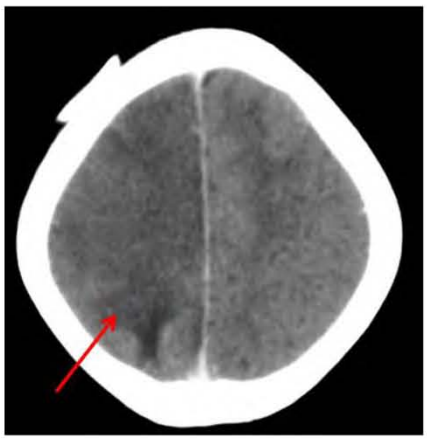


Figure 12.11 illustrates the NIRS trace in a patient who suffered an aggressive disease process and died by day 10. The drop in the right trace on day 3 likely represents an ischaemic process which progressed to infarction in the right hemisphere as indicated on the CT on that same day (image included below). The reversal of the right and left trends seen on day 5 may have signified luxury perfusion in the infarcted right hemisphere and a progressive ischemic response in the left hemisphere indicated by the decline in the left NIRS trace. This pattern continued until day 7 when treatment was ceased and the patient died soon after. There were no problems with NIRS sensor contact in this patient

Figure 12.11: NIRS responds to ischaemia and luxury perfusion



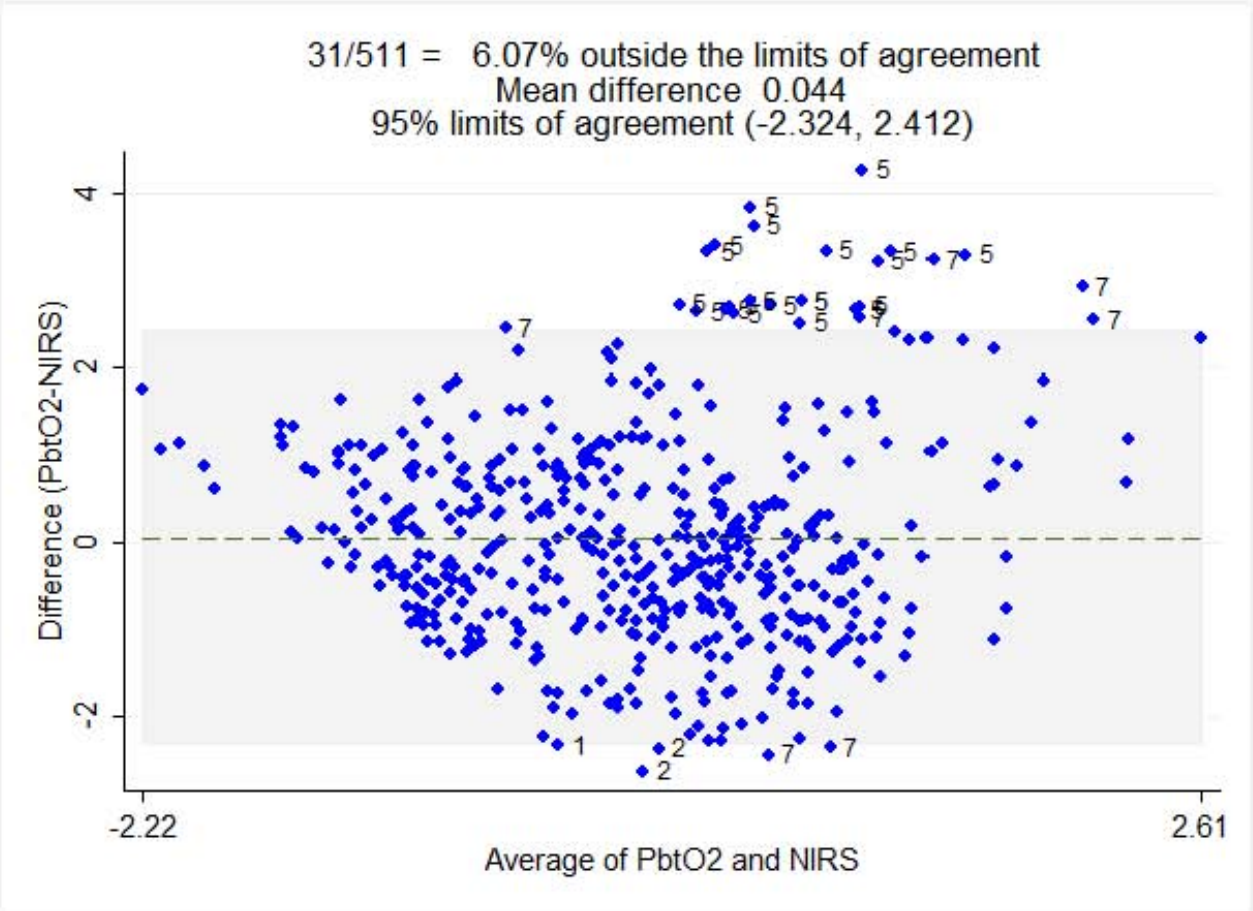


More severe infarction in right hemisphere

NIRS and PbtO2 monitors

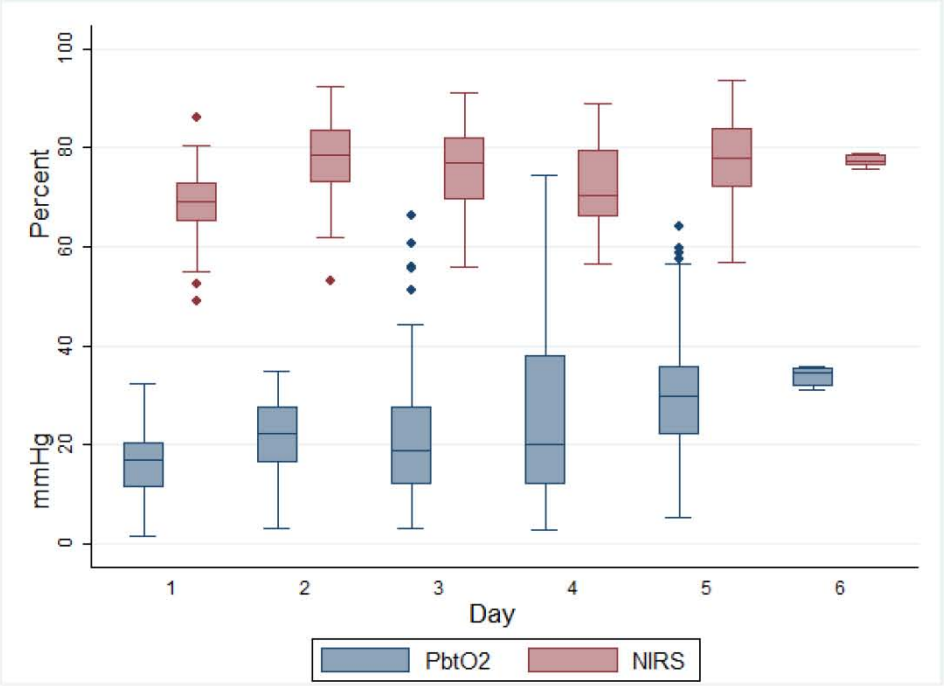
The Bland-Altman plot (Figure 12.12) of standardised NIRS and PbtO2 scores demonstrated that the mean difference between these 2 brain oxygenation monitors was 0.024 (ideal is 0) and 6.07 % of the data points fell outside the limits of agreement; these data points mostly belonged to patients 5 and 7 who both had increasing PbtO2 and NIRS over the duration of monitoring. A proportional increase in the data suggests that another factor is playing a role in the relationship between the PbtO2 and NIRS. This factor was likely time, as both variables increased with time after admission. This increasing temporal profile is depicted in Figure 12.13 and 12.14, which illustrate that PbtO2 and NIRS trends follow similar patterns across the duration of monitoring, although the variability of PbtO2 within patients was greater.

Figure 12.12: Bland-Altman plot for PbtO2 and NIRS standardised scores



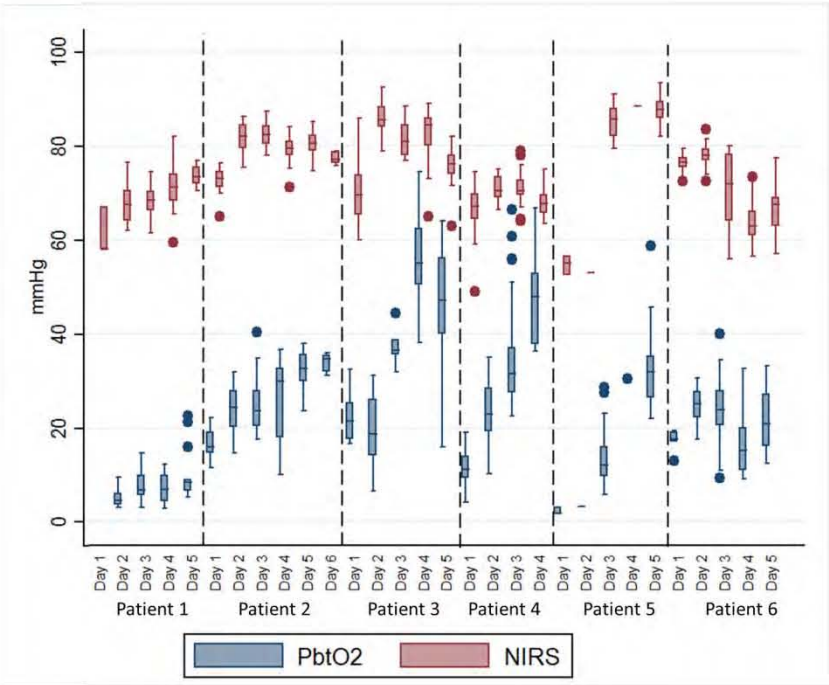
This figure depicts the Bland-Altman plot testing the levels of agreement between PbtO2 and NIRS data in all patients who received both monitors

Figure 12.13: Temporal profile of PbtO2 and NIRS for the monitored cohort overall



This figure illustrates the temporal profile of PbtO2 and NIRS over the duration of monitoring for the cohort of 6 patients, indicated by box-and-whisker plots: boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile. Both monitors demonstrated an increasing trend over time.

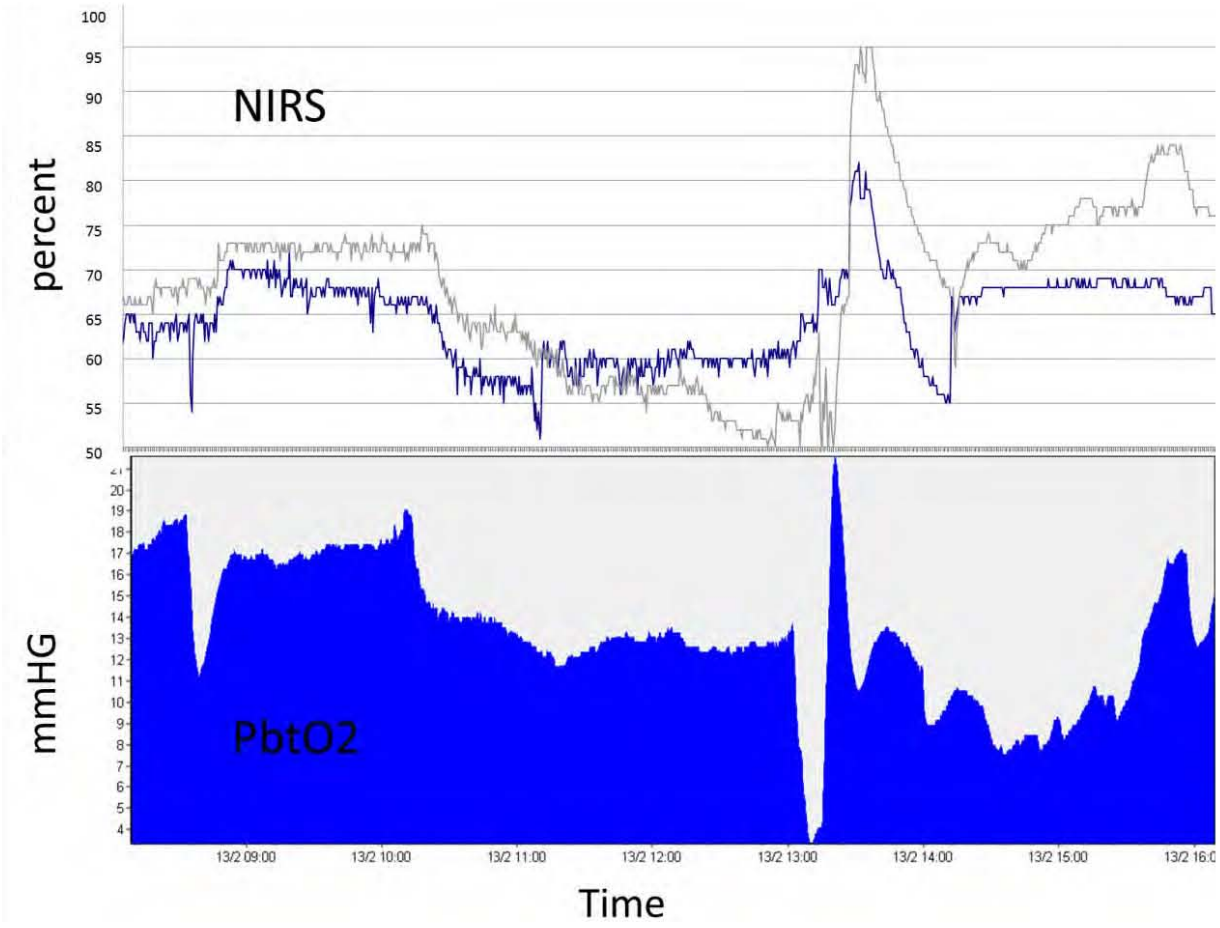
Figure 12.4: Temporal profile of PbtO2 and NIRS per patient



This figure demonstrates the temporal profile of PbtO2 and NIRS over the duration of monitoring for each individual patient who had both PbtO2 and NIRS monitoring. Box-and-whisker plots are used; boxes demonstrate median and inter-quartile range, whiskers denote 5th and 95th percentile. Both monitors followed a similar trend in individual patients.

The similarity in the patterns of PbtO2 and NIRS were also observed within individual patients at selected time points. Figure 12.15 demonstrates how the trend in PbtO2 was mirrored by the trend in NIRS over a 7 hour time period.

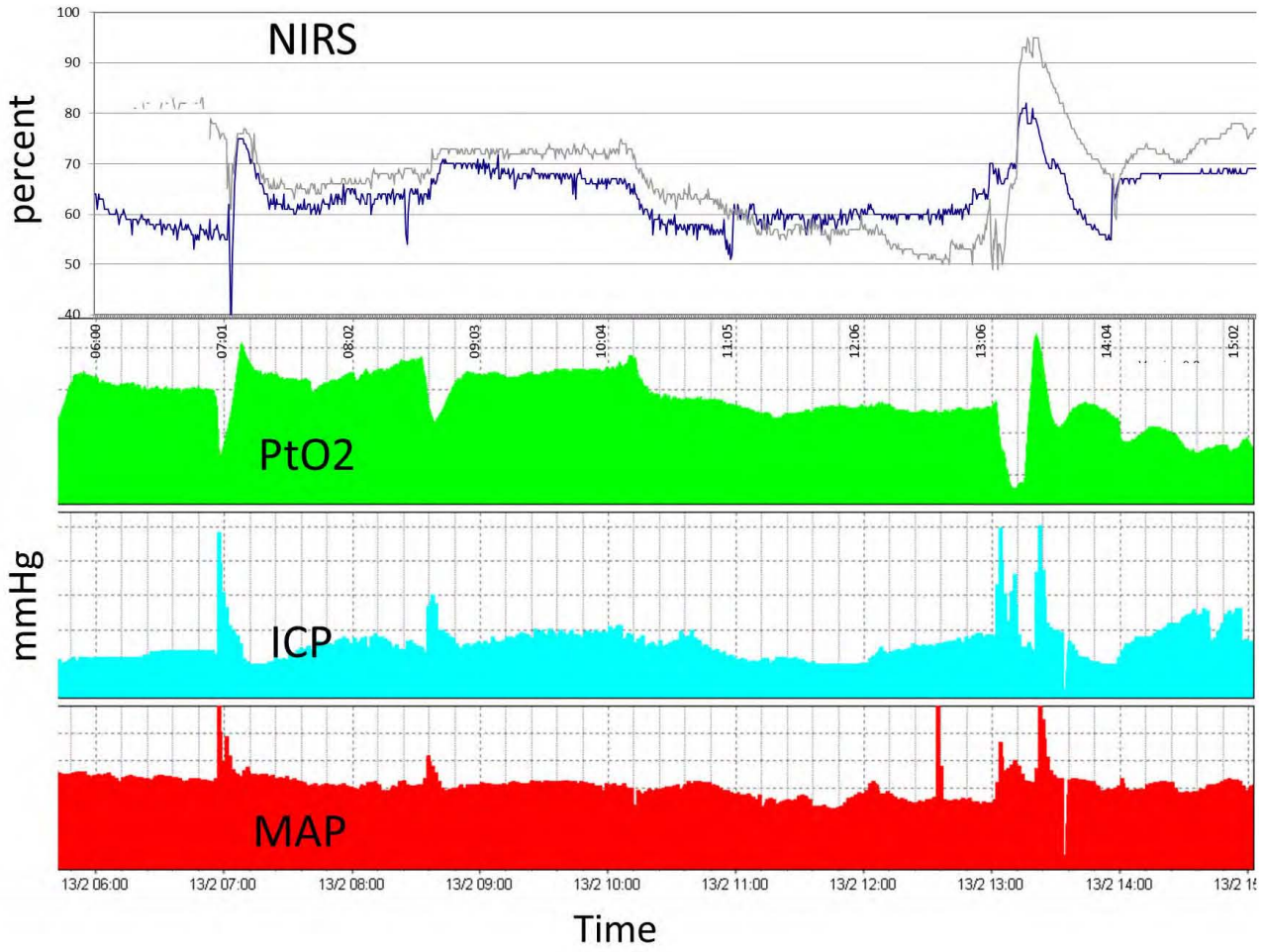
Figure 12.15: NIRS mirrors the trend in PbtO2



This figure illustrates the parallel trends in PbtO2 and NIRS (left in blue and right in grey) over a 7 hour period. Data extracted from high frequency recording of PbtO2 and NIRS.

Figure 12.16 illustrates how the 2 monitors followed a similar pattern of response to the dynamic relationship with other physiological variables which may influence brain oxygenation. Increases in MAP and ICP, likely due to suctioning or patient handling, resulted in immediate declines in both PbtO2 and NIRS.

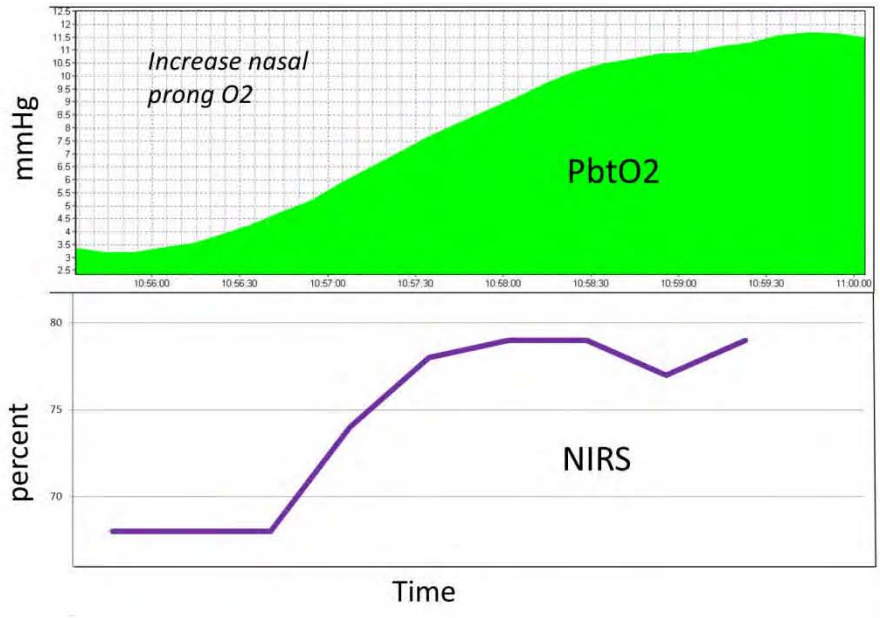
Figure 12.16: PbtO2 and NIRS in response to ICP and MAP



This figure illustrates the parallel response in PbtO2 and NIRS (left in blue, right in grey) to increases in ICP and MAP.

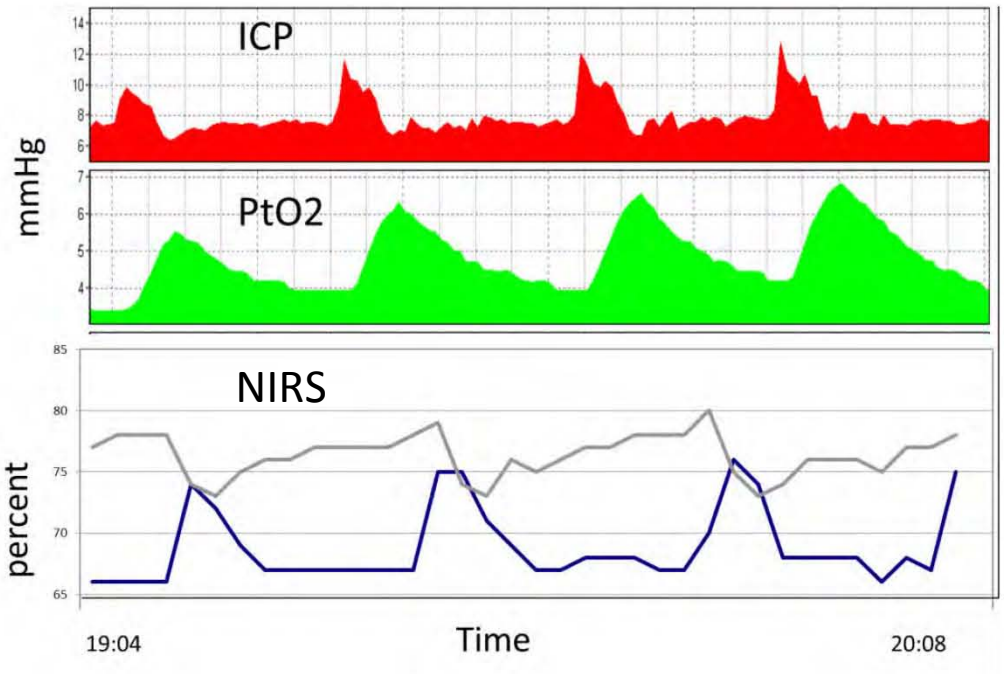
Figure 12.17 illustrates that both monitors responded positively to interventions targeted at increasing brain oxygenation, such as nasal prong oxygen.

Figure 12.17: PbtO2 and NIRS both increase in response to nasal prong oxygen



Additionally, PbtO2 and NIRS monitors also provided information on pathophysiological events that may be difficult to diagnose, such sub-clinical seizure activity as demonstrated by the cycling pattern of ICP, PbtO2 and NIRS demonstrated in Figure 12.18. These cycling episodes corresponded with twitching of the hand and were treated as sub-clinical seizures.

Figure 12.18: Brain monitoring raises the suspicion of sub-clinical seizures



Association with neuromarkers

No significant correlations were demonstrated between the indices of PbtO2 and NIRS and admission neuromarkers or their trend over time.

CHAPTER 12: MONITORING BRAIN OXYGENATION IN TBM

Discussion

Brain oxygenation monitors PbtO2 and NIRS demonstrated that brain oxygenation (as measured by these monitors) is compromised in TBM, and that low brain oxygenation can be detected in these patients in real time, is sensitive to raised ICP and responds to interventions. In individual patients there appears to be reasonable agreement in trends between the 2 monitors although the small patient numbers limited the analysis that could be done. Importantly, although these monitors may assist in detecting reduced oxygenation of the brain and provide insight into TBM, whether using these monitors to guide treatment improves outcomes awaits more evidence.

Temporal profile

Overall both PbtO2 and NIRS demonstrated the lowest readings on admission or day 1, followed by an increase thereafter (likely due to treatment), except in patients who suffered poor outcomes and in whom low PbtO2 and NIRS values were recorded on day 5 or 6. This suggests that TBM patients are at high risk of ischemia early in the first week after admission and the window of opportunity to intervene is likely greatest in this acute phase. This parallels the radiology findings which suggested that ischemia progressed to infarction within the first couple of weeks such that infarcts not visible on admission were already established by the 3 week MRI scan.

PbtO2 and ICP

An important factor contributing to compromised CBF and brain oxygenation in this early phase of disease is raised ICP, often due to HCP in these patients. This was demonstrated in the case illustrations depicted in Figures 12.3 and 12.4 where elevations in ICP, even within the normal range and over short time periods, resulted in an immediate and marked decrease in the PbtO2. This relationship was likely more pronounced due to the fact that baseline PbtO2 was already compromised in these patients; probably as a result of ischemia caused by direct vascular involvement. This suggests that in TBM patients the likely poor baseline brain oxygenation is particularly sensitive to increases in ICP. Possibly, a lower ICP treatment threshold and careful continuous ICP control may be indicated to maximise perfusion in the already compromised brain.

Overall, the relationship between ICP and PbtO2 was not well demonstrated by the correlation between these two variables and in some instances their temporal profiles were increasing in concert whereas ICP is expected to have a negative relationship with PbtO2. This is likely due to a number of factors; *first*, PbtO2 may be influenced by a number of variables independently of ICP, especially in

these children in whom HCP was treated. The most important of these factors would be ischaemia resulting from vasospasm or vascular occlusion in response to basal exudate coating the vessels in the Circle of Willis. *Second*, a third factor may negatively or positively affect both ICP and PbtO2 simultaneously. This does not preclude that the change in ICP will in turn have its own effect on PbtO2, but emphasizes that it is not the only factor causing a decrease in PbtO2. *Third*, both raised ICP and low PbtO2 may reflect injury severity. The more injured the brain, the greater the perturbation in cerebral physiology. This is associated with not only a greater likelihood of ICP and oxygenation problems, but also a stronger probability of other complications, like impaired pressure autoregulation and vascular reactivity, poor cerebral perfusion and disturbed metabolic function. *Fourth*, an increase in both ICP and PbtO2 may be precipitated by phenomena such as increased blood pressure when pressure autoregulation is impaired or hyperaemia which may occur in patients who suffered infarction leading to luxury perfusion in the infarcted territories. The absence of viable tissue to consume the oxygen provided would lead to an increase in PbtO2 as it measures oxygen tension and not utilisation, and the increased cerebral blood volume may precipitate an increase in ICP. Brain pathophysiology is also likely to be dynamic in these patients and therefore relationships between physiological variables may vary from day to day, and from patient to patient. This heterogeneity would be masked when data is pooled for analysis. The absence of an association observed between CPP (measured once weekly) and outcome in a TBM study by Nourse et al [453] may similarly have been due to the dynamic nature of brain physiology in the context of disease. However, despite the potential for variability in the relationship between ICP and PbtO2 it is clear that raised ICP may have a negative impact on PbtO2 in individual patients and at individual time points; therefore, ICP control remains an important component of TBM management.

NIRS

NIRS demonstrated episodes of low brain oxygenation and was responsive to changes in brain oxygenation levels due to pathology and intervention. Differences in the recordings from the right and left sensors appeared to provide additional information about interhemispheric brain oxygenation and pathology as they were occurring. A drop in the NIRS trace was likely associated with ischaemia, but the monitor was also sensitive to post-ischaemic changes such as luxury perfusion following infarction, indicated by a rise in the NIRS trend, demonstrated in Figure 12.11. This suggests that the interpretation of the NIRS readings are best made in the context of the recording as a whole and in conjunction with clinical and radiological indicators of injury; an elevated NIRS recording in a patient who has demonstrated compromised levels of NIRS and has clinical or radiological signs of severe disease does not necessarily suggest healthy brain oxygenation and may indicate hyperaemia in infarcted tissue.

PbtO2 and NIRS in relation to outcome and intervention

For patients with poor outcomes the frequency of both low PbtO2 and NIRS was more common than in patients with good outcomes. While this could not be statistically tested for PbtO2 due to the small sample size, the descriptive picture is still valuable in providing insight into the prevalence and potential consequences of compromised brain oxygenation in TBM. NIRS values <50% were predictive of poor outcomes in these children and this provides some indication of what may be considered compromised NIRS in TBM patients undergoing long term monitoring. NIRS was sensitive to fatally declining brain oxygenation and offered a visibly appreciable picture of deterioration leading to death in real-time; CT brain scans confirming the extent of infarction were often only conducted several hours later.

The case illustrations demonstrated that PbtO2 and NIRS were sensitive to changes in brain oxygenation in response to changes in ICP and MAP and also in response to interventions. This adds insight into the dynamic relationship between intracranial and systemic physiological variables in TBM and may aid in tailoring patient management (such as targeting an optimal MAP or controlling the threshold of ICP) such that brain oxygenation may be optimised. Additionally, the response in PbtO2 and NIRS to interventions, some as simple as nasal prong oxygen, is promising and suggests that there may be potential simple methods to treat impaired oxygenation of the brain and mitigate its damaging effects on the brain; however, this would require formal study.

PbtO2 and NIRS

The data suggest that these two monitors demonstrate an acceptable degree of agreement and reflect similar patterns for brain oxygenation over time. NIRS may however be less sensitive than PbtO2 and more prone to error introduced by difficulties around sensor contact. The absolute values of NIRS require further investigation and thresholds indicative of injury and injury severity are unclear. This monitor is frequently used in the operative environment for short term monitoring where deviation from an established baseline in response to a surgical intervention (usually cardiac surgery) is measured as an indicator of compromised CBF [246,247]. The pathology of a chronic illness like TBM has unique requirements from a brain oxygenation monitor including sensitivity to the effect of intracranial pathological on brain oxygenation, stability of signal for the duration of monitoring and clear treatment thresholds. NIRS demonstrated potential as a non-invasive monitor in TBM but further study into its long term utility, sensitivity to brain injury and critical thresholds are required.

PbtO2, NIRS and neuromarkers

The absence of an association between brain oxygenation monitors and neuromarkers may be due to a number of factors. Firstly, the sample size was small and limited analysis. Secondly, brain oxygen

monitors and neuromarkers are measuring different processes; neuromarker concentrations provide an index of tissue damage that has occurred, while brain oxygenation monitors provide a moving index of dynamic brain perfusion, perturbations in which may or may not lead to actual tissue injury. Thirdly, brain monitoring data and biomarker data were not time-linked; in a study in TBI a significant negative correlation was found between serum S100B concentrations and NIRS readings 6 hours prior to sample collection [242]. However, in this study CSF or serum samples were taken immediately after admission before the initiation of treatment, usually many hours before PbtO2 or NIRS monitoring were begun. Additionally, HCP treatment with EVDs or LPs would have commenced by the time brain monitoring was set up and the reduction in ICP would likely have lead to improved cerebral perfusion relative to admission when biomarker samples were taken. Fourthly, episodes of low brain oxygenation (in the case of PbtO2) during the time of monitoring would have been treated thereby limiting the impending tissue injury.

Limitations

PbtO2 monitoring was only performed in patients who were at high risk of ischaemia based on clinical and radiological findings. Therefore, the relationship with PbtO2 and outcome would have been biased by patient selection. Although the NIRS was non-invasive and could be used in all patients, this was not always feasible in children who were active and mobile (discussed further below), and therefore, there was likely some selection bias in this group as well.

