

Biomarkers of cerebral injury and inflammation in pediatric tuberculous meningitis

Ursula K Rohlwink, PhD ^{a, c}, Katya Mauff ^b, Katalin A Wilkinson, PhD ^{c, d}, Nico Enslin, MBChB, FCS ^a, Emmanuel Wegoye, MBChB, FCS ^a, Robert J Wilkinson, FRCP ^{c, e}, Anthony A Figaji, MBChB, FCS, PhD ^a

Affiliations: ^a Division of Neurosurgery, University of Cape Town, South Africa; ^b Department of Statistical Science, University of Cape Town, South Africa, ^c Clinical Infectious Diseases Research Initiative, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, South Africa, ^d Francis Crick Institute, London, NW1 2AT, United Kingdom, ^e Department of Medicine, Imperial College London, W2 1PG, United Kingdom

Address correspondence to: Ursula Rohlwink, ursulakarin@gmail.com, 6th Floor, ICH Building, Red Cross War Memorial Children's Hospital, Klipfontein Road, Rondebosch, Cape Town, South Africa, 7700, Tel: 00 27 21 658 5341, Fax: 00 27 21 685 6632

Alternative contact: Anthony Figaji, Anthony.Figaji@uct.ac.za, 6th Floor, ICH Building, Red Cross War Memorial Children's Hospital, Klipfontein Road, Rondebosch, Cape Town, South Africa, 7700, Tel: 00 27 21 658 5340, Fax: 00 27 21 685 6632

Author Addresses:

Ursula Rohlwink: 6th Floor, ICH Building, Red Cross War Memorial Children's Hospital, Klipfontein Road, Rondebosch, Cape Town, South Africa, 7700

Katya Mauff: Erasmus MC, Department of Biostatistics, NA 2418, PO Box 2040, 3000 CA Rotterdam, The Netherlands

Katalin Wilkinson: Institute of Infectious Disease and Molecular Medicine, Room N2.09.B3 Wernher Beit North Building, UCT Faculty of Health Sciences, Observatory 7925, South Africa

Nico Enslin: Division of Neurosurgery, Paediatric Neurosurgery Unit, Red Cross War Memorial Children's Hospital, Klipfontein Road, Rondebosch, University of Cape Town, South Africa, 7700

Emmanuel Wegoye: Division of Neurosurgery, University of Cape Town, South Africa, 7700

Robert Wilkinson: Clinical Infectious Diseases Research Initiative, Room 3.03.05, Wolfson Pavilion, UCT Faculty of Health Sciences, Observatory 7925, South Africa

Anthony Figaji: 6th Floor, ICH Building, Red Cross war Memorial Children's Hospital, Klipfontein Road, Rondebosch, Cape Town, South Africa, 7700

Running title: Biomarkers in TBM

Keywords: pediatric tuberculous meningitis, biomarkers, S100B, NSE, GFAP

Key points: Neuro-specific biomarkers were elevated in pediatric TBM and showed an increasing temporal profile in patients who died, whereas markers of inflammation decreased in all patients regardless of outcome. Secondary injury mechanisms initiated by inflammation likely cause ongoing brain damage.

Abstract

Background

Tuberculous meningitis (TBM) leads to death or disability in half the affected individuals. Tools to assess severity and predict outcome are lacking. Neuro-specific biomarkers could serve as markers of the severity and evolution of brain injury, but have not been widely explored in TBM. We examined biomarkers of neurological injury (neuromarkers) and inflammation in pediatric TBM and their association with outcome.

Methods

Blood and cerebrospinal fluid (CSF) of children with TBM and hydrocephalus taken on admission and over 3 weeks were analysed for neuromarkers S100B, neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP), and multiple inflammatory markers. Results were compared with 2 control groups; patients with 1) a fatty filum (abnormal filum terminale of the spinal cord), and 2) pulmonary tuberculosis (pTB). Imaging was conducted on admission and at 3 weeks. Outcome was assessed at 6 months.

Results

Data were collected from 44 TBM cases (median age 3.3 [0.3-13.1] years), 11 fatty filum (median age 2.8 [0.8-8] years) and 9 pTB controls (median age 3.7 [1.3-11.8] years). Seven cases (16%) died and 16 (36%) had disabilities. Neuromarkers and inflammatory markers were elevated in CSF on admission and for up to 3 weeks, but not in serum. Initial and highest concentrations in week 1 of S100B and NSE were associated with poor outcome, as were highest concentration overall and an increasing profile over time in S100B, NSE and GFAP. Combined neuromarker concentrations increased over time in patients who died, whereas inflammatory markers decreased. Cerebral infarcts were associated with highest overall neuromarker concentrations and an increasing profile over time. Tuberculomas were associated with elevated IL-12p40, IP-10 and MCP-1 concentrations, whereas infarcts were associated with elevated TNF- α , MIP-1 α , IL-6 and IL-8.

Conclusion

CSF neuromarkers are promising biomarkers of injury severity and are predictive of mortality. An increasing trend suggested ongoing brain injury, even though markers of inflammation declined with treatment. These findings could offer novel insight into the pathophysiology of TBM..

Introduction

Tuberculous meningitis is a severe form of cerebral tuberculosis leading to high rates of death and disability in children [1-3]. The resulting cerebral tissue injury is an important determinant of poor outcome and is considered a consequence of the host immune response. The prolific inflammatory process causes extensive basal subarachnoid exudate and vascular involvement, with external compression, vasospasm, and endovascularitis [4]. Additionally, it often obstructs the flow of cerebrospinal fluid (CSF), precipitating hydrocephalus. The consequent raised intracranial pressure (ICP) aggravates the already decreased cerebral blood flow (CBF) and increases the risk of brain ischaemia and infarction.

Quantification of the degree of cerebral injury is inexact: disease severity is commonly evaluated by clinical and radiological findings. However, these have limited utility - several reversible and irreversible factors influence clinical status and imaging findings usually manifest once the damage is already permanent. Advanced tools to assess cerebral injury severity and prognosis are lacking. Inflammatory markers have been studied in TBM [5-12]; however, the extent of inflammation as detected by these markers may not correlate with the extent of cerebral tissue injury. Direct markers of cerebral tissue injury are elevated in TBM [13, 14] but have not been examined in detail or in association with markers of inflammation. Reliable markers of neurological tissue injury could serve as biomarkers to assess injury severity, monitor disease resolution or deterioration, direct treatment, prognosticate, and as surrogate end points to help develop novel treatments. Literature on neuro-tissue markers in various central nervous system (CNS) pathologies (including infection, trauma, stroke, hemorrhage and degenerative disease) has identified significant relationships between brain injury and/or poor clinical outcome and elevated concentrations of neuron-specific enolase (NSE – a marker of neuronal injury), S100B and glial-fibrillary acidic protein (GFAP – both markers of astroglial injury) [15]. Given their promise in other forms of brain injury we examined the concentrations and temporal profiles of these three markers of neurological tissue injury in serum, and lumbar and ventricular CSF compared with controls. These were combined with markers of inflammation, and examined in association with clinical and radiological outcome in children with TBM.

Methods

This study was approved by the Institutional Review Board of the University of Cape Town (HREC 318/2010). Informed consent was obtained from the parents of all participants.

Patient cohort

Over a three year period (October 2010 – August 2013) study participants from the Red Cross War Memorial Children's Hospital Cape Town, were enrolled in this prospective study. TBM cases comprised children admitted for definite or probable TBM according to a published case definition.[16] Cases were limited to patients with associated hydrocephalus as they undergo repeated CSF diversion procedures as part of treatment. All cases were treated with the standard antimicrobial regimen and adjunctive prednisone [17]. Hydrocephalus was treated according to a published protocol [18]. Demographic and clinical data were collected as previously described [19]. Clinical outcome according to the Pediatric Cerebral Performance Category Scale [20] (poor outcome [PCSP 4-6: severe disability - death], good outcome [PCPS 1-3: normal – mild/moderate disability]) and mortality at six months were recorded. Radiological data were collected from admission and follow up computed tomography (CT) brain scans, three week magnetic resonance imaging (MRI) of the brain and spine and MR angiogram of the cerebral vessels [21]. Control patients included: 1) children admitted for elective surgery for a fatty filum terminale of the spinal cord (a congenital abnormality not associated with neurological injury or inflammation); 2) children clinically diagnosed with and treated for pulmonary TB (pTB).

Sample collection (Supplement 1)

Samples from cases included lumbar CSF, ventricular CSF (from the lateral ventricles) and serum collected concurrently with clinically indicated procedures on admission and once weekly for 3-4 weeks. For the fatty filum controls blood was drawn prior to surgery and lumbar CSF was taken before the dura was opened. Blood from pTB controls was taken before treatment initiation or within 48 hours thereof. Samples were stored at -80°C for batch analysis. Commercially available enzyme-

linked immunoassays were used to analyse neuromarkers S100B (Merck Millipore, Billerica, USA), NSE (DRG Diagnostics, Marburg, Germany) and GFAP (Merck Millipore, Billerica, USA) according to manufacturer instructions. Interleukin (IL)-1 β , IL-1 receptor antagonist (RA), IL-6, IL-10, IL-12p40, tumour necrosis factor (TNF)- α , interferon (IFN)- γ , IL-8, growth regulated oncogene (GRO), monocyte chemoattractant protein (MCP)-1, interferon-inducible protein (IP)-10, macrophage inflammatory protein (MIP) -1 α , vascular endothelial growth factor (VEGF) and regulated upon-activation, normal T-cell-expressed and secreted protein (RANTES) were analysed on the Bio-Plex platform (Bio-Rad Laboratories, Hercules, CA, USA), using customised MilliplexTM kits (Millipore, St Charles, MO, USA), according to manufacturer instructions.

Samples were excluded when they were taken within 48 hours after neurosurgical procedures, were not immediately refrigerated or processed within 12 hours of collection, exhibited hemolysis or xanthochromia (NSE only) – Supplement 1.

Imaging data (Supplement 3 with images included)

Hydrocephalus was defined as mild (visible temporal horns, rounding of the third ventricle [V3]), moderate (all ventricles dilated, or at least triventricular in the case of aqueduct obstruction, no transependymal fluid shift) or severe (markedly dilated ventricles, fluid shift and loss of sulcal markings). Infarcts were recorded as unilateral or bilateral, and their size was graded as single (lacunar), multiple/large (multiple or large branch infarcts) and large territory (encompassing MCA, ACA, PCA territories). *Tuberculomas* were classified by number (single or multiple when >1). Scans were evaluated by 3 assessors blinded to patient outcome.

Data Analysis

Descriptive statistics were compiled for samples at each time point. Neuromarker concentrations of TBM cases were compared to controls using Mann Whitney's U with a Holm's p-value adjustment. The association between initial (admission or week 1 when no admission data was available)

neuromarker concentrations and patient characteristics (age, sex, admission symptoms) was evaluated using the Chi-squared or Fisher's exact test. The correlation between neuro- and inflammatory markers and CSF glucose, chloride, protein and white cell count was examined using Spearman's correlation. Using the Kruskal Wallis test findings on admission CT scan (Supplement 3) were analysed in association with initial (admission) CSF neuro- and inflammatory marker concentrations. Further, radiology features overall (admission and follow-up CT/MRI) were examined in association with highest overall concentrations of the neuro- and inflammatory markers, and the change in neuromarker concentrations between admission and week 2 (Δ S100B, Δ NSE, Δ GFAP) as an indicator of early change.