No monitoring modality is without its limitations nor does it present the full picture. Although PbtO2 appears to be a useful tool for monitoring an aspect of brain oxygenation, it is not a precise measure of the adequacy of brain oxygenation and not a monitor of ischaemia per se as it is sensitive to several other variables, especially arterial PaO2 [454]. Positron emission tomography (PET) scanning is the gold standard for measuring brain perfusion, but it can only be done as a 'snapshot' study at one point in time, and continuous monitors are required in the clinical setting. Although PbtO2 is a focal monitor and is not placed in the region of the brain most affected by TBM, there is data to suggest that when placed in uninjured white matter PbtO2 provides an approximation of global brain saturation [224,237]. Furthermore, the infarcts in this cohort were distributed throughout the brain and episodes of vascular pathology were reported to occur in all of the major cerebral vessels (Chapter 11), suggesting that ischemic processes are not limited to a single domain. Arguably, the process is global, even though the eventual infarct is focal; this may represent a vulnerability of certain vessels and territories to an ischaemic process. Raised ICP due to unresolved HCP is also likely to cause diffuse rather than focal ischaemia.

Similarly, the near-infrared beam of NIRS interrogates only the frontal lobes and only superficially. The light signals may be affected by skull thickness, CSF and ambient light, and the relative

contributions of the venous, arterial and capillary compartments remains unclear (Chapter 4). Long term NIRS monitoring is also compromised by the technical limitations of the monitor probes. The sensors easily peeled off in response to perspiration, patient handling and patients pulling at them. Their design is not conducive to long term monitoring in children who are awake as the discomfort created by the sensors on the forehead awkwardly connecting to the bedside monitor increased the desire to pull them off, and made patient mobility difficult. Consequently, frequent disconnections or poor sensor contact compromised the reliability of the monitor and it performed better in patients with a depressed level of consciousness. This limits its utility in TBM patients who are on the milder spectrum of disease but still at risk of ischaemia. However, it may be argued that children with mild disease generally had good outcomes and NIRS may not have made a meaningful contribution to their outcome.

CHAPTER 13: SUMMARY AND CONCLUSION

TBM is a frequent and potentially devastating disease in children from high TB prevalence areas. The disease remains challenging to diagnose and treatment is often delayed, increasing the risk of poor outcome. Currently clinical tools lack the sensitivity and specificity to assess injury severity on admission and radiological examination only illustrates injury once it is irreversible. Identifying patients at greatest risk of poor outcomes and predicting outcome can, therefore, be challenging. Reliable markers of neurological injury may offer clinicians the tools to assess injury severity, prognosticate, anticipate deterioration and direct treatment, and could serve as surrogate markers to evaluate the efficacy of novel interventions.

This study aimed to examine three candidate markers of neurological injury (S100B, NSE and GFAP) together with a panel of inflammatory markers in the CSF and serum of paediatric patients with TBM in terms of their concentrations relative to controls, their temporal profile, their association with clinical, radiological and physiological indicators of injury severity, and their association with clinical and neurodevelopmental outcome.

The main findings of this study in 44 paediatric TBM patients with HCP are summarised as follows:

1. This cohort of patients with HCP represented the more severe spectrum of TBM admission to RXH. The mortality rate was 16% and 36% of patients were left with disability. All patients demonstrated neurodevelopmental deficits and the impact of TBM on development was likely multidimensional comprising the organic injury, as well as environmental, social and educational limitations imposed by prolonged treatment.
2. Neuro- and inflammatory markers were elevated in the lumbar and ventricular CSF of TBM cases relative to controls on admission and for up to 3 weeks, however, serum concentrations were not. This suggests that CSF S100B, NSE and GFAP are indicators of TBM-induced injury and that the immune response in TBM is compartmentalised to the brain.
3. CSF neuromarker concentrations were highest within the first week and declined thereafter, likely due to treatment, except in patients who died. Inflammatory markers demonstrated an early downward trajectory over time, suggesting that the inflammatory response was subsiding.
4. Neuromarker concentrations were higher in the ventricular than lumbar CSF ngand inflammatory concentrations were higher in lumbar than ventricular CSF, likely reflect the cerebral origin of neuromarkers and the largely subarachnoid location of inflammation

5. Neuromarkers were poorly associated with patient and admission clinical characteristics suggesting that they provide information about brain injury that is not reflected in the history and clinical examination.
6. Elevated neuromarker concentrations within the first week and overall were prognostic of poor outcome. The strongest predictor of outcome was an increasing trend in neuromarker concentrations over time.
7. PCA combining all 3 neuromarkers over time demonstrated that concentrations continued to increase in patients who died, while combined inflammatory concentrations decreased. This suggests that it is not the ongoing immune response which is responsible for the progressive brain injury associated with TBM, but rather the secondary pathological processes that are initiated by the inflammatory process.
8. Neuromarkers were not predictive of neurodevelopmental deficits, likely because they reflect the quantitative rather than the qualitative aspects of brain injury.
9. GFAP was elevated in response to brain injury induced by HCP and raised ICP, tuberculomas and infarcts across the spectrum of injury severity. S100B was elevated in response to HCP and severe tissue injury, and small increases over time were strongly predictive of poor outcome suggesting that it is sensitive to deterioration. NSE was elevated in response to severe neurological injury and was prognostic of survivor outcome on admission.
10. Inflammatory markers were poorly associated with clinical and radiological outcome.
11. Novel descriptions of radiology in TBM included spinal pathology (arachnoiditis and tuberculomas) in 75% of patients, and attenuation/occlusion of the major cerebral vessels (particularly the MCA) in 55% of patients on MRA.
12. Brain oxygenation monitors (PbtO2 and NIRS) demonstrated that brain oxygenation was compromised in TBM, sensitive to fluctuations in ICP, even within the normal range, and responsive to interventions.

This project found that biomarkers of neurological tissue injury S100B, NSE and GFAP were elevated in TBM and associated with outcome. This suggests that these neuromarkers could be used to assess injury severity, to track disease course and prognosticate, and that they could be useful as surrogate end points in assessing the efficacy of novel interventions. The results demonstrated that the inflammatory process in TBM declines after the initiation of treatment, but that it likely sets up a cascade of secondary injury mechanisms that continue to perpetuate brain tissue injury. The radiological findings suggest that every child with TBM should have spinal imaging performed and that caution must be exercised when interpreting lumbar CSF results as they may not be reflective of the condition inside the cranium. Low brain oxygenation can be detected with brain oxygenation monitors in TBM and can respond to intervention.

Recommendations for future work

This study's findings demonstrated that S100B, NSE and GFAP are indicators of cerebral injury in response to pathology associated with TBM (whether it be injury due to ischaemia, raised ICP and HCP, or other mechanisms), and are prognostic of poor outcome. Further work in larger patient cohorts are recommended to validate and expand on the findings of this pilot project, including investigating other potential biomarkers of neurological injury. Research on the clinical value that neuromarkers may have to prognosticate and potentially to help guide therapeutic interventions would be an area for further research. The identification of high risk patients may allow for more aggressive targeted interventions. The feasibility of neuromarker testing as part of clinical routine would also need to be examined.

S100B, NSE and GFAP were not well represented in the serum. While all TBM patients will eventually undergo a LP, identifying a marker of intracranial injury that is detectable in the serum during the early phase of injury when TBM may not yet be suspected, would be beneficial in raising the suspicion of meningitis early and hastening diagnosis and treatment initiation, especially because the symptoms are often non-specific.

The panel of inflammatory markers selected offered limited insight into the immunology of the disease and were not associated with outcome. The temporal course of the disease is an important determinant of the immunological profile as measured by immune mediators such as cytokines and chemokines. However, the timing of infection and injury is not clear in TBM and there appears to be heterogeneity in the disease course between patients. Patients often present late and so the peak of the inflammatory response may have been missed. The later clinical deterioration, therefore, may be less associated with ongoing inflammation and more with secondary cerebral events set in motion by that inflammatory response. However, many questions still exist, so further characterization of the host response is important and research into the host transcriptomic response to TBM may offer insight into the immunology underlying TBM and generate biomarkers for diagnosis and treatment efficacy.

The radiological findings of spinal disease accompanying TBM have important clinical implications in terms of diagnosis, management of HCP and lumbar CSF investigation for chemistry, drug assays and biomarker investigation. Therefore, establishing the prevalence of spinal disease in children with TBM in a larger sample size is important and may inform patient management. The recognition of the extent of spinal disease accompanying TBM, as well as the ependymal involvement, suggests further research on the pathophysiology of TBM and spread of disease through the central nervous system is warranted. Cerebrovascular pathology on MRA was surprisingly not associated with infarcts on 3 week MRI. It may be that early pathology, including vasospasm, was missed. Therefore, earlier imaging may provide greater insight into the evolution of infarcts. However, other mechanisms may also contribute to the development of infarcts. The immunology of tuberculomas in TBM may be

valuable to examine further, especially in light of the fact that they were negatively associated with infarction. For these many reasons, we would recommend that MRI be part of the routine management of TBM in children. With the growing concerns about CT-induced radiation effects in children, it would seem safer and of greater value for MRI to be the imaging of choice in paediatric TBM, preferably on admission and at follow-up where needed. Given the frequency of intradural spinal pathology, the addition of spinal imaging would be ideal. The role of MRA still needs to be better defined.

Brain oxygenation monitoring results suggested that PbtO2 is often compromised and is sensitive to changes in ICP in TBM, even within an apparently normal range. This may reflect the already narrowed cerebral perfusion pressure due to vasculitis. Further work into clarifying critical thresholds of ICP and blood pressure management in patients with TBM could be important in directing ICP management so as to maximise cerebral perfusion. These data also suggested that ischaemia or compromised brain oxygen is detectable in real time, and further research into the utility of routine brain oxygenation monitoring in TBM may yield worthwhile benefits to patient care. Similarly, investigation into interventions for poor brain oxygenation and their effect on patient outcome may be important steps to improving management of TBM. NIRS provided valuable insights, but further research into critical thresholds, instability of the signal and the practical issues of sensor and monitor design for long term monitoring in awake patients would need to be addressed.

Microdialysis monitoring has potential to provide further insight into the inflammatory mileu and extent of brain tissue death by enabling the sampling and analysis of fluid derived from the cerebral extracellular space. There is further potential to conduct metabolic analysis, as well as drug recovery testing which may be particularly useful in a condition like TBM where brain injury continues in spite of full antibiotic and steroid treatment and better therapeutic options are urgently needed.

In conclusion, although it is widely known that Christiaan Barnard performed the first heart transplant in Cape Town 47 years ago, it is less well known that prior to this his MD thesis was, in fact, on TBM. His discussion on the poor outcomes of TBM was sobering and remains as true today as it was so many years ago. Much work has been done by excellent and dedicated researchers in an attempt to make progress in changing the outcomes of this disease, but the challenges still loom largely, in part because the brain is notoriously difficult to study. However, with the recent advances in technology and laboratory technique and the potential that these have created to open new windows of insight into the brain, there is hope and promise that the years to come will witness greater progress in understanding and treating this disease that currently devastates so many.

Appendix 1: TBM case definition criteria

Case definition: TBM Diagnosis *(adapted from Marais et al (2010) [69] – Table 2)*

Required criteria

Symptoms and signs of meningitis including 1 or more of the following:
headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits or altered consciousness, or lethargy.

Definite TB

Required criteria PLUS culture-positive CSF for TB or acid-fast bacilli visualised in CSF

Probable TBM

Required criteria PLUS a total diagnostic score of ≥10 points (when cerebral imaging is not available) or ≥ 12 points (when cerebral imaging is available) plus exclusion of alternative diagnoses.

Possible TBM

Required criteria PLUS a total diagnostic score of 6-9 points (when cerebral imaging is not available) or 6-11 points (when cerebral imaging is available) plus exclusion of alternative diagnoses.

Not TBM

Alternative diagnosis established, without a definitive diagnosis of TBM or other convincing signs of dual disease.

Different categories	**Diagnostic score**
A) Clinical criteria	Maximum category score=6
Symptom duration >5 days	4
Systemic symptoms suggestive of TB (1 or more of the following): Weight loss/ failure to thrive (in children), night sweats, persistent cough >2 weeks	2
History of recent (within past year) close contact with pulmonary TB, positive TST or IGRA	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
Altered consciousness	1

B) Cerebrospinal fluid (CSF) criteria	Maximum category score=4
Clear appearance	1
Cells: 10- 500/µl	1
Lymphocytic predominance (>50%)	1
Protein >1 g/l	1
CSF: plasma glucose ratio <50% and/or absolute glucose concentration <2.2mmol/L	1
C) Cerebral imaging criteria	Maximum category score=6
Hydrocephalus	1
Basal meningeal enhancement	2
Tuberculoma	2
Infarct	1
Pre-contrast basal hyperdensity	2
D) Evidence of TB elsewhere	Maximum category score=4
CXR suggestive of active TB Signs of TB = 2; Miliary TB = 4	2 or 4
CT/ MRI/ ultrasound evidence for TB outside the CNS	2
Acid-fast bacilli identified or *M.tb* cultured from another source i.e. sputum, lymph node, gastric washing, urine, blood culture	4
Positive commercial sputum *M.tb* NAAT	4
E) Exclusion of alternative diagnoses An alternative diagnosis must be confirmed microbiologically (by stain, culture or NAAT when appropriate), serologically (e.g. syphilis), or histopathologically (e.g. lymphoma). The list of alternative diagnoses that should be considered, dependent upon age, immune status, and geographical region, include:	

1. Pyogenic bacterial meningitis 2. Cryptococcal meningitis 3. Syphilitic meningitis 4. Viral meningo-encephalitis 5. Cerebral malaria 6. Parasitic/eosinophilic meningitis (*Angiostrongylus cantonesis, Gnathostoma spinigerum*, toxocariasis, cysticercosis) 7. Cerebral toxoplasmosis and bacterial brain abscess (space-occupying lesion on cerebral imaging) 8. Malignancy (e.g. lymphoma)	

TB: tuberculosis, *M.tb: Mycobacterium tuberculosis,* TST: tuberculin skin test, IGRA: Interferon–gamma release assay, NAAT: nucleic acid amplification test, CXR: chest radiograph, CT: computed tomography, MRI: magnetic resonance imaging, *M.tb: Mycobacterium tuberculosis.*

*The individual points for each criterion (*1,2 or 4 points)* were determined by consensus, considering its perceived diagnostic value in previous studies.

Appendix 2: Ethics approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • **Facsimile** [021] 406 6411
e-mail: lamees.emjedi@uct.ac.za

16 July 2010

HREC REF: 318/2010

Prof A Figaji
Neurosurgery
OMB

Dear Prof Figaji

PROJECT TITLE: BIOMARKERS OF INJURY AND INFLAMMATION IN CHILDREN WITH TUBERCULOSIS MENINGITIS

Thank you for your ethics submission to the Faculty of Health Sciences Human Research Ethics Committee.

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

Approval is granted for one year until 28 July 2011.

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

Please quote the REC. REF in all your correspondence.

Yours sincerely

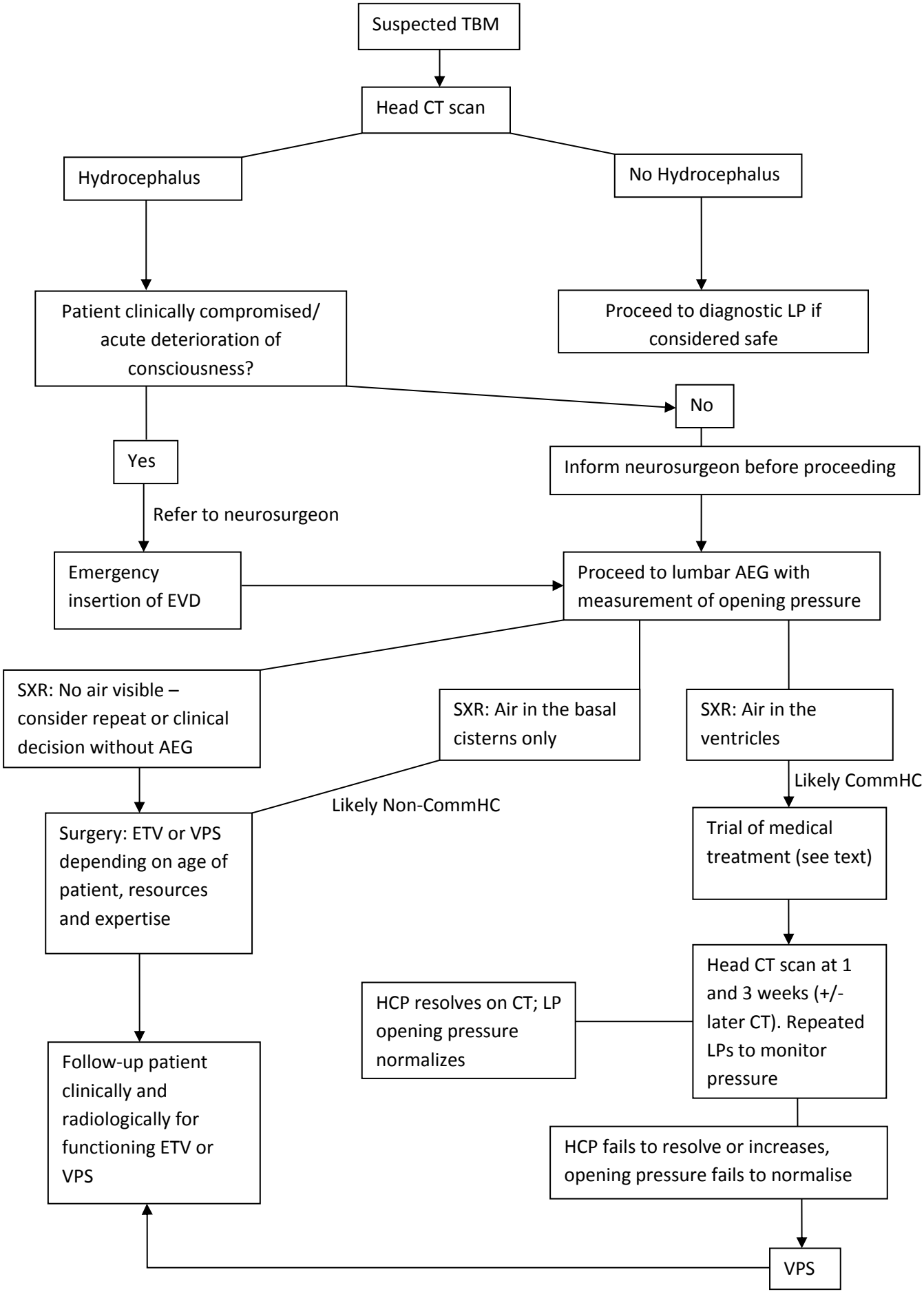
Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
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-

Appendix 3: Institutional Protocol for the Management of Hydrocephalus in TBM (extracted from Figaji et al (2010) [110])



Appendix 4: Paediatric Cerebral Performance Category Scale (PCPS) – Fiser et al (1992)[419]

Pediatric Cerebral Performance Category Scale

Overview: The Pediatric Cerebral Performance Category Scale was described by Fiser and can be used t summarize the level of neurologic function seen in a pediatric patient.

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 or vegetative state	5
• apnea OR • areflexia OR • electroencephalographic (EEG) silence	brain death	6

Appendix 5: Griffiths Mental Scales-Extended Version

RECORD BOOK

for use with the

REVISED EXTENDED GRIFFITHS SCALES OF MENTAL DEVELOPMENT

for testing babies and young children

from birth to the eighth year

Name: _____ **Gender: M/F** _____

Address: _____

_____

_____ **Telephone:** _____

Examiner: _____

Date of Assessment	_____	Year	_____	Month	_____ Day
Date of Birth	_____	Year	_____	Month	_____ Day
Chronological Age	_____	Year	_____	Month	_____ Day

Age at Testing: _____ **Months** _____ **Days**

_____ **Months**

© Association for Research in Infant and Child Development (ARICD)
Not to be reproduced without ARICD permission.

BACKGROUND INFORMATION:

CHILD:

DATE:

Birth weight:

Type of Delivery:

Gestation: wks

Position in family:

Age of siblings:

Relevant medical history:

Relevant social/family history:

Reason for referral:

Referral source:

Vision assessed: Yes/No

Hearing assessed: Yes/No

Test Used:

Test Used:

MOTHER:

FATHER:

Name:

Name:

Age:

Age:

Occupation:

Occupation:

Nationality:

Nationality:

Summary of Clinical Observations/Test Behaviour:

SUMMARY OF TEST RESULTS (Date of test: _____)

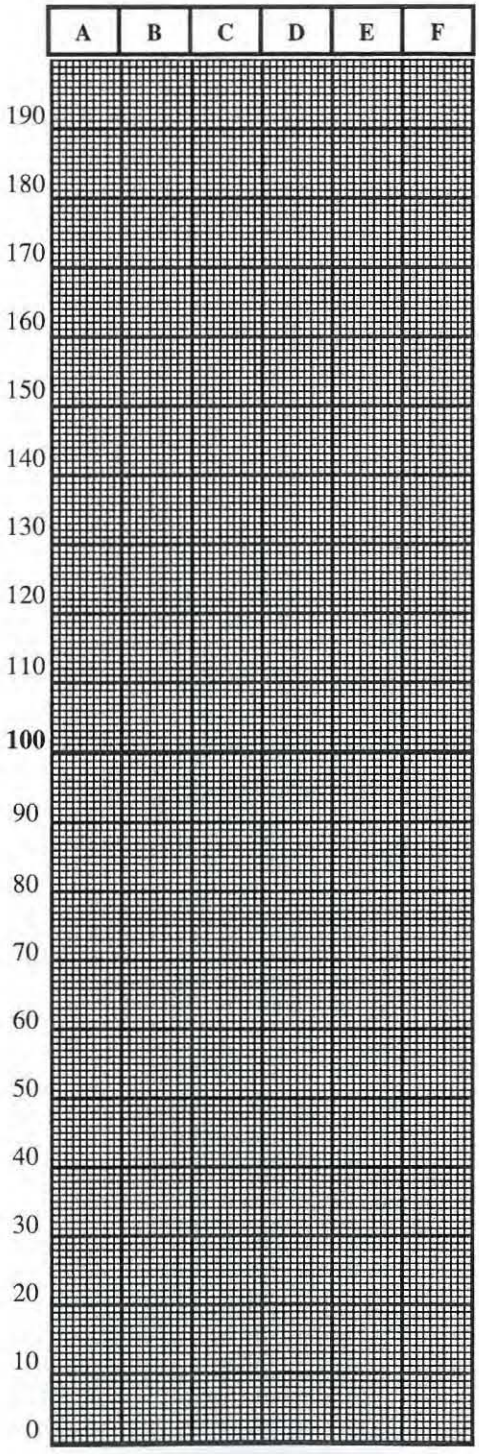
MA Credits in Months

Subscales:	A	B	C	D	E	F
YEARS I						*
II						*
III						
IV						
V						
VI						
VII						
VIII						
Extra Months						
Total MA's (months)						
CA (months)						
Raw Sub-Quotients MA X 100 CA						
Standard Scores						
Percentiles						

Summary in Months

Subscales A to F	Months
I ... items passed / 175 X 12 =	
II ... items passed / 101 X 12 =	
III ... items passed / 3 =	
IV ... items passed / 3 =	
V ... items passed / 3 =	
VI ... items passed / 3 =	
VII ... items passed / 3 =	
VIII ... items passed / 3 =	
Extra ... items passed / 3 =	
Total MA (months) =	
CA (months) =	
Raw GQ =	
Standard Score =	
Percentile =	

Profile



Note: The Raw General Quotient or GQ is obtained by taking the average of all the six Sub-Quotients.

*The average of A, B, C, D and E.

SUBSCALES **FIRST**

YEAR I Months of Age	A LOCOMOTOR		B PERSONAL-SOCIAL		C HEARING & LANGUAGE	
	Lifts chin when prone	1	Regards person – fleeting glance	1	Startled by sound R	1
	Pushes with feet against examiner's hands	2	Quieted when picked up R	2	Listens to bell	2
1	Holds head erect for few seconds	3	Enjoys bath. R	3	Vocalization other than crying	3
	Kicks vigorously	4	Visually recognizes mother	4	Cooing – one syllable R	4
	Lifts head up when prone	5	Follows moving persons with eyes	5	Makes two different sounds	5
	Active in bath – kicks R	6	Smiles	6	Listens to music.	6
2	Rolls from side to back	7	Vocalizes when talked to	7	Searches for sound with eyes	7
	Back firm when held in sitting position.	8	Returns examiner's glance with smiling or cooing	8	Searches for sound with head movements	8
3	Lifts head when in dorsal position	9	Friendly to strangers R	9	Laughs aloud R	9
	Lifts head, shoulders and chest when prone	10	Expresses two or more recognizable emotions, e.g. pleasure, fear, sadness, distress or anger	10	Talks (babbles) to persons	10
4	Holds head erect continuously	11	Stops crying when talked to R	11	Coos or stops crying on hearing music	11
	Lifts head and shoulders, dorsal	12	Frolics when played with	12	Turns head deliberately to bell	12
	Crawling reaction 1: Draws up knees, etc.	13	Regards mirror image: 1. Looks at	13	Makes 4 different sounds	13
5	Rolls from side to side via dorsal position.	14	Resists adult who tries playfully to take ring	14	Listens to tuning fork	14
	Sits with slight support.	15	Turns head to person talking or singing.	15	Responds when called	15
	Plays with own toes R	16	Holds a spoon	16	Manipulates bell	16
	Stepping reaction: 1. Dancing movements	17	Anticipatory movements when about to be lifted	17	Shouts for attention R	17
	Sits alone for a short time	18	Knows strangers from familiar friends	18	Two syllable babble	18
6	Crawling reaction: 2. Can turn around when left on floor (pivoting)	19	Prompt reaction to situation, e.g., at table, waiting to be fed	19	Listens to conversations	19
	Crawling reaction: 3. Tries vigorously to crawl	20	Manipulates cup or spoon in play	20	Rings bell	20
7	Can roll from stomach to back (or from back to stomach)	21	Displeased if toy is taken.	21	Looks at pictures for a few seconds	21
	Crawling reaction: 4. Makes some progress forward or backwards.	22	Holds and bites biscuits, rusks, ice – cream wafers, etc.	22	Singing tones R	22
8	Stepping reaction: 2. One foot in front of the other	23	Interested in small children other than own siblings R	23	One word (mama, dada, etc.) definite and meaningful	23
	Can be left sitting on floor R	24	Helps to hold cup or mug for drinking	24	Babbled phrases: 4+ syllables R	24
	"Stands" when held up	25	Pulls off hat	25	Likes rhymes and jingles	25
9	Sits well in a chair	26	Drinks from any open cup or mug if held to lips	26	Knows own name	26
	Crawling reaction: 5. Creeps on hands and knees, etc.	27	Stretches to be taken up.	27	Babbled monologue when alone	27
	Pulls self up by furniture.	28	Finger feeds (thumb and forefinger) e.g. sultanas, "Smarties", etc. R	28	Shakes head for" No"	28
10	Can stand holding on to furniture	29	Picks up and drinks from lidded and closed feeder–cup unaided R	29	Use 2 definite words	29
	Side – steps round inside of cot or playpen holding rails	30	Responds socially to mirror image: 2. Smiles at or plays with	30	Reacts to music vocally	30
	Climbs on a low ledge or step	31	Gives affection R	31	Short babbled sentences	31
11	Can walk when led	32	Plays very simple interactive games with others R	32	Uses 3 words	32
	Climbs stairs (up).	33	Plays with cup, spoon and saucer	33	Identifies objects (1)	33
12	Likes pushing pram, toy, horse etc. R	34	Waves bye – bye R	34	Tries definitely to sing R	34
	Stands alone	35	An interest in the activities of others is shown R	35	Identifies objects (2)	35
	Months Credit: Year I-A Items = (/ 35) x 12	... ...	Months Credit: Year I-B Items = (/ 35) x 12	... ...	Months Credit: Year I-C Items = (/ 35) x 12	... ...

YEAR

YEAR I Months of Age	D EYE & HAND CO-ORDINATION		E PERFORMANCE		NOTES AND COMMENTS
	Follows moving light with eyes	1	Reflex grasp of examiner's finger	1	
	Looks at bell-ring or toy momentarily	2	Reacts to paper: 1. Generalised physical movements	2	
	Looks steadily at bell-ring held still	3	Energetic arm movements	3	
	Follows moving bell-ring horizontally	4	Hand goes to mouth R	4	
	Follows moving bell-ring vertically	5	Holds rod	5	
	Glances from one object to another	6	Plays with own fingers R	6	
	Follows moving bell-ring in a circle	7	Reacts to paper: 2. Vigorous head turning	7	
	Watches objects pulled along by string	8	Resists withdrawal of rod	8	
1	Grasps ring when given	9	Looks at yellow box on table	9	
	Visually explores new environment	10	Clasps cube put in hand and holds it	10	
	Reaches for ring and grasps	11	Shows interest in box	11	
2	Carries ring to mouth	12	Drops first cube for second	12	
	Clutches at dangling ring	13	Reacts to paper: 3. Pulls it away	13	
3	Secures dangling ring	14	Takes cube or toy from table	14	
	Hands explore table surface	15	Holds two cubes	15	
4	Plays with ring – shaking, banging etc.	16	Manipulates cube or toy	16	
	Reaches for and picks up string	17	Grasps box	17	
	Looks for fallen object	18	Passes toy or cube from hand to hand	18	
	Strikes one object with another	19	Reacts to paper: 4. Reaches for and takes	19	
5	Secures ring by means of string	20	Manipulates two objects at once	20	
	Watches examiner scribble	21	Reacts to paper: 5. Plays with – tears, crumples	21	
6	Forefinger and thumb partly specialized.	22	Lifts cup inverted over toy	22	
7	Dangles ring by string	23	Drops one cube for third	23	
	Fine prehension	24	Rattles box	24	
	Interested in motor car R	25	Lifts lid off box	25	
8	Likes holding little toys	26	Finds toy under cup	26	
9	Throws objects (record how child throws)	27	Tries to take cubes out of box	27	
	Thumb opposition complete	28	Holds third cube	28	
10	Can hold pencil as if to mark on paper	29	Clicks two bricks together (in imitation) TX2	29	
11	Can point with index finger	30	Manipulates box, lid and both cubes	30	
	Plays pulling ring or toy by string	31	Removes both cubes from box	31	
12	Uses pencil on paper a little	32	Unwraps and finds toy or cube	32	
	Preference for one hand R	33	One-circle board TX2	33	
	Plays pushing little cars along	34	Removes lids & both bricks from the other two boxes	34	
	Can hold four cubes in hands at once	35	Puts 2 bricks back into any 1 box when encouraged to do so	35	
	Months Credit: Year I-A Items = (/ 35) x 12	... ...	Months Credit: Year I-B Items = (/ 35) x 12	... ...	