Outcome analysis focussed on neuromarkers. *Univariate analysis:* The relationship between neuromarker concentrations and outcome was examined using Mann-Whitney's U. Various neuromarker indices including initial, highest in week 1, highest overall throughout hospitalisation, Δ were examined to control for the limitations of each individual index. *PCA:* Z-scores combining all 3 neuromarkers (Z-Neuro) or all 14 inflammatory markers (Z-Inflammatory) for each patient were derived as a statistical approximation of a single index for neurological injury or inflammation; time was added to this analysis to generate 3-dimensional PCA biplots, and mortality was indicated on the plot (Supplement 2). *Receiver operating characteristic (ROC) analysis:* ROC curves were constructed to identify the thresholds of highest S100B, NSE and GFAP as well as Δ S100B, Δ NSE and Δ GFAP which best predicted outcome. *Multivariate analysis:* Logistic regression models for poor outcome were constructed for Δ S100B, Δ NSE and Δ GFAP since these indices demonstrated the strongest outcome association on univariate analysis. Covariates were drawn from admission and radiology characteristics which were significantly associated with clinical outcome.[19]

Results

Forty-four TBM cases (median age 3.3[0.3-13.1] years) were enrolled (Table 1). The culture positivity yield was 56.4%, no drug resistance was reported. Hydrocephalus was non-communicating in 7% of patients. Thirty-two patients (72.7%) had a good clinical outcome, seven patients died

(16%). Infarcts were reported in 66% of patients, tuberculomas in 11%, and spinal pathology in 76% (Table 2). Eleven fatty filum controls (median age 2.75 [0.75-8] years) and nine pTB controls (median age 3.67 [1.25-11.83] years) were enrolled and were similar to cases in age and sex distribution. PTB controls were treated according to the standard medical regimen.

Descriptive statistics for neuromarkers for each time point are shown in Figure 1 and included in Supplements 4 and 5 (inflammatory data also presented). CSF neuro- and inflammatory marker concentrations were greater in cases than controls, but serum values were not (Supplement 4). CSF neuro- and inflammatory marker concentrations in the CSF remained greater than controls for up to 4 weeks. Overall neuromarker concentrations were greater in ventricular than lumbar CSF, whereas inflammatory marker concentrations were greater in lumbar than ventricular CSF (Figure 1 and Supplements 5 and 6).

Neuromarker concentrations were not significantly associated with patient demographic or admission characteristics. Only lumbar GFAP was significantly more elevated in patients who were culture positive ($p<0.05$). Neuro- and inflammatory markers were not significantly associated with CSF glucose, chloride, protein or white cell count.

Association with Radiology

Admission CT scan: The highest lumbar GFAP and S100B concentrations were greater in moderate-severe than mild hydrocephalus ($p<0.01$ and $p=0.02$ respectively) – Figure 2. Ventricular TNF- α and IFN- γ concentrations were higher 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).

Radiology overall (Figure 2): The highest lumbar GFAP and NSE were associated with multiple ($p=0.02$ and $p<0.01$ respectively) and large infarcts compared to small ones ($p<0.01$ and $p=0.05$ respectively). Highest ventricular GFAP and NSE were also positively associated with larger infarcts ($p=0.04$ and $p=0.02$). Lumbar GFAP and NSE concentrations increased from admission to week 2 in patients with infarcts ($p=0.01$ and $p=0.03$ respectively), particularly with large infarcts relative to

small ($p=0.01$ and $p=0.01$ respectively). Similarly S100B concentrations increased in patients with larger infarcts ($p=0.01$). Highest lumbar and ventricular GFAP were associated with multiple tuberculomas ($p=0.04$ and $p=0.02$ respectively). No association was demonstrated between neuromarkers and spinal disease.

Parenchymal tuberculomas were positively associated with highest lumbar CSF IL-12p40 ($p=0.04$), IP-10 ($p<0.01$) and MCP-1 ($p=0.02$). Bilateral infarcts was positively associated with highest lumbar and ventricular CSF TNF- α ($p=0.05$ and $p=0.01$ respectively), MIP-1 α ($p=0.03$ and $p<0.01$ respectively) and IL-6 ($p=0.05$ and $p=0.01$ respectively) and IL-8 (ventricular CSF only, $p=0.02$) concentrations - *Figure 3* (multiple testing was not controlled for).

Association with outcome

Univariate analysis (Figure 4): The highest lumbar S100B concentrations recorded in week 1 and overall were associated with outcome at 6 months ($p=0.04$ and $p=0.03$ respectively), and an increasing trend was significantly predictive of mortality and six month outcome ($p<0.001$ and $p=0.02$ respectively). Initial and highest in week 1 ventricular S100B concentrations were associated with mortality ($p=0.02$ and $p=0.03$ respectively) and 6 month outcome ($p=0.01$ and $p=0.01$), and highest overall with mortality ($p=0.02$). Highest lumbar NSE overall and an increasing trend were predictive of mortality ($p=0.01$ and $p=0.01$ respectively) and 6 month outcome ($p=0.01$ and $p=0.02$ respectively). All ventricular NSE indices were strong predictors of mortality and 6 month outcome ($p\leq 0.01$). Highest lumbar GFAP overall and an increasing trend were associated with mortality ($p=0.01$ and $p=0.003$ respectively). Ventricular GFAP was not a predictor of mortality or outcome.

PCA analysis: PCA plots of the temporal profile of combined neuromarkers (Z-Neuro) illustrated that neuromarker concentrations in the lumbar CSF decreased over time in patients who survived, but increased in those who died (*Figure 5A*). For neuromarkers in lumbar CSF PC1+PC2 explained 86.6% of variability in the data over time, in ventricular CSF PC1+PC2 explained 92.5%. For the combined inflammatory markers (Z-Inflammatory) patients who died followed a similar downward

trajectory to those who survived (Figure 5B); 52.5% of the variability was described by the first 2 PCs in lumbar CSF and 52.6% in ventricular CSF.

ROC analysis: An increase in neuromarker concentrations from admission to week 2 predicted mortality with the best combination of sensitivity and specificity (Table 3); in particular Δ S100B as little as 0.06 $\mu\text{g/L}$ had 100% sensitivity and 93.1% specificity for predicting mortality. ROCs were not constructed for inflammatory markers due to the poor outcome association.

Multivariate analysis (Table 4): An increase between admission and week 2 in S100B, NSE, GFAP and Z-Neuro all demonstrated associations with death in multiple logistic regression models. The odds of death were 23 times greater with a 1 unit increase in Z-Neuro Δ (CI: 1.57-324.66, $p=0.02$) and nine times greater per unit increase in Δ S100B (CI: 1.9-42.1, $p=0.005$). Patients with an increase in Δ NSE and Δ GFAP had only slightly higher odds of death (CI: 1-1.1, $p=0.02$) and (CI: 1-1.2, $p=0.01$) respectively. Covariates included in the models were MRC staging, symptom duration, age, gender, HIV status, fever, seizures, culture positivity, hydrocephalus severity and tuberculomas. Infarcts could not be added because they perfectly predicted death. Only the inclusion of seizures and tuberculomas altered these models (results shown in Table 3) Some of the multivariate models were also predictive of dichotomized clinical outcome at six months; the odds of a poor outcome were 4.1 times greater per unit increase in Z-Neuro Δ (CI: 1.31-13.04, $p=0.02$), and only slightly higher in patients with an increase in Δ NSE (CI: 1.01-1.11, $p=0.02$). These relationships were not affected by clinical, admission or radiology characteristics.

Discussion

This study demonstrated the prolonged elevation of CSF S100B, NSE and GFAP and their association with poor outcome, in particular when they increased over time. These markers may help elucidate the causes of progressive injury in these patients. Neither neuro- nor inflammatory markers were elevated in serum suggesting that serum reflects little of the injury in the central nervous system, and that the

immune response is compartmentalised to the site of disease. Elevated inflammatory markers in the CSF decreased in all patients regardless of outcome.

Biomarker elevations in TBM cases

The highest CSF neuromarker concentrations were observed in the ventricular CSF, consistent with their cerebral origin. Lower lumbar concentrations were likely due to degradation by proteinases as CSF flows down the rostro-caudal gradient of the brain-spine axis [22-24]. However, cytokine concentrations were greater in lumbar CSF, possibly because much of the inflammatory process occurs in the subarachnoid space and because 76% of patients also had spinal disease. These differences are important in interpreting results from different compartments, especially where lumbar CSF results are reported without concurrent spinal imaging. Determining treatment/prognostic thresholds for biomarkers is challenging; studies demonstrate variability in biomarker concentrations across brain injury etiologies, testing platforms, and age of patients [15].

Serum neuromarker concentrations were low. Various factors may determine the concentration of brain-derived proteins in the blood, including their intrathecal concentration, response to pathology, diffusion across the blood-brain and blood-CSF barrier, and half-life in the blood. S100B, NSE and GFAP are detectable in the blood early after traumatic brain injury or stroke [25-27]; however, their half-lives are estimated between 30 minutes-48 hours [28, 29]. Consequently, serum concentrations likely reflect only a fraction of CSF concentrations and the timing of sampling relative to the onset of injury is important, but difficult to identify in a chronic condition like TBM where injury may also not be a single event. Episodes of ischemia and peaks in serum concentrations may, therefore, have been missed, especially if patients presented late.

Association with radiology

Neuromarker concentrations were not associated with spinal disease, possibly because neuromarkers of spinal origin may be masked by larger concentrations of cerebral origin. The association between severity of hydrocephalus and high GFAP and S100B concentrations suggests that these markers may be sensitive to transient injury due to the mechanical effect of dilating ventricles on the parenchyma,

often accompanied by the opposing pressure from brain oedema [30, 31]. The lack of significant differences in highest neuromarker concentrations between patients with and without infarcts may reflect the fact that 35% of patients with infarcts had only small lesions. Overall neuromarker concentrations were greater with large infarcts; their increasing profiles over time likely reflected ischaemia-induced progressive injury. Because infarcts detected on imaging are already established, tracking these biomarkers could highlight patients at high risk of deterioration before irreversible events occur and increase surveillance and supportive measures (including supplemental oxygen, blood pressure augmentation, and more aggressive ICP control). In this way the neuromarkers could complement imaging and other clinical measures of disease severity and progression. They may also help us better understand the pathophysiological process and the dynamic nature of the disease. Additionally markers may also act as a proxy for disease response to intervention.

The association between high initial concentrations of ventricular CSF IFN- γ and TNF- α with mild enhancement and an absence of infarcts on admission scan may represent the early phases of the inflammatory process. In this cohort tuberculomas and infarcts were negatively correlated, and tuberculomas were predictive of survival whereas infarcts were predictive of death [21]. This may represent the evolution of disease with tuberculomas representing an early phase of the disease which progresses to more severe disease with infarcts if treatment is delayed. Alternatively, it may be that the underlying pathophysiology leading to the development of tuberculomas is different from that leading to infarction, as demonstrated by the differential cytokine profiles. A localised Th1 response promoted by IL-12p40 and reflected in elevated IP-10 and MCP-1 concentrations may promote tuberculoma formation, whereas elevated chemokines TNF- α , MIP-1 α , IL-6 and IL-8 may provoke diffuse cellular infiltration and inflammation contributing to more extensive infarction.

Association with clinical outcome

Increasing neuromarker concentrations in CSF were the best indication of poor outcome, especially Δ S100B, suggesting that cerebral injury in TBM continues to evolve in some patients and leads to poor outcome despite the initiation of anti-TB and steroid treatment. The associated decreasing trend

of 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 the secondary pathological processes that are initiated by the inflammatory process and continue to cause tissue injury. These processes may include biochemical and inflammatory mechanisms of secondary brain injury. This highlights the need for not only anti-TB and immunomodulatory drugs, but also better brain protection. More evidence of specific pathophysiological processes leading to an increase in neuromarkers is needed in order to direct brain management appropriately. If these processes are associated with brain ischemia, simple interventions such as more aggressive treatment of ICP, augmentation of blood pressure, and supplemental systemic oxygenation may improve oxygenation of the brain. Ongoing studies of pharmacological intervention in other forms of brain injury [32] may be applicable also in meningitis if there are similar underlying mechanisms.

Neuromarkers have predictive potential, either as point in time measures or, more particularly, over time. The profile over time may also be valuable in evaluating response to novel interventions. Ventricular CSF neuromarker concentrations appeared to be more predictive than lumbar CSF, likely because they arise from the predominant site of injury. In the resource limited setting assessment of injury may rely on a single marker; S100B is promising - an increase between admission and week 2 was associated with infarct severity and also strongly predicted mortality.

Limitations

The sample size was modest and the age range too variable to adequately compare cases with age-matched controls; however, the role of age in normative biomarker concentrations remains contentious and there is no clear indication of how age influences normal ranges [15]. More frequent sampling during the acute phase (admission-week 2) may have provided greater insight into the dynamic nature of disease evolution. However, patients presented at different stages of the disease course and so peak concentrations may have been missed. The cytokines measured were not associated with injury severity or outcome; it may be that an unbiased exploration such as transcriptomic profiling would generate a better understanding of the association between the immune

response and outcome. The complexity of the data set limited the statistical tools for examining outcome association and correction for multiple comparisons for the association between the inflammatory markers and radiology was not conducted. PCA enabled combining multiple biomarkers over time but does not provide insight into the biology of the disease process; however, this study aimed to collect pilot data and generate hypotheses. This study was limited to patients with associated hydrocephalus and findings may not be generalizable to patients without hydrocephalus. Comparing neuromarker concentrations in TBM cases to a control group of patients with non-TB CNS infections would be valuable to discern the specificity of these neuromarkers and the range of elevation for TBM. This was beyond the scope of this pilot study and would be a valuable addition to a follow up study.

Conclusion

Neuromarkers in the CSF of patients with TBM provide insight into ongoing cerebral injury and prognosis. They may help direct limited resources and act as surrogate markers for the efficacy of novel interventions. Treatment or injury thresholds should be established for the ventricular and lumbar CSF compartments. Serum is uninformative for examining cerebral inflammation and injury. The peak of inflammation likely occurs early and decreases on treatment. Further examination of the secondary injury processes initiated by the inflammatory process is needed.

Acknowledgements

This work was supported by the Wellcome Trust [grant number 084323 to U.K.R.] The authors have no conflicts of interest to declare.

References

1. 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.
2. 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): 30-5.
3. Yaramis A, Gurkan F, Elevli M, et al. Central nervous system tuberculosis in children: A review of 214 cases. *Pediatrics*, **1998**; 102(5): E49.
4. Rich A, McCordock H. The pathogenesis of tuberculous meningitis. *Bull John Hopkins Hosp*, **1933**; 52: 5-37.
5. 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): 171-6.
6. 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): 924-9.
7. Kashyap RS, Deshpande PS, Ramteke SR, et al. Changes in cerebrospinal fluid cytokine expression in tuberculous meningitis patients with treatment. *Neuroimmunomodulation*, **2010**; 17(5): 333-9.
8. 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.

9. 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.
10. 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): 2007-14.
11. 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.
12. 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): 1008-10.
13. Zhang CX, Zhang DJ, Wang YL, Han W, Shi GC, Zhang HQ. Expression level of NSE, S100B and NPY in children with acute miliary phthisis and secondary tubercular meningitis. *Eur Rev Med Pharmacol Sci*, **2016**; 20(8): 1474-8.
14. Song F, Sun X, Wang X, Nai Y, Liu Z. Early diagnosis of tuberculous meningitis by an indirect ELISA protocol based on the detection of the antigen ESAT-6 in cerebrospinal fluid. *Ir J Med Sci*, **2013**; 183(1):85-88.
15. Rohlwink UK, Figaji AA. Biomarkers of brain injury in cerebral infections. *Clin Chem*, **2014**; 60(6): 823-34.
16. 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): 803-12.

17. 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): 248-52.
18. Figaji AA, Fieggen AG. The neurosurgical and acute care management of tuberculous meningitis: Evidence and current practice. *Tuberculosis (Edinb)*, **2010**; 90(6): 393-40.
19. Rohlwink UK, Donald K, Gavine B, et al. Clinical characteristics and neurodevelopmental outcomes of children with tuberculous meningitis and hydrocephalus. *Dev Med Child Neurol*, **2016**; 58(5): 461-8.
20. Fiser DH. Assessing the outcome of pediatric intensive care. *J Pediatr*, **1992**; 121: 68-74.
21. Rohlwink UK, Kilborn T, Wieselthaler N, Banderker E, Zwane E, Figaji AA. Imaging features of the brain, cerebral vessels and spine in pediatric tuberculous meningitis with associated hydrocephalus. *Pediatr Infect Dis J*, **2016**; 35(10): e301-10.
22. Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. *Clin Chim Acta*, **2001**; 310(2): 173-86.
23. Tarnaris A, Toma AK, Chapman MD, et al. Rostrocaudal dynamics of CSF biomarkers. *Neurochem Res*, **2011**; 36(3): 528-32.
24. 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): 261-4.
25. 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): 61-8.

26. 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): 732-8.
27. 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.
28. 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): 586-90.
29. Dvorak F, Haberer I, Sitzler M, Foersch 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.
30. 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.
31. 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.
32. Kochanek PM, Bramlett HM, Dixon CE, et al. Approach to modeling, therapy evaluation, drug selection, and biomarker assessments for a multicenter pre-clinical drug screening consortium for acute therapies in severe traumatic brain injury: Operation brain trauma therapy. *J Neurotrauma*, **2016**; 33(6): 513-22.

Table 1: Demographic and clinical characteristics

Characteristic	Value
TBM Cases	
Demographic characteristics	
Age	3.3 (0.3 – 13.1) years
0-2 years	19 (43.2)
3- 5 years	18 (40.9)
> 5 years	7 (15.9)
Sex	Male 28 (63.6)
Admission characteristics	
MRC Staging admission	
1	4 (9)
2	31 (70.5)
3	9 (20.5)
Symptom duration	7.5 (1 – 90) days
Vomiting	24 (54.5)
Headache (n=37) ^a	15 (40.1)
Fever	30 (68.2)
Seizures	14 (31.2)
Focal neurology ^b	21 (47.7)
Altered level of consciousness	40 (90.1)
Meningism	34 (77.2)
Bulging fontanelle (n=9) ^c	5 (55.6)
HIV infection (n=43) ^d	2 (4.7)
Diagnostics	
CSF TB culture or AFB positive (n= 39) ^e	22 (56.4)
Outcome	
Good clinical outcome ^f	32 (72.7)
Poor clinical outcome ^g	12 (27.2)
Mortality ^h	7 (16)
Controls	
Age	
Fatty Filum controls	2.75 (0.8-8) years
Pulmonary TB controls	3.67 (1.3-11.8) years
Sex	
Fatty Filum controls	Male 8 (72.7)
Pulmonary TB controls	Male 5 (55.6)

Values reported as median (min-max range) or number (percentage), MRC Staging = British Medical Research Council TBM severity staging, ^a Pre-verbal children under the age of 1.5 years excluded, ^b Focal neurology included aphasia, absence of pupillary response, paresis, cranial nerve palsies, and paresis, ^c For children with open fontanelles, ^d Only 43 patients had HIV testing performed, ^e Only 39 patients had CSF sent for TB diagnostics, in 5 patients TB culture was not requested in error or the volume of CSF was insufficient, ^f Pediatric Cerebral Performance Category Scale (PCPS) 1-3: normal

to mild/moderate disability, ^g PCPS 4-6: severe-disability and death, ^h 4 of these patients died within 10 days of hospital admission

Table 2: Summary of radiology features overall

Radiology features	Admission CT (n=44)	Follow-up CT/MRI (n=43)
Hydrocephalus present	n = 44 (100)	n=24 (61.5) ^a
Severity		
Mild	11 (25)	18 (75)
Moderate	24 (54.5)	6 (25)
Severe	9 (20.5)	2 (8.3)
Basal enhancement	n = 41 (95.5)	n=38 (100) ^b
Infarcts present	9 (20.5)	29 (67.4)
Unilateral	6 (66.7)	14 (48.3)
Bilateral	3 (33.3)	15 (51.2)
Infarct size		
<i>Small</i>	3 (33.3)	10 (34.5)
<i>Moderate</i>	6 (66.7)	16 (55.2)
<i>Large</i>	0	3 (10.3)
MRA pathology detected		16 (55.2) ^c
Tuberculoma present	3 (6.8)	23 (59) ^d
Single	1 (33.3)	10 (43.5)
Multiple	2 (66.7)	13 (56.5)
Spinal pathology present		
Spinal arachnoiditis and/or exudate		24 (75.8)
Spinal tuberculoma		6 (18.2)

Frequency of radiology features reported on admission CT scans and at follow up - 43 patients had follow up imaging during their hospital stay. Follow Up CT/MRI include CT scans of 4 patients who died within 10 days and MRI of 39 patients at 26 ±11 days post-admission, 1 patient only had a follow up MRI at 3 months. Data are presented as number (percent) of patients. ^a Hydrocephalus was treated with external ventricular drains or ventriculo-peritoneal shunts in all 4 patients who died early, ^b 38 of the 39 patients with follow up MRI received contrast, ^c MRA was performed in 29 patients at 3 weeks as this imaging was a late addition to the study protocol, ^d Follow up head CT scans of the 4 deceased patients were not contrast-enhanced, tuberculomas were only reported on follow up MRI in the remaining 39 patients.