SUBSCALES **SECOND**

YEAR II Months of Age	A LOCOMOTOR		B PERSONAL-SOCIAL		C HEARING & LANGUAGE	
13	Climbs into a low chair	36	Claps hands in imitation	36	Uses 4 words	36
	Walks alone	37	Puts small objects in and out of cup in play	37	Uses 5 words	37
	Kneels on floor or chair	38	Tries to help dressing - arms into coat etc. R	38	Identifies objects (3)	38
14	Stoops	39	Obeys simple requests – "give me the cup" R	39	Uses 6 or 7 words	39
	Trots about well	40	Can hold open cup for drinking R	40	Enjoys picture book	40
15	Can walk backwards	41	Tries to turn doorknob or handle R	41	Identifies objects (4)	41
	Climbs to stand on a chair	42	Shows shoes	42	Uses 9 words	42
16	Climbs stairs – up and down R	43	Uses spoon himself: Spills some R	43	Names objects (1)	43
	Walks backwards pulling toy on string	44	Likes adult to show book	44	Long babbled sentences – some words clear	44
17	Can seat self at table	45	Parts of doll's body (1) hands, hair, feet, eyes, nose and mouth	45	Names objects (2)	45
18	Walks upstairs	46	Cleanliness – indicates when wet or dirty	46	Uses 12 words	46
	Runs	47	Uses spoon well	47	Uses 20+ words	47
19	Can kick a ball (tennis ball size)	48	Manages cup well – half full	48	Identifies objects (5 or 6)	48
20	Goes alone on stairs	49	Can open a door	49	Uses word combinations	49
21	Walks up and down stairs	50	Can take off shoes and socks	50	Identifies objects (7)	50
22	Jumps	51	Parts of doll's body (2) hands, hair, feet, eyes, nose and mouth	51	Listens to stories	51
23						
24	Can jump off a step	52	Parts of doll's body (3)	52	Names objects (3)	52
	Jumps off one step – both feet together and land together	53	Helps actively to dress or undress R	53	Identifies objects (8)	53
	Walks upstairs – one foot on each step – adult *	54	Parts of doll's body (4) hands, hair, feet, eyes, nose and mouth	54	Names objects (4)	54
			Puts away toys or objects when encouraged to do so R	55	Names objects (5)	55
			Asks for things at table by name – at least two articles of food or drink R	56	Uses sentences of 4+ syllables	56
			Begins to cooperate in play with other children R	57		
			At table uses spoon and fork together without help * R	58		
	Months Credit: Year II-A Items = ... (/ 19) x 12		Months Credit: Year II-B Items = ... (/ 23) x 12 ...		Months Credit: Year II-C Items = ... (/ 21) x 12	

* Note: Please refer to the Revised instructions which provides the instructions for items A54, B58 & E54.

YEAR

YEAR II Months of Age	D EYE & HAND CO-ORDINATION		E PERFORMANCE		OBJECTS		
	Plays at rolling a ball	36	Two-circle board – one in	36		**IDENTIFIED**	**NAMED**
13	Places one lid, box or brick upon another	37	Puts bricks in and out of boxes in play	37	**BALL**		
	Pulls cloth to get toy	38	Square board TX2	38	**SPOON**		
14	Scribbles more freely	39	Two-circle board – two in	39	**BRUSH**		
	Constructive play with boxes or other materials	40	Can put lid back on box	40	**CAR**		
	Can throw a ball towards person	41	Three-hole board – 1 in TX2	41	**DOLL**		
15	Tower of 3 bricks	42	Circle and square board together TX2	42	**CUP**		
16	Tower of 4 bricks	43	Three-hole board – 2 in	43	**SOCK**		
	Enjoys vigorous straight scribble	44	Puts 2 bricks into 1 box, and the lid on; all complete	44	**BRICK**		
17	Can transfer cube from one container to another	45	Three-hole board – 3 in	45	**TOTAL**		
18	Can pour water from one container to another	46	Circle and square boards - rotated	46	Toileting Questions (See Scale B)		
	Tower of 5 bricks	47	Two-circle board - rotated	47	**A Bowel control complete** Is this child usually quite bowel-continent and reliable as regards this function, except for an occasional mishap? YES/NO		
19	Makes a brick or toy "walk"	48	Puts two bricks in each box	48			
20	Circular scribble (in imitation)	49	Three-hole board rotated – 2 in	49			
21	Tower of 6 bricks	50	Three-hole board rotated – 3 in	50	**B Bladder control by day** Is this child usually reliably dry by day in regard to bladder function, but not necessarily at night? YES/NO		
22 23	Throws ball into basket	51	Puts lids on all three, filled boxes	51			
24	Train of three (bricks)	52	Can open screw toy	52			
	Perpendicular stroke	53	Reassembles screw toy	53			
	Horizontal stroke	54	Returns 9 bricks to box and replaces lid within 60 secs. (Time.....secs.) *	54			
	Months Credit: Year II-D Items = (/ 19) x 12 =	....	Months Credit: Year II-E Items = (/ 19) x 12 =	... ...	Total Months Credit: Year II (Total items passed / 101) x 12 = Months		

RESULTS				
SCALE	RAW SCORE	AGE EQUIVALENT SCORE (months)	SUB- and GENERAL QUOTIENTS	PERCENTILE SCORE
A				
B				
C				
D				
E				
TOTAL				

SUBSCALES **THIRD, FOURTH**

	A 1/2/3/4 LOCOMOTOR		B PERSONAL-SOCIAL		C LANGUAGE	
YEAR III ①	Jumps off 1 step D; TX2 one foot after the other	1	Puts away toys when encouraged to do so R	1	Names 12 objects in box say what something is	1
3/20	Static balance I: Can stand on one foot for 3+ seconds D; TX2(C)	2	Gives first name	2	Picture vocabulary (12) (NB: Administer item after item FIV.4)	2
	Can rise from kneeling without using hands D(C); TX2	3	Assists with small household tasks on request R	3	Defines by use (2+)	3
	Can run fast indoors or in a small outside space	4	Uses spoon and fork together, without help R	4	Names 6+ objects in large picture	4
	Can stand and walk tip-toe: 6+ steps D(C); TX2	5	Knows own gender	5	Uses two or more descriptive words ≥2	5
	Walks upstairs: One foot on each step, adult manner D; TX2	6	Plays well with other children R	6	Talks well in sentences of 6+ syllables	6
	Months credit: YEAR III – A Items = ... x 2 = ...		Months credit: YEAR III – B Items = ... x 2 = ...		Months credit: YEAR III – C Items = ... X 2 = ...	
YEAR IV	Can ride a tricycle or other pedal toy R	1	Can undo buttons D(C); TX2	1	Names 18 objects in box	1
	Can cross both feet and both knees when seated D(C); TX2	2	Can undress self R	2	Names six or more colours ≥6	2
②	Jumps off 2 steps D; TX2 feet together	3	Washes own hands and face, with some assistance R	3	Repeats one six-syllable sentence	3
	Can walk a chalk line or painted line at least 1.2m (4ft.) long D; TX2	4	Knows age	4	Comprehension (2+ items)	4
	Can run and kick a medium-size ball D; TX2	5	Can do up buttons D(C); TX2	5	Defines by use (6+)	5
3X	Can jump over 15cm (6in.) foam block hurdle D; TX2	6	Gives family name	6	Uses 2+ personal pronouns correctly ≥2	6
	Months credit: YEAR IV – A Items = ... x 2 = ...		Months credit: YEAR IV – B Items = ... x 2 = ...		Months credit: YEAR IV – C Items = ... X 2 = ...	
YEAR V	Walks downstairs: One foot on each step, adult manner D; TX2	1	Can put on socks and shoes, unaided R	1	Names 12 objects in large picture	1
	Can hop on one foot: 3+ steps D; TX2	2	Can dress and undress self R	2	Picture vocabulary (18+) (NB: Administer item after item FIV.4)	2
	Can run fast out of doors R	3	Manages topcoat, cardigan or raincoat unaided R	3	Opposites (2)	3
	Touches toes, knees straight D; TX2	4	Brushes own teeth, without assistance R	4	Repeats one ten- syllable sentence	4
	Broad jump 37.5cm (15in.) over foam blocks D; TX2	5	Can fetch item in a shop on request R	5	Picture description: One or more descriptive sentences	5
	Kangaroo jumps over three foam blocks D(C); TX2	6	Can fasten shoe buckles D	6	Materials (2+) (See below)	6
	Months credit: YEAR V – A Items = ... x 2 = ...		Months credit: YEAR V – B Items = ... x 2 = ...		Months credit: YEAR V – C Items = ... X 2 = ...	

Item CV.6: Materials (2+)

1. What is a table made of?
2. What is a window made of?
3. What is a house made of?

Items CIV.2, CVI.5: Names Colours
Tick if known

red	brown
white	green
blue	yellow
orange	grey
lilac/purple	black

Don't include if answer is repeat

& FIFTH YEARS

D EYE & HAND CO-ORDINATION		E PERFORMANCE		F PRACTICAL REASONING		TOTALS A-F
Builds a tower of 8+ bricks D(C); TX2	1	Four-squares board: 50 sec TX2(C)	1	Knows 'penny' or 'money'	1	
Copies a horizontal stroke D	2	Six-hole board: 50 sec TX2(C)	2	Repeats 1 digit (8; 2; 7) P	2	
Handles scissors: Tries to cut D(C)	3	Returns 9 bricks to box and puts the lid on: 50 sec TX2(C)	3	Compares two insets for size	3	
Threads 6 beads D(C)	4	Re-assembles screw toy D(C); TX2	4	Repeats 2 digits (1-6; 5-3; 9-4) P	4	
Copies a circle: Stage I	5	Four-squares board: 40 sec TX2(C)	5	Knows 'big' and 'little'	5	
Folds a 10.2cm (4in.) square in half D(C)	6	Six-hole board: 40 sec TX2(C)	6	Preliminary counting to 4+	6	
Months Credit: YEAR III-D Items = ... X 2 = ...		Months Credit: YEAR III-E Items = ... X 2 = ...		Months Credit: YEAR III-F Items = ... X 2 = ...		YEAR III A to F Items 3 = ...
Threads 11+ beads D(C)	1	Assembles brick-boxes by colour: All 12 pieces	1	Compares two towers for height	1	
Copies a cross: Stage I	2	Eleven-hole board: 60 sec TX2(C)	2	Compares two lines for length D	2	
Draws a person: Stage I	3	Builds bridge with 3 boxes: Inferior model D(C)	3	Can count 4 bricks correctly TX2	3	
Scissors: Can cut a square into two fairly equal halves D(C)	4	Four-squares board: 20 sec TX2(C)	4	Visual memory (3)	4	
Folds a 10.2cm (4in.) square twice D(C)	5	Returns 9 bricks to box and puts the lid on: 35 sec TX2(C)	5	Compares two weights	5	
Copies a ladder: Stage I	6	Six-hole board: 20 sec TX2(C)	6	Knows right from wrong "Is it right or wrong to hurt someone?" "Is it right or wrong to lie to someone?"	6	
Months Credit: YEAR IV-D Items = ... X 2 = ...		Months Credit: YEAR IV-E Items = ... X 2 = ...		Months Credit: YEAR IV-F Items = ... X 2 = ...		YEAR IV A to F Items 3 = ...
Copies a square: Stage I	1	Pattern-making No. 2: 50 sec D; P(C)	1	Can count 10 bricks correctly TX2	1	
Draws a house: Stage I	2	Train under bridge successfully D(C)	2	Knows number of fingers on each hand	2	
Copies a circle: Stage II	3	Pattern-making No. 2: 40 sec D; P(C)	3	Can take out the middle brick	3	
Threads 11 beads to colour pattern D(C)	4	Pattern-making No. 5: 50 sec D; P(C)	4	Repeats 4 digits (3-7-2-9; 5-8-1-6; 4-9-5-2) P	4	
Copies 6+ letters	5	Builds 'gate' to model, using 3 boxes and lids D(C)	5	Can count 15 bricks correctly TX2	5	
Scissors: Can strip edge of paper D(C)	6	Eleven-hole board: 40 sec TX2(C)	6	"Which costs more?" P(C) A bicycle or a ball? (practice example) A watch or an ice-cream? (no.1)	6	
Months Credit: YEAR V-D Items = ... X 2 = ...		Months Credit: YEAR V-E Items = ... X 2 = ...		Months Credit: YEAR V-F Items = ... X 2 = ...		YEAR V A to F Items 3 = ...

FORMBOARD	4-square	6-hole	11-hole
TRIAL 1	"	"	"
TRIAL 2	"	"	"

PATTERN MAKING	No. 2	No.3	No.4	No.5
TRIAL 1	"	"	"	"

9 BRICKS TO BOX	
TRIAL 1	"
TRIAL 2	"

SUBSCALES **SIXTH, SEVENTH**

	A LOCOMOTOR		B PERSONAL-SOCIAL		C LANGUAGE	
YEAR VI	Can run upstairs	1	Has a special playmate R	1	Comprehension (4+ items)	1
	(3) Jumps off 3 steps D; TX2	2	Can get a drink of water from the tap or bottle, without assistance R	2	Talks in sentences of 10+ syllables	2
	Can bounce and catch a tennis ball D(C); TX2	3	Can wash and dry own hands and face, without any assistance R	3	Names 10+ capital letters	3
	30cm apart Hopscotch I kick 1 D(C); TX2	4	Can choose own clothes R	4	Similarities (1) P(C)	4
	Can jog at a steady pace all around playground	5	Can shampoo hair, with some assistance R	5	Names 10 colours	5
	Can hopskip, recognisable by skipping down hard D(C); TX2	6	Knows address >2 parts thereof	6	Differences (2) P(C)	6
	Months credit: YEAR VI – A Items = ... x 2 = ...		Months credit: YEAR VI – B Items = ... x 2 = ...		Months credit: YEAR VI – C Items = ... X 2 = ...	
YEAR VII	Can jump over 25cm (10in.) foam block hurdle D; TX2	1	Can tie a single knot D(C); TX2	1	Names 20+ capital letters	1
	Marches in time to tambourine TX2	2	Eats without assistance R	2	Similarities (2) P(C)	2
	Can throw a tennis ball up and catch it D(C); TX2	3	Can lay a table completely, with some supervision R	3	Differences (3) P(C)	3
	Runs downstairs	4	Can dress and undress completely, without help R	4	Similarities (3) P(C)	4
	Hopscotch II kicks 2 D(C); TX2	5	Has one special school friend R	5	Picture Description: 3 descriptive sentences	5
	Can skip with a rope: 3+ single skips D; TX2	6	Knows full address	6	Repeats one sixteen-syllable sentence	6
	Months credit: YEAR VII – A Items = ... x 2 = ...		Months credit: YEAR VII – B Items = ... x 2 = ...		Months credit: YEAR VII – C Items = ... X 2 = ...	
YEAR VIII	Rides a bicycle (2 wheeler) R	1	Knows birthday I day + month	1	Names 26 capital letters	1
	Static balance II: Can stand on one foot for 20+ seconds D; TX2(C)	2	Can tie a bow-knot D(C); TX2	2	Uses 6+ descriptive words	2
	Hopskips some distance in an open area D(C); TX2	3	Can tie own shoe-laces R	3	Picture Description: 4 or more descriptive sentences	3
	Hopscotch III kicks 3 D(C); TX2	4	Can shampoo hair, without any assistance R	4	Comprehension (6+ items)	4
	Rides a bicycle (2 wheeler) with skill R	5	Can tie a double bow-knot D(C); TX2	5	Differences (4) P(C)	5
	Jumps off 4 steps D; TX2	6	Baths or showers and dries self, without assistance R	6	Uses 6+ personal pronouns correctly	6
Extra	Fast single skipping with rope: 12+ skips D; TX2	7	Can lay a table completely, without help or supervision, on all ordinary occasions R	7	Differences (5) P(C)	7
Items	Skips well with rope: 12+ double skips D; TX2	8	Knows birthday II day, month, year	8	Opposites (3)	8
	Months credit: YEAR VIII – A Items = ... X 2 = ... Extra items:+ X 2 A =		Months credit: YEAR VIII – B Items = ... X 2 = ... Extra items:+ X 2 B =		Months credit: YEAR VIII – C Items = ... X 2 = ... Extra items:+ X 2 C =	

Notes and Comments:

& EIGHTH YEARS

D EYE & HAND CO-ORDINATION		E PERFORMANCE		F PRACTICAL REASONING		TOTALS A-F
Copies 10+ letters No reversal	1	Pattern-making No. 5: 40 sec D; P(C)	1	Knows number of fingers on both hands together	1	
Copies 6+ numbers	2	Pattern-making No. 3: 50 sec D; P(C)	2	Can count backwards from 10 D; TX2	2	
Can write own first name	3	Returns 9 bricks to box and puts the lid on: 20 sec TX2(C)	3	Knows morning and afternoon	3	
Copies a cross: Stage II +‡+	4	Pattern-making No. 4: 50 sec D; P(C)	4	"Which goes faster?" (3)	4	
Copies a triangle: Stage I recognisable	5	Builds bridge with 3 boxes: Superior model D(C)	5	Can say six of the seven days of the week	5	
Draws a person: Stage II Circle + 3/6 features	6	Four-squares board: 7 sec TX2(C)	6	"Which costs more?" P(C) A bicycle or a ball? (Practice example) A cool-drink or shoes? (no.2)	6	
Months Credit: YEAR VI-D Items = ... X 2 = ...		Months Credit: YEAR VI-E Items = ... X 2 = ...		Months Credit: YEAR VI-F Items = ... X 2 = ...		YEAR VI A to F Items 3 = ...
Draws a house: Stage II neat, 3/6 features	1	Pattern-making No. 3: 40 sec D; P(C)	1	Can count up to 30	1	
Copies 24+ letters	2	Pattern- making No. 2: 25 sec D; P(C)	2	Picture Arrangement I: Bird on nest P(C) get child to stand	2	
Copies a window: Stage I recognisable, square + cross	3	Pattern-making No. 3: 30 sec D; P(C) - demonstrate fingers working up stairs	3	Knows right and left (6+) TX2 1. Right hand X 5. Left hand 2. Left ear X 6. Right ear 3. Right foot X 7. Left foot 4. Right eye X 8. Left eye	3	
Copies a diamond: Stage I recognisable	4	Ten-brick memory stairs D(C)	4	Picture Arrangement II: Pouring a drink P(C)	4	
Copies 9 numbers	5	Pattern-making No. 4: 40 sec D; P(C)	5	Knows 'long' and 'short'	5	
Copies a triangle: Stage II neat, ✡	6	Eleven-hole board: 30 sec TX2(C)	6	Days of the week: (2+) "What day comes after Tuesday?" "What day comes before Saturday?" "What day comes after Sunday?"	6	
Months Credit: YEAR VII-D Items = ... X 2 = ...		Months Credit: YEAR VII-E Items = ... X 2 = ...		Months Credit: YEAR VII-F Items = ... X 2 = ...		YEAR VII A to F Items 3 = ...
Can write full name	1	Pattern-making No. 2: 20 sec D; P(C)	1	Series P(C)	1	
Copies a square: Stage II neat	2	Six-hole board: 10 sec TX2(C)	2	Can count backwards from 20 D; TX2	2	
Copies a ladder: Stage II ≤5 rungs, neat	3	Pattern-making No. 4: 30 sec D; P(C)	3	Knows 'heavy' and 'light'	3	
Copies a window: Stage II neat	4	Returns 9 bricks to box and puts the lid on: 15 sec TX2 (C)	4	Knows 'high' and 'low'	4	
Copies a diamond: Stage II neat ✡	5	Pattern-making No. 3: 25 sec D; P(C)	5	Repeats 5 digits (6-1-3-8-4; 5-9-2-7-1; 9-2-7-8-6)	5	
Draws a person: Stage III + creativity, originality clothes clothes	6	Pattern-making No. 5: 20 sec D; P(C)	6	Repeats 3 digits backwards (1-8-6; 7-2-5; 4-9-3) P	6	
Draws a house: Stage III + creativity, originality garden fence...	7	Pattern- making No. 4: 20 sec D; P(C)	7	Picture Arrangement III: Building a house P(C)	7	YEAR VIII A to F
(Credit as 2 items) (See DV.2 & DVII.1)	8	Pattern-making No. 5: 15 sec D; P(C)	8	Directional Arrows	8	Items 3 = ...
Months Credit: YEAR VIII-D Items = ... X 2 = ... Extra items:+ X 2 D =		Months Credit: YEAR VIII-E Items = ... X 2 = ... Extra items:+ X 2 E =		Months Credit: YEAR VIII-F Items = ... X 2 = ... Extra items:+ X 2 F =		EXTRA Items 3 = ...

Item FVI.4: "Which goes faster?

1. A big dog running or a puppy (baby dog) running?
2. A bird flying or an aeroplane?
3. A car or a bicycle?

SUBSCALE C

18 OBJECTS IN A BOX **Please tick if named correctly** (Items CIII.1, CIV.1)

☐ Chair	☐ Cat	☐ Brick	☐ Watch
☐ Doll	☐ Cup	☐ Coin	☐ Key
☐ Ball	☐ Spoon	☐ Knife	☐ Pencil
☐ Horse	☐ Button	☐ Fork	
☐ Dog	☐ Car	☐ Plate	

DEFINES BY USE (Items CIII.3, CIV.5)

Cup	Knife	Chair
Coin	Car	House
Pencil	Watch	Key

PICTURE CARDS **Please tick if named correctly** (Items CIII.2, CV.2)

☐ Ball	☐ Cap/Hat	☐ Bird	☐ Tea Pot/Kettle
☐ Shoe	☐ Flower	☐ Key	☐ Flag
☐ Dog	☐ Horse	☐ Umbrella	☐ Shop
☐ Train	☐ Spoon	☐ Hammer	☐ Wheel-barrow
☐ Boy	☐ Bed	☐ Cup	☐ Owl

LARGE PICTURE

1. **Full Verbatim** Report: Record everything the child says.

2. **Objects** named (nouns) N =
   (Items CIII.4, CV.1)

3. **Descriptive words** used (adjectives, adverbs) N =
   (Items CIII.5, CVIII.2)

4. **Personal pronouns** and **possessive pronouns** N =
   (Items CIV.6, CVIII.6)

   try to prompt

5. **Descriptive sentences** of 6 or more syllables N =
   (Items CV.5, CVII.5, CVIII.3)

SPONTANEOUS SENTENCES

1. Six or more syllables (Item CIII.6)

2. Ten or more syllables (Item CVI.2)

REPETITION OF SENTENCES

a) **Six** syllable sentences (Item CIV.3). One correct sentence scores as a pass.
   - i " I have a little cat."
   - ii " My kitty caught a mouse."
   - iii "The mouse had a long tail."

b) Repeats **10** syllables (Item CV.4).) One correct sentence scores as a pass.
   - i "My dog is a very good friend to me."
   - ii "I take my dog when I go for a walk."

c) Repeats a sentence of **16** syllables (Item CVII.6). One correct sentence scores as a pass.
   - i "It will be my birthday next week, Mummy will give me a party."
   - ii "The children were playing a game in the park and then they went home."

COMPREHENSION: (Item CIV.4, CVI.1, CVIII.4) 2|4|6

1. " What should you do if you feel tired?"

2. " What should you do if you are cold?"

3. " What is the thing to do if it is raining and you have to go out?"

4. " What should you do if you are going somewhere and you missed the bus?"

5. " What do you do if you feel lonely?"

6. " What is the best thing to do if you are on your way to school, and you find its getting late?"

7. " What would you do if you were lost?"

2 | 3

OPPOSITES: (Items CV.3, CVIII.8)

1. " A boy is big, a baby is _____ ?"
2. " Coal is black, snow is _____ ?"
3. " A lion is fierce, a lamb _____ ?"

1 | 2 | 3

SIMILARITIES: (Items CVI.4, CVII.2, CVII.4)

" How are the **moon** and the **stars** the same?" **(PRACTICE EXAMPLE)**

1. " How are a **bird** and an **aeroplane** the same?"
2. " How are a **car** and a **bus** the same?"
3. " How are a **door** and a **window** the same?"
4. " How are a **pen** and **pencil (or crayon)** the same?"

2 | 3 | 4 | 5

DIFFERENCES: (Item CVI.6, CVII.3, CVIII.5, CVIII.7)

" How are a **fly** and a **bee** different?" **(PRACTICE EXAMPLE)**

1. "How are the **morning** and **night** different?"
2. " How are a **fish** and a **dog** different?"
3. " How are **salt** and **sugar** different?"
4. " How are a **triangle** and a **square** different?"
5. " How are **winning** and **losing** different?"

Re-test page

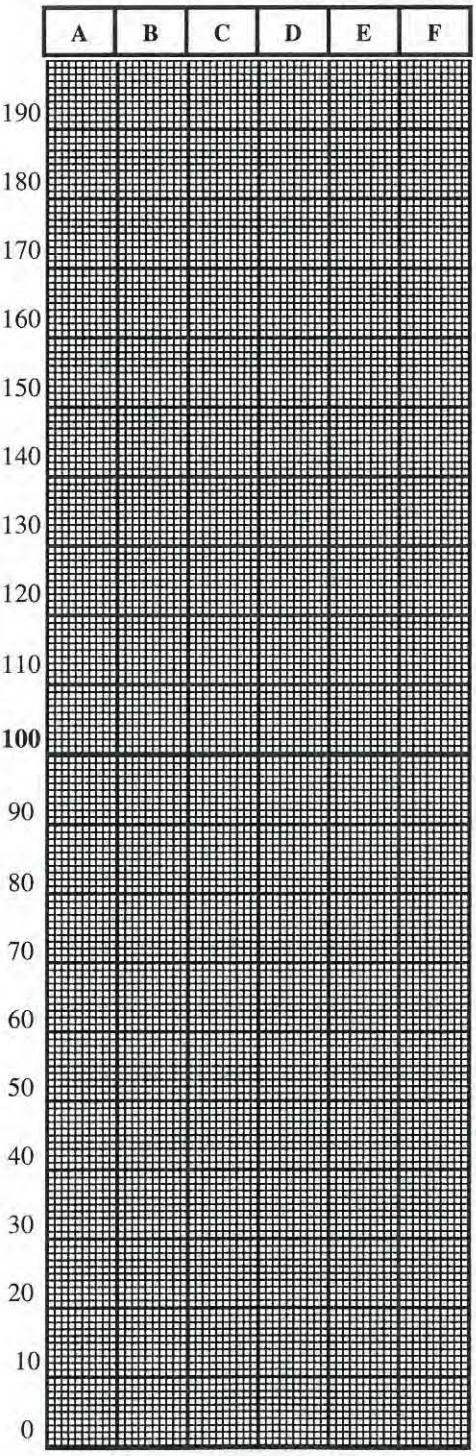
MA Credits in Months

Subscales:	A	B	C	D	E	F
YEARS I						*
II						*
III						
IV						
V						
VI						
VII						
VIII						
Extra Months						
Total MA's (months)						
CA (months)						
Raw Sub-Quotients MA X 100 / CA						
Standard Scores						
Percentiles						

Summary in Months

Subscales A to F Months	
I ... items passed / 175 X 12 =	
II ... items passed / 101 X 12 =	
III ... items passed / 3 =	
IV ... items passed / 3 =	
V ... items passed / 3 =	
VI ... items passed / 3 =	
VII ... items passed / 3 =	
VIII ... items passed / 3 =	
Extra ... items passed / 3 =	
Total MA (months) =	
CA (months) =	
Raw GQ =	
Standard Score =	
Percentile =	

Profile



Note: The Raw General Quotient or GQ is obtained by taking the average of all the six Sub-Quotients.

*The average of A, B, C, D and E.

Perpendicular Stroke
Item DII.53

Young Child's Attempt at Drawing
Items DI.21, 29, 32; DII.39, 44, 49

Horizontal Stroke
Items DII.54; DIII.2

SUBSCALE D

Appendix 6: TBM patients not eligible for study enrolment

This cohort of 30 patients had a median age of 4.3 years (6 months-12 years). Most patients presented with Stage I TBM (n= 14, 46.7%). HIV co-infection was recorded in 4 (13.3%) of the 28 patients who were tested. The culture positivity yield was 33.3% (n=10), 2 patients were drug resistant (6.7% - 1 had INH resistance, and 1 had INH and rifampicin resistance) and 2 patients (6.7%) had confirmed TB cultured from sputum. Three patients (10%) died during their hospital stay. Demographic, admission clinical and laboratory characteristics for the excluded patients are tabulated below.

Admission demographic and clinical characteristics for ineligible patients

Demographic characteristics		**Value**
Age		4.3 (0.5-12) years
	0-2 years	9 (30)
	3- 5 years	7 (23.33)
	>5 years	14 (46.67)
Gender: male/female		15/15
Admission characteristics		
Admission MRC Staging		
	1	14 (46.7)
	2a/2b	5 (16.7)/ 5 (16.7)
	3	6 (20)
Vaccination (n= 21)		17 (81)
TST (n= 20)		13 (65)
HIV infection (n= 28)		4 (13.3)
HIV exposure		6 (21.4)
Diagnostics		
Positive CSF TB culture		10 (33.33)
Confirmed TB in other specimens		2 (6.67)
In-hospital mortality		3 (10)
CSF chemistry		
Glucose (mmol/l)		2.55 (0.8-5.0)
Abnormal (< 2.3)		11 (36.67)
Chloride (mmol/l)		112 (95-126)
Abnormal (< 120)		19 (63.33)
Protein g/l [a]		0.77 (0.1-9.28)
Abnormal (> 0.45/ >0.8)		26 (86.67)
Polymorphonuclear Cells (/cu mm)		5.5 (0-365)
Lymphocytes (/cu mm)		93 (1-560)
Total white cell count		117.5 (1-700)
Abnormal (> 10)		28 (93.33)
Lymphocyte predominance		
>50%		25 (83.33)
>90%		14 (46.67)

Values reported as median and range, number (percent), CSF chemistry reported for admission LP, [a] Protein reference ranges are age dependent: >1 year and <1 year respectively

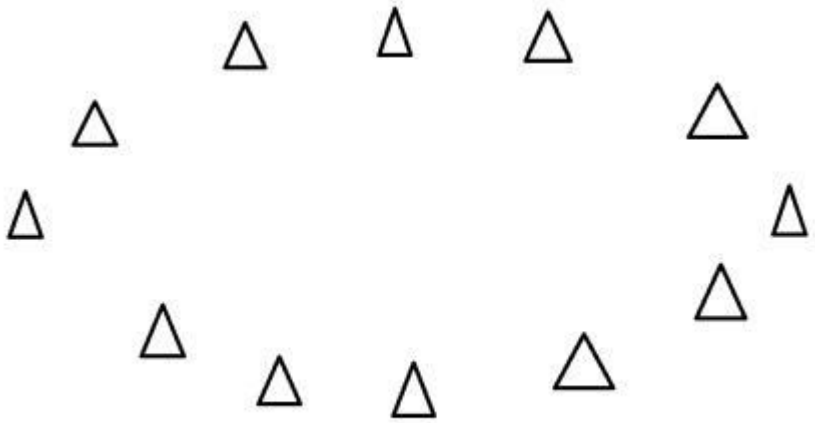
Imaging findings

Admission CT brain scans demonstrated HCP in 9 patients (30%), basal meningeal enhancement in 16 patients (64% - 5 patients did not have a contrast enhanced scan), pre-contrast hyperdensity in 4 patients (13.3%), tuberculomas in 3 patients (10%) and infarcts in 3 patients (10%). CXRs were suggestive of TB in 9 patients (32% - only 28 patients had a CXR). No evidence of miliary TB was reported.

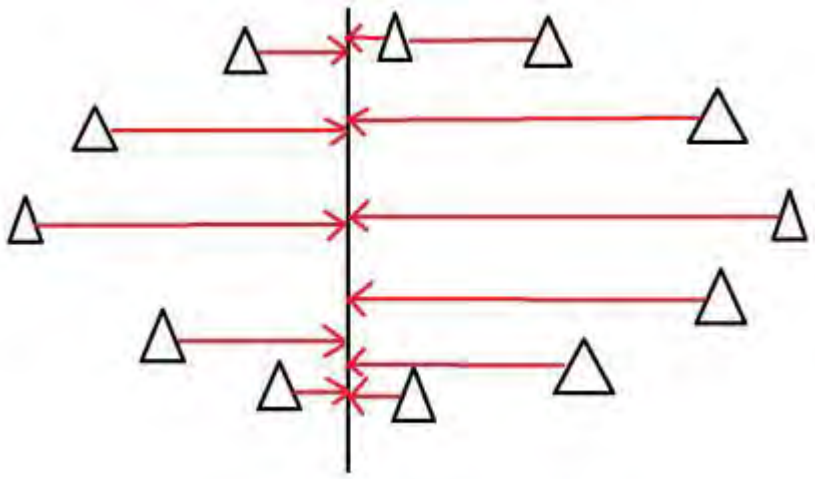
Appendix 7: What is Principal Component Analysis?

(Extracted from: http://georgemdallas.wordpress.com/2013/10/30/principal-component-analysis-4-dummies-eigenvectors-eigenvalues-and-dimension-reduction/)

It is often useful to measure data in terms of its principal components rather than on a normal x-y axis. So what are principal components then? They're the underlying structure in the data. They are the directions where there is the most variance, the directions where the data is most spread out. This is easiest to explain by way of example. Here's some triangles in the shape of an oval:

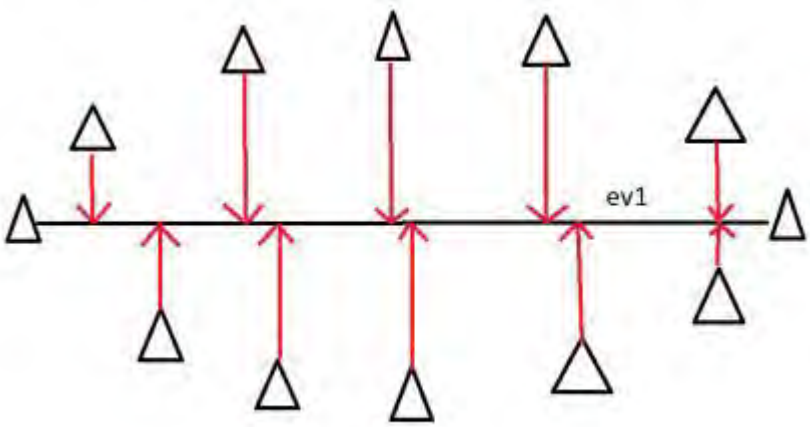


Imagine that the triangles are points of data. To find the direction where there is most variance, find the straight line where the data is most spread out when projected onto it. A vertical straight line with the points projected on to it will look like this:



The data isn't very spread out here, therefore it doesn't have a large variance. It is probably not the principal component.

A horizontal line are with lines projected on will look like this:



On this line the data is way more spread out, it has a large variance. In fact there isn't a straight line you can draw that has a larger variance than a horizontal one. A horizontal line is therefore the principal component in this example.

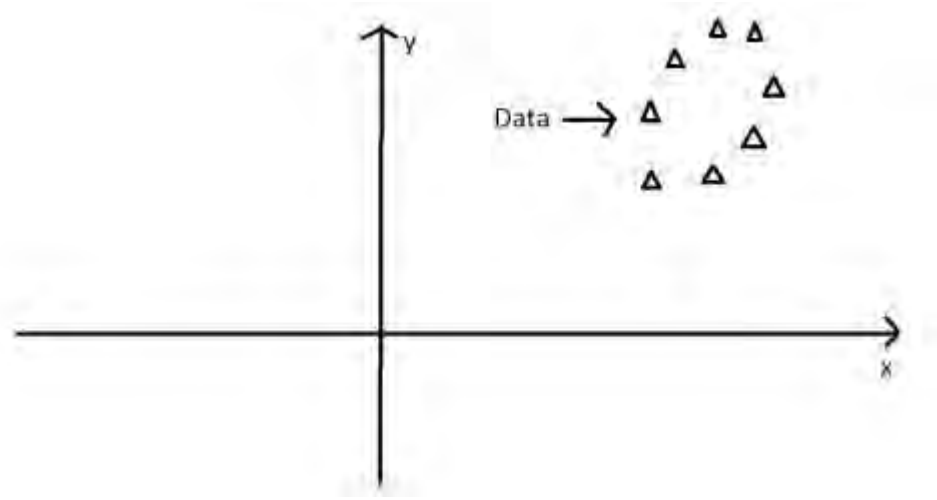
Luckily we can use maths to find the principal component rather than drawing lines and unevenly shaped triangles. This is where eigenvectors and eigenvalues come in.

Eigenvectors and Eigenvalues

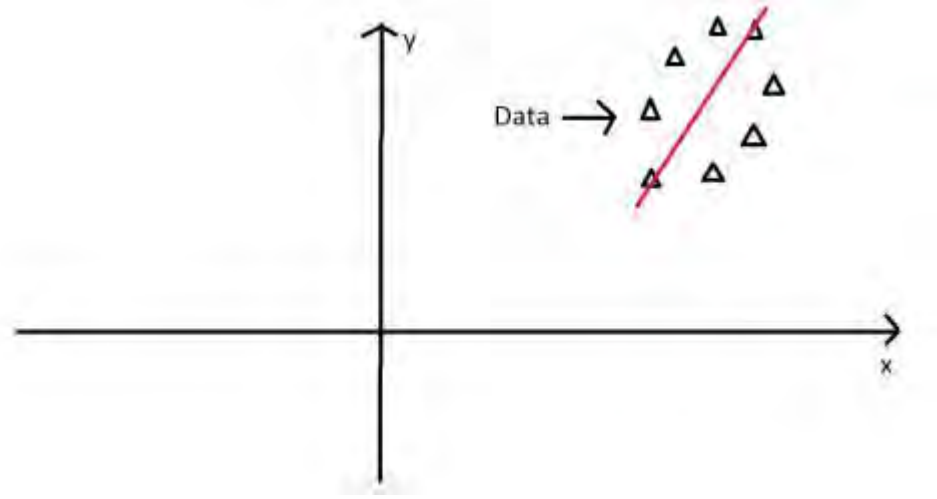
When we get a set of data points, like the triangles above, we can deconstruct the set into eigenvectors and eigenvalues. Eigenvectors and values exist in pairs: every eigenvector has a corresponding eigenvalue. An eigenvector is a direction, in the example above the eigenvector was the direction of the line (vertical, horizontal, 45 degrees etc.) . An eigenvalue is a number, telling you how much variance there is in the data in that direction, in the example above the eigenvalue is a number telling us how spread out the data is on the line. The eigenvector with the highest eigenvalue is therefore the principal component.

Okay, so even though in the last example I could point my line in any direction, it turns out there are not many eigenvectors/values in a data set. In fact the amount of eigenvectors/values that exist equals the number of dimensions the data set has. Say i'm measuring age and hours on the internet. there are 2 variables, it's a 2 dimensional data set, therefore there are 2 eigenvectors/values. If i'm measuring age, hours on internet and hours on mobile phone there's 3 variables, 3-D data set, so 3 eigenvectors/values. The reason for this is that eigenvectors put the data into a new set of dimensions, and these new dimensions have to be equal to the original amount of dimensions. This sounds complicated, but again an example should make it clear.

Here's a graph with the oval:



At the moment the oval is on an x-y axis. x could be age and y hours on the internet. These are the two dimensions that my data set is currently being measured in. Now remember that the principal component of the oval was a line splitting it longways:

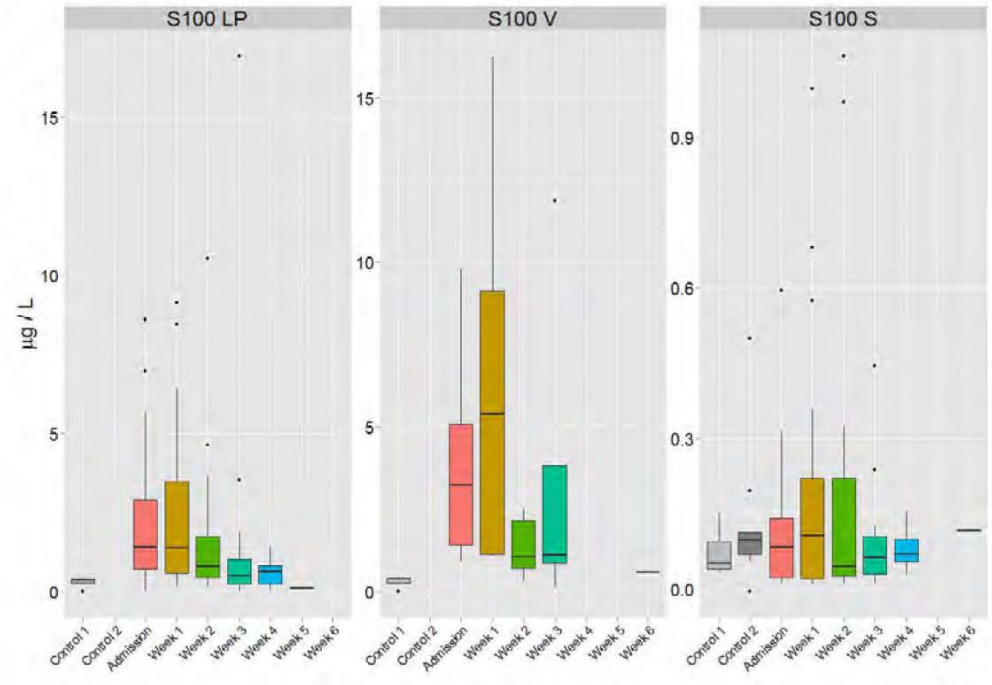


It turns out the other eigenvector (remember there are only two of them as it's a 2-D problem) is perpendicular to the principal component. As we said, the eigenvectors have to be able to span the whole x-y area, in order to do this (most effectively), the two directions need to be orthogonal (i.e. 90 degrees) to one another. This why the x and y axis are orthogonal to each other in the first place. It would be really awkward if the y axis was at 45 degrees to the x axis. So the second eigenvector would look like this:

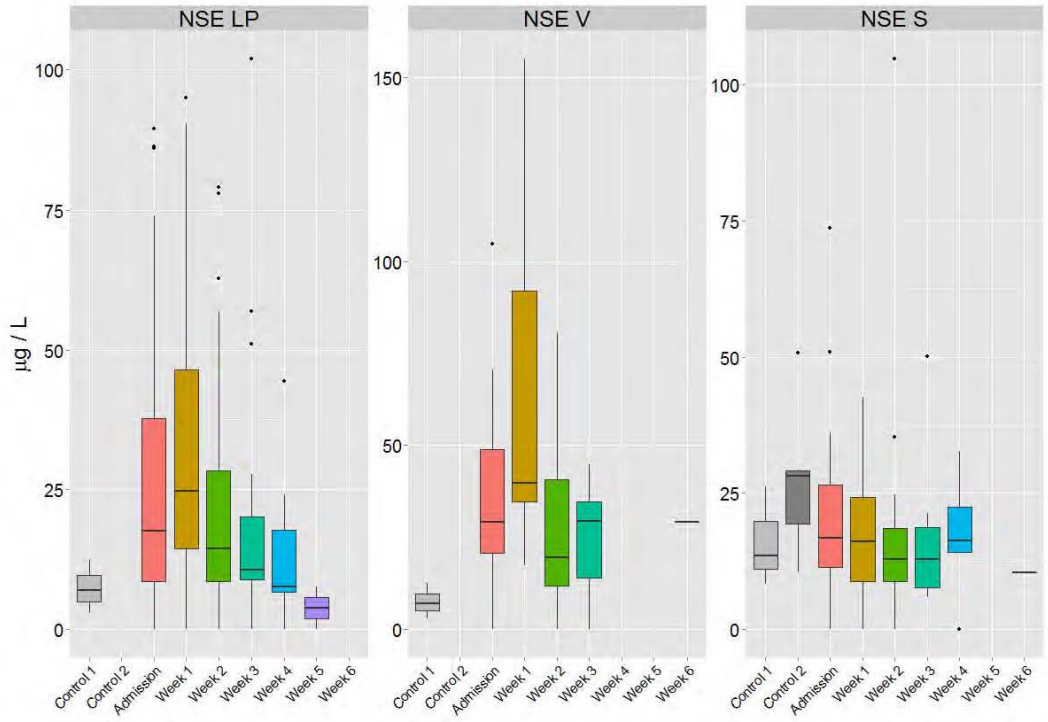
Appendix 8: Box-and-whisker plots over time, including outliers

Neuromarkers

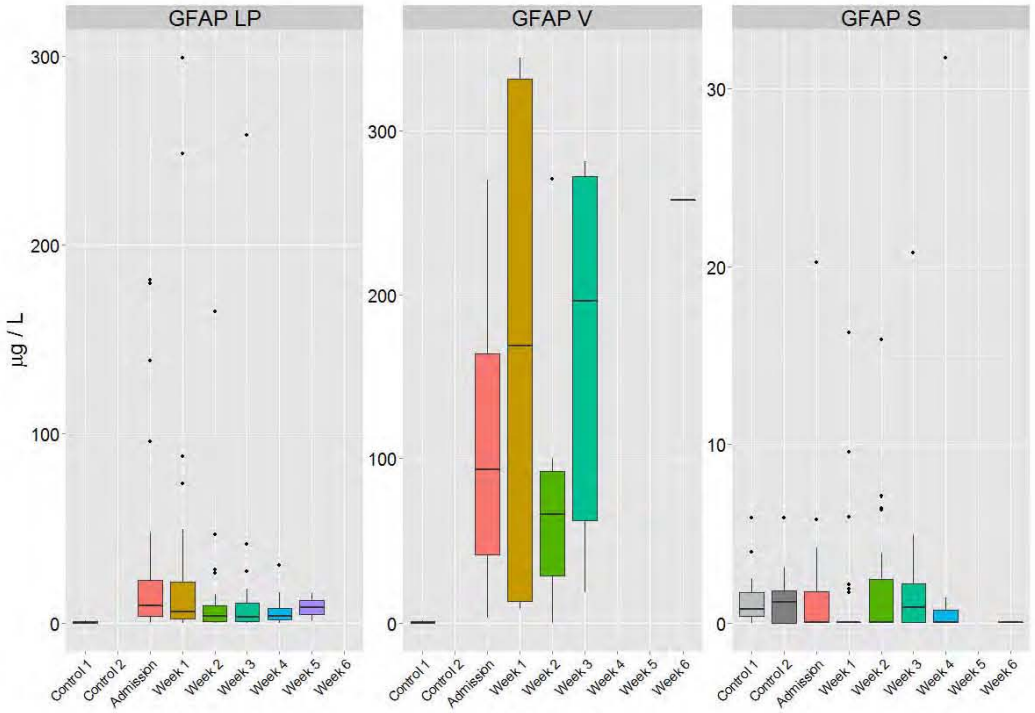
S100B



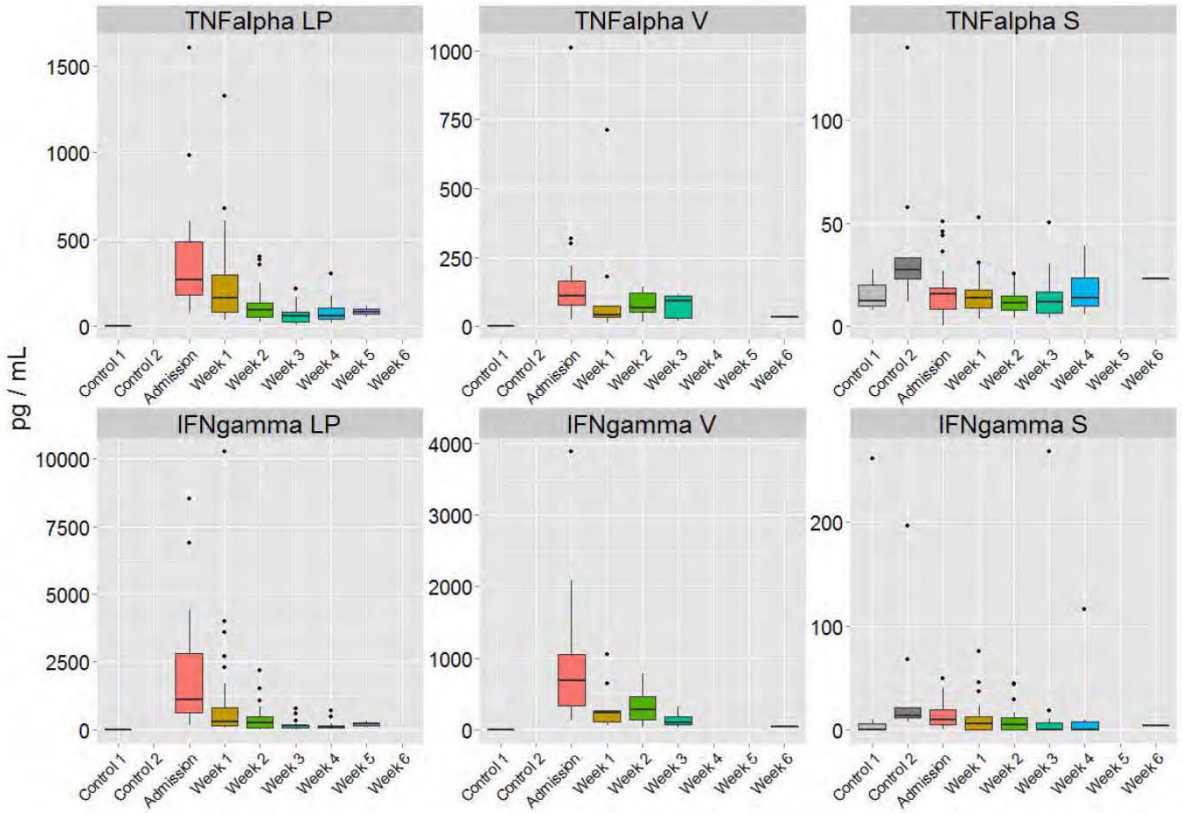
NSE

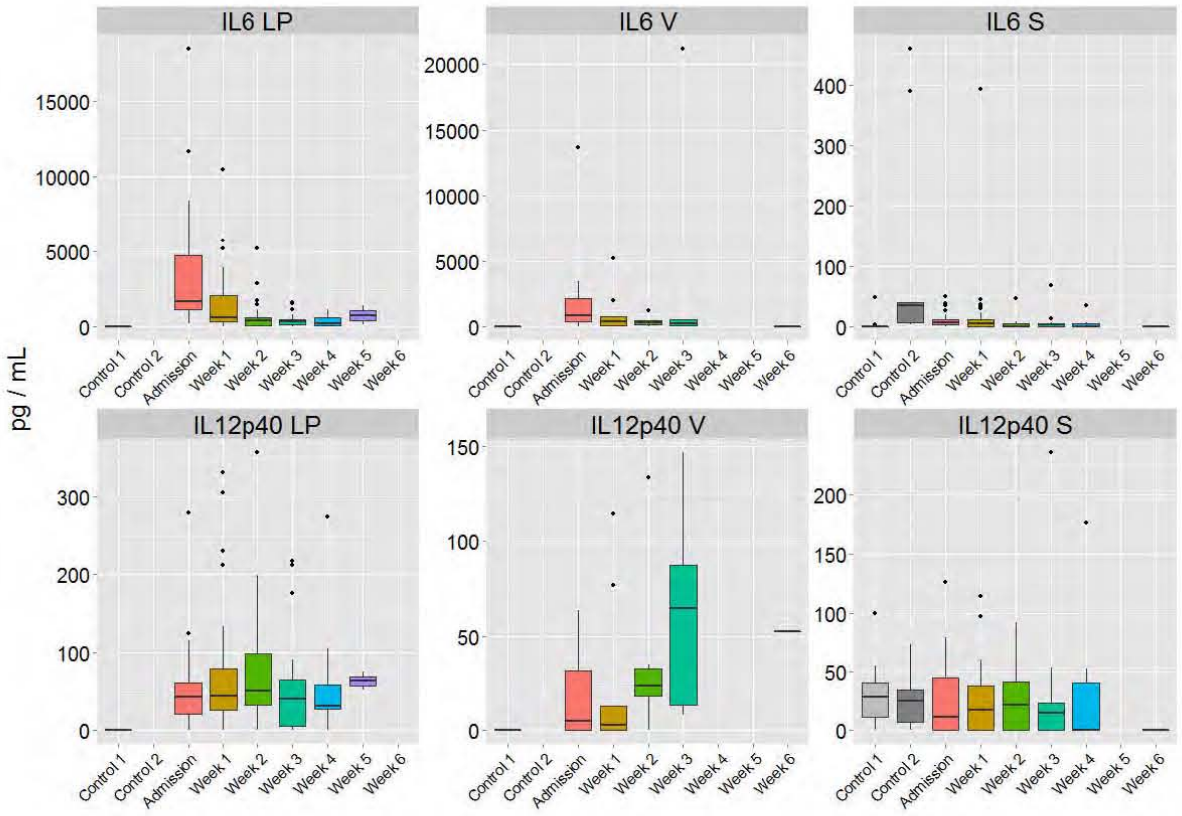


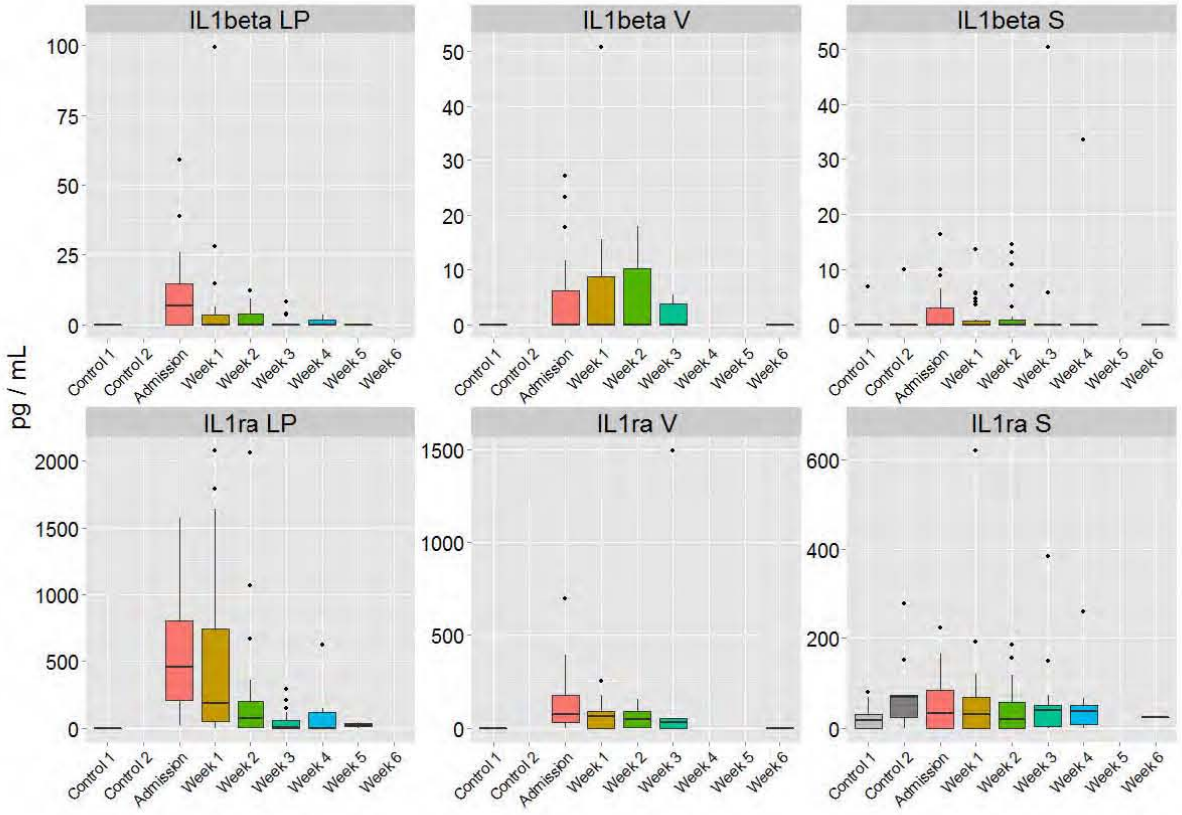
GFAP

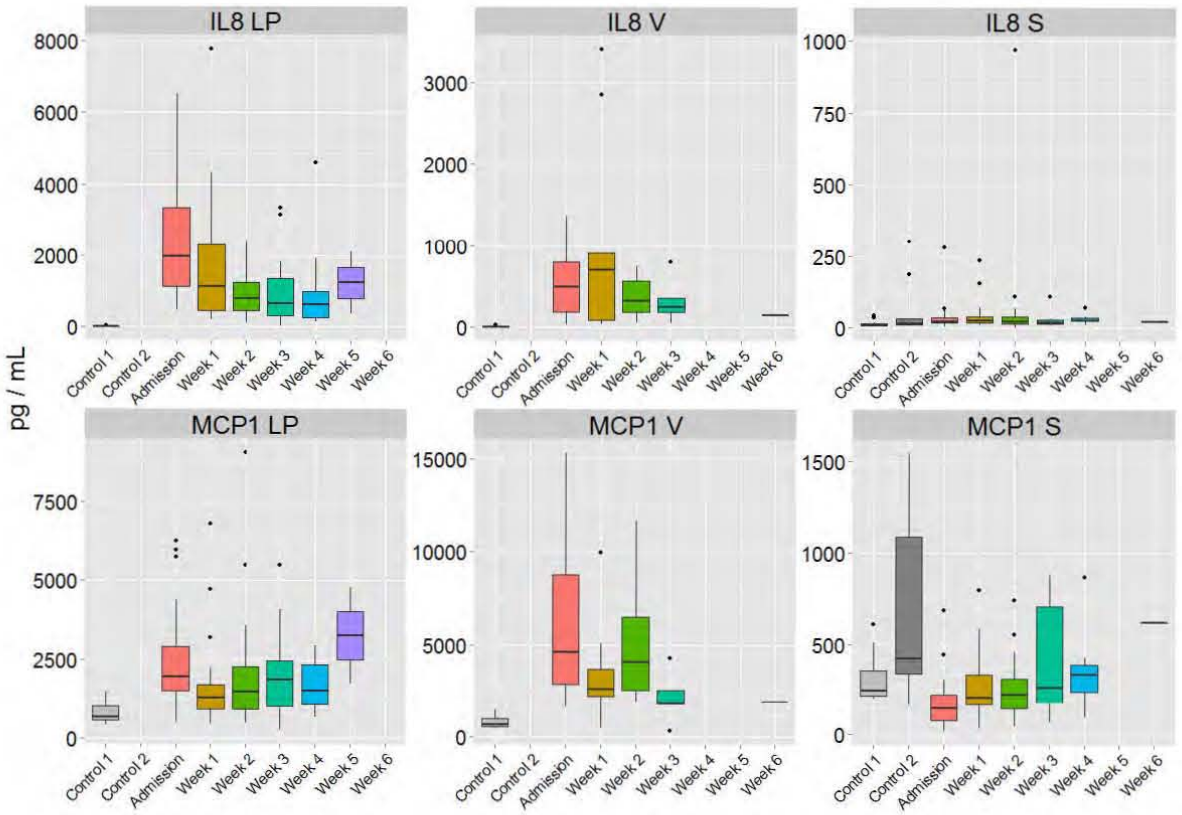


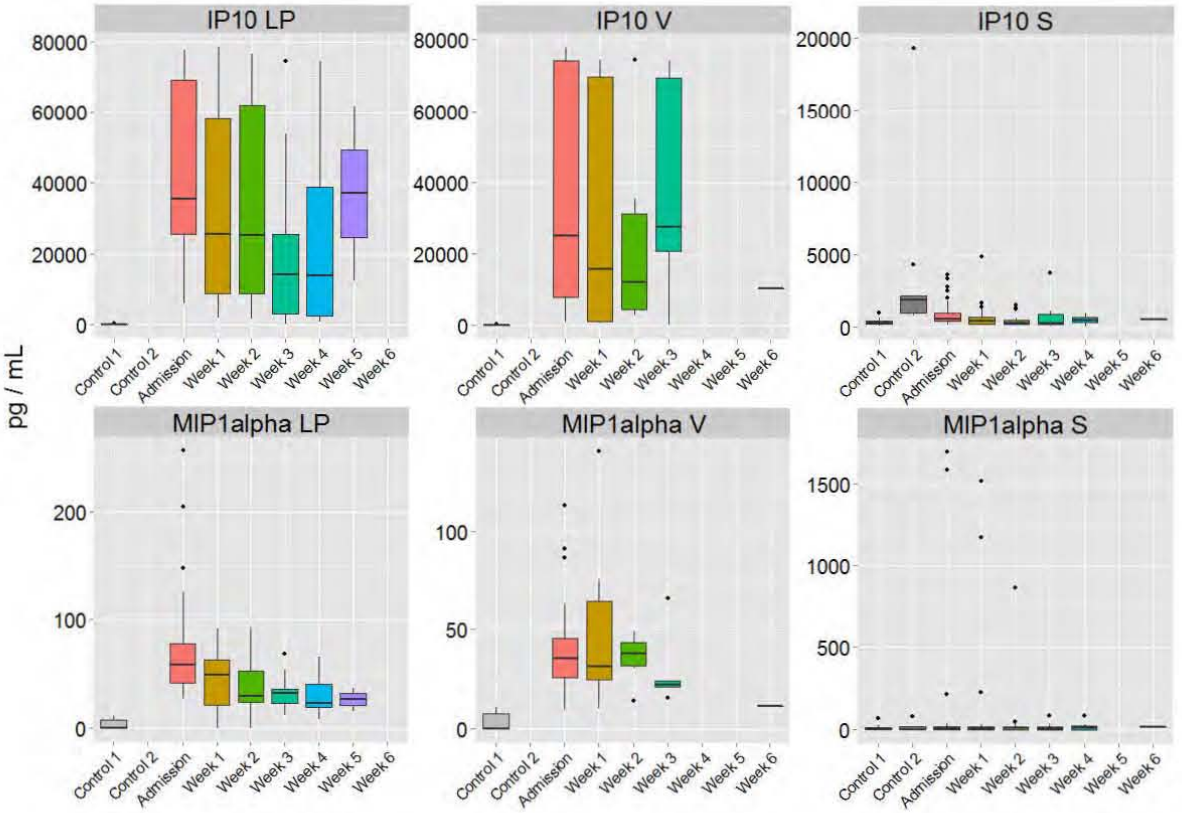
Inflammatory markers

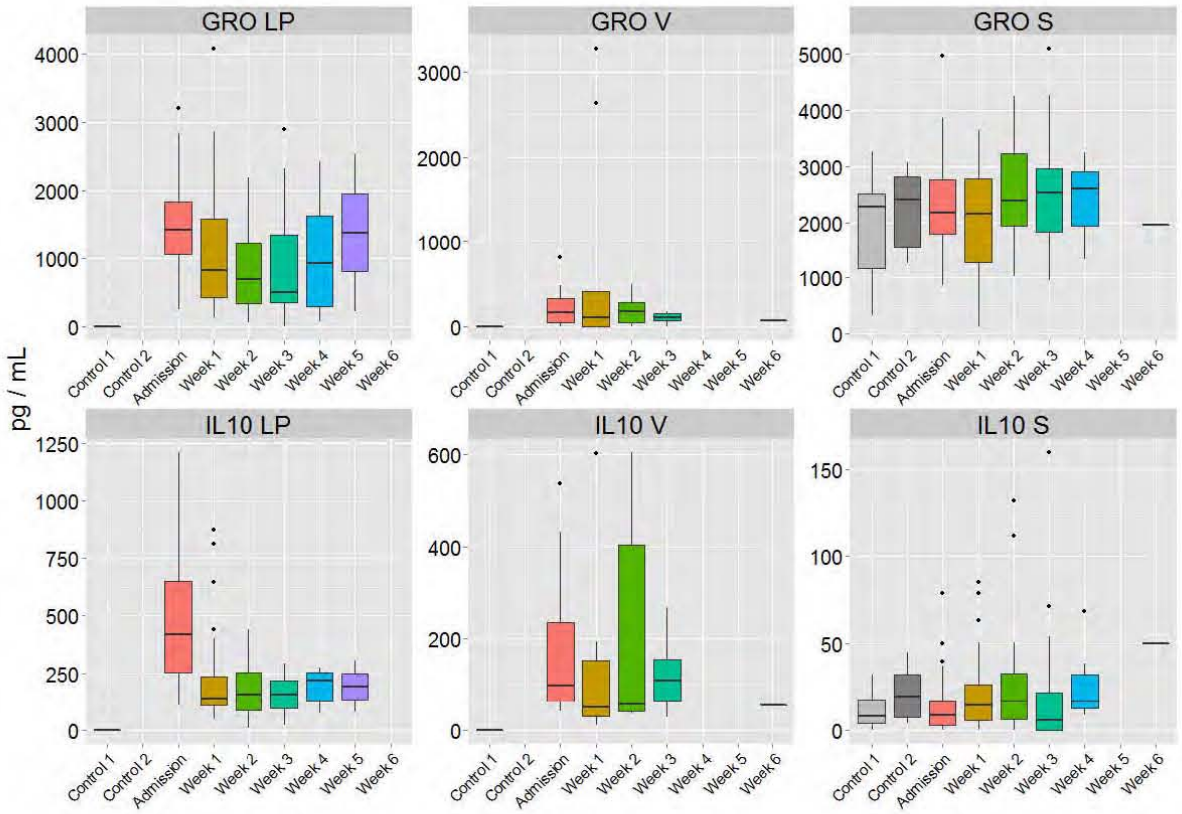


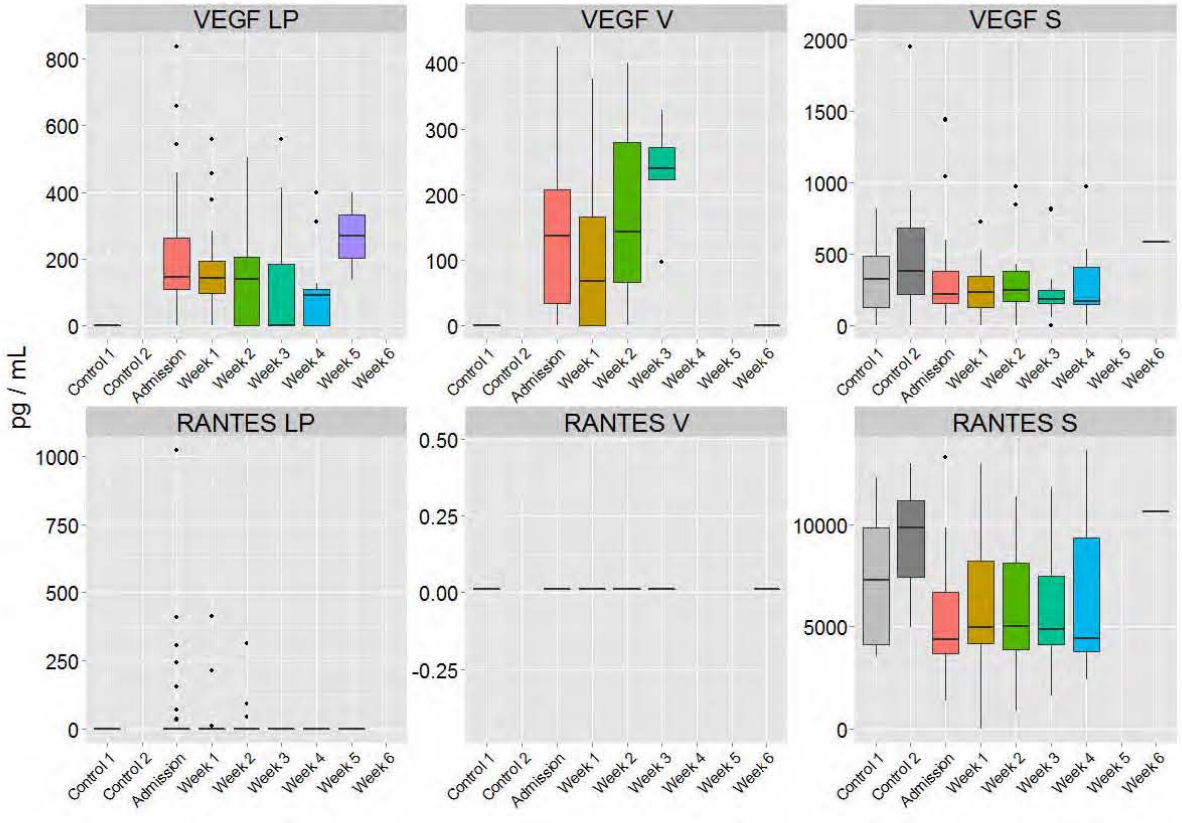

IL6 LP
IL6 V
IL6 S
IL12p40 LP
IL12p40 V
IL12p40 S
pg / mL
Control 1
Control 2
Admission
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6