Table 3: ROC and outcome analysis

Neuromarker threshold	Sensitivity	Specificity	AUC
Mortality			
S100B >2.75 µg/L	71.43%	70.97%,	0.69, CI:0.4-0.97
NSE > 56.84 µg/L	77.42%	78.95%	0.81, CI: 0.63-0.99
GFAP >27.25 µg/L	85.71%	73.68%	0.84, CI: 0.71-0.98
ΔS100B > 0.06 µg/L	100%	93.1%	0.97, CI: 0.92-1
ΔNSE > 2.07 µg/L	85.71%	65.52%	0.81, CI: 0.56-1
ΔGFAP > 12.54 µg/L	85.7%	96.5%	0.87, CI: 0.64-1
Morbidity			
S100B >2 µg/L	83.33%	61.54%	0.72, CI:0.53-0.9
NSE > 38.86 µg/L	75%	65.38%	0.77, CI: 0.62-0.92
GFAP > 27.25 µg/L	50%	65.38%	0.69, CI: 0.51-0.86
ΔS100B >0.06 µg/L	66.67%	95.83%	0.73, CI:0.51-0.96
ΔNSE >8.67 µg/L	58.33%	91.67%	0.75, CI: 0.54-0.95
ΔGFAP > 12.54 µg/L	85.7%	96.5%	0.68, CI:0.48-0.88

The table demonstrates concentrations of S100B, NSE, GFAP and increases in their concentrations over the first 2 weeks (Δ), which predicted mortality and morbidity (dichotomized clinical outcome at 6 months) with the best combination of sensitivity and specificity. AUC: area under the curve, CI: confidence interval

Table 4: Multivariate outcome analysis

Variable	Outcome	Odds Ratio	Confidence interval	p-value
Z-Neuro Δ	Mortality	22.5	1.57-324.66	0.02
<i>with seizure covariate added to model</i>	Mortality	26.3	1.8 – 383.05	0.02
	6 month clinical outcome	4.1	1.31-13.04	0.02
Δ S100B	Mortality	8.9	1.9-42.1	0.005
	6 month clinical outcome	1.9	0.96-3.65	0.06
Δ NSE	Mortality	1.07	1-1.1	0.02
	6 month clinical outcome	1.06	1.01-1.11	0.02
Δ GFAP	Mortality	1.09	1-1.2	0.01
<i>with seizures added as a covariate to model</i>		1.1	1.02-1.21	0.01
<i>with tuberculomas added as a covariate</i>		0.5	1.01-1.2	0.02
	6 month clinical outcome	1.04	0.1-1.09	0.06

This table demonstrates the odds ratios, confidence intervals and p-values per unit increase in Z-Neuro Δ , Δ S100B, Δ NSE and Δ GFAP for mortality (grey panels) and dichotomized clinical outcome at 6 months (white panels). Z-Neuro represents a combined z-score for all 3 neuromarkers, Δ represents the change from admission to week 2 concentrations.

Figure 1: Neuromarker concentrations for cases over time

Concentrations (ng/ml) for neuromarkers S100B (pink), NSE (blue) and GFAP (green) in ventricular CSF, lumbar CSF and serum are plotted from admission to week 4 for all TBM cases. Ventricular samples from week 4 represent only 2 patients – the elevated concentrations belonged to a patient who had failed medical treatment for their hydrocephalus and required a ventriculo-peritoneal shunt. Concentrations for fatty filum and pulmonary TB (pTB) controls are also shown in purple.

Figure 2: Association between radiology overall and neuromarker concentrations

These box and whisker plots demonstrate the median, interquartile range and range of the highest neuromarker concentrations (panel 1) and the change between admission and week 2 (panel 2- lumbar CSF only, a positive value indicates an increase over time) for various radiological features recorded across admission and in-hospital follow-up scans (overall CT and MRI). Lumbar CSF is indicated in blue, ventricular CSF in green, statistically significant results are indicated by a square bracket and *. Hydrocephalus (HCP) was scored on admission scans and dichotomized as mild and moderate/severe. There were very few patients with mild hydrocephalus.

.

Figure 3: Association between overall radiology and inflammatory marker concentrations

These box and whisker plots demonstrate the median, interquartile range and range of the highest inflammatory marker concentrations for various radiological features recorded across admission and in-hospital follow-up scans (overall CT and MRI). Lumbar CSF is indicated in blue, ventricular CSF in green, statistically significant results are indicated by a square bracket and *. Each row represents the data for individual cytokines (IL-12p40, IP-10, MCP-1, TNF-a, MIP-1a, IL-6 and IL-8) in the presence of parenchymal tuberculomas (top panel) or infarcts (bottom panel).

Figure 4: Univariate analysis between outcome and neuromarker indices

These box and whisker plots demonstrate the median, interquartile range and range of various neuromarker indices (initial/admission concentration, highest concentration in week 1, highest concentration overall during hospitalization and the change between week 2 and admission) in association with mortality (top panel) and dichotomized clinical outcome (bottom panel – PCPS 1-3: normal – mild/moderate disability = good outcome, PCSP 4-6: severe disability - death = poor outcome). Lumbar CSF is indicated in blue, ventricular CSF in green, statistically significant results are indicated by a square bracket and *

Figure 5: PCA plot: Temporal profile of combined neuromarkers in lumbar CSF

These 3-dimensional PCA plots depicts the z-scores for **A:** combined neuromarkers and **B:** combined inflammatory markers in lumbar CSF over the duration of sampling (admission – week 5). Each pink circle represents a patient's z-score at that time point, and the blue circles represent the fatty filum control patients for whom only 1 sample was collected. The axes represent principal component 1 (PC1), principal component 2 (PC2) and time. Plot A demonstrates that patients who died (indicated by black circles and lines) had increasing neuromarker trends, whereas survivors demonstrated a decline in concentrations over time. Plot B demonstrates that, overall, patients who died (indicated by black circles and lines) had decreasing neuromarker trends, similar to survivors.

Figure 1

[Click here to download Figure \(.tif and .eps files only\)](#) [Figure 1.tif](#)

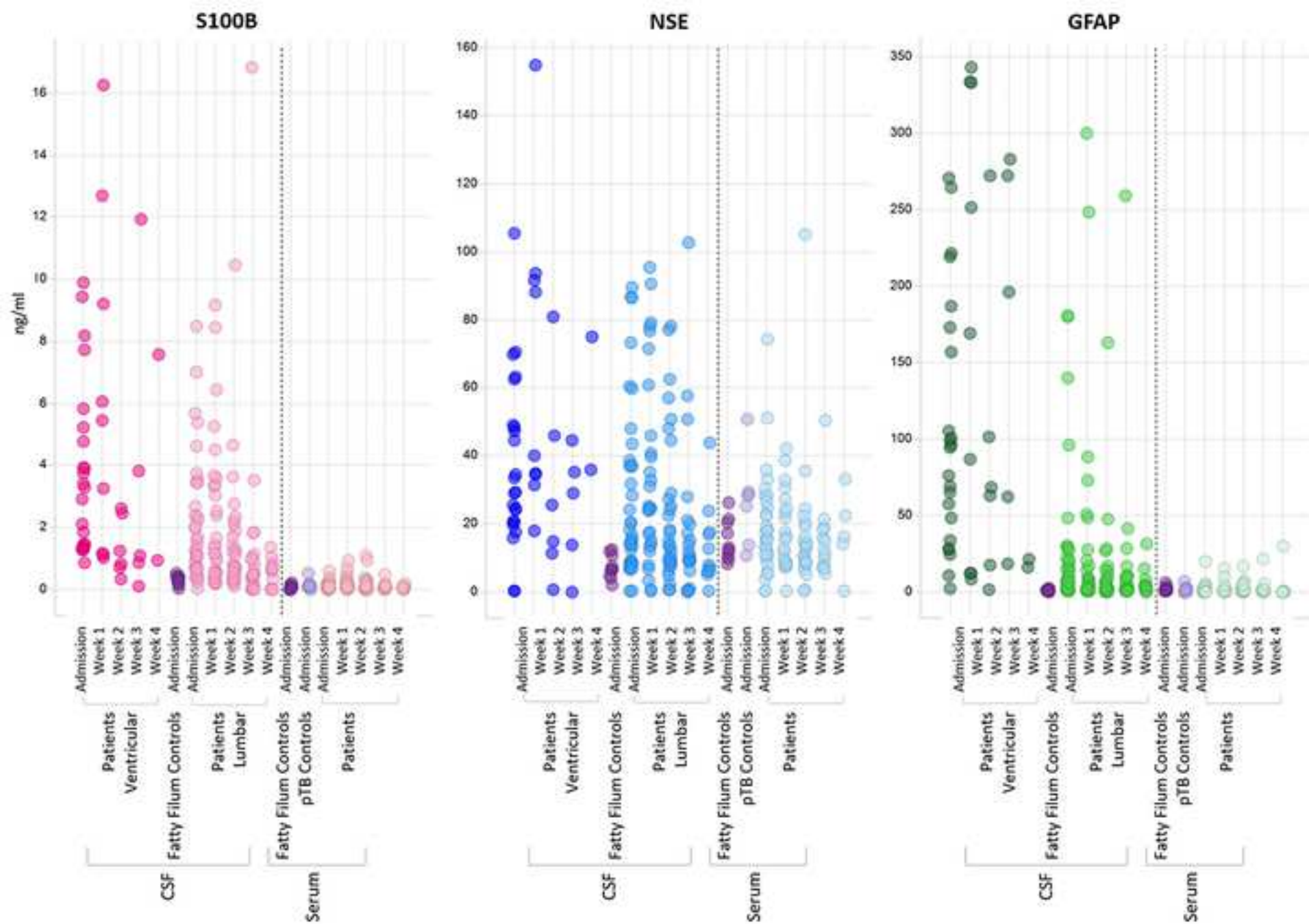


Figure 2

[Click here to download Figure \(.tif and .eps files only\) Figure 5.tif](#)