IL1beta LP
IL1beta V
IL1beta S
IL1ra LP
IL1ra V
IL1ra S
pg / mL
Control 1
Control 2
Admission
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6


IL8 LP
IL8 V
IL8 S
MCP1 LP
MCP1 V
MCP1 S
pg / mL
Control 1
Control 2
Admission
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6


IP10 LP
IP10 V
IP10 S
MIP1alpha LP
MIP1alpha V
MIP1alpha S
pg / mL
Control 1
Control 2
Admission
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6


GRO LP
GRO V
GRO S
IL10 LP
IL10 V
IL10 S
pg / mL
Control 1
Control 2
Admission
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6


VEGF LP
VEGF V
VEGF S
RANTES LP
RANTES V
RANTES S
pg / mL
Control 1
Control 2
Admission
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6

Appendix 9: GEE coefficients for biomarker concentrations according to sample type

Sample type	S100B		NSE		GFAP	
Rostro-caudal gradient overall						
	Coef	**CI**	**Coef**	**CI**	**Coef**	**CI**
Vent CSF	2.2	1.47-2.93	13.55	5.99-21.1	91.18	71.22-111.14
Serum	-1.59	1.22-2.38	-7.19	-12.83- -1.54	-21.59	-36.07- -7.11
Patients with spinal arachnoiditis						
Vent CSF	1.18	0.46-2.33	8.9	-1.9-19.9	87.74	56.48-119

Coef = GEE coefficient – coefficients represent number of μg/L by which ventricular CSF (vent CSF) is greater (positive coefficient) than lumbar CSF concentrations, and the number of μg/L by which serum is less than lumbar CSF concentrations (negative coefficient). All coefficients were $p < 0.05$ except for NSE in patients with spinal arachnoiditis – lumbar and ventricular CSF were no longer significantly different in those patients. The GEE compared ventricular CSF and blood to lumbar CSF as the constant in the model.

Appendix 10: Correlation matrix: Neuro- and inflammatory markers

	S100	NSE	GFAP	GRO	IL-1ra	IL-12p40	IP-10	MCP-1	MIP1-α	TNF-α	VEGF	IFN-γ	IL-10	IL-1β	IL-6	IL-8	RANTES
S100	1.00																
NSE	0.62	1.00															
p-value	*0.00*																
GFAP	-0.12	-0.18	1.00														
p-value	*0.53*	*0.33*															
GRO	0.06	0.03	0.36	1.00													
p-value	*0.74*	*0.86*	*0.05*														
IL-1ra	0.38	0.56	-0.05	-0.01	1.00												
p-value	*0.04*	*0.00*	*0.77*	*0.94*													
IL-12p40	0.30	0.26	0.20	0.23	0.65	1.00											
p-value	*0.11*	*0.17*	*0.28*	*0.22*	*0.00*												
IP-10	0.30	0.23	-0.02	-0.12	0.29	0.25	1.00										
p-value	*0.11*	*0.22*	*0.94*	*0.53*	*0.12*	*0.19*											
MCP-1	0.03	0.22	-0.24	-0.43	0.33	0.02	0.23	1.00									
p-value	*0.87*	*0.25*	*0.20*	*0.02*	*0.08*	*0.92*	*0.22*										
MIP1-α	0.01	0.14	0.30	0.19	0.08	0.07	0.05	0.08	1.00								
p-value	*0.95*	*0.45*	*0.10*	*0.33*	*0.69*	*0.70*	*0.80*	*0.67*									
TNF-α	0.24	0.53	0.13	0.16	0.65	0.57	0.32	0.24	0.27	1.00							
p-value	*0.20*	*0.00*	*0.48*	*0.41*	*0.00*	*0.00*	*0.08*	*0.21*	*0.14*								
VEGF	0.34	0.55	0.14	0.26	0.58	0.54	0.13	-0.04	0.41	0.39	1.00						
p-value	*0.06*	*0.00*	*0.47*	*0.16*	*0.00*	*0.00*	*0.51*	*0.84*	*0.02*	*0.03*							
IFN-γ	0.06	0.17	-0.03	-0.23	0.36	0.45	0.58	0.31	-0.07	0.49	0.28	1.00					
p-value	*0.74*	*0.36*	*0.87*	*0.23*	*0.05*	*0.01*	*0.00*	*0.10*	*0.69*	*0.01*	*0.13*						
IL-10	0.24	0.35	0.06	0.12	0.30	0.60	0.26	-0.10	0.11	0.39	0.44	0.40	1.00				
p-value	*0.20*	*0.06*	*0.74*	*0.52*	*0.10*	*0.00*	*0.17*	*0.60*	*0.58*	*0.04*	*0.02*	*0.03*					
IL-1β	0.18	0.11	-0.21	0.05	0.40	0.48	0.04	-0.09	-0.26	0.50	-0.05	0.24	0.31	1.00			
p-value	*0.35*	*0.57*	*0.28*	*0.80*	*0.03*	*0.01*	*0.84*	*0.64*	*0.16*	*0.00*	*0.78*	*0.20*	*0.10*				
IL-6	0.37	0.15	-0.23	-0.05	0.25	0.28	0.59	0.38	-0.19	0.13	0.03	0.61	0.18	0.18	1.00		
p-value	*0.04*	*0.44*	*0.23*	*0.80*	*0.19*	*0.13*	*0.00*	*0.04*	*0.31*	*0.49*	*0.89*	*0.00*	*0.33*	*0.34*			
IL-8	0.15	0.22	0.05	-0.06	0.34	0.14	0.31	0.22	0.00	0.20	0.14	0.39	0.21	-0.09	0.39	1.00	
p-value	*0.44*	*0.24*	*0.81*	*0.74*	*0.07*	*0.46*	*0.09*	*0.24*	*0.99*	*0.29*	*0.44*	*0.03*	*0.26*	*0.62*	*0.03*		
RANTES	0.22	0.29	0.02	0.29	0.23	0.10	0.05	-0.04	0.09	0.18	0.56	0.10	0.08	-0.21	-0.02	0.08	1.00
p-value	*0.23*	*0.12*	*0.92*	*0.13*	*0.21*	*0.59*	*0.79*	*0.81*	*0.63*	*0.33*	*0.00*	*0.59*	*0.66*	*0.26*	*0.91*	*0.66*	

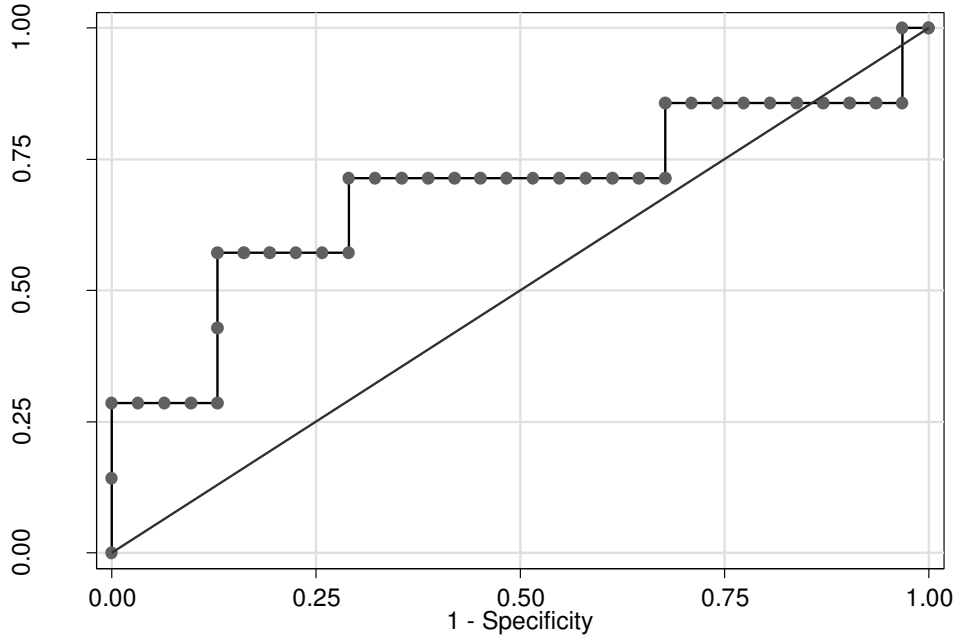
This matrix demonstrates the correlation coefficients and p-values for correlations between neuro- and inflammatory markers, significant correlations marked in red

Appendix 11: Summary of neuromarker univariate outcome analysis p-values

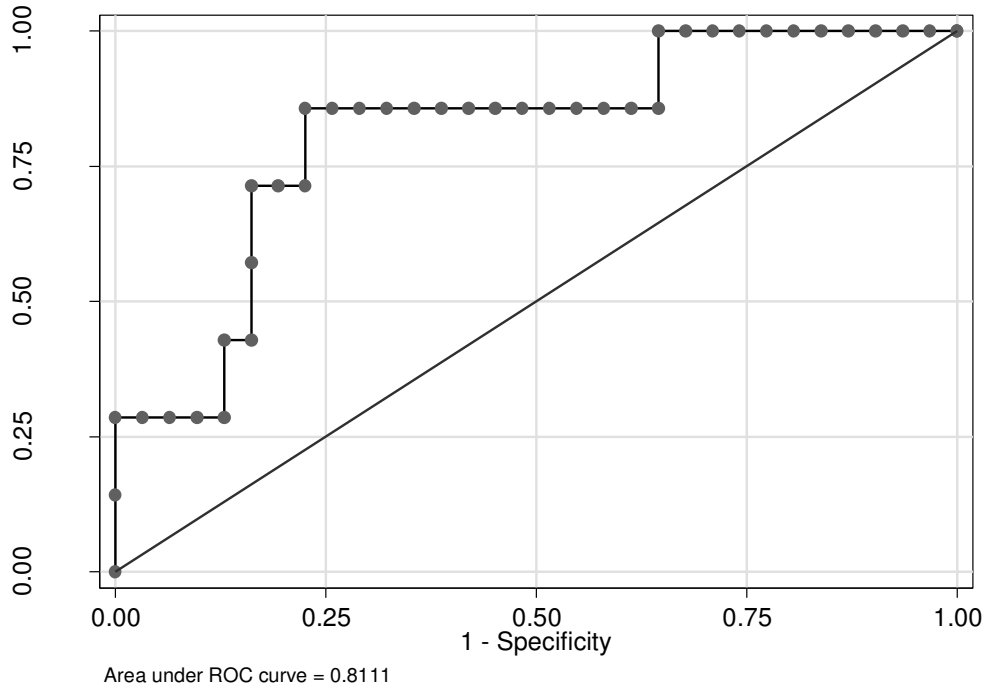
		Mortality	Outcome	Morbidity 3 month	6 months
S100B					
Lumbar	Initial	0.42	0.15	0.03*	0.26
Ventricular		0.02*	0.01*	0.08	0.18
Lumbar	Highest in week 1	0.19	0.04*	0.03*	0.18
Ventricular		0.03*	0.01*	0.08	0.18
Lumbar	Highest overall	0.13	0.03*	0.04*	0.2
Ventricular		0.02*	0.06	0.65	0.8
Lumbar	Absolute Δ	<0.001	0.02	0.79	0.6
NSE					
Lumbar	Initial	0.18	0.07	0.03*	0.02*
Ventricular		0.01*	0.01*	0.28	0.32
Lumbar	Highest in week 1	0.18	0.08	0.05*	0.02*
Ventricular		0.003*	0.01*	0.3	0.18
Lumbar	Highest overall	0.01*	0.01*	0.04*	0.02*
Ventricular		0.01*	0.01*	0.13	0.44
Lumbar	Absolute Δ	0.01	0.02	0.24	0.14
GFAP					
Lumbar	Initial	0.53	0.59	*0.06*	0.09
Ventricular		0.23	0.27	0.96	0.9
Lumbar	Highest in week 1	0.07	0.12	0.07	0.09
Ventricular		0.08	0.14	0.98	0.95
Lumbar	Highest overall	0.01*	0.07	0.02*	0.06
Ventricular		0.09	0.33	0.92	0.96
Lumbar	Absolute Δ	0.003	*0.08*	0.96	0.6

Appendix 12: ROC curves: Analysis neuromarkers and mortality

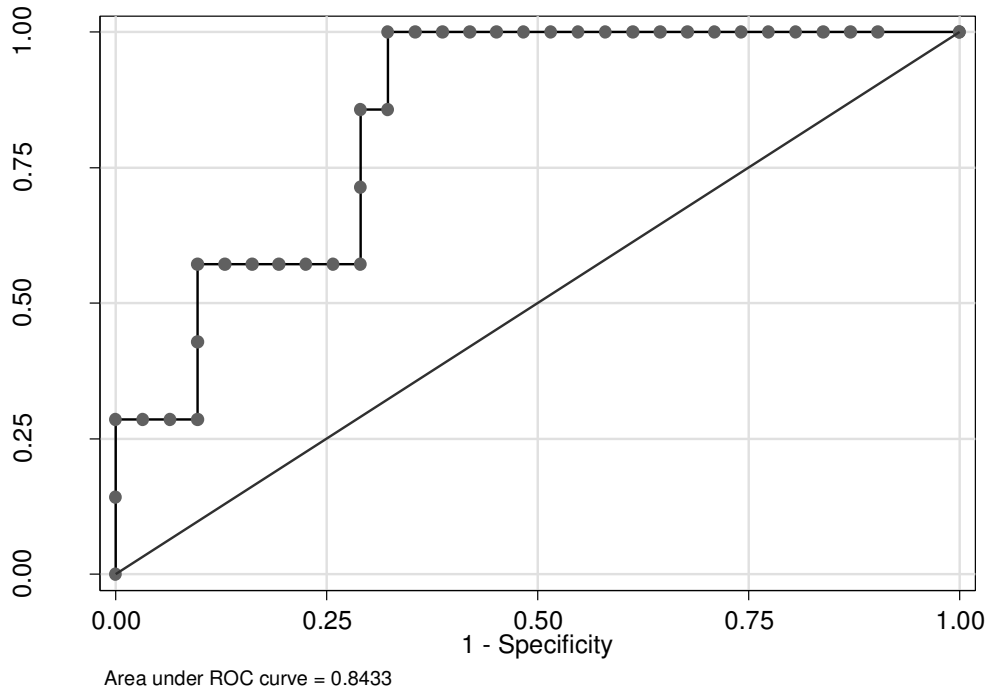
ROC S100B and Mortality



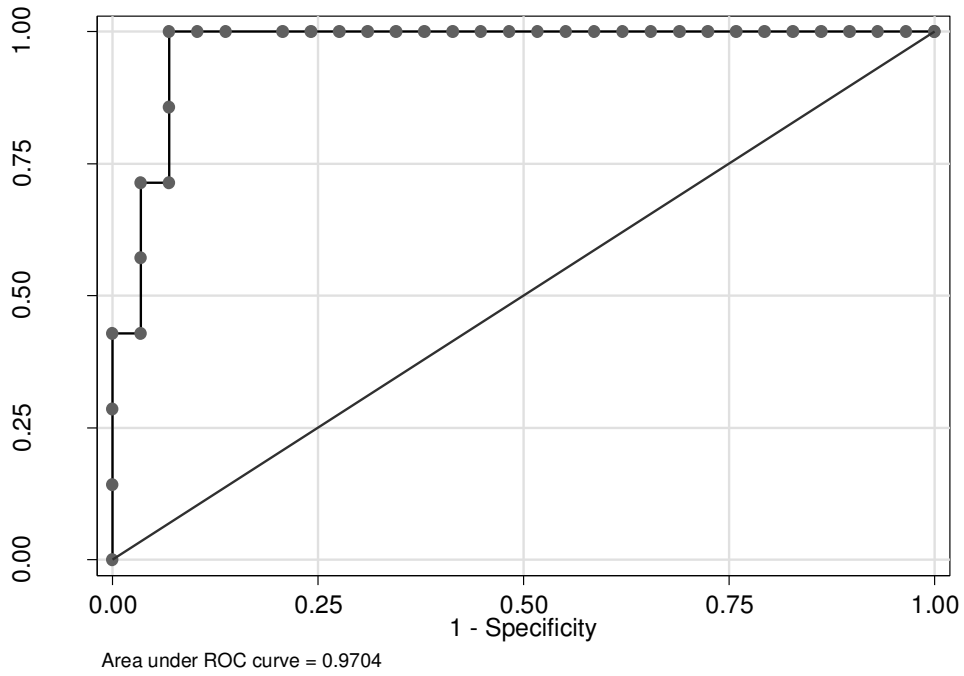
ROC NSE and Mortality



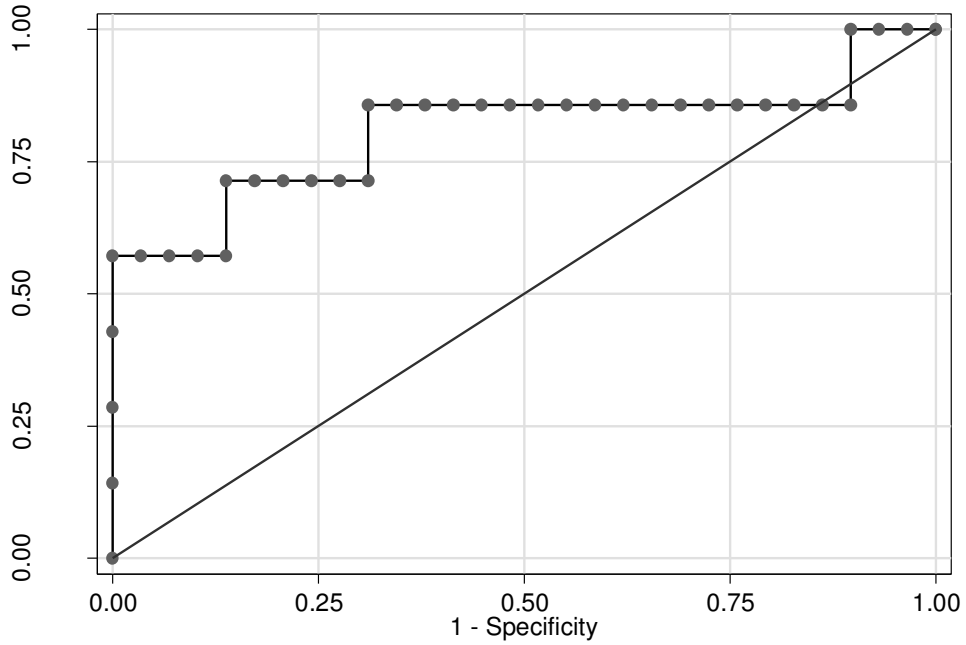
ROC: GFAP and mortality



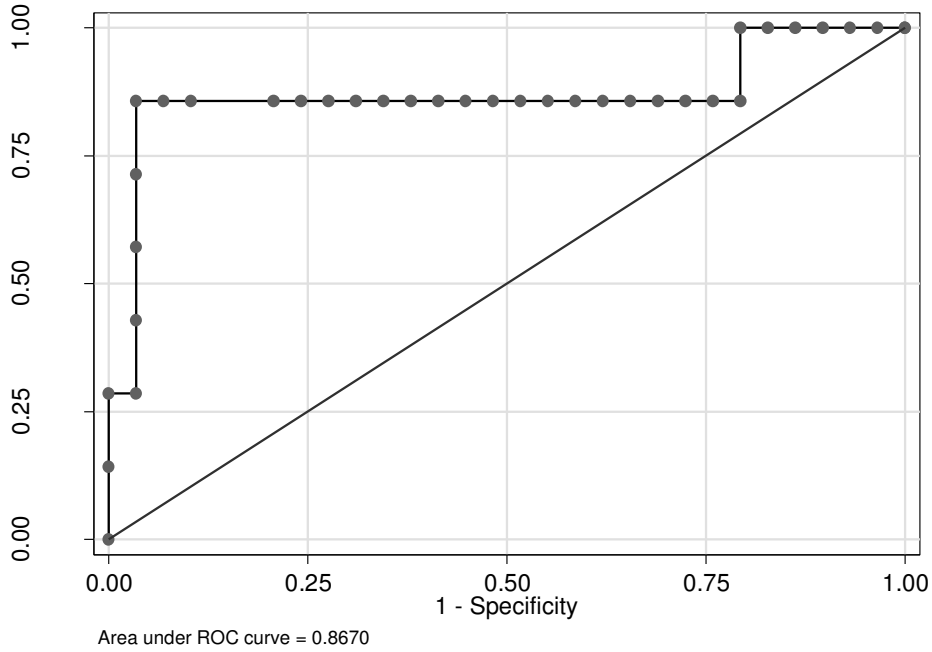
ROC Δ S100B and Mortality



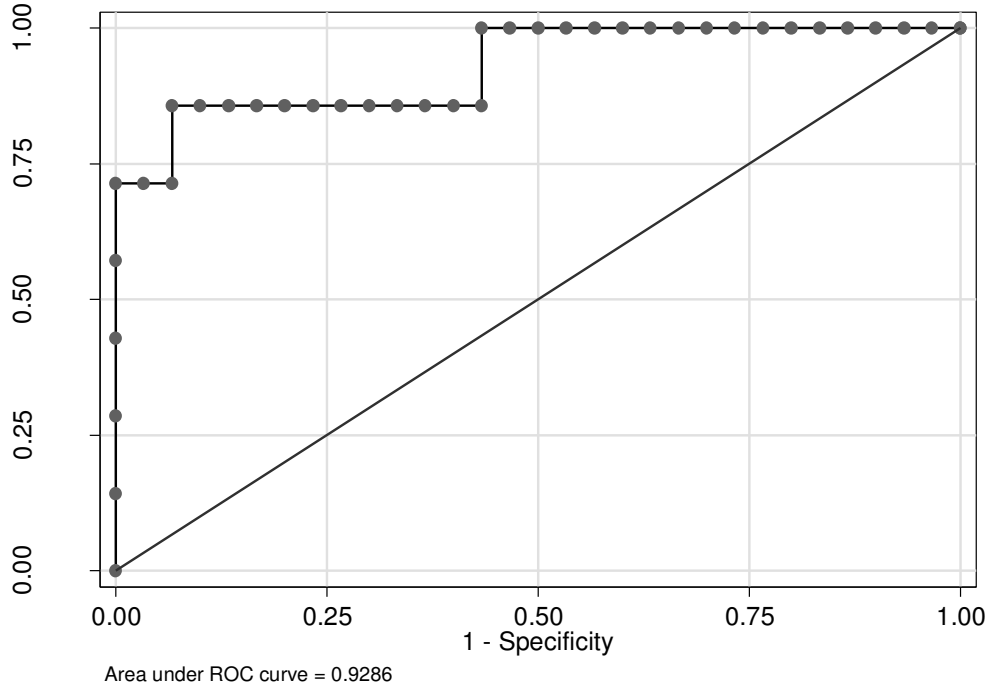
ROC Δ NSE and mortality



ROC Δ GFAP and Mortality



ROC Z-Neuro Δ and Mortality



Appendix 13: p-values for the association between neuromarkers and admission radiology

Admission CT	S100B		NSE		GFAP		Z-Neuro	
	LP	**V**	**LP**	**V**	**LP**	**V**	**LP**	**V**
HCP severity	*0.05**	*0.56*	*0.22*	*0.93*	*0.01**	*0.05**	*0.01**	*0.6*
Basal enhancement	*0.63*	*0.29*	*0.89*	*0.55*	*0.94*	*0.23*	*0.68*	*0.29*
Enhancement severity	*0.37*	*0.26*	*0.26*	*0.38*	*0.54*	*0.23*	*0.61*	*0.2*
Tuberculoma (n=3)	*0.27*	*x*	*0.29*	*x*	*0.06*	*x*	*0.09*	*x*
Infarcts (n=3)	*0.4*	*0.65*	*0.33*	*0.24*	*0.64*	*0.55*	*0.61*	*0.24*

The relationship between ventricular neuromarkers and tuberculomas could not be investigated as no patients with ventricular samples had tuberculomas

Appendix 14: p-values for the relationship between overall radiology features and neuromarker concentrations

	S100B			NSE			GFAP			Z-NeuroΔ
	Highest		Δ	Highest		Δ	Highest		Δ	
	LP	V		LP	V		LP	V		
HCP severity	*0.06*	*0.4*	*0.44*	*0.24*	*0.5*	*0.55*	*0.02**	*0.53*	*0.63*	*0.66*
Infarcts	*0.9*	*0.39*	*0.12*	*0.8*	*0.62*	*0.03**	*0.05**	*0.14*	*0.01**	*0.02**
Uni/bilat	*0.1*	*0.07*	*0.04**	*0.02**	*0.01**	*0.08*	*0.01**	*0.18*	*0.06*	*0.06*
Single/multi	*0.09*	*0.46*	*0.08*	*0.002**	*0.02**	*0.09*	*0.02**	*0.28*	*0.18*	*0.08*
Size	*0.37*	*0.17*	*0.02**	*0.02**	*0.003**	*0.03**	*0.002**	*0.06*	*0.01**	*0.01**
MRA pathology	*0.87*	*0.22*	*0.19*	*0.83*	*0.39*	*0.07*	*0.62*	*0.84*	*0.67*	*0.17*
Tuberculoma	*0.09*	*0.29*	*0.1*	*0.94*	*0.27*	*0.17*	*0.6*	*0.7*	*0.1*	*0.19*
Uni/bilat	*0.85*	*0.12*	*0.84*	*0.1*	*0.83*	*0.77*	*0.05**	*0.1*	*0.03**	*0.49*
Single/multi	*0.76*	*0.13*	*0.69*	*1*	*0.66*	*0.82*	*0.04**	*0.2*	*0.02**	*0.3*
Spinal disease	*0.67*	*0.13*	*1*	*0.26*	*0.59*	*0.56*	*0.28*	*0.82*	*0.95*	*0.51*
Arachnoiditis	*0.96*	*0.08*	*0.53*	*0.24*	*0.64*	*0.73*	*0.59*	*0.78*	*0.57*	*0.89*
Tuberculoma	*0.87*	*0.78*	*0.55*	*0.15*	*0.74*	*0.59*	*0.62*	*0.62*	*0.54*	*0.91*

Appendix 15: Prevalence of compromised PbtO2 values between outcome groups

Pt No	Outcome	$PbtO2_{24}$	Lowest PbtO2	T<5	T<10	T<20	Days
1	0	5.43	1.29	2556	5831	22179	4
2	0	3.93	1.78	5436	18326	23072	4
3	0	6.77	1.27	2813	8850	18130	6
4	1	23.05	4.49	86	578	5540	6
5	1	4.66	3.97	252	3064	12078	6
6	1	17.30	7.38	0	1010	14485	5

This table demonstrates the median PbtO2 recorded within the first 24 hours ($PbtO2_{24}$), the lowest PbtO2 recorded during the monitoring period, and the time spent with PbtO2 <5mmHg, < 10mmHg and < 20mmHg for patients with poor (0) and good outcomes (1). The duration of monitoring is also included.

References

1. Kumar V, Abbas A, Fausto N, Aster J. *Robbins and Cotran Pathologic Basis of Disease.* 8th ed. Philadelphia, PA: Saunders Elsevier; 2010.

2. Coico R, Sunshine G. *Immunology: A Short Course.* 6th ed. Hoboken, New Jersey: Wiley & Sons, Inc; 2009.

3. Donald PR, Schaaf HS, Schoeman JF. Tuberculous meningitis and miliary tuberculosis: the Rich focus revisited. *J Infect*. 2005;50(3):193-195.

4. van den Bos F, Terken M, Ypma L, et al. Tuberculous meningitis and miliary tuberculosis in young children. *Trop Med Int Health*. 2004;9(2):309-313.

5. Thwaites GE, van Toorn R, Schoeman J. Tuberculous meningitis: more questions, still too few answers. *Lancet Neurol*. 2013;12(10):999-1010.

6. Castro-Garza J, King CH, Swords WE, Quinn FD. Demonstration of spread by Mycobacterium tuberculosis bacilli in A549 epithelial cell monolayers. *FEMS Microbiol Lett*. 2002;212(2):145-149.

7. Krishnan N, Robertson BD, Thwaites G. The mechanisms and consequences of the extra-pulmonary dissemination of Mycobacterium tuberculosis. *Tuberculosis (Edinb)*. 2010;90(6):361-366.

8. Jain SK, Paul-Satyaseela M, Lamichhane G, Kim KS, Bishai WR. Mycobacterium tuberculosis invasion and traversal across an in vitro human blood-brain barrier as a pathogenic mechanism for central nervous system tuberculosis. *J Infect Dis*. 2006;193(9):1287-1295.

9. Nguyen L, Pieters J. The Trojan horse: survival tactics of pathogenic mycobacteria in macrophages. *Trends Cell Biol*. 2005;15(5):269-276.

10. Hawn TR, Dunstan SJ, Thwaites GE, et al. A polymorphism in Toll-interleukin 1 receptor domain containing adaptor protein is associated with susceptibility to meningeal tuberculosis. *J Infect Dis*. 2006;194(8):1127-1134.

11. Caws M, Thwaites G, Dunstan S, et al. The influence of host and bacterial genotype on the development of disseminated disease with Mycobacterium tuberculosis. *PLoS Pathog*. 2008;4(3):e1000034.

12. Fernando SL, Saunders BM, Sluyter R, et al. A polymorphism in the P2X7 gene increases susceptibility to extrapulmonary tuberculosis. *Am J Respir Crit Care Med*. 2007;175(4):360-366.

13. Caws M, Thwaites G, Stepniewska K, et al. Beijing genotype of Mycobacterium tuberculosis is significantly associated with human immunodeficiency virus infection and multidrug resistance in cases of tuberculous meningitis. *J Clin Microbiol*. 2006;44(11):3934-3939.

14. Visser DH, Schoeman JF, VAN Furth AM. Seasonal variation in the incidence rate of tuberculous meningitis is associated with sunshine hours. *Epidemiol Infect*. 2012;31:1-4.

15. Be NA, Kim KS, Bishai WR, Jain SK. Pathogenesis of central nervous system tuberculosis. *Curr Mol Med*. 2009;9(2):94-99.

16. Pulzova L, Bhide MR, Andrej K. Pathogen translocation across the blood-brain barrier. *FEMS Immunol Med Microbiol*. 2009;57(3):203-213.

17. Be NA, Bishai WR, Jain SK. Role of Mycobacterium tuberculosis pknD in the pathogenesis of central nervous system tuberculosis. *BMC Microbiol*. 2012;12:7-2180-12-7.

18. Tauber MG, Moser B. Cytokines and chemokines in meningeal inflammation: biology and clinical implications. *Clin Infect Dis*. 1999;28(1):1-11.

19. Rock RB, Olin M, Baker CA, Molitor TW, Peterson PK. Central nervous system tuberculosis: pathogenesis and clinical aspects. *Clin Microbiol Rev*. 2008;21(2):243-61.

20. Rich A, McCordock H. The Pathogenesis of Tuberculous Meningitis. *Bull John Hopkins Hosp*. 1933;52:5-37.

21. Dastur DK, Manghani DK, Udani PM. Pathology and pathogenetic mechanisms in neurotuberculosis. *Radiol Clin North Am*. 1995;33(4):733-752.

22. Lamprecht D, Schoeman J, Donald P, Hartzenberg H. Ventriculoperitoneal shunting in childhood tuberculous meningitis. *Br J Neurosurg*. 2001;15(2):119-125.

23. Donald PR, Schoeman JF. Tuberculous meningitis. *N Engl J Med*. 2004;351(17):1719-1720.

24. Bernaerts A, Vanhoenacker FM, Parizel PM, et al. Tuberculosis of the central nervous system: overview of neuroradiological findings. *Eur Radiol*. 2003;13(8):1876-1890.

25. Mastroianni CM, Paoletti F, Rivosecchi RM, et al. Cerebrospinal fluid interleukin 8 in children with purulent bacterial and tuberculous meningitis. *Pediatr Infect Dis J*. 1994;13(11):1008-1010.

26. Koedel U, Klein M, Pfister HW. New understandings on the pathophysiology of bacterial meningitis. *Curr Opin Infect Dis*. 2010;23(3):217-223.

27. Sellner J, Tauber M, Lieb S. Pathogeneis and pathophysiology of bacterial CNS infections. In: K.L Roos ART, ed. *Handbook of Clinical Neurology, Bacterial Infections.* Vol 96. 3rd ed. ; 2010:1.

28. Daniel PM. Gross morbid anatomy of the central nervous system of cases of tuberculous meningitis treated with streptomycin. *Proc R Soc Med*. 1949;42(3):169-174.

29. Shinoyama M, Suzuki M, Nomura S. Fulminant tuberculous meningitis. *Neurol Med Chir (Tokyo)*. 2012;52(10):761-764.

30. Lammie GA, Hewlett RH, Schoeman JF, Donald PR. Tuberculous cerebrovascular disease: a review. *J Infect*. 2009;59(3):156-166.

31. Schoeman J, Mansvelt E, Springer P, van Rensburg AJ, Carlini S, Fourie E. Coagulant and fibrinolytic status in tuberculous meningitis. *Pediatr Infect Dis J*. 2007;26(5):428-431.

32. Dastur DK, Lalitha VS, Udani PM, Parekh U. The brain and meninges in tuberculous meningitis-gross pathology in 100 cases and pathogenesis. *Neurol India*. 1970;18(2):86-100.

33. Kumar R, Pandey CK, Bose N, Sahay S. Tuberculous brain abscess: clinical presentation, pathophysiology and treatment (in children). *Childs Nerv Syst*. 2002;18(3-4):118-123.

34. Schoeman JF, Fieggen G, Seller N, Mendelson M, Hartzenberg B. Intractable intracranial tuberculous infection responsive to thalidomide: report of four cases. *J Child Neurol*. 2006;21(4):301-308.

35. Kumar R, Singhi V. Tuberculous brain stem abscesses in children. *Journal of Pediatric Neurology*. 2004;2(2):101-106.

36. Schoeman JF, Morkel A, Seifart HI, et al. Massive posterior fossa tuberculous abscess developing in a young child treated for miliary tuberculosis. Possible role of very rapid acetylation of isoniazid. *Pediatr Neurosurg*. 1998;29(2):64-68.

37. Saini AG, Dogra S, Kumar R, Nada R, Singh M. Primary tuberculous cerebellar abscess: case report. *Ann Trop Paediatr*. 2011;31(4):367-369.

38. Moghtaderi A, Alavi Naini R. Tuberculous radiculomyelitis: review and presentation of five patients. *Int J Tuberc Lung Dis*. 2003;7(12):1186-1190.

39. Hernandez Pando R, Aguilar D, Cohen I, et al. Specific bacterial genotypes of Mycobacterium tuberculosis cause extensive dissemination and brain infection in an experimental model. *Tuberculosis*. 2010;90(4):268-277.

40. Srivastava T, Kochar DK. Asymptomatic spinal arachnoiditis in patients with tuberculous meningitis. *Neuroradiology*. 2003;45(10):727-729.

41. Skendros P, Kamaria F, Kontopoulos V, Tsitouridis I, Sidiropoulos L. Intradural, eextramedullary tuberculoma of the spinal cord as a complication of tuberculous meningitis. *Infection*. 2003;31(2):115-117.

42. Lim YS, Kim SB, Kim MK, Lim YJ. Disseminated tuberculosis of central nervous system : spinal intramedullary and intracranial tuberculomas. *J Korean Neurosurg Soc*. 2013;54(1):61-64.

43. Kato M, Mochizuki T, Negaro K, Fukusako T, Nogaki H, Morimatsu M. Magnetic resonance imaging of a case of central nervous system tuberculosis with tuberculous arachnoiditis and multiple tuberculomas. *Nihon Ronen Igakkai Zasshi*. 1997;34(10):818-824.

44. World Health Organisation. Global tuberculosis report. 2013. http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf.

45. van Well GT, Paes BF, Terwee CB, et al. Twenty years of pediatric tuberculous meningitis: a retrospective cohort study in the western cape of South Africa. *Pediatrics*. 2009;123(1):e1-8.

46. van Toorn R, Schaaf HS, Laubscher JA, Elsland SL, Donald PR, Schoeman JF. Short Intensified Treatment in Children with Drug-Susceptible Tuberculous Meningitis. *Pediatr Infect Dis J*. 2013;33(3):248-252.

47. Ramzan A, Nayil K, Asimi R, Wani A, Makhdoomi R, Jain A. Childhood tubercular meningitis: an institutional experience and analysis of predictors of outcome. *Pediatr Neurol*. 2013;48(1):30-35.

48. Yaramis A, Gurkan F, Elevli M, et al. Central nervous system tuberculosis in children: a review of 214 cases. *Pediatrics*. 1998;102(5):E49.

49. Farinha NJ, Razali KA, Holzel H, Morgan G, Novelli VM. Tuberculosis of the central nervous system in children: a 20-year survey. *J Infect*. 2000;41(1):61-68.

50. Marais S, Pepper DJ, Schutz C, Wilkinson RJ, Meintjes G. Presentation and Outcome of Tuberculous Meningitis in a High HIV Prevalence Setting. *PLoS One*. 2011;6(5):e20077.

51. Luma HN, Tchaleu BC, Ngahanne BH, et al. Tuberculous meningitis: presentation, diagnosis and outcome in hiv-infected patients at the douala general hospital, cameroon: a cross sectional study. *AIDS Res Ther*. 2013;10(1):16.

52. Lu CH, Chang WN, Chang HW. The prognostic factors of adult tuberculous meningitis. *Infection*. 2001;29(6):299-304.

53. Thwaites G, Fisher M, Hemingway C, Scott G, Solomon T, Innes J. British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *J Infect*. 2009;59(3):167-187.

54. Wolzak NK, Cooke ML, Orth H, van Toorn R. The Changing Profile of Pediatric Meningitis at a Referral Centre in Cape Town, South Africa. *J Trop Pediatr*. 2012;58(6):491-495.

55. Donald PR, Cotton MF, Hendricks MK, Schaaf HS, de Villiers JN, Willemse TE. Pediatric meningitis in the Western Cape Province of South Africa. *J Trop Pediatr*. 1996;42(5):256-261.

56. Krauss-Mars AH, Lachman PI. Social factors associated with tuberculous meningitis. A study of children and their families in the western Cape. *S Afr Med J*. 1992;81(1):16-19.

57. Schoeman CJ. The epidemiology and outcome of childhood tuberculous meningitis. The Pelonomi Hospital experience. *S Afr Med J*. 1990;78(5):245-247.

58. Thwaites GE, Nguyen DB, Nguyen HD, et al. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. *N Engl J Med*. 2004;351(17):1741-1751.

59. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet*. 2006;367(9517):1173-1180.

60. Kumar P, Kumar R, Srivastava KL, Kumar M. Protective role of BCG vaccination against tuberculous meningitis in Indian children: a reappraisal. *Natl Med J India*. 2005;18(1):7-11.

61. Wasay M, Arif H, Khealani B, Ahsan H. Neuroimaging of tuberculous myelitis: analysis of ten cases and review of literature. *J Neuroimaging*. 2006;16(3):197-205.

62. Donald PR, van Zyl LE, de Villiers J. BCG vaccination status of children with tuberculous meningitis and the use of unsupervised isoniazid prophylaxis. *S Afr Med J*. 1995;85(3):167-170.

63. Parida SK, Kaufmann SH. Novel tuberculosis vaccines on the horizon. *Curr Opin Immunol*. 2010;22(3):374-384.

64. Schoeman JF, Van Zyl LE, Laubscher JA, Donald PR. Serial CT scanning in childhood tuberculous meningitis: prognostic features in 198 cases. *J Child Neurol*. 1995;10(4):320-329.

65. Donald PR, Schoeman JF, Cotton MF, van Zyl LE, Strachan G. Missed opportunities for the prevention and early diagnosis of tuberculous meningitis in children. *S Afr J Epidemiol Infect*. 1990;5:76-78.

66. Thwaites GE, Chau TT, Stepniewska K, et al. Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features. *Lancet*. 2002;360(9342):1287-1292.

67. Kumar R, Singh SN, Kohli N. A diagnostic rule for tuberculous meningitis. *Arch Dis Child*. 1999;81(3):221-224.

68. Youssef FG, Afifi SA, Azab AM, et al. Differentiation of tuberculous meningitis from acute bacterial meningitis using simple clinical and laboratory parameters. *Diagn Microbiol Infect Dis*. 2006;55(4):275-278.

69. Marais S, Thwaites G, Schoeman JF, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis*. 2010;10(11):803-812.

70. Hsu PC, Yang CC, Ye JJ, Huang PY, Chiang PC, Lee MH. Prognostic Factors of Tuberculous Meningitis in Adults: A 6-Year Retrospective Study at a Tertiary Hospital in Northern Taiwan. *J Microbiol Immunol Infect*. 2010;43(2):111-118.

71. Salekeen S, Mahmood K, Naqvi IH, Baig MY, Akhter ST, Abbasi A. Clinical course, complications and predictors of mortality in patients with tuberculous meningitis--an experience of fifty two cases at Civil Hospital Karachi, Pakistan. *J Pak Med Assoc*. 2013;63(5):563-567.

72. Yasar KK, Pehlivanoglu F, Sengoz G. Predictors of mortality in tuberculous meningitis: a multivariate analysis of 160 cases. *Int J Tuberc Lung Dis*. 2010;14(10):1330-1335.

73. Donald PR, Schoeman JF, van Schalkwyk HJ. The 'Road to Health' card in tuberculous meningitis. *J Trop Pediatr*. 1985;31(2):117-120.

74. Sharma P, Garg RK, Verma R, Singh MK, Shukla R. Incidence, predictors and prognostic value of cranial nerve involvement in patients with tuberculous meningitis: A retrospective evaluation. *Eur J Intern Med*. 2011;22(3):289-295.

75. Tung YR, Lai MC, Lui CC, et al. Tuberculous meningitis in infancy. *Pediatr Neurol*. 2002;27(4):262-266.

76. MRC. Streptomycin treatment of tuberculous meningitis. *Br Med J*. 1948:582-597.

77. Chou CH, Lin GM, Ku CH, Chang FY. Comparison of the APACHE II, GCS and MRC scores in predicting outcomes in patients with tuberculous meningitis. *Int J Tuberc Lung Dis*. 2010;14(1):86-92.

78. Saitoh A, Pong A, Waecker NJ,Jr, Leake JA, Nespeca MP, Bradley JS. Prediction of neurologic sequelae in childhood tuberculous meningitis: a review of 20 cases and proposal of a novel scoring system. *Pediatr Infect Dis J*. 2005;24(3):207-212.

79. Mathew JM, Rajshekhar V, Chandy MJ. Shunt surgery in poor grade patients with tuberculous meningitis and hydrocephalus: effects of response to external ventricular drainage and other variables on long term outcome. *J Neurol Neurosurg Psychiatry*. 1998;65(1):115-118.

80. Rajshekhar V. Management of hydrocephalus in patients with tuberculous meningitis. *Neurol India*. 2009;57(4):368-374.

81. van Toorn R, Springer P, Laubscher JA, Schoeman JF. Value of different staging systems for predicting neurological outcome in childhood tuberculous meningitis. *Int J Tuberc Lung Dis*. 2012;16(5):628-32.

82. Venkataswamy MM, Rafi W, Nagarathna S, Ravi V, Chandramuki A. Comparative evaluation of BACTEC 460TB system and Lowenstein-Jensen medium for the isolation of M. tuberculosis from cerebrospinal fluid samples of tuberculous meningitis patients. *Indian J Med Microbiol*. 2007;25(3):236-240.

83. Thwaites GE, Caws M, Chau TT, et al. Comparison of conventional bacteriology with nucleic acid amplification (amplified mycobacterium direct test) for diagnosis of tuberculous meningitis before and after inception of antituberculosis chemotherapy. *J Clin Microbiol*. 2004;42(3):996-1002.

84. Chen P, Shi M, Feng GD, et al. A Highly Efficient Ziehl-Neelsen Stain: Identifying De Novo Intracellular and Improving Detection of Extracellular Mycobacterium Tuberculosis in Cerebrospinal Fluid. *J Clin Microbiol*. 2012;50(4):1166-1170.

85. Takahashi T, Tamura M, Takasu T. The PCR-Based Diagnosis of Central Nervous System Tuberculosis: Up to Date. *Tuberc Res Treat*. 2012;2012:831292.

86. Pai M, Flores LL, Pai N, Hubbard A, Riley LW, Colford JM,Jr. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2003;3(10):633-643.

87. Donald PR, Victor TC, Jordaan AM, Schoeman JF, van Helden PD. Polymerase chain reaction in the diagnosis of tuberculous meningitis. *Scand J Infect Dis*. 1993;25(5):613-617.

88. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol*. 2010;48(1):229-237.

89. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet*. 2011;377(9776):1495-1505.

90. Alvarez-Uria G, Azcona JM, Midde M, Naik PK, Reddy S, Reddy R. Rapid Diagnosis of Pulmonary and Extrapulmonary Tuberculosis in HIV-Infected Patients. Comparison of LED Fluorescent Microscopy and the GeneXpert MTB/RIF Assay in a District Hospital in India. *Tuberc Res Treat*. 2012;2012:932862.

91. Sinner SW. Approach to the diagnosis and management of tuberculous meningitis. *Curr Infect Dis Rep*. 2010;12(4):291-298.

92. Sun Q, Sha W, Xiao HP, Tian Q, Zhu H. Evaluation of cerebrospinal fluid adenosine deaminase activity for the differential diagnosis of tuberculous and nontuberculous meningitis. *Am J Med Sci*. 2012;344(2):116-121.

93. Cho BH, Kim BC, Yoon GJ, et al. Adenosine deaminase activity in cerebrospinal fluid and serum for the diagnosis of tuberculous meningitis. *Clin Neurol Neurosurg*. 2013;115(9):1831-1836.

94. Soeters M, de Vries AM, Kimpen JL, Donald PR, Schaaf HS. Clinical features and outcome in children admitted to a TB hospital in the Western Cape--the influence of HIV infection and drug resistance. *S Afr Med J*. 2005;95(8):602-606.

95. Ferrara G, Losi M, D'Amico R, et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with Mycobacterium tuberculosis: a prospective study. *Lancet*. 2006;367(9519):1328-1334.

96. Donald PR, Schoeman JF, Cotton MF, van Zyl LE. Cerebrospinal fluid investigations in tuberculous meningitis. *Ann Trop Paediatr*. 1991;11(3):241-246.

97. World Health Organisation. Rapid Advice: treatment of tuberculosis in children. 2010. http://apps.who.int/iris/handle/10665/44444.

98. van Loenhout-Rooyackers JH, Keyser A, Laheij RJ, Verbeek AL, van der Meer JW. Tuberculous meningitis: is a 6-month treatment regimen sufficient? *Int J Tuberc Lung Dis*. 2001;5(11):1028-1035.

99. Donald PR, Schoeman JF, Van Zyl LE, De Villiers JN, Pretorius M, Springer P. Intensive short course chemotherapy in the management of tuberculous meningitis. *Int J Tuberc Lung Dis*. 1998;2(9):704-711.

100. Ruslami R, Ganiem AR, Dian S, et al. Intensified regimen containing rifampicin and moxifloxacin for tuberculous meningitis: an open-label, randomised controlled phase 2 trial. *Lancet Infect Dis*. 2012;13(1):27-35.

101. Schoeman JF, Van Zyl LE, Laubscher JA, Donald PR. Effect of corticosteroids on intracranial pressure, computed tomographic findings, and clinical outcome in young children with tuberculous meningitis. *Pediatrics*. 1997;99(2):226-231.

102. Prasad K, Singh MB. Corticosteroids for managing tuberculous meningitis. *Cochrane Database Syst Rev*. 2008;(1)(1):CD002244.

103. Schoeman JF, Springer P, Ravenscroft A, et al. Adjunctive thalidomide therapy of childhood tuberculous meningitis: possible anti-inflammatory role. *J Child Neurol*. 2000;15(8):497-503.

104. Schoeman JF, Springer P, van Rensburg AJ, et al. Adjunctive thalidomide therapy for childhood tuberculous meningitis: results of a randomized study. *J Child Neurol*. 2004;19(4):250-257.

105. Misra UK, Kalita J, Nair PP. Role of aspirin in tuberculous meningitis: A randomized open label placebo controlled trial. *J Neurol Sci*. 2010;293(1-2):12-17.

106. Schoeman JF, Janse van Rensburg A, Laubscher JA, Springer P. The Role of Aspirin in Childhood Tuberculous Meningitis. *J Child Neurol*. 2011;26(8):956-962.

107. Byrd TF, Davis LE. Multidrug-resistant tuberculous meningitis. *Curr Neurol Neurosci Rep*. 2007;7(6):470-475.

108. Patel VB, Padayatchi N, Bhigjee AI, et al. Multidrug-resistant tuberculous meningitis in KwaZulu-Natal, South Africa. *Clin Infect Dis*. 2004;38(6):851-856.

109. Thwaites GE, Lan NT, Dung NH, et al. Effect of antituberculosis drug resistance on response to treatment and outcome in adults with tuberculous meningitis. *J Infect Dis*. 2005;192(1):79-88.

110. Meintjes G, Lawn SD, Scano F, et al. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. *Lancet Infect Dis*. 2008;8(8):516-523.

111. Marais S, Meintjes G, Pepper DJ, et al. Frequency, severity and prediction of tuberculous meningitis immune reconstitution inflammatory syndrome. *Clin Infect Dis*. 2012;56(3):450-60.

112. Figaji AA, Fieggen AG. The neurosurgical and acute care management of tuberculous meningitis: Evidence and current practice. *Tuberculosis (Edinb)*. 2010;90(6):393-340.

113. Lindvall P, Ahlm C, Ericsson M, Gothefors L, Naredi S, Koskinen LO. Reducing intracranial pressure may increase survival among patients with bacterial meningitis. *Clin Infect Dis*. 2004;38(3):384-390.

114. Thwaites GE, Macmullen-Price J, Tran TH, et al. Serial MRI to determine the effect of dexamethasone on the cerebral pathology of tuberculous meningitis: an observational study. *Lancet Neurol*. 2007;6(3):230-236.

115. Theron S, Andronikou S, Grobbelaar M, Steyn F, Mapukata A, du Plessis J. Localized basal meningeal enhancement in tuberculous meningitis. *Pediatr Radiol*. 2006;36(11):1182-1185.

116. Singh B, Garg RK, Singh MK, et al. Computed tomography angiography in patients with tuberculous meningitis. *J Infect*. 2012;64(6):565-572.

117. Schoeman J, Hewlett R, Donald P. MR of childhood tuberculous meningitis. *Neuroradiology*. 1988;30(6):473-477.

118. Bhargava S, Gupta AK, Tandon PN. Tuberculous meningitis - a CT study. *Br J radiol*. 1982;55:189-196.

119. Figaji AA, Fieggen AG, Peter JC. Air encephalography for hydrocephalus in the era of neuroendoscopy. *Childs Nerv Syst*. 2005;21(7):559-565.

120. Figaji AA, Fieggen AG, Peter JC. Endoscopic third ventriculostomy in tuberculous meningitis. *Childs Nerv Syst*. 2003;19(4):217-225.

121. Singh D, Kumar S. Ventriculoperitoneal shunt in post tubercular hydrocephalus. *Indian Pediatr*. 1996;33(10):854-855.

122. Palur R, Rajshekhar V, Chandy MJ, Joseph T, Abraham J. Shunt surgery for hydrocephalus in tuberculous meningitis: a long-term follow-up study. *J Neurosurg*. 1991;74(1):64-69.

123. Srikantha U, Morab JV, Sastry S, et al. Outcome of ventriculoperitoneal shunt placement in Grade IV tubercular meningitis with hydrocephalus: a retrospective analysis in 95 patients. Clinical article. *J Neurosurg Pediatr*. 2009;4(2):176-183.

124. Peng J, Deng X, He F, et al. Role of ventriculoperitoneal shunt surgery in grade IV tubercular meningitis with hydrocephalus. *Childs Nerv Syst*. 2012;28(2):209-215.

125. Savardekar A, Chatterji D, Singhi S, Mohindra S, Gupta S, Chhabra R. The role of ventriculoperitoneal shunt placement in patients of tubercular meningitis with hydrocephalus in poor neurological grade: a prospective study in the pediatric population and review of literature. *Childs Nerv Syst*. 2013;29(5):719-725.

126. Schoeman J, Donald P, van Zyl L, Keet M, Wait J. Tuberculous hydrocephalus: comparison of different treatments with regard to ICP, ventricular size and clinical outcome. *Dev Med Child Neurol*. 1991;33(5):396-405.

127. Sil K, Chatterjee S. Shunting in tuberculous meningitis: a neurosurgeon's nightmare. *Childs Nerv Syst*. 2008;24(9):1029-1032.

128. Ambekar S, Dwarakanath S, Chandramouli BA, Sampath S, Devi BI, Pandey P. Does CSF composition predict shunt malfunction in tuberculous meningitis? *Indian J Tuberc*. 2011;58(2):77-81.

129. Figaji AA, Fieggen AG, Peter JC. Endoscopy for tuberculous hydrocephalus. *Childs Nerv Syst*. 2007;23(1):79-84.

130. Figaji AA, Fieggen AG. Endoscopic Challenges and Applications in Tuberculous Meningitis. *World Neurosurg*. 2012;79(2 Suppl):S24.e9-14.

131. Bhagwati S, Mehta N, Shah S. Use of endoscopic third ventriculostomy in hydrocephalus of tubercular origin. *Childs Nerv Syst*. 2010;26(12):1675-1682.

132. Figaji AA, Fieggen AG. Endoscopic challenges and applications in tuberculous meningitis. *World Neurosurg*. 2013;79(2 Suppl):S24.e9-14.

133. Chugh A, Husain M, Gupta RK, Ojha BK, Chandra A, Rastogi M. Surgical outcome of tuberculous meningitis hydrocephalus treated by endoscopic third ventriculostomy: prognostic factors and postoperative neuroimaging for functional assessment of ventriculostomy. *J Neurosurg Pediatr*. 2009;3(5):371-377.

134. Singh I, Haris M, Husain M, Husain N, Rastogi M, Gupta RK. Role of endoscopic third ventriculostomy in patients with communicating hydrocephalus: an evaluation by MR ventriculography. *Neurosurg Rev*. 2008;31(3):319-325.

135. Jonathan A, Rajshekhar V. Endoscopic third ventriculostomy for chronic hydrocephalus after tuberculous meningitis. *Surg Neurol*. 2005;63(1):32-4; discussion 34-5.

136. Li H, Liu W, You C. Central nervous system tuberculoma. *J Clin Neurosci*. 2012;19(5):691-695.

137. Ravenscroft A, Schoeman JF, Donald PR. Tuberculous granulomas in childhood tuberculous meningitis: radiological features and course. *J Trop Pediatr*. 2001;47(1):5-12.

138. Andronikou S, Greyling PJ. Devastating yet treatable complication of tuberculous meningitis: the resistant TB abscess. *Childs Nerv Syst*. 2009;25(9):1105-6; discussion 1107, 1109-10.

139. Cotton MF, Donald PR, Schoeman JF, Van Zyl LE, Aalbers C, Lombard CJ. Raised intracranial pressure, the syndrome of inappropriate antidiuretic hormone secretion, and arginine vasopressin in tuberculous meningitis. *Childs Nerv Syst*. 1993;9(1):10-5; discussion 15-6.

140. Murthy JM. Management of intracranial pressure in tuberculous meningitis. *Neurocrit Care*. 2005;2(3):306-312.

141. Harrigan MR. Cerebral salt wasting syndrome. *Crit Care Clin*. 2001;17(1):125-138.

142. Peters JP, Welt LG, Sims EA, Orloff J, Needham J. A salt-wasting syndrome associated with cerebral disease. *Trans Assoc Am Physicians*. 1950;63:57-64.

143. Narotam PK, Kemp M, Buck R, Gouws E, van Dellen JR, Bhoola KD. Hyponatremic natriuretic syndrome in tuberculous meningitis: the probable role of atrial natriuretic peptide. *Neurosurgery*. 1994;34(6):982-8; discussion 988.

144. Sinha MK, Garg RK, Anuradha H, et al. Vision impairment in tuberculous meningitis: predictors and prognosis. *J Neurol Sci*. 2010;290(1-2):27-32.

145. Springer P, Swanevelder S, van Toorn R, van Rensburg AJ, Schoeman J. Cerebral infarction and neurodevelopmental outcome in childhood tuberculous meningitis. *Eur J Paediatr Neurol*. 2009;13(4):343-349.

146. Misra UK, Kalita J, Roy AK, Mandal SK, Srivastava M. Role of clinical, radiological, and neurophysiological changes in predicting the outcome of tuberculous meningitis: a multivariable analysis. *J Neurol Neurosurg Psychiatry*. 2000;68(3):300-303.

147. Donald PR, Burger PJ, Becker WB. Paediatric meningitis inthe Western Cape. *SAMJ*. 1986;70:391-395.

148. Health Systems Trust Health Indicators. HIV prevalence % population. http://indicators.hst.org.za/healthstats/84/data. Accessed 19 January, 2014.

149. Blusse van Oud-Alblas HJ, van Vliet ME, Kimpen JL, de Villiers GS, Schaaf HS, Donald PR. Human immunodeficiency virus infection in children hospitalised with tuberculosis. *Ann Trop Paediatr*. 2002;22(2):115-123.

150. Berman S, Kibel MA, Fourie PB, Strebel PM. Childhood tuberculosis and tuberculous meningitis: high incidence rates in the Western Cape of South Africa. *Tuber Lung Dis*. 1992;73(6):349-355.

151. Weyer K, Brand J, Lancaster J, Levin J, van der Walt M. Determinants of multidrug-resistant tuberculosis in South Africa: results from a national survey. *S Afr Med J*. 2007;97(11 Pt 3):1120-1128.

152. Andronikou S, Wieselthaler N, Smith B, et al. Value of early follow-up CT in paediatric tuberculous meningitis. *Pediatr Radiol*. 2005;35(11):1092-1099.

153. Andronikou S, Smith B, Hatherhill M, Douis H, Wilmshurst J. Definitive neuroradiological diagnostic features of tuberculous meningitis in children. *Pediatr Radiol*. 2004;34(11):876-885.

154. Brenner D, Elliston C, Hall E, Berdon W. Estimated risks of radiation-induced fatal cancer from pediatric CT. *AJR Am J Roentgenol*. 2001;176(2):289-296.

155. Berrington de Gonzalez A, Mahesh M, Kim KP, et al. Projected cancer risks from computed tomographic scans performed in the United States in 2007. *Arch Intern Med*. 2009;169(22):2071-2077.

156. Pienaar M, Andronikou S, van Toorn R. MRI to demonstrate diagnostic features and complications of TBM not seen with CT. *Childs Nerv Syst*. 2009;25(8):941-947.

157. Desai SB, Shah VC, Tavri OJ, Roa P. MRI: more specific than CT in cranial tuberculomas. *Neuroradiology*. 1991;33(Suppl):216-218.

158. Ranjan P, Kalita J, Misra UK. Serial study of clinical and CT changes in tuberculous meningitis. *Neuroradiology*. 2003;45(5):277-282.

159. Bruwer GE, Van der Westhuizen S, Lombard CJ, Schoeman JF. Can CT predict the level of CSF block in tuberculous hydrocephalus? *Childs Nerv Syst*. 2004;20(3):183-187.

160. Raut T, Garg RK, Jain A, et al. Hydrocephalus in tuberculous meningitis: Incidence, its predictive factors and impact on the prognosis. *J Infect*. 2013;66(4):330-337.

161. Chan KH, Cheung RT, Fong CY, Tsang KL, Mak W, Ho SL. Clinical relevance of hydrocephalus as a presenting feature of tuberculous meningitis. *QJM*. 2003;96(9):643-648.