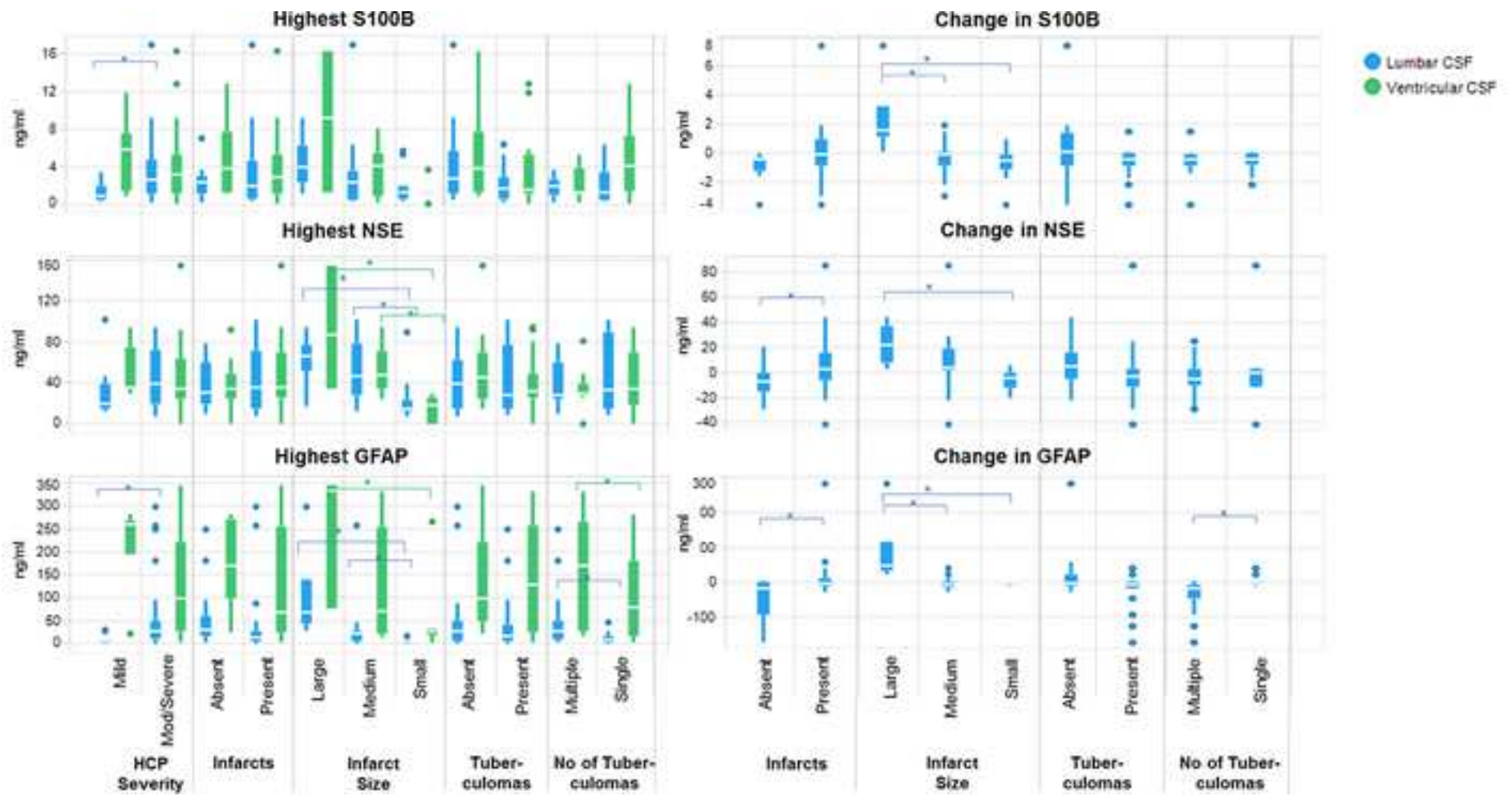


Figure 3

[Click here to download Figure \(.tif and .eps files only\) Figure 6.tif](#)

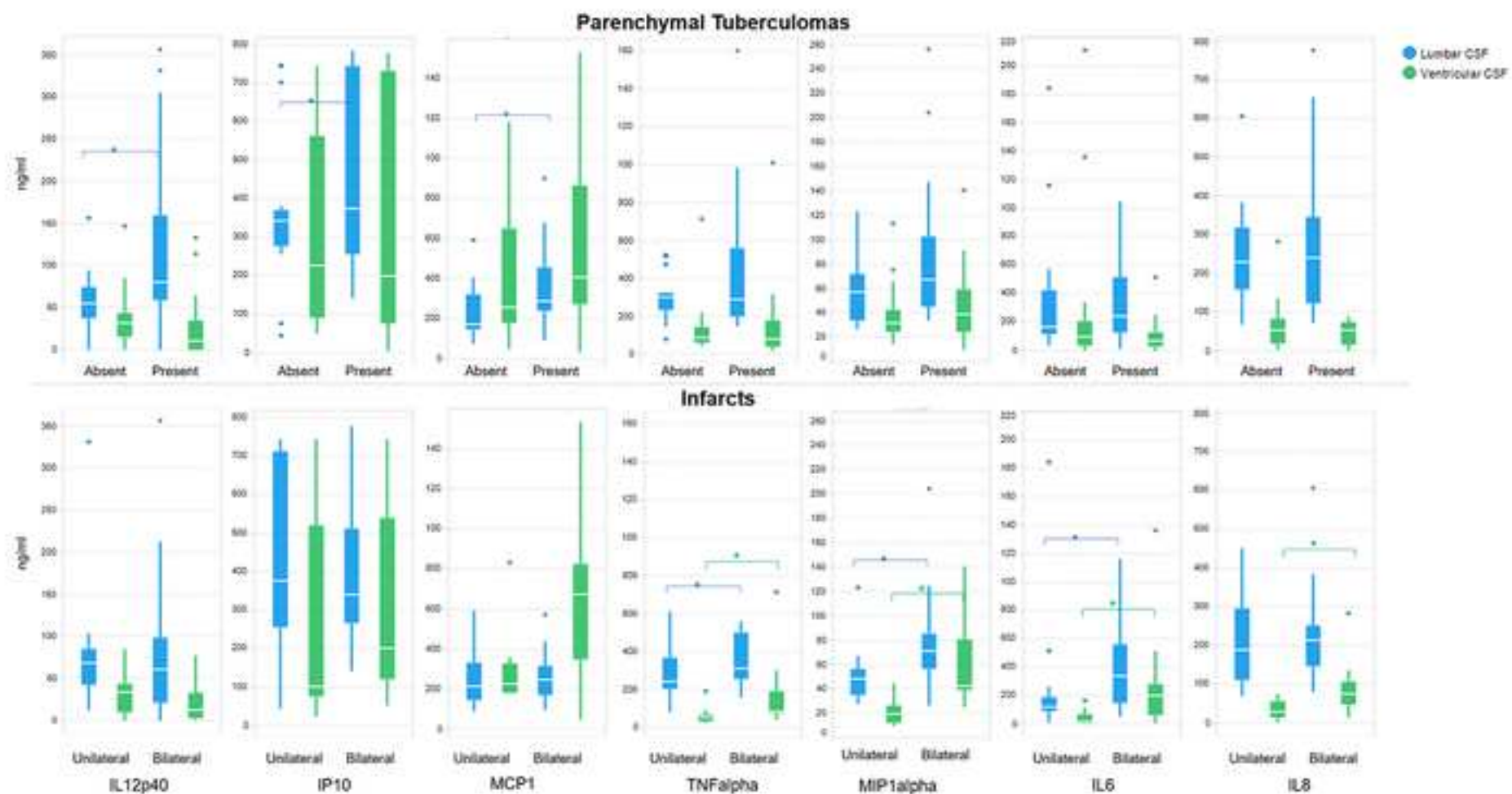


Figure 4

[Click here to download Figure \(.tif and .eps files only\) Figure 7.tif](#)

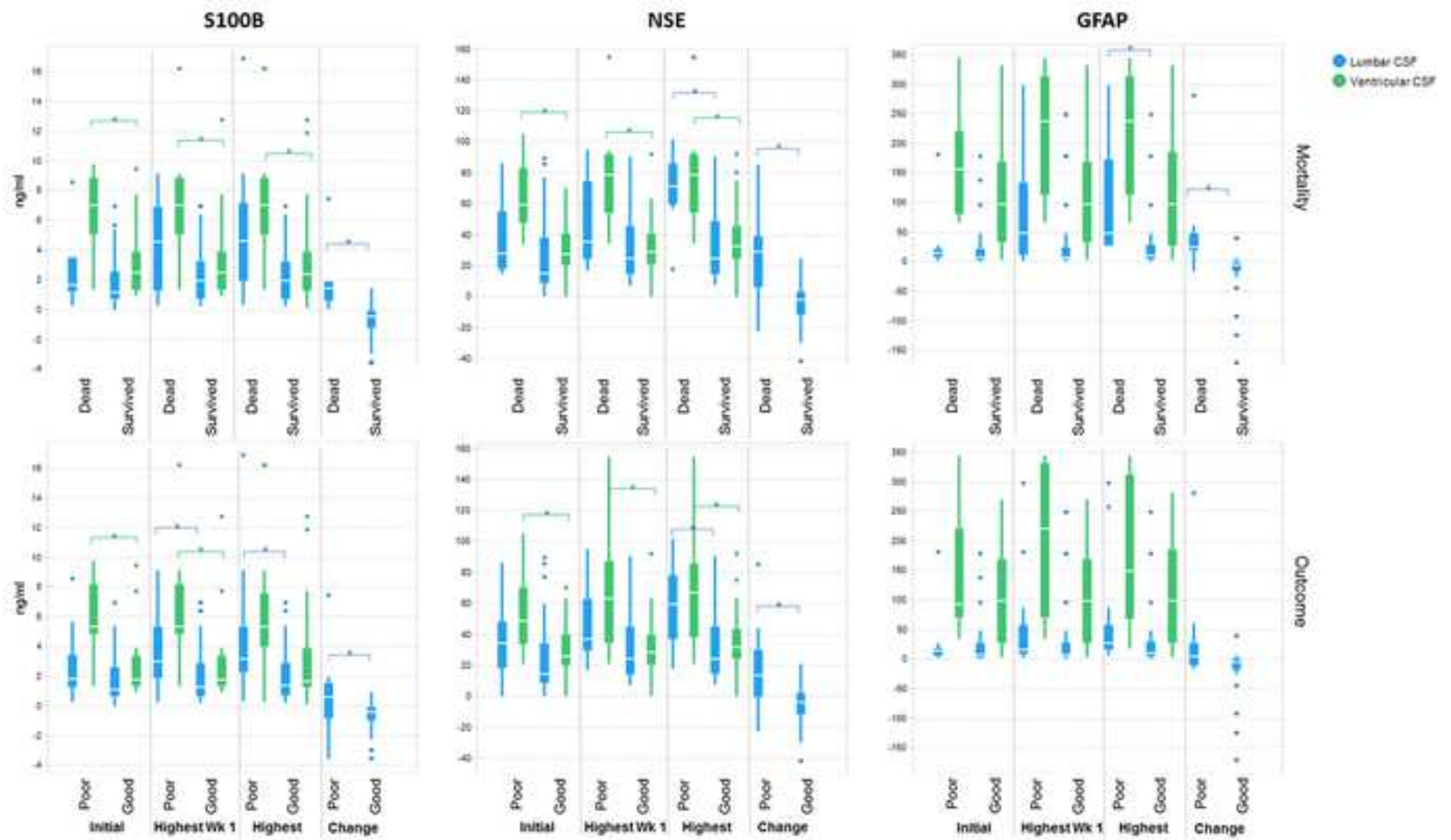
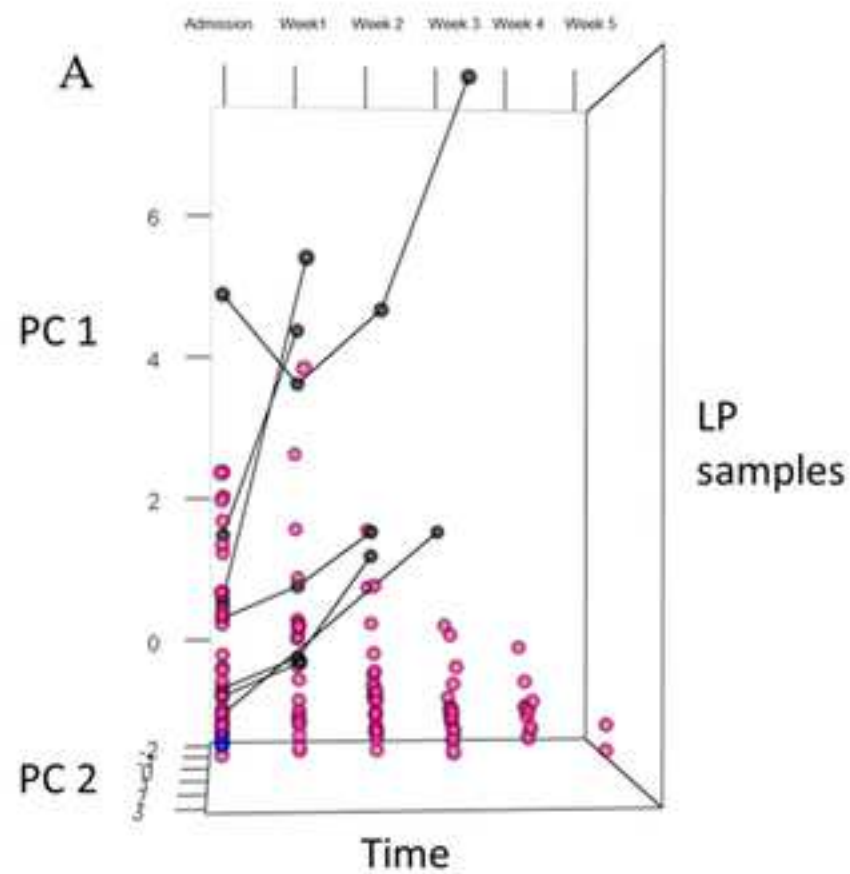