162. Misra UK, Kalita J, Srivastava R, Nair PP, Mishra MK, Basu A. A study of cytokines in tuberculous meningitis: clinical and MRI correlation. *Neurosci Lett*. 2010;483(1):6-10.

163. Patwari AK, Aneja S, Ravi RN, Singhal PK, Arora SK. Convulsions in tuberculous meningitis. *J Trop Pediatr*. 1996;42(2):91-97.

164. Ahmadinejad Z, Ziaee V, Reiskarami SR, Aghsaeifar M. Tuberculous meningitis: Role of Hydrocephalus in Prognosis. *MJIRC*. 2004;7(1):62-65.

165. Clemente Morgado T, Kinsky M, Carrara H, Rothemeyer S, Semple P. Prognostic Value of Computed Tomography-Evident Cerebral Infarcts in Adult Patients with Tuberculous Meningitis and Hydrocephalus Treated with an External Ventricular Drain. *World Neurosurg*. 2012;80(6):e255-260.

166. Gupta RK, Gupta S, Singh D, Sharma B, Kohli A, Gujral RB. MR imaging and angiography in tuberculous meningitis. *Neuroradiology*. 1994;36(2):87-92.

167. Shukla R, Abbas A, Kumar P, Gupta RK, Jha S, Prasad KN. Evaluation of cerebral infarction in tuberculous meningitis by diffusion weighted imaging. *J Infect*. 2008;57(4):298-306.

168. Przybojewski S, Andronikou S, Wilmshurst J. Objective CT criteria to determine the presence of abnormal basal enhancement in children with suspected tuberculous meningitis. *Pediatr Radiol*. 2006;36(7):687-696.

169. Kalita J, Prasad S, Maurya PK, Kumar S, Misra UK. MR angiography in tuberculous meningitis. *Acta Radiol*. 2012;3(3):324-329.

170. Mastroianni CM, Paoletti F, Lichtner M, D'Agostino C, Vullo V, Delia S. Cerebrospinal fluid cytokines in patients with tuberculous meningitis. *Clin Immunol Immunopathol*. 1997;84(2):171-176.

171. Akalin H, Akdis AC, Mistik R, Helvaci S, Kilicturgay K. Cerebrospinal fluid interleukin-1 beta/interleukin-1 receptor antagonist balance and tumor necrosis factor-alpha concentrations in tuberculous, viral and acute bacterial meningitis. *Scand J Infect Dis*. 1994;26(6):667-674.

172. Simmons CP, Thwaites GE, Quyen NT, et al. The clinical benefit of adjunctive dexamethasone in tuberculous meningitis is not associated with measurable attenuation of peripheral or local immune responses. *J Immunol*. 2005;175(1):579-590.

173. Koh SB, Kim BJ, Park MH, Yu SW, Park KW, Lee DH. Clinical and laboratory characteristics of cerebral infarction in tuberculous meningitis: a comparative study. *J Clin Neurosci*. 2007;14(11):1073-1077.

174. Anuradha HK, Garg RK, Agarwal A, et al. Predictors of stroke in patients of tuberculous meningitis and its effect on the outcome. *QJM*. 2010;103(9):671-678.

175. Kalita J, Misra UK, Nair PP. Predictors of stroke and its significance in the outcome of tuberculous meningitis. *J Stroke Cerebrovasc Dis*. 2009;18(4):251-258.

176. Misra UK, Kalita J, Maurya PK. Stroke in tuberculous meningitis. *J Neurol Sci*. 2011;303(1-2):22-30.

177. Leiguarda R, Berthier M, Starkstein S, Nogues M, Lylyk P. Ischemic infarction in 25 children with tuberculous meningitis. *Stroke*. 1988;19(2):200-204.

178. Andronikou S, Wilmshurst J, Hatherill M, VanToorn R. Distribution of brain infarction in children with tuberculous meningitis and correlation with outcome score at 6 months. *Pediatr Radiol*. 2006;36(12):1289-1294.

179. van der Merwe DJ, Andronikou S, Van Toorn R, Pienaar M. Brainstem ischemic lesions on MRI in children with tuberculous meningitis: with diffusion weighted confirmation. *Childs Nerv Syst*. 2009;25(8):949-954.

180. Omar N, Andronikou S, van Toorn R, Pienaar M. Diffusion-weighted magnetic resonance imaging of borderzone necrosis in paediatric tuberculous meningitis. *J Med Imaging Radiat Oncol*. 2011;55(6):563-570.

181. Airon RK, Jain AX, Mishra DS, Dua S, Sen J. Carotid angiography in tuberculous and pyogenic meningitis. *Ind J Tub*. 1991;38:143-147.

182. Rojas-Echeverri LA, Soto-Hernandez JL, Garza S, et al. Predictive value of digital subtraction angiography in patients with tuberculous meningitis. *Neuroradiology*. 1996;38(1):20-24.

183. Shah I, Borse S. Paradoxical tuberculomas after completion of antituberculous treatment. *Trop Med Health*. 2012;40(1):15-17.

184. Delance AR, Safaee M, Oh MC, et al. Tuberculoma of the central nervous system. *J Clin Neurosci*. 2013;20(10):1333-1341.

185. Anuradha HK, Garg RK, Sinha MK, et al. Intracranial tuberculomas in patients with tuberculous meningitis: predictors and prognostic significance. *Int J Tuberc Lung Dis*. 2011;15(2):234-239.

186. Hernandez-Albujar S, Arribas JR, Royo A, Gonzalez-Garcia JJ, Pena JM, Vazquez JJ. Tuberculous radiculomyelitis complicating tuberculous meningitis: case report and review. *Clin Infect Dis*. 2000;30(6):915-921.

187. Lan SH, Chang WN, Lu CH, Lui CC, Chang HW. Cerebral infarction in chronic meningitis: a comparison of tuberculous meningitis and cryptococcal meningitis. *QJM*. 2001;94(5):247-253.

188. Moghtaderi A, Alavi-Naini R, Rashki S. Cranial nerve palsy as a factor to differentiate tuberculous meningitis from acute bacterial meningitis. *Acta Med Iran*. 2013;51(2):113-118.

189. Daif AK, al Rajeh S, Ogunniyi A, al Boukai A, al Tahan A. Syringomyelia developing as an acute complication of tuberculous meningitis. *Can J Neurol Sci*. 1997;24(1):73-76.

190. Paganini H, Gonzalez F, Santander C, Casimir L, Berberian G, Rosanova MT. Tuberculous meningitis in children: clinical features and outcome in 40 cases. *Scand J Infect Dis*. 2000;32(1):41-45.

191. Kalita J, Misra UK, Ranjan P. Tuberculous meningitis with pulmonary miliary tuberculosis: a clinicoradiological study. *Neurol India*. 2004;52(2):194-196.

192. van der Weert EM, Hartgers NM, Schaaf HS, et al. Comparison of diagnostic criteria of tuberculous meningitis in human immunodeficiency virus-infected and uninfected children. *Pediatr Infect Dis J*. 2006;25(1):65-69.

193. Katrak SM, Shembalkar PK, Bijwe SR, Bhandarkar LD. The clinical, radiological and pathological profile of tuberculous meningitis in patients with and without human immunodeficiency virus infection. *J Neurol Sci*. 2000;181(1-2):118-126.

194. Dekker G, Andronikou S, van Toorn R, Scheepers S, Brandt A, Ackermann C. MRI findings in children with tuberculous meningitis: a comparison of HIV-infected and non-infected patients. *Childs Nerv Syst*. 2011;27(11):1943-9.

195. Schutte CM. Clinical, cerebrospinal fluid and pathological findings and outcomes in HIV-positive and HIV-negative patients with tuberculous meningitis. *Infection*. 2001;29(4):213-217.

196. Bandyopadhyay SK, Bandyopadhyay R, Dutta A. Profile of tuberculous meningitis with or without HIV infection and the predicators of adverse outcome. *West Indian Med J*. 2009;58(6):589-592.

197. Marais S, Scholtz P, Pepper DJ, Meintjes G, Wilkinson RJ, Candy S. Neuroradiological features of the tuberculosis-associated immune reconstitution inflammatory syndrome. *Int J Tuberc Lung Dis*. 2010;14(2):188-196.

198. Figaji AA, Zwane E, Thompson C, et al. Brain tissue oxygen tension monitoring in pediatric severe traumatic brain injury : Part 1: Relationship with outcome. *Childs Nerv Syst*. 2009;25(10):1325-1333.

199. Stiefel MF, Spiotta A, Gracias VH, et al. Reduced mortality rate in patients with severe traumatic brain injury treated with brain tissue oxygen monitoring. *J Neurosurg*. 2005;103(5):805-811.

200. Lang E, Matthias J. Systematic and Comprehensive Literature Review of Publications on Direct Cerebral Oxygenation Monitoring. *The Open Critical Care Medicine Journal*. 2013;6:1-24.

201. Weinstein JD, Langfitt TW, Bruno L, Zaren HA, Jackson JL. Experimental study of patterns of brain distortion and ischemia produced by an intracranial mass. *J Neurosurg*. 1968;28(6):513-521.

202. Langfitt TW, Weinstein JD, Kassell NF, Gagliardi LJ. Transmission of increased intracranial pressure within the craniospinal axis. *J Neurosurg*. 1964;21:989-997.

203. Astrup J, Siesjo BK, Symon L. Thresholds in cerebral ischemia - the ischemic penumbra. *Stroke*. 1981;12(6):723-725.

204. Siesjo BK. Pathophysiology and treatment of focal cerebral ischemia. Part I: Pathophysiology. (1992). *J Neurosurg*. 2008;108(3):616-631.

205. Marshall RS. The functional relevance of cerebral hemodynamics: why blood flow matters to the injured and recovering brain. *Curr Opin Neurol*. 2004;17(6):705-709.

206. Tureen JH, Dworkin RJ, Kennedy SL, Sachdeva M, Sande MA. Loss of cerebrovascular autoregulation in experimental meningitis in rabbits. *J Clin Invest*. 1990;85(2):577-581.

207. Leib SL, Kim YS, Chow LL, Sheldon RA, Tauber MG. Reactive oxygen intermediates contribute to necrotic and apoptotic neuronal injury in an infant rat model of bacterial meningitis due to group B streptococci. *J Clin Invest*. 1996;98(11):2632-2639.

208. Menon DK, Coles JP, Gupta AK, et al. Diffusion limited oxygen delivery following head injury. *Crit Care Med*. 2004;32(6):1384-1390.

209. Tureen J. Effect of recombinant human tumor necrosis factor-alpha on cerebral oxygen uptake, cerebrospinal fluid lactate, and cerebral blood flow in the rabbit: role of nitric oxide. *J Clin Invest*. 1995;95(3):1086-1091.

210. Fassbender K, Ries S, Schminke U, Schneider S, Hennerici M. Inflammatory cytokines in CSF in bacterial meningitis: association with altered blood flow velocities in basal cerebral arteries. *J Neurol Neurosurg Psychiatry*. 1996;61(1):57-61.

211. Rohlwink UK, Figaji AA. Methods of monitoring brain oxygenation. *Childs Nerv Syst*. 2010;26(4):453-464.

212. Lang EW, Mulvey JM, Mudaliar Y, Dorsch NW. Direct cerebral oxygenation monitoring--a systematic review of recent publications. *Neurosurg Rev*. 2007;30(2):99-106; discussion 106-7.

213. Dings J, Meixensberger J, Amschler J, Hamelbeck B, Roosen K. Brain tissue pO2 in relation to cerebral perfusion pressure, TCD findings and TCD-CO2-reactivity after severe head injury. *Acta Neurochir (Wien)*. 1996;138(4):425-434.

214. Kiening KL, Unterberg AW, Bardt TF, Schneider GH, Lanksch WR. Monitoring of cerebral oxygenation in patients with severe head injuries: brain tissue PO2 versus jugular vein oxygen saturation. *J Neurosurg*. 1996;85(5):751-757.

215. Maas AI, Fleckenstein W, de Jong DA, van Santbrink H. Monitoring cerebral oxygenation: experimental studies and preliminary clinical results of continuous monitoring of cerebrospinal fluid and brain tissue oxygen tension. *Acta Neurochir Suppl (Wien)*. 1993;59:50-57.

216. Doppenberg EM, Watson JC, Broaddus WC, Holloway KL, Young HF, Bullock R. Intraoperative monitoring of substrate delivery during aneurysm and hematoma surgery: initial experience in 16 patients. *J Neurosurg*. 1997;87(6):809-816.

217. Hoffman WE, Charbel FT, Gonzalez-Portillo G, Ausman JI. Measurement of ischemia by changes in tissue oxygen, carbon dioxide, and pH. *Surg Neurol*. 1999;51(6):654-658.

218. Kett-White R, Hutchinson PJ, Al-Rawi PG, et al. Cerebral oxygen and microdialysis monitoring during aneurysm surgery: effects of blood pressure, cerebrospinal fluid drainage, and temporary clipping on infarction. *J Neurosurg*. 2002;96(6):1013-1019.

219. Valadka AB, Gopinath SP, Contant CF, Uzura M, Robertson CS. Relationship of brain tissue PO2 to outcome after severe head injury. *Crit Care Med*. 1998;26(9):1576-1581.

220. Wartenberg KE, Schmidt JM, Mayer SA. Multimodality monitoring in neurocritical care. *Crit Care Clin*. 2007;23(3):507-538.

221. Brain Trauma Foundation, American Association of Neurological Surgeons, Congress of Neurological Surgeons, et al. Guidelines for the management of severe traumatic brain injury. X. Brain oxygen monitoring and thresholds. *J Neurotrauma*. 2007;24 Suppl 1:S65-70.

222. Hlatky R, Valadka AB, Goodman JC, Contant CF, Robertson CS. Patterns of energy substrates during ischemia measured in the brain by microdialysis. *J Neurotrauma*. 2004;21(7):894-906.

223. Huchzermeyer C, Albus K, Gabriel HJ, et al. Gamma oscillations and spontaneous network activity in the hippocampus are highly sensitive to decreases in pO2 and concomitant changes in mitochondrial redox state. *J Neurosci*. 2008;28(5):1153-1162.

224. Scheufler KM, Lehnert A, Rohrborn HJ, Nadstawek J, Thees C. Individual value of brain tissue oxygen pressure, microvascular oxygen saturation, cytochrome redox level, and energy metabolites in detecting critically reduced cerebral energy state during acute changes in global cerebral perfusion. *J Neurosurg Anesthesiol*. 2004;16(3):210-219.

225. Valadka AB, Hlatky R, Furuya Y, Robertson CS. Brain tissue PO2: correlation with cerebral blood flow. *Acta Neurochir Suppl*. 2002;81:299-301.

226. Gupta AK, Hutchinson PJ, Al-Rawi P, et al. Measuring brain tissue oxygenation compared with jugular venous oxygen saturation for monitoring cerebral oxygenation after traumatic brain injury. *Anesth Analg*. 1999;88(3):549-553.

227. van den Brink WA, van Santbrink H, Steyerberg EW, et al. Brain oxygen tension in severe head injury. *Neurosurgery*. 2000;46(4):868-76; discussion 876-8.

228. Kochanek PM, Carney N, Adelson PD, et al. Guidelines for the acute medical management of severe traumatic brain injury in infants, children, and adolescents--second edition. *Pediatr Crit Care Med*. 2012;13 Suppl 1:S1-82.

229. Narotam PK, Morrison JF, Nathoo N. Brain tissue oxygen monitoring in traumatic brain injury and major trauma: outcome analysis of a brain tissue oxygen-directed therapy. *J Neurosurg*. 2009;111(4):672-682.

230. Maloney-Wilensky E, Gracias V, Itkin A, et al. Brain tissue oxygen and outcome after severe traumatic brain injury: a systematic review. *Crit Care Med*. 2009;37(6):2057-2063.

231. Strege RJ, Lang EW, Stark AM, et al. Cerebral edema leading to decompressive craniectomy: an assessment of the preceding clinical and neuromonitoring trends. *Neurol Res*. 2003;25(5):510-515.

232. Barth M, Woitzik J, Weiss C, et al. Correlation of clinical outcome with pressure-, oxygen-, and flow-related indices of cerebrovascular reactivity in patients following aneurysmal SAH. *Neurocrit Care*. 2010;12(2):234-243.

233. Stuart RM, Claassen J, Schmidt M, et al. Multimodality neuromonitoring and decompressive hemicraniectomy after subarachnoid hemorrhage. *Neurocrit Care*. 2011;15(1):146-150.

234. Allen BB, Hoffman CE, Traube CS, Weinstein SL, Greenfield JP. Continuous brain tissue oxygenation monitoring in the management of pediatric stroke. *Neurocrit Care*. 2011;15(3):529-536.

235. Bordes J, Boret H, Lacroix G, Prunet B, Meaudre E, Kaiser E. Decompressive craniectomy guided by cerebral microdialysis and brain tissue oxygenation in a patient with meningitis. *Acta Anaesthesiol Scand*. 2011;55(1):130-133.

236. Figaji AA, Sandler SI, Fieggen AG, Le Roux PD, Peter JC, Argent AC. Continuous monitoring and intervention for cerebral ischemia in tuberculous meningitis. *Pediatr Crit Care Med*. 2008;9(4):e25-30.

237. Carmona Suazo JA, Maas AI, van den Brink WA, van Santbrink H, Steyerberg EW, Avezaat CJ. CO2 reactivity and brain oxygen pressure monitoring in severe head injury. *Crit Care Med*. 2000;28(9):3268-3274.

238. Smith M, Elwell C. Near-infrared spectroscopy: shedding light on the injured brain. *Anesth Analg*. 2009;108(4):1055-1057.

239. Kurth CD, McCann JC, Wu J, Miles L, Loepke AW. Cerebral oxygen saturation-time threshold for hypoxic-ischemic injury in piglets. *Anesth Analg*. 2009;108(4):1268-1277.

240. Al-Rawi PG, Smielewski P, Kirkpatrick PJ. Evaluation of a near-infrared spectrometer (NIRO 300) for the detection of intracranial oxygenation changes in the adult head. *Stroke*. 2001;32(11):2492-2500.

241. Brady KM, Lee JK, Kibler KK, et al. Continuous time-domain analysis of cerebrovascular autoregulation using near-infrared spectroscopy. *Stroke*. 2007;38(10):2818-2825.

242. Subbaswamy A, Hsu AA, Weinstein S, Bell MJ. Correlation of cerebral Near-infrared spectroscopy (cNIRS) and neurological markers in critically ill children. *Neurocrit Care*. 2009;10(1):129-135.

243. Misra M, Stark J, Dujovny M, Widman R, Ausman JI. Transcranial cerebral oximetry in random normal subjects. *Neurol Res*. 1998;20(2):137-141.

244. Hirtz DG. Report of the National Institute of Neurological Disorders and Stroke workshop on near infrared spectroscopy. *Pediatrics*. 1993;91(2):414-417.

245. Al-Rawi PG, Kirkpatrick PJ. Tissue oxygen index: thresholds for cerebral ischemia using near-infrared spectroscopy. *Stroke*. 2006;37(11):2720-2725.

246. Edmonds HL,Jr. Multi-modality neurophysiologic monitoring for cardiac surgery. *Heart Surg Forum*. 2002;5(3):225-228.

247. Nollert G, Jonas RA, Reichart B. Optimizing cerebral oxygenation during cardiac surgery: a review of experimental and clinical investigations with near infrared spectrophotometry. *Thorac Cardiovasc Surg*. 2000;48(4):247-253.

248. Adelson PD, Nemoto E, Colak A, Painter M. The use of near infrared spectroscopy (NIRS) in children after traumatic brain injury: a preliminary report. *Acta Neurochir Suppl*. 1998;71:250-254.

249. Hull R, Bortfeld H, Koons S. Near-Infrared Spectroscopy and Cortical Responses to Speech Production. *Open Neuroimag J*. 2009;3:26-30.

250. van Bel F, Dorrepaal CA, Benders MJ, Zeeuwe PE, van de Bor M, Berger HM. Changes in cerebral hemodynamics and oxygenation in the first 24 hours after birth asphyxia. *Pediatrics*. 1993;92(3):365-372.

251. Shojima M, Watanabe E, Mayanagi Y. Cerebral blood oxygenation after cerebrospinal fluid removal in hydrocephalus measured by near infrared spectroscopy. *Surg Neurol*. 2004;62(4):312-8; discussion 318.

252. Weerakkody RA, Czosnyka M, Zweifel C, et al. Near infrared spectroscopy as possible non-invasive monitor of slow vasogenic ICP waves. *Acta Neurochir Suppl*. 2012;114:181-185.

253. Tureen J, Liu Q, Chow L. Near-infrared spectroscopy in experimental pneumococcal meningitis in the rabbit: cerebral hemodynamics and metabolism. *Pediatr Res*. 1996;40(5):759-763.

254. Park WS, Chang YS, Ko SY, Kang MJ, Han JM, Lee M. Effects of microbial invasion on cerebral hemodynamics and oxygenation monitored by near infrared spectroscopy in experimental Escherichia coli meningitis in the newborn piglet. *Neurol Res*. 1999;21(4):391-398.

255. Diedler J, Czosnyka M. Merits and Pitfalls of Multimodality Brain Monitoring. *Neurocrit Care*. 2010;12(3):313-316.

256. Knirsch W, Stutz K, Kretschmar O, et al. Regional cerebral oxygenation by NIRS does not correlate with central or jugular venous oxygen saturation during interventional catheterisation in children. *Acta Anaesthesiol Scand*. 2008;52(10):1370-1374.

257. Gatto R, Hoffman W, Mueller M, Flores A, Valyi-Nagy T, Charbel FT. Frequency domain near-infrared spectroscopy technique in the assessment of brain oxygenation: a validation study in live subjects and cadavers. *J Neurosci Methods*. 2006;157(2):274-277.

258. Hamed SA, Hamed EA, Zakary MM. Oxidative stress and S-100B protein in children with bacterial meningitis. *BMC Neurol*. 2009;9:51.

259. Coyle PK. Overview of acute and chronic meningitis. *Neurol Clin*. 1999;17(4):691-710.

260. Petzold A. CSF biomarkers for improved prognostic accuracy in acute CNS disease. *Neurol Res*. 2007;29(7):691-708.

261. Berger RP. The use of serum biomarkers to predict outcome after traumatic brain injury in adults and children. *J Head Trauma Rehabil*. 2006;21(4):315-333.

262. Marangos PJ, Schmechel DE. Neuron specific enolase, a clinically useful marker for neurons and neuroendocrine cells. *Annu Rev Neurosci*. 1987;10:269-295.

263. Harpio R, Einarsson R. S100 proteins as cancer biomarkers with focus on S100B in malignant melanoma. *Clin Biochem*. 2004;37(7):512-518.

264. Lewandrowski K, Chen A, Januzzi J. Cardiac markers for myocardial infarction. A brief review. *Am J Clin Pathol*. 2002;118 Suppl:S93-9.

265. Kuhle J, Petzold A. What makes a prognostic biomarker in CNS diseases: Strategies for targeted biomarker discovery? Part 2: Chronic progressive and relapsing disease. *Expert Opinion on Medical Diagnostics*. 2011;5(5):393-410.

266. Lamers KJ, Vos P, Verbeek MM, Rosmalen F, van Geel WJ, van Engelen BG. Protein S-100B, neuron-specific enolase (NSE), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) in cerebrospinal fluid (CSF) and blood of neurological patients. *Brain Res Bull*. 2003;61(3):261-264.

267. Persson L, Hardemark HG, Gustafsson J, et al. S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. *Stroke*. 1987;18(5):911-918.

268. Bakay RA, Ward AA,Jr. Enzymatic changes in serum and cerebrospinal fluid in neurological injury. *J Neurosurg*. 1983;58(1):27-37.

269. Lamers KJ, van Engelen BG, Gabreels FJ, Hommes OR, Borm GF, Wevers RA. Cerebrospinal neuron-specific enolase, S-100 and myelin basic protein in neurological disorders. *Acta Neurol Scand*. 1995;92(3):247-251.

270. Sandler SJ, Figaji AA, Adelson PD. Clinical applications of biomarkers in pediatric traumatic brain injury. *Childs Nerv Syst*. 2010;26(2):205-213.

271. Lo TY, Jones PA, Minns RA. Pediatric brain trauma outcome prediction using paired serum levels of inflammatory mediators and brain-specific proteins. *J Neurotrauma*. 2009;26(9):1479-1487.

272. Berger RP, Beers SR, Richichi R, Wiesman D, Adelson PD. Serum biomarker concentrations and outcome after pediatric traumatic brain injury. *J Neurotrauma*. 2007;24(12):1793-1801.

273. Wiesmann M, Missler U, Hagenstrom H, Gottmann D. S-100 protein plasma levels after aneurysmal subarachnoid haemorrhage. *Acta Neurochir (Wien)*. 1997;139(12):1155-1160.

274. Mokuno K, Kato K, Kawai K, Matsuoka Y, Yanagi T, Sobue I. Neuron-specific enolase and S-100 protein levels in cerebrospinal fluid of patients with various neurological diseases. *J Neurol Sci*. 1983;60(3):443-451.

275. Sheng JG, Mrak RE, Rovnaghi CR, Kozlowska E, Van Eldik LJ, Griffin WS. Human brain S100 beta and S100 beta mRNA expression increases with age: pathogenic implications for Alzheimer's disease. *Neurobiol Aging*. 1996;17(3):359-363.

276. Bottiger BW, Mobes S, Glatzer R, et al. Astroglial protein S-100 is an early and sensitive marker of hypoxic brain damage and outcome after cardiac arrest in humans. *Circulation*. 2001;103(22):2694-2698.

277. Lima JE, Takayanagui OM, Garcia LV, Leite JP. Use of neuron-specific enolase for assessing the severity and outcome in patients with neurological disorders. *Braz J Med Biol Res*. 2004;37(1):19-26.

278. Teunissen CE, Tumani H, Bennett JL, et al. Consensus Guidelines for CSF and Blood Biobanking for CNS Biomarker Studies. *Mult Scler Int*. 2011;2011:246412.

279. Butterfield LH, Potter DM, Kirkwood JM. Multiplex Serum Biomarker Assessments: Technical and Biostatistical Issues. *J Transl Med*. 2011;9(1):173.

280. Warsinke A, Benkert A, Scheller FW. Electrochemical immunoassays. *Fresenius J Anal Chem*. 2000;366(6-7):622-634.

281. Gosling JP. A decade of development in immunoassay methodology. *Clin Chem*. 1990;36(8 Pt 1):1408-1427.

282. Voller A, Bartlett A, Bidwell DE. Enzyme immunoassays with special reference to ELISA techniques. *Journal of Clinical Pathology, JCP*. 1978;31:507-520.

283. Infante JR, Martinez A, Ochoa J, et al. Cerebrospinal fluid S-100 protein levels in neurological pathologies. *J Physiol Biochem*. 2003;59(4):255-261.

284. Heizmann CW, Fritz G, Schafer BW. S100 proteins: structure, functions and pathology. *Front Biosci*. 2002;7:d1356-68.

285. Steiner J, Bernstein HG, Bielau H, et al. Evidence for a wide extra-astrocytic distribution of S100B in human brain. *BMC Neurosci*. 2007;8:2.

286. Raabe A, Seifert V. Protein S-100B as a serum marker of brain damage in severe head injury: preliminary results. *Neurosurg Rev*. 2000;23(3):136-138.

287. Raabe A, Seifert V. Fatal secondary increase in serum S-100B protein after severe head injury. Report of three cases. *J Neurosurg*. 1999;91(5):875-877.

288. Spinella PC, Donoghue A, Rajendra A, Drott HR, Dominguez TE, Helfaer M. Cerebrospinal fluid levels of S-100beta in children and its elevation in pediatric meningitis. *Pediatr Crit Care Med*. 2004;5(1):53-57.

289. Raabe A, Kopetsch O, Gross U, Zimmermann M, Gebhart P. Measurements of serum S-100B protein: effects of storage time and temperature on pre-analytical stability. *Clin Chem Lab Med*. 2003;41(5):700-703.

290. Gazzolo D, Frulio R, Bruschettini P, Lituania M, Michetti F. Effects of temperature on pre-analytical stability of S100B protein concentrations in urine of healthy full-term infants. *Clin Chim Acta*. 2004;350(1-2):231-232.

291. Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. *Stroke*. 1997;28(10):1956-1960.

292. Bertsch T, Casarin W, Kretschmar M, et al. Protein S-100B: a serum marker for ischemic and infectious injury of cerebral tissue. *Clin Chem Lab Med*. 2001;39(4):319-323.

293. Goncalves CA, Leite MC, Nardin P. Biological and methodological features of the measurement of S100B, a putative marker of brain injury. *Clin Biochem*. 2008;41(10-11):755-763.

294. Wright NT, Inman KG, Levine JA, Cannon BR, Varney KM, Weber DJ. Refinement of the solution structure and dynamic properties of Ca(2+)-bound rat S100B. *J Biomol NMR*. 2008;42(4):279-286.

295. Mazzini GS, Souza DO, Portela LV. The ischemic heart as an extracerebral source for S100B. *Resuscitation*. 2009;80(1):144.

296. Tiu SC, Chan WY, Heizmann CW, Schafer BW, Shu SY, Yew DT. Differential expression of S100B and S100A6(1) in the human fetal and aged cerebral cortex. *Brain Res Dev Brain Res*. 2000;119(2):159-168.

297. Gazzolo D, Bruschettini M, Corvino V, et al. S100b protein concentrations in amniotic fluid correlate with gestational age and with cerebral ultrasound scanning results in healthy fetuses. *Clin Chem*. 2001;47(5):954-956.

298. Gazzolo D, Vinesi P, Marinoni E, et al. S100B protein concentrations in cord blood: correlations with gestational age in term and preterm deliveries. *Clin Chem*. 2000;46(7):998-1000.

299. Portela LV, Tort AB, Schaf DV, et al. The serum S100B concentration is age dependent. *Clin Chem*. 2002;48(6 Pt 1):950-952.

300. Gazzolo D, Michetti F, Bruschettini M, et al. Pediatric concentrations of S100B protein in blood: age- and sex-related changes. *Clin Chem*. 2003;49(6 Pt 1):967-970.

301. Bouvier D, Castellani C, Fournier M, et al. Reference ranges for serum S100B protein during the first three years of life. *Clin Biochem*. 2011;44(10-11):927-929.

302. Nygaard O, Langbakk B, Romner B. Age- and sex-related changes of S-100 protein concentrations in cerebrospinal fluid and serum in patients with no previous history of neurological disorder. *Clin Chem*. 1997;43(3):541-543.

303. Wiesmann M, Missler U, Gottmann D, Gehring S. Plasma S-100b protein concentration in healthy adults is age- and sex-independent. *Clin Chem*. 1998;44(5):1056-1058.

304. Garnier Y, Frigiola A, Li Volti G, et al. Increased maternal/fetal blood S100B levels following systemic endotoxin administration and periventricular white matter injury in preterm fetal sheep. *Reprod Sci*. 2009;16(8):758-766.

305. Giussani DA, Thakor AS, Frulio R, Gazzolo D. Acute hypoxia increases S100beta protein in association with blood flow redistribution away from peripheral circulations in fetal sheep. *Pediatr Res*. 2005;58(2):179-184.

306. Unden J, Christensson B, Bellner J, Alling C, Romner B. Serum S100B levels in patients with cerebral and extracerebral infectious disease. *Scand J Infect Dis*. 2004;36(1):10-13.

307. Lins H, Wallesch CW, Wunderlich MT. Sequential analyses of neurobiochemical markers of cerebral damage in cerebrospinal fluid and serum in CNS infections. *Acta Neurol Scand*. 2005;112(5):303-308.

308. Gazzolo D, Grutzfeld D, Michetti F, et al. Increased S100B in cerebrospinal fluid of infants with bacterial meningitis: relationship to brain damage and routine cerebrospinal fluid findings. *Clin Chem*. 2004;50(5):941-944.

309. Berger RP, Adelson PD, Pierce MC, Dulani T, Cassidy LD, Kochanek PM. Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. *J Neurosurg*. 2005;103(1 Suppl):61-68.

310. Berger RP, Pierce MC, Wisniewski SR, et al. Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. *Pediatrics*. 2002;109(2):E31.

311. van Engelen BG, Lamers KJ, Gabreels FJ, Wevers RA, van Geel WJ, Borm GF. Age-related changes of neuron-specific enolase, S-100 protein, and myelin basic protein concentrations in cerebrospinal fluid. *Clin Chem*. 1992;38(6):813-816.

312. Nygaard O, Langbakk B, Romner B. Neuron-specific enolase concentrations in serum and cerebrospinal fluid in patients with no previous history of neurological disorder. *Scand J Clin Lab Invest*. 1998;58(3):183-186.

313. Rodriguez-Nunez A, Cid E, Eiris J, et al. Neuron-specific enolase levels in the cerebrospinal fluid of neurologically healthy children. *Brain Dev*. 1999;21(1):16-19.

314. Casmiro M, Maitan S, De Pasquale F, et al. Cerebrospinal fluid and serum neuron-specific enolase concentrations in a normal population. *Eur J Neurol*. 2005;12(5):369-374.

315. Hardemark HG, Persson L, Bolander HG, Hillered L, Olsson Y, Pahlman S. Neuron-specific enolase is a marker of cerebral ischemia and infarct size in rat cerebrospinal fluid. *Stroke*. 1988;19(9):1140-1144.

316. Song TJ, Choi YC, Lee KY, Kim WJ. Serum and cerebrospinal fluid neuron-specific enolase for diagnosis of tuberculous meningitis. *Yonsei Med J*. 2012;53(6):1068-1072.

317. Bandyopadhyay S, Hennes H, Gorelick MH, Wells RG, Walsh-Kelly CM. Serum neuron-specific enolase as a predictor of short-term outcome in children with closed traumatic brain injury. *Acad Emerg Med*. 2005;12(8):732-738.

318. Rodriguez-Nunez A, Cid E, Rodriguez-Garcia J, Camina F, Rodriguez-Segade S, Castro-Gago M. Neuron-specific enolase, nucleotides, nucleosides, purine bases, oxypurines and uric acid concentrations in cerebrospinal fluid of children with meningitis. *Brain Dev*. 2003;25(2):102-106.

319. Nara T, Nozaki H, Nakae Y, Arai T, Ohashi T. Neuron-specific enolase in comatose children. *Am J Dis Child*. 1988;142(2):173-174.

320. Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem Res*. 2000;25(9-10):1439-1451.

321. Schiff L, Hadker N, Weiser S, Rausch C. A literature review of the feasibility of glial fibrillary acidic protein as a biomarker for stroke and traumatic brain injury. *Mol Diagn Ther*. 2012;16(2):79-92.

322. Petzold A, Keir G, Green AJ, Giovannoni G, Thompson EJ. An ELISA for glial fibrillary acidic protein. *J Immunol Methods*. 2004;287(1-2):169-177.

323. Liedtke W, Edelmann W, Bieri PL, et al. GFAP is necessary for the integrity of CNS white matter architecture and long-term maintenance of myelination. *Neuron*. 1996;17(4):607-615.

324. Balasingam V, Tejada-Berges T, Wright E, Bouckova R, Yong VW. Reactive astrogliosis in the neonatal mouse brain and its modulation by cytokines. *J Neurosci*. 1994;14(2):846-856.

325. Lumpkins KM, Bochicchio GV, Keledjian K, Simard JM, McCunn M, Scalea T. Glial fibrillary acidic protein is highly correlated with brain injury. *J Trauma*. 2008;65(4):778-82; discussion 782-4.

326. Wiesmann M, Steinmeier E, Magerkurth O, Linn J, Gottmann D, Missler U. Outcome prediction in traumatic brain injury: comparison of neurological status, CT findings, and blood levels of S100B and GFAP. *Acta Neurol Scand*. 2009;121(3):178-185.

327. Kelley TW, Prayson RA, Ruiz AI, Isada CM, Gordon SM. The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature. *Am J Clin Pathol*. 2003;119(5):749-753.

328. Canova C, Neal JW, Gasque P. Expression of innate immune complement regulators on brain epithelial cells during human bacterial meningitis. *J Neuroinflammation*. 2006;3:22.

329. Gerber J, Raivich G, Wellmer A, et al. A mouse model of Streptococcus pneumoniae meningitis mimicking several features of human disease. *Acta Neuropathol*. 2001;101(5):499-508.

330. Vissers JL, Mersch ME, Rosmalen CF, et al. Rapid immunoassay for the determination of glial fibrillary acidic protein (GFAP) in serum. *Clin Chim Acta*. 2006;366(1-2):336-340.

331. van Geel WJ, de Reus HP, Nijzing H, Verbeek MM, Vos PE, Lamers KJ. Measurement of glial fibrillary acidic protein in blood: an analytical method. *Clin Chim Acta*. 2002;326(1-2):151-154.

332. Pelinka LE, Kroepfl A, Leixnering M, Buchinger W, Raabe A, Redl H. GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome. *J Neurotrauma*. 2004;21(11):1553-1561.

333. Nylen K, Csajbok LZ, Ost M, et al. Serum glial fibrillary acidic protein is related to focal brain injury and outcome after aneurysmal subarachnoid hemorrhage. *Stroke*. 2007;38(5):1489-1494.

334. Unden J, Strandberg K, Malm J, et al. Explorative investigation of biomarkers of brain damage and coagulation system activation in clinical stroke differentiation. *J Neurol*. 2009;256(1):72-77.

335. Foerch C, Niessner M, Back T, et al. Diagnostic accuracy of plasma glial fibrillary acidic protein for differentiating intracerebral hemorrhage and cerebral ischemia in patients with symptoms of acute stroke. *Clin Chem*. 2012;58(1):237-245.

336. Vos PE, Lamers KJ, Hendriks JC, et al. Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. *Neurology*. 2004;62(8):1303-1310.

337. Dvorak F, Haberer I, Sitzer M, Foerch C. Characterisation of the diagnostic window of serum glial fibrillary acidic protein for the differentiation of intracerebral haemorrhage and ischaemic stroke. *Cerebrovasc Dis*. 2009;27(1):37-41.

338. Wunderlich MT, Wallesch CW, Goertler M. Release of glial fibrillary acidic protein is related to the neurovascular status in acute ischemic stroke. *Eur J Neurol*. 2006;13(10):1118-1123.

339. Deck JH, Eng LF, Bigbee J, Woodcock SM. The role of glial fibrillary acidic protein in the diagnosis of central nervous system tumors. *Acta Neuropathol*. 1978;42(3):183-190.

340. Brommeland T, Rosengren L, Fridlund S, Hennig R, Isaksen V. Serum levels of glial fibrillary acidic protein correlate to tumour volume of high-grade gliomas. *Acta Neurol Scand*. 2007;116(6):380-384.

341. Fukuyama R, Izumoto T, Fushiki S. The cerebrospinal fluid level of glial fibrillary acidic protein is increased in cerebrospinal fluid from Alzheimer's disease patients and correlates with severity of dementia. *Eur Neurol*. 2001;46(1):35-38.

342. Wallin A, Blennow K, Rosengren LE. Glial fibrillary acidic protein in the cerebrospinal fluid of patients with dementia. *Dementia*. 1996;7(5):267-272.

343. Rosengren LE, Wikkelso C, Hagberg L. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of adults. *J Neurosci Methods*. 1994;51(2):197-204.

344. Rosengren LE, Ahlsen G, Belfrage M, Gillberg C, Haglid KG, Hamberger A. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of children. *J Neurosci Methods*. 1992;44(2-3):113-119.

345. Jesse S, Steinacker P, Lehnert S, et al. A proteomic approach for the diagnosis of bacterial meningitis. *PLoS One*. 2010;5(4):e10079.

346. Dotevall L, Rosengren LE, Hagberg L. Increased cerebrospinal fluid levels of glial fibrillary acidic protein (GFAp) in Lyme neuroborreliosis. *Infection*. 1996;24(2):125-129.

347. Grahn A, Hagberg L, Nilsson S, Blennow K, Zetterberg H, Studahl M. Cerebrospinal fluid biomarkers in patients with varicella-zoster virus CNS infections. *J Neurol*. 2013;260(7):1813-21.

348. Bonneh-Barkay D. Hayasaka D, ed. *Pathogenesis of Encephalitis.* InTech; 2011:1-17. http://www.intechopen.com/books/pathogenesis-of-encephalitis/biomarkers-of-encephalitis. ISBN: 978-953-307-741-3,.

349. Shahim P, Darin N, Andreasson U, et al. Cerebrospinal fluid brain injury biomarkers in children: a multicenter study. *Pediatr Neurol*. 2013;49(1):31-39.e2.

350. Tsukahara H, Fujii Y, Matsubara K, et al. Prognostic value of brain injury biomarkers in acute encephalitis/encephalopathy. *Pediatr Int*. 2013;55(4):461-464.

351. Brand HK, Hermans PW, de Groot R. Host biomarkers and paediatric infectious diseases: from molecular profiles to clinical application. *Adv Exp Med Biol*. 2010;659:19-31.

352. Goonetilleke UR, Scarborough M, Ward SA, Gordon SB. Proteomic analysis of cerebrospinal fluid in pneumococcal meningitis reveals potential biomarkers associated with survival. *J Infect Dis*. 2010;202(4):542-550.

353. Kataria J, Rukmangadachar LA, Hariprasad G, O J, Triapthi M, Srinivasan A. Two dimensional difference gel electrophoresis analysis of cerebrospinal fluid in tuberculous meningitis patients. *J Proteomics*. 2011;74(10):2194-2203.

354. Zhang A, Sun H, Wang P, Han Y, Wang X. Recent and potential developments of biofluid analyses in metabolomics. *J Proteomics*. 2012;75(4):1079-1088.

355. Coen M, O'Sullivan M, Bubb WA, Kuchel PW, Sorrell T. Proton nuclear magnetic resonance-based metabonomics for rapid diagnosis of meningitis and ventriculitis. *Clin Infect Dis*. 2005;41(11):1582-1590.

356. Subramanian A, Gupta A, Saxena S, et al. Proton MR CSF analysis and a new software as predictors for the differentiation of meningitis in children. *NMR Biomed*. 2005;18(4):213-225.

357. Townend W, Ingebrigtsen T. Head injury outcome prediction: a role for protein S-100B? *Injury*. 2006;37(12):1098-1108.

358. Spinella PC, Dominguez T, Drott HR, et al. S-100beta protein-serum levels in healthy children and its association with outcome in pediatric traumatic brain injury. *Crit Care Med*. 2003;31(3):939-945.

359. Martiney JA, Cuff C, Litwak M, Berman J, Brosnan CF. Cytokine-induced inflammation in the central nervous system revisited. *Neurochem Res*. 1998;23(3):349-359.

360. Mastroianni CM, Lancella L, Mengoni F, et al. Chemokine profiles in the cerebrospinal fluid (CSF) during the course of pyogenic and tuberculous meningitis. *Clin Exp Immunol*. 1998;114(2):210-214.

361. Mendez-Samperio P. Expression and regulation of chemokines in mycobacterial infection. *J Infect*. 2008;57(5):374-384.

362. Stamatovic SM, Shakui P, Keep RF, et al. Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. *J Cereb Blood Flow Metab*. 2005;25(5):593-606.

363. Stamatovic SM, Dimitrijevic OB, Keep RF, Andjelkovic AV. Inflammation and brain edema: new insights into the role of chemokines and their receptors. *Acta Neurochir Suppl*. 2006;96:444-450.

364. Kielian T, Drew P. Cytokines and Brain, Health and Disease. In: Minagar, A., Alexander, J.S., ed. *Current Clinical Neurology: Inflammatory Disorders of the Nervous System: Pathogenesis, Immunology, and Clinical Management.* Totowa, NJ: Humana Press Inc; 2005:41-80.

365. Maier B, Laurer HL, Rose S, Buurman WA, Marzi I. Physiological levels of pro- and anti-inflammatory mediators in cerebrospinal fluid and plasma: a normative study. *J Neurotrauma*. 2005;22(7):822-835.

366. Bell MJ, Kochanek PM, Doughty LA, et al. Interleukin-6 and interleukin-10 in cerebrospinal fluid after severe traumatic brain injury in children. *J Neurotrauma*. 1997;14(7):451-457.

367. Richens JL, Urbanowicz RA, Metcalf R, Corne J, O'Shea P, Fairclough L. Quantitative validation and comparison of multiplex cytokine kits. *J Biomol Screen*. 2010;15(5):562-568.

368. dupont NC, Wang K, Wadhwa PD, Culhane JF, Nelson EL. Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants. *J Reprod Immunol*. 2005;66(2):175-191.

369. Himmerich H, Fulda S, Linseisen J, et al. TNF-alpha, soluble TNF receptor and interleukin-6 plasma levels in the general population. *Eur Cytokine Netw*. 2006;17(3):196-201.

370. Pantsulaia I, Trofimov S, Kobyliansky E, Livshits G. Genetic and environmental influences on IL-6 and TNF-alpha plasma levels in apparently healthy general population. *Cytokine*. 2002;19(3):138-146.

371. de Vries HE, Kuiper J, de Boer AG, Van Berkel TJ, Breimer DD. The blood-brain barrier in neuroinflammatory diseases. *Pharmacol Rev*. 1997;49(2):143-155.

372. Thwaites GE, Simmons CP, Than Ha Quyen N, et al. Pathophysiology and prognosis in vietnamese adults with tuberculous meningitis. *J Infect Dis*. 2003;188(8):1105-1115.

373. Blamire AM, Anthony DC, Rajagopalan B, Sibson NR, Perry VH, Styles P. Interleukin-1beta -induced changes in blood-brain barrier permeability, apparent diffusion coefficient, and cerebral blood volume in the rat brain: a magnetic resonance study. *J Neurosci*. 2000;20(21):8153-8159.

374. Ramilo O, Saez-Llorens X, Mertsola J, et al. Tumor necrosis factor alpha/cachectin and interleukin 1 beta initiate meningeal inflammation. *J Exp Med*. 1990;172(2):497-507.

375. Hackett SJ, Thomson AP, Hart CA. Cytokines, chemokines and other effector molecules involved in meningococcal disease. *J Med Microbiol*. 2001;50(10):847-859.

376. Mansour AM, Frenck RW,Jr, Darville T, et al. Relationship between intracranial granulomas and cerebrospinal fluid levels of gamma interferon and interleukin-10 in patients with tuberculous meningitis. *Clin Diagn Lab Immunol*. 2005;12(2):363-365.

377. Coulter JB, Baretto RL, Mallucci CL, et al. Tuberculous meningitis: protracted course and clinical response to interferon-gamma. *Lancet Infect Dis*. 2007;7(3):225-232.

378. Lee SC, Dickson DW, Liu W, Brosnan CF. Induction of nitric oxide synthase activity in human astrocytes by interleukin-1 beta and interferon-gamma. *J Neuroimmunol*. 1993;46(1-2):19-24.

379. Chen L, Jiang H, Wei C. Nitric oxide, TNF-alpha and IL-8 in cerebrospinal fluids of tuberculous and cryptococcic meningitis. *Hunan Yi Ke Da Xue Xue Bao*. 1997;22(6):514-516.

380. Dinarello CA, ed. *Immunological and inflammatory functions of the interleukin-1 family. Annual Review of Immunology*. 2009: No. 27.

381. Simmons CP, Thwaites GE, Quyen NT, et al. Pretreatment intracerebral and peripheral blood immune responses in Vietnamese adults with tuberculous meningitis: diagnostic value and relationship to disease severity and outcome. *J Immunol*. 2006;176(3):2007-2014.

382. Hasan Z, Jamil B, Khan J, et al. Relationship between circulating levels of IFN-gamma, IL-10, CXCL9 and CCL2 in pulmonary and extrapulmonary tuberculosis is dependent on disease severity. *Scand J Immunol*. 2009;69(3):259-267.

383. Sheng WS, Hu S, Kravitz FH, Peterson PK, Chao CC. Tumor necrosis factor alpha upregulates human microglial cell production of interleukin-10 in vitro. *Clin Diagn Lab Immunol*. 1995;2(5):604-608.

384. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med*. 1991;174(5):1209-1220.

385. Oh JW, Drabik K, Kutsch O, Choi C, Tousson A, Benveniste EN. CXC chemokine receptor 4 expression and function in human astroglioma cells. *J Immunol*. 2001;166(4):2695-2704.

386. Zwijnenburg PJ, de Bie HM, Roord JJ, van der Poll T, van Furth AM. Chemotactic activity of CXCL5 in cerebrospinal fluid of children with bacterial meningitis. *J Neuroimmunol*. 2003;145(1-2):148-153.

387. Spanaus KS, Nadal D, Pfister HW, et al. C-X-C and C-C chemokines are expressed in the cerebrospinal fluid in bacterial meningitis and mediate chemotactic activity on peripheral blood-derived polymorphonuclear and mononuclear cells in vitro. *J Immunol*. 1997;158(4):1956-1964.

388. Robinson S, Tani M, Strieter RM, Ransohoff RM, Miller RH. The chemokine growth-regulated oncogene-alpha promotes spinal cord oligodendrocyte precursor proliferation. *J Neurosci*. 1998;18(24):10457-10463.

389. Hasan Z, Jamil B, Ashraf M, et al. ESAT6-induced IFNgamma and CXCL9 can differentiate severity of tuberculosis. *PLoS One*. 2009;4(4):e5158.

390. Mendez A, Hernandez-Pando R, Contreras S, Aguilar D, Rook GA. CCL2, CCL18 and sIL-4R in renal, meningeal and pulmonary TB; a 2 year study of patients and contacts. *Tuberculosis (Edinb)*. 2011;91(2)140-5.

391. Diab A, Abdalla H, Li HL, et al. Neutralization of macrophage inflammatory protein 2 (MIP-2) and MIP-1alpha attenuates neutrophil recruitment in the central nervous system during experimental bacterial meningitis. *Infect Immun*. 1999;67(5):2590-2601.

392. Van Well G. *Innate immune responses in (myco) bacterial meningitis.* [Doctoral dissertation]. Vrije Universiteir Amsterdam; 2012.

393. Tsenova L, Bergtold A, Freedman VH, Young RA, Kaplan G. Tumor necrosis factor alpha is a determinant of pathogenesis and disease progression in mycobacterial infection in the central nervous system. *Proc Natl Acad Sci U S A*. 1999;96(10):5657-5662.

394. Tsenova L, Sokol K, Freedman VH, Kaplan G. A combination of thalidomide plus antibiotics protects rabbits from mycobacterial meningitis-associated death. *J Infect Dis*. 1998;177(6):1563-1572.

395. Tsenova L, Mangaliso B, Muller G, et al. Use of IMiD3, a thalidomide analog, as an adjunct to therapy for experimental tuberculous meningitis. *Antimicrob Agents Chemother*. 2002;46(6):1887-1895.

396. Mazzolla R, Puliti M, Barluzzi R, et al. Differential microbial clearance and immunoresponse of Balb/c (Nramp1 susceptible) and DBA2 (Nramp1 resistant) mice intracerebrally infected with Mycobacterium bovis BCG (BCG). *FEMS Immunol Med Microbiol*. 2002;32(2):149-158.

397. van Well GT, Wieland CW, Florquin S, Roord JJ, van der Poll T, van Furth AM. A new murine model to study the pathogenesis of tuberculous meningitis. *J Infect Dis*. 2007;195(5):694-697.

398. Be NA, Lamichhane G, Grosset J, et al. Murine model to study the invasion and survival of Mycobacterium tuberculosis in the central nervous system. *J Infect Dis*. 2008;198(10):1520-1528.

399. Lee J, Ling C, Kosmalski MM, et al. Intracerebral Mycobacterium bovis bacilli Calmette-Guerin infection-induced immune responses in the CNS. *J Neuroimmunol*. 2009;213(1-2):112-122.

400. Bolin CA, Whipple DL, Khanna KV, Risdahl JM, Peterson PK, Molitor TW. Infection of swine with Mycobacterium bovis as a model of human tuberculosis. *J Infect Dis*. 1997;176(6):1559-1566.

401. Donald PR, Schoeman JF, Beyers N, et al. Concentrations of interferon gamma, tumor necrosis factor alpha, and interleukin-1 beta in the cerebrospinal fluid of children treated for tuberculous meningitis. *Clin Infect Dis*. 1995;21(4):924-929.

402. Kashyap RS, Deshpande PS, Ramteke SR, et al. Changes in Cerebrospinal Fluid Cytokine Expression in Tuberculous Meningitis Patients with Treatment. *Neuroimmunomodulation*. 2010;17(5):333-339.

403. Nagesh Babu G, Kumar A, Kalita J, Misra UK. Proinflammatory cytokine levels in the serum and cerebrospinal fluid of tuberculous meningitis patients. *Neurosci Lett*. 2008;436(1):48-51.

404. Yaramis A, Colpan L, Tas MA. Diagnostic value of cytokine measurement in cerebrospinal fluid in children with central nervous system tuberculosis. *Pediatrics*. 2001;107(5):1236.

405. Adams LB, Mason CM, Kolls JK, Scollard D, Krahenbuhl JL, Nelson S. Exacerbation of acute and chronic murine tuberculosis by administration of a tumor necrosis factor receptor-expressing adenovirus. *J Infect Dis*. 1995;171(2):400-405.

406. Curto M, Reali C, Palmieri G, et al. Inhibition of cytokines expression in human microglia infected by virulent and non-virulent mycobacteria. *Neurochem Int*. 2004;44(6):381-392.

407. Patel VB, Bhigjee AL, Bill PLA. Cytokine profiles in HIV seropositive patients with tuberculous meningitis. *J Neurol Neurosurg Psychiatry*. 2002;73:597-603.

408. Rock RB, Hu S, Gekker G, et al. Mycobacterium tuberculosis-induced cytokine and chemokine expression by human microglia and astrocytes: effects of dexamethasone. *J Infect Dis*. 2005;192(12):2054-2058.

409. Seneviratne SL, Doffinger R, Macfarlane J, et al. Disseminated Mycobacterium tuberculosis infection due to interferon gamma deficiency. Response to replacement therapy. *Thorax*. 2007;62(1):97-99.

410. Raad I, Hachem R, Leeds N, Sawaya R, Salem Z, Atweh S. Use of adjunctive treatment with interferon-gamma in an immunocompromised patient who had refractory multidrug-resistant tuberculosis of the brain. *Clin Infect Dis*. 1996;22(3):572-574.

411. Lee JY, Yim JJ, Yoon BW. Adjuvant interferon-gamma treatment in two cases of refractory tuberculosis of the brain. *Clin Neurol Neurosurg*. 2012;114(6):732-734.

412. Waage A, Halstensen A, Shalaby R, Brandtzaeg P, Kierulf P, Espevik T. Local production of tumor necrosis factor alpha, interleukin 1, and interleukin 6 in meningococcal meningitis. Relation to the inflammatory response. *J Exp Med*. 1989;170(6):1859-1867.

413. Kornelisse RF, Savelkoul HF, Mulder PH, et al. Interleukin-10 and soluble tumor necrosis factor receptors in cerebrospinal fluid of children with bacterial meningitis. *J Infect Dis*. 1996;173(6):1498-1502.

414. Yilmaz E, Gurgoze MK, Ilhan N, Dogan Y, Aydinoglu H. Interleukin-8 levels in children with bacterial, tuberculous and aseptic meningitis. *Indian J Pediatr*. 2002;69(3):219-221.

415. Vermont CL, Hazelzet JA, de Kleijn ED, van den Dobbelsteen GP, de Groot R. CC and CXC chemokine levels in children with meningococcal sepsis accurately predict mortality and disease severity. *Crit Care*. 2006;10(1):R33.

416. Arditi M, Manogue KR, Caplan M, Yogev R. Cerebrospinal fluid cachectin/tumor necrosis factor-alpha and platelet-activating factor concentrations and severity of bacterial meningitis in children. *J Infect Dis*. 1990;162(1):139-147.

417. van Furth AM, Roord JJ, van Furth R. Roles of proinflammatory and anti-inflammatory cytokines in pathophysiology of bacterial meningitis and effect of adjunctive therapy. *Infect Immun*. 1996;64(12):4883-4890.

418. Jain M, Aneja S, Mehta G, Ray GN, Batra S, Randhava VS. CSF interleukin-1 beta, tumor necrosis factor-alpha and free radicals production in relation to clinical outcome in acute bacterial meningitis. *Indian Pediatr*. 2000;37(6):608-614.

419. Ceyhan M, Kanra G, Ecevit Z, et al. Tumor necrosis factor-alpha and interleukin-1 beta levels in children with bacterial, tuberculous and aseptic meningitis. *Turk J Pediatr*. 1997;39(2):177-184.

420. Juan RS, Sanchez-Suarez C, Rebollo MJ, et al. Interferon gamma quantification in cerebrospinal fluid compared with PCR for the diagnosis of tuberculous meningitis. *J Neurol*. 2006;253(10):1323-1330.

421. Yu Y, Zhang Y, Hu S, et al. Different Patterns of Cytokines and Chemokines Combined with IFN-γ Production Reflect Mycobacterium tuberculosis Infection and Disease. *PLoS ONE*. 2012;7(9).

422. Molteno CD, Hollingshead J, Moodie AD, Bradshaw D, Bowie MD, Willoughby W. Preschool development of coloured children in Cape Town. *South African Medical Journal*. 1991;79(11):665-670.

423. Laughton B, Springer PE, Grove D, et al. Longitudinal developmental profile of children from low socio-economic circumstances in Cape Town, using the 1996 Griffiths Mental Development Scales. *SAJCH South African Journal of Child Health*. 2010;4(4):106-111.

424. Berger RP, Bazaco MC, Wagner AK, Kochanek PM, Fabio A. Trajectory analysis of serum biomarker concentrations facilitates outcome prediction after pediatric traumatic and hypoxemic brain injury. *Dev Neurosci*. 2010;32(5-6):396-405.

425. Berger RP, Ta'asan S, Rand A, Lokshin A, Kochanek P. Multiplex assessment of serum biomarker concentrations in well-appearing children with inflicted traumatic brain injury. *Pediatr Res*. 2009;65(1):97-102.

426. Figaji AA, Fieggen AG, Schoeman JF, Peter JC. Endoscopic third ventriculostomy in post-tubercular meningitic hydrocephalus. *Minim Invasive Neurosurg*. 2006;49(1):60-61.

427. Fiser DH. Assessing the outcome of pediatric intensive care. *J Pediatr*. 1992;121:68-74.

428. Luiz DM, Foxcroft CD, Stewart R. The construct validity of the Griffiths Scales of Mental Development. *Child Care Health Dev*. 2001;27(1):73-83.

429. Meyers T, Moultrie H, Naidoo K, Cotton M, Eley B, Sherman G. Challenges to pediatric HIV care and treatment in South Africa. *J Infect Dis*. 2007;196 Suppl 3:S474-81.

430. Thwaites GE, Chau TT, Farrar JJ. Improving the bacteriological diagnosis of tuberculous meningitis. *J Clin Microbiol*. 2004;42(1):378-379.

431. Reiber H. Proteins in cerebrospinal fluid and blood: barriers, CSF flow rate and source-related dynamics. *Restor Neurol Neurosci*. 2003;21(3-4):79-96.

432. Naija W, Mateo J, Raskine L, et al. Case report: greater meningeal inflammation in lumbar than in ventricular region in human bacterial meningitis. *Crit Care*. 2004;8(6):R491-4.

433. Sommer JB, Gaul C, Heckmann J, Neundorfer B, Erbguth FJ. Does lumbar cerebrospinal fluid reflect ventricular cerebrospinal fluid? A prospective study in patients with external ventricular drainage. *Eur Neurol*. 2002;47(4):224-232.

434. Gerber J, Tumani H, Kolenda H, Nau R. Lumbar and ventricular CSF protein, leukocytes, and lactate in suspected bacterial CNS infections. *Neurology*. 1998;51(6):1710-1714.

435. Lowe DM, Redford PS, Wilkinson RJ, O'Garra A, Martineau AR. Neutrophils in tuberculosis: friend or foe? *Trends Immunol*. 2012;33(1):14-25.

436. Venter A. 'Cross-cultural' Issues in Child Development and Assessment within the South African context. *Southern African Journal of Child and Adolescent Mental Health*. 2000;12(2):162-177.

437. Helmy A, Antoniades CA, Guilfoyle MR, Carpenter KL, Hutchinson PJ. Principal component analysis of the cytokine and chemokine response to human traumatic brain injury. *PLoS One*. 2012;7(6):e39677.

438. Hair J, Black W, Babin B, Anderson R. *Multivariate data analysis.* 7th ed. Upper Saddle River, NJ: Prentice Hall; 2010.

439. Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. *Clin Chim Acta*. 2001;310(2):173-186.

440. Ghanem G, Loir B, Morandini R, et al. On the release and half-life of S100B protein in the peripheral blood of melanoma patients. *Int J Cancer*. 2001;94(4):586-590.

441. Tolan NV, Vidal-Folch N, Algeciras-Schimnich A, Singh RJ, Grebe SK. Individualized correction of neuron-specific enolase (NSE) measurement in hemolyzed serum samples. *Clin Chim Acta*. 2013;424:216-221.

442. Albrechtsen M, Sorensen PS, Gjerris F, Bock E. High cerebrospinal fluid concentration of glial fibrillary acidic protein (GFAP) in patients with normal pressure hydrocephalus. *J Neurol Sci*. 1985;70(3):269-274.

443. Beems T, Simons KS, Van Geel WJ, De Reus HP, Vos PE, Verbeek MM. Serum- and CSF-concentrations of brain specific proteins in hydrocephalus. *Acta Neurochir (Wien)*. 2003;145(1):37-43.

444. Stewart A, Tekes A, Huisman TA, et al. Glial fibrillary acidic protein as a biomarker for periventricular white matter injury. *Am J Obstet Gynecol*. 2013;209(1):27.e1-27.e7.

445. Tullberg M, Rosengren L, Blomsterwall E, Karlsson JE, Wikkelso C. CSF neurofilament and glial fibrillary acidic protein in normal pressure hydrocephalus. *Neurology*. 1998;50(4):1122-1127.

446. Tarnaris A, Toma AK, Chapman MD, et al. Rostrocaudal dynamics of CSF biomarkers. *Neurochem Res*. 2011;36(3):528-532.

447. Greenfield JG. Original Papers: ON FROIN'S SYNDROME, AND ITS RELATION TO ALLIED CONDITIONS IN THE CEREBROSPINAL FLUID. *J Neurol Psychopathol*. 1921;2(6):105-141.

448. Schoeman JF, Laubscher JA, Donald PR. Serial lumbar CSF presure measurements and cranial computed tomographic findings in childhood tuberculous meningitits. *Childs Nerv Syst*. 2000;16(4):203-8; discussion 209.

449. Marais BJ, Gie RP, Schaaf HS, Beyers N, Donald PR, Starke JR. Childhood pulmonary tuberculosis: old wisdom and new challenges. *Am J Respir Crit Care Med*. 2006;173(10):1078-1090.

450. Lamont AC, Cremin BJ, Pelteret RM. Radiological patterns of pulmonary tuberculosis in the paediatric age group. *Pediatr Radiol*. 1986;16(1):2-7.

451. Padayachy LC, Figaji AA, Bullock MR. Intracranial pressure monitoring for traumatic brain injury in the modern era. *Childs Nerv Syst*. 2010;26(4):441-452.

452. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1(8476):307-310.

453. Nourse P, Schoeman JF, van der Merwe PL. Does cerebral perfusion pressure influence outcome in children with tuberculous meningitis? *Dev Med Child Neurol*. 2004;46(5):358-360.

454. Rosenthal G, Hemphill JC,3rd, Sorani M, et al. Brain tissue oxygen tension is more indicative of oxygen diffusion than oxygen delivery and metabolism in patients with traumatic brain injury. *Crit Care Med*. 2008;36(6):1917-1924.