Figure 5

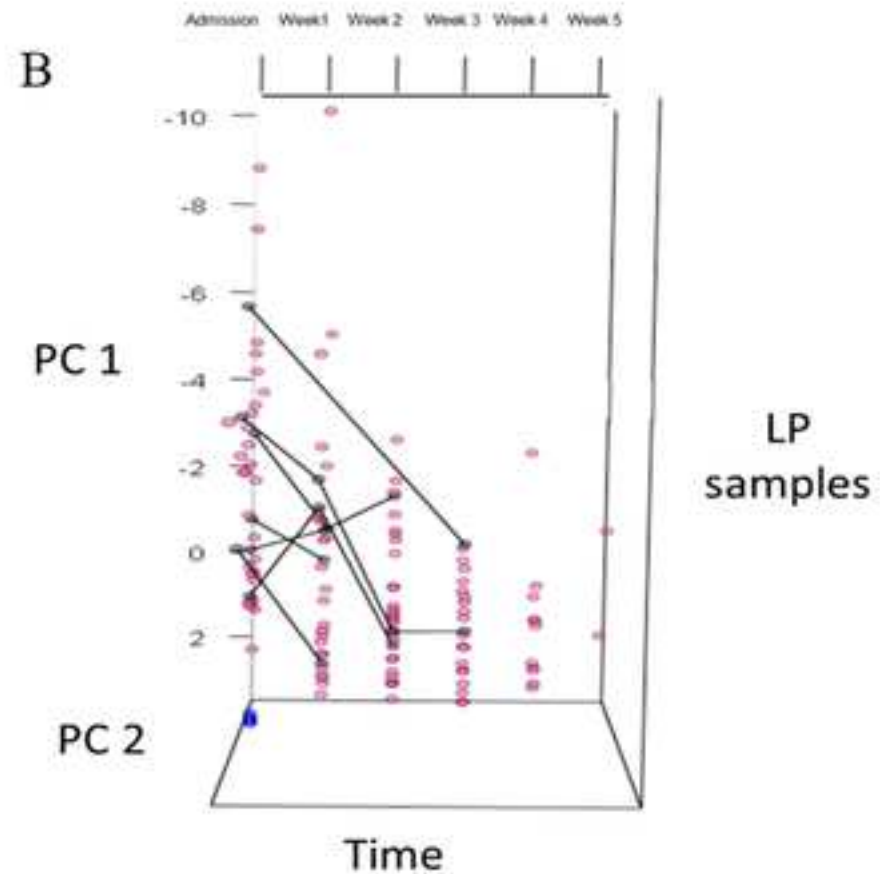
[Click here to download Figure \(.tif and .eps files only\) Figure 8.tif](#)



● Control samples

● TBM survivors

● Deceased TBM patients



Supplementary material

Supplement 1: Biomarker 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. All assays were carried out according to manufacturer's instructions. 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 were 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-1 β , IL-1Ra, IL-6, IL-10, IL-12p40, TNF- α , IFN- γ , IL-8, GRO, MCP-1, IP-10, MIP -1 α , 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. 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 that 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.

Sample exclusion criteria

Biomarker data were reviewed in the clinical context of each patient. Seventeen samples were excluded for the following reasons: samples taken within 24-48 hours after procedures like insertion of external ventricular drains, endoscopic third ventriculostomies, CSF shunts and air encephalograms 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. Hemolyzed or xanthochromic samples were excluded from NSE analysis to exclude NSE of erythrocyte origin.

Supplement 2: PCA analysis

Principal component analysis (PCA) was used as a data reduction technique to allow for the 2-dimensional visualization and simultaneous assessment of multiple biomarker values (p), taken in multiple sample types over time in a small sample size ¹.

In PCA, the data are transformed to a set of $r < p$ principal components, which are (linear) combinations of the original variables that explain as much of the variation in the data as possible.

$$\begin{aligned} Z_1 &= w_{11} \times \text{variable}_1 + w_{12} \times \text{variable}_2 + w_{13} \times \text{variable}_3 \dots + w_{1p} \times \text{variable}_p \\ &\vdots \\ Z_r &= w_{r1} \times \text{variable}_1 + w_{r2} \times \text{variable}_2 + w_{r3} \times \text{variable}_3 \dots + w_{rp} \times \text{variable}_p \end{aligned}$$

The coefficients ($w_{ij}, i = 1 \dots r, j = 1 \dots p$) are called the principal component loadings. Substituting participant specific values for each of the original variables, we obtain a set of principal component scores (also known as Z-scores).

The first principal component is selected to represent as much of the variation in the original data as possible by a single component. The second principal component represents as much of the variation remaining after forming the first principal component and so forth. We can therefore define the quality of the PCA fit as a proportion of the total variance represented.

Plotting the first two principal component scores on a two dimensional co-ordinate system to represent the (n) sample points, together with information on the original variables, we obtain a biplot. This plot is interpreted in terms of distances and orthogonal projections to the axes.

For each of the sample types (lumbar and ventricular CSF and serum), PCA was performed using the admission neuro-and inflammatory marker data. The Z-scores for each participant for the first two principal components were plotted on a biplot, and markers were used to differentiate between the cases and the controls (Figure 2 and 3).

Similarly, PCA biplots were obtained for admission neuro- and inflammatory marker data for cases only, with markers representing each of the three sample types (Figure 4).

These scores were further used as a proxy for a single index of neurological injury or inflammation, incorporating the contribution of multiple neuro- or inflammatory markers respectively (Z-Neuro and Z-Inflammatory).

Finally, 3-dimensional PCA biplots were obtained for the lumbar CSF neuro-and inflammatory markers, incorporating time (Figure 8). Patients who died were indicated in black and those who survived were indicated in pink.

1 Helmy A, Antoniadou 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:e39677.

Supplement 3: Radiological criteria

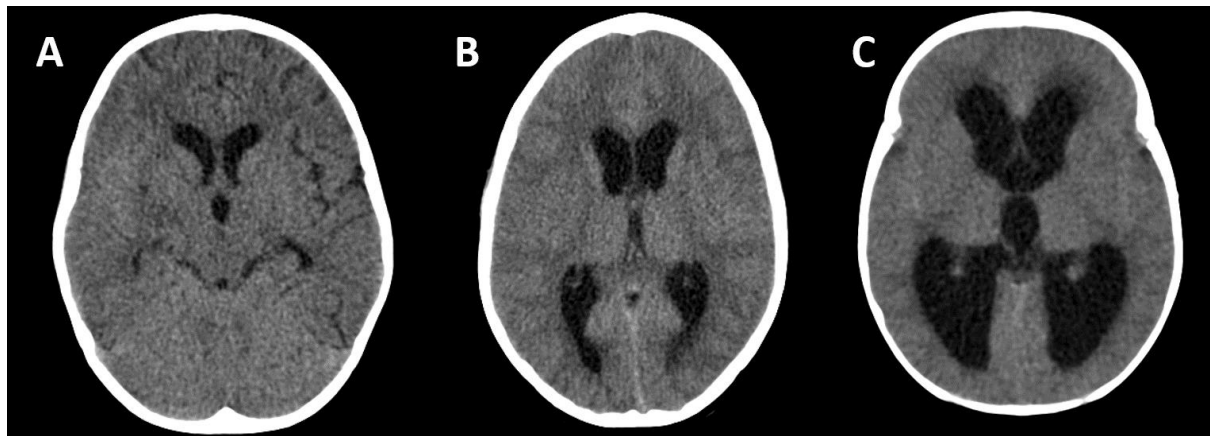
Currently no standardized criteria exist to classify radiological characteristics like the severity of hydrocephalus or the size of infarcts. These criteria were determined by 3 senior pediatric radiologists and 1 senior pediatric neurosurgeon until consensus was reached. Reviewers were blind to patient outcome.

Brain imaging - *Hydrocephalus* was defined as mild (visible temporal horns, rounding of the third ventricle [V3]), moderate (all ventricles dilated, no transependymal fluid shift) or severe (dilated ventricles, fluid shift and loss of sulcal markings). *Basal enhancement* was classified as focal (localized enhancement) or diffuse (enhancement not limited to a focal region). *Infarcts* were recorded as unilateral or bilateral, and their size was graded as single (lacunar), multiple/large (multiple or large branch infarcts) and large territory (encompassing MCA, ACA, PCA territories). *Tuberculomas* were classified by number.

Spinal imaging (MRI with contrast) - *Arachnoiditis* was defined as enhancement of the spinal cord or roots; with nodules, exudate and clumping of nerve roots, and the presence of plaques (extra or intradural collections of exudate) noted. *Tuberculoma or TB abscesses* were categorized as surface or intramedullary.

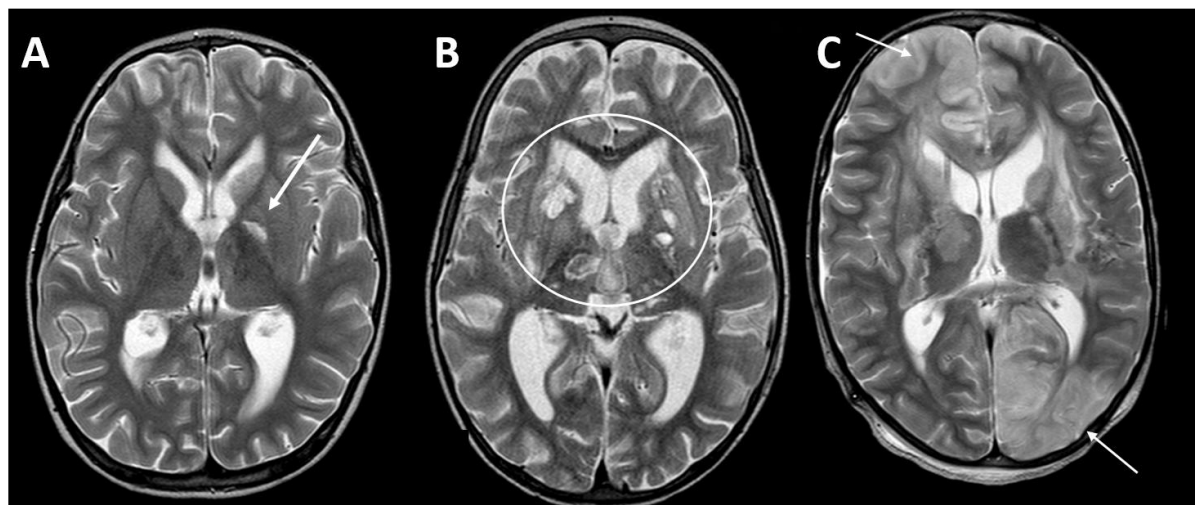
Some examples of these radiological characteristics are included below:

Hydrocephalus



Axial uncontrasted CT brain scans in patients with TBM showing (A) mild hydrocephalus with a rounded 3rd ventricle, (B) moderate hydrocephalus with ventricular dilatation but no transependymal fluid shift, (C) severe hydrocephalus with diffuse ventricular dilatation and transependymal fluid shift.

Infarcts



Axial T2-weighted MRIs in patients with TBM showing (A) small focal infarct involving the basal ganglia (arrow), (B) multiple bilateral basal ganglia infarcts (circled), (C) multiple hemispheric (arrows) and basal ganglia infarcts.

Tuberculoma



Axial post-contrast CT brain scan showing a peripherally enhancing tuberculoma in a TBM patient

Spinal disease



Sagittal T1 weighted MRI postcontrast in a TBM patient showing (1) diffuse enhancement surrounding the cord, (2) enhancing extramedullary exudate plaque-like collection, (3) diffuse hyperintense exudate filling the thecal sac

A total of 130 lumbar CSF, 46 ventricular CSF and 107 serum samples were analyzed from TBM cases, 17 samples were excluded (n=7 lumbar CSF, 4 ventricular CSF, 6 serum) because samples were taken within 24-48 hours after neurosurgical procedures or were not frozen within 12 hours. Hemolyzed serum samples (n=15) were excluded. An additional lumbar CSF and serum sample were excluded from Luminex analysis due to a technical error. Eleven lumbar CSF and serum sample pairs from fatty filum controls, and 9 serum samples from pTB controls were analysed. Hemolyzed samples were excluded from NSE analysis (n=1 per control group).

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. Descriptive statistics for neuro- and inflammatory marker concentrations for cases and controls, and the results of the Mann-Whitney's U comparison are tabulated below.

Marker	S100B Cases (ug/L)		S100B controls (ug/L)	Cases vs controls Lumbar	Cases vs controls Vent	S100B Cases (ug/L)	Fatty filum controls (ug/L)	pTB controls (ug/L)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	0.002*	0.002*	n=36	n=11	n=9	1	1
Median	1.37	3.24	0.36			0.08	0.05	0.1		
Min	0.01	0.91	0.01			0.01	0.03	0.01		
Max	8.57	9.82	0.44			0.59	0.15	0.5		
p25	0.7	1.34	0.21			0.02	0.04	0.07		
p75	3.07	5.30	0.39			0.14	0.1	0.11		
p95			0.44				0.15	0.5		
Week 1	n=29	n=9		0.003*	0.003*	n=26			1	1
Median	1.37	5.37				0.11				
Min	0.16	1.07				0.01				
Max	9.13	16.25				1.00				
p25	0.57	1.13				0.02				
p75	3.46	9.12				0.23				
Week 2	n=31	n=6		0.01*	0.09	n=24			1	1
Median	0.77	1.05				0.04				
Min	0.16	0.29				0.01				
Max	10.53	2.53				1.06				
p25	0.4	0.62				0.03				
p75	1.84	2.47				0.22				
Week 3	n=21	n=5		0.43	0.28	n=14			1	1
Median	0.48	1.10				0.06				
Min	0.01	0.12				0.01				
Max	16.9	11.86				0.44				
p25	0.22	0.86				0.03				
p75	1.01	3.81				0.11				
Week 4	n=11			0.4		n=7			1	1
Median	0.62					0.07				
Min	0.01					0.03				
Max	1.41					0.15				
p25	0.03					0.05				
p75	0.83					0.12				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p < 0.05$, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	NSE Cases (ug/L)		NSE controls (ug/L)	Cases vs controls Lumbar	Cases vs controls Vent	NSE Cases (ug/L)	Fatty filum controls (ug/L)	pTB controls (ug/L)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=10	0.01*	0.003*	n=30	n=10	n=7	1	1
Median	17.62	29.07	6.88			16.74	13.4	28.12		
Min	0.01	0.01	2.87			0.01	8.2	10.42		
Max	89.49	104.86	12.46			73.75	26.23	50.82		
p25	8.48	20.53	4.51			11.36	10.9	13.39		
p75	38.12	48.89	10.42			27.08	20.9	29.34		
p95			12.46				26.23	50.82		
Week 1	n=29	n=9		0.01*	0.003*	n=21			1	1
Median	24.67	39.61				16.09				
Min	0.01	17.38				0.01				
Max	94.97	154.86				42.60				
p25	14.32	34.61				8.8				
p75	46.42	91.97				24.21				
Week 2	n=31	n=6		0.06	0.28	n=23			1	0.79
Median	14.44	19.42				12.86				
Min	0.01	0.01				0.01				
Max	78.92	80.64				104.67				
p25	8.21	10.82				7.99				
p75	28.8	46.01				19.88				
Week 3	n=21	n=5		0.43	0.28	n=13			1	0.1
Median	10.52	29.20				12.74				
Min	0.01	0.01				5.82				
Max	101.92	44.64				50.08				
p25	8.75	13.86				7.66				
p75	20.08	34.52				18.62				
Week 4	n=11			0.46	0.24	n=5			1	1
Median	7.54					16.28				
Min	0.01					0.01				
Max	44.34					32.70				
p25	6.51					14.07				
p75	18.34					22.45				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p < 0.05$, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	GFAP Cases (ug/L)		GFAP controls (ug/L)	Cases vs controls Lumbar	Cases vs controls Vent	GFAP Cases (ug/L)	Fatty filum controls (ug/L)	pTB controls (ug/L)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	0.002*	0.002*	n=36	n=11	n=9	0.68	1
Median	8.92	93.31	0.03			0.01	0.76	1.17		
Min	0.01	2.73	0			0.01	0.01	0.01		
Max	181.09	269.96	1.19			20.25	5.90	5.90		
p25	3.25	33.86	0			0.02	0.24	0.01		
p75	24.16	172.37	0.62			1.77	2.51	1.79		
p95			1.19				5.9	5.79		
Week 1	n=29	n=9		0.002*	0.003*	n=26			1	1
Median	6.06	168.64				0.01				
Min	0.01	8.23				0.01				
Max	298.66	344.40				16.29				
p25	2.1	13.37				0.01				
p75	21.84	331.87				0.01				
Week 2	n=31	n=6		0.01*	0.07	n=24			1	1
Median	3.38	66.30				0.01				
Min	0.01	0.01				0.01				
Max	164.43	270.50				15.88				
p25	0.03	17.09				0.01				
p75	9.38	100.01				2.72				
Week 3	n=21	n=5		0.002*	0.02*	n=14			1	1
Median	3.26	196.12				0.89				
Min	0.01	18.55				0.01				
Max	257.8	281.20				20.79				
p25	0.67	62.29				0.01				
p75	10.27	271.99				2.21				
Week 4	n=11			0.01*	0.23	n=7			1	1
Median	3.46					0.01				
Min	0.01					0.01				
Max	30.64					31.72				
p25	1.62					0.01				
p75	9.4					1.4				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p < 0.05$, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	TNF- α Cases (pg/mL)		TNF- α controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	TNF- α Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.78	p=0.01*
Median	266.32	109	0.01			15.46	12.09	27.4		
Min	76.97	20.63	0.01			0.01	7.7	11.84		
Max	1604.31	1010.17	0.01			50.88	27.82	135.02		
p25	180.14	75.49	0.01			8.42	9.75	23.16		
p75	493.68	171.6	0.01			19.11	20.84	33.06		
p95			0.01				27.82	135.02		
Week 1	n=29	n=9				n=26				
Median	160.16	39.89				13.48				
Min	34.63	12.09				3.36				
Max	1329.57	712.6				52.55				
p25	83.49	32.65				8.86				
p75	295.86	74.18				17.94				
Week 2	n=30	n=6				n=23				
Median	95.15	67.55				11.45				
Min	25.48	15.8				3.99				
Max	402.75	141.27				25.31				
p25	48.62	50.58				7.19				
p75	138.29	137.06				14.94				
Week 3	n=21	n=5				n=14				
Median	56.84	90.7				11.7				
Min	8.49	17.74				4.12				
Max	216.71	118.5				50.26				
p25	26.16	29.96				5.52				
p75	84	110.26				16.64				
Week 4	n=11					n=7				
Median	56.05					13.8				
Min	20.5					5.35				
Max	299.88					39.14				
p25	34.94					6.48				
p75	129.88					29.52				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	IFN- γ Cases (pg/mL)		IFN- γ controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	IFN- γ Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.02*	p=0.08
Median	1095.67	684.79	0.01			9.66	12.09	27.4		
Min	140.99	118.89	0.01			0.01	7.7	11.84		
Max	8541.02	3888.62	0.01			48.9	27.82	135.02		
p25	619.97	307.39	0.01			4.12	9.75	23.16		
p75	2809.42	1080.8	0.01			19.58	20.84	33.06		
p95			0.01				27.82	135.02		
Week 1	n=29	n=9				n=26				
Median	278.44	239.02				6.07				
Min	48.44	57.14				0.01				
Max	10275.4	1055.49				75.84				
p25	149.23	112.76				0.01				
p75	793.26	259.64				13.05				
Week 2	n=30	n=6				n=23				
Median	244.51	271.32				4.43				
Min	0.01	25.92				0.01				
Max	2197.81	787.56				44.78				
p25	80.71	108.88				0.01				
p75	465.49	507.03				13.57				
Week 3	n=21	n=5				n=14				
Median	126.68	93.92				0.01				
Min	0.01	31.33				0.01				
Max	757.17	315.17				268.25				
p25	66.08	67.74				0.01				
p75	154.25	184.12				7.04				
Week 4	n=11					n=7				
Median	69.71					0.01				
Min	21.1					0.01				
Max	715.18					116.18				
p25	49.34					0.01				
p75	209.65					9.15				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	IL-6 Cases (pg/mL)		IL-6 controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	IL-6 Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p<0.01*	p=0.04*
Median	1700.91	873.54	0.01			7.61	0.01	34.39		
Min	218.79	38.35	0.01			49.95	0.01	3.82		
Max	18448.02	13643.62	0.01			10.20	47.63	458.64		
p25	1101.84	415.62	0.01			1.96	0.01	7.21		
p75	4863.87	2272.35	0.01			12.35	0.01	40.48		
p95			0.01				47.63	458.64		
Week 1	n=29	n=9				n=26				
Median	595.86	377.59				4.73				
Min	0.01	43.08				0.01				
Max	10464.67	5174.25				392.83				
p25	328.33	58.56				0.01				
p75	2066.4	791.99				12.43				
Week 2	n=30	n=6				n=23				
Median	373.81	343.71				0.01				
Min	21.68	18.89				0.01				
Max	5175.25	1210.65				46.97				
p25	91.83	170.51				0.01				
p75	604.53	500.6				5.73				
Week 3	n=21	n=5				n=14				
Median	351.6	244.88				2.78				
Min	5.65	5.22				0.01				
Max	1586.25	21146.37				67.69				
p25	120.81	59.89				0.01				
p75	482.87	561.8				4.74				
Week 4	n=11					n=7				
Median	216.2					0.01				
Min	12.87					0.01				
Max	1117.09					35.62				
p25	62.02					0.01				
p75	678.55					6.8				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	IL-12p40 Cases (pg/mL)		IL-12p40 controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	IL-12p40 Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p=0.01*	n=36	n=11	n=9	p=0.4	p=0.54
Median	43.34	4.85	0.01			10.69	29.25	25.69		
Min	0.01	0.01	0.01			0.01	0.01	0.01		
Max	279.46	63.99	0.01			126.18	99.76	74.31		
p25	20.25	0.01	0.01			0.01	5.74	6.51		
p75	62.8	32.03	0.01			45.2	41.58	35.11		
p95			0.01				99.76	74.31		
Week 1	n=29	n=9				n=26				
Median	44.96	2.39				17.29				
Min	0.01	0.01				0.01				
Max	330.96	114.41				114.21				
p25	25.2	0.01				0.01				
p75	79.95	12.54				38.13				
Week 2	n=30	n=6				n=23				
Median	50.99	23.7				21.47				
Min	0.01	0.01				0.01				
Max	356.42	133.38				92.16				
p25	30.99	16.74				0.01				
p75	100.02	35.15				44.04				
Week 3	n=21	n=5				n=14				
Median	41.37	64.48				13.99				
Min	0.01	8.08				0.01				
Max	217.54	146.87				236.27				
p25	4.87	12.89				0.01				
p75	65.95	87.23				23.44				
Week 4	n=11					n=7				
Median	30.99					0.01				
Min	0.01					0.01				
Max	273.4					176.57				
p25	25.2					0.01				
p75	59.27					52.47				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	IL-1 β Cases (pg/mL)		IL-1 β controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	IL-1 β Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p=0.02*	n=36	n=11	n=9	p=0.2	p=0.31
Median	6.7	0.01	0.01			0.01	0.01	0.01		
Min	0.01	0.01	0.01			0.01	0.01	0.01		
Max	58.76	27.14	0.01			16.37	6.89	9.95		
p25	0.01	0.01	0.01			0.01	0.01	0.01		
p75	15.04	6.89	0.01			3.31	0.01	0.01		
p95			0.01				6.89	9.95		
Week 1	n=29	n=9				n=26				
Median	0.01	0.01				0.01				
Min	0.01	0.01				0.01				
Max	99.39	50.68				13.62				
p25	0.01	0.01				0.01				
p75	3.7	8.86				1.01				
Week 2	n=30	n=6				n=23				
Median	0.01	0.01				0.01				
Min	0.01	0.01				0.01				
Max	12.31	18				14.6				
p25	0.01	0.01				0.01				
p75	4.1	13.79				1.52				
Week 3	n=21	n=5				n=14				
Median	0.01	0.01				0.01				
Min	0.01	0.01				0.01				
Max	8.47	5.58				50.42				
p25	0.01	0.01				0.01				
p75	0.01	3.8				0.01				
Week 4	n=11					n=7				
Median	0.01					0.01				
Min	0.01					0.01				
Max	3.7					33.5				
p25	0.01					0.01				
p75	3.41					0.01				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	IL-Ra Cases (pg/mL)		IL-Ra controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	IL-Ra Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.11	p=0.3
Median	456.2	72.65	0.01			33.31	16.53	69.36		
Min	19.02	0.01	0.01			0.01	0.01	0.01		
Max	1572.11	699.77	0.01			223.29	80.09	279.49		
p25	204.17	28.42	0.01			0.01	0.01	23.65		
p75	810.35	195.84	0.01			84.48	30.74	72.85		
p95			0.01				80.09	279.49		
Week 1	n=29	n=9				n=26				
Median	182.06	62.91				31.18				
Min	0.01	0.01				0.01				
Max	2074.47	252.14				619.99				
p25	47.26	0.01				0.01				
p75	736.48	88.53				68.73				
Week 2	n=30	n=6				n=23				
Median	71.99	46.68				19.02				
Min	0.01	0.01				0.01				
Max	2060.31	154.38				184.79				
p25	2.53	0.01				0.01				
p75	210.34	89.94				69.48				
Week 3	n=21	n=5				n=14				
Median	2.53	31.57				38.74				
Min	0.01	0.01				0.01				
Max	293.77	1492.95				384.11				
p25	0.01	0.01				0.01				
p75	59.28	51.87				52.69				
Week 4	n=11					n=7				
Median	0.01					37.8				
Min	0.01					0.01				
Max	618.6					261.35				
p25	0.01					0.01				
p75	130.97					66.3				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	IL-8 Cases (pg/mL)		IL-8 controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	IL-8 Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.01*	p=0.55
Median	1981.46	490.57	6.58			20.14	9.93	15.37		
Min	487.48	23.41	3.26			4.31	3.47	7.41		
Max	6539.84	1370.34	30.11			280.93	42.59	300.62		
p25	1122.93	177.8	5.07			16.68	6.28	12.52		
p75	3396.45	806.4	13.65			36.2	18.75	30.44		
p95			30.11				42.59	300.62		
Week 1	n=29	n=9				n=26				
Median	1115.03	706.78				26.27				
Min	197.78	35.16				5.73				
Max	7766.9	3399.34				236.15				
p25	467.65	94.41				17.43				
p75	2307.95	906.85				40.62				
Week 2	n=30	n=6				n=23				
Median	804.1	323.85				21.77				
Min	126.07	48.82				0.01				
Max	2424.95	749.45				969.47				
p25	407.35	182.55				13.82				
p75	1291.6	608.44				40.12				
Week 3	n=21	n=5				n=14				
Median	651.52	244.96				17.42				
Min	37.35	57.55				6.72				
Max	3329.57	805.35				108.11				
p25	301.26	187.91				13.91				
p75	1355.66	354.89				28.05				
Week 4	n=11					n=7				
Median	635.77					25.91				
Min	133.58					10.84				
Max	4602.41					69.42				
p25	253.15					22.19				
p75	1099.52					39.96				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	MCP-1 Cases (pg/mL)		MCP-1 controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	MCP-1 Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p<0.01*	p<0.01*
Median	1947.4	4597.47	651.38			143.21	246.54	418.17		
Min	507.8	1627.85	412.88			20.22	198.02	160.26		
Max	6257.75	15327.69	1475.21			684.11	610.11	1543.51		
p25	1510.62	2678.13	533.75			78.21	216.59	339.71		
p75	2954.85	9227.79	1175.25			225.95	365.65	1084.61		
p95			1475.21				610.11	1543.51		
Week 1	n=29	n=9				n=26				
Median	1280.36	2581.72				207.33				
Min	421.86	500.45				34.4				
Max	6799.66	9936.64				792.72				
p25	906.29	2238.52				164.24				
p75	1702.15	3655.25				348.78				
Week 2	n=30	n=6				n=23				
Median	1466.31	4045.46				225.1				
Min	467.46	1894.41				45.62				
Max	9046.45	11696.31				740.67				
p25	892.7	2395.89				143.34				
p75	2295.7	6962.93				326.09				
Week 3	n=21	n=5				n=14				
Median	1866.22	1821.05				263.32				
Min	245.94	336.85				71.19				
Max	5474.77	4258.12				878.79				
p25	1006.69	1796.2				159.45				
p75	2455.82	2537.43				791.14				
Week 4	n=11					n=7				
Median	1506.78					331.02				
Min	648.07					95.63				
Max	2938.7					866.52				
p25	1016.79					232.79				
p75	2539.85					428.2				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	IP-10 Cases (pg/mL)		IP-10 controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	IP-10 Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.01*	p<0.01*
Median	35505.4	24924.98	43.42			550.97	272.16	1931.02		
Min	5919.14	970.83	7.76			165.43	91.43	807.44		
Max	77800.54	77800.54	364.18			3654.24	1012.02	19318.78		
p25	25618.52	7422.5	14.44			395.26	186.21	996.4		
p75	69388.13	74447.62	106.29			1080.1	470.15	2188.88		
p95			364.18				1012.02	19318.78		
Week 1	n=29	n=9				n=26				
Median	25618.52	15672.62				463.92				
Min	1959.68	488.21				146.81				
Max	78500.87	74531.67				4861.21				
p25	8734.63	1217.97				226.1				
p75	58255.83	69676.58				704.59				
Week 2	n=30	n=6				n=23				
Median	25357.92	12102.38				324				
Min	1847.29	2804.47				162.49				
Max	76541.2	74447.62				1582.56				
p25	8481.32	4147.57				194.98				
p75	63333.72	35471.74				507.72				
Week 3	n=21	n=5				n=14				
Median	14252.21	27490.36				312.1				
Min	445.64	396.94				113.99				
Max	74447.62	74447.62				3768.51				
p25	3284.14	20746.36				205.44				
p75	25618.52	69382.67				969.06				
Week 4	n=11					n=7				
Median	14017.15					532.95				
Min	980.38					84.56				
Max	74447.62					1002.57				
p25	2476.6					358.18				
p75	51893.2					782.34				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	MIP-1 α Cases (pg/mL)		MIP-1 α controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	MIP-1 α Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.77	p=0.76
Median	57.79	35.2	0.01			3.26	6.63	0.01		
Min	26.48	9.54	0.01			0.01	0.01	0.01		
Max	256.37	113.17	10.61			1694.16	63.47	78.87		
p25	41.39	24.38	0.01			0.01	0.01	0.01		
p75	78.92	46.58	7.41			17.34	13.14	15.49		
p95			10.61				63.47	78.87		
Week 1	n=29	n=9				n=26				
Median	48.46	31.27				2.49				
Min	0.01	10.08				0.01				
Max	91.08	140.46				1516.58				
p25	21.5	24.62				0.01				
p75	62.71	64.35				19.59				
Week 2	n=30	n=6				n=23				
Median	37.4	37.94				9.54				
Min	0.01	14.01				0.01				
Max	92	49.28				864.09				
p25	23.3	30.09				0.01				
p75	53.81	45.13				20.3				
Week 3	n=21	n=5				n=14				
Median	32.61	22.18				2.44				
Min	11.87	15.49				0.01				
Max	68.5	66.05				82.85				
p25	22.59	21.07				0.01				
p75	36.28	24.27				19				
Week 4	n=11					n=7				
Median	23.06					8.81				
Min	8.49					0.01				
Max	65.32					86.67				
p25	17.5					0.01				
p75	46.58					30.89				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	GRO Cases (pg/mL)		GRO controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	GRO Cases	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.28	p=0.67
Median	1431.18	166.77	0.01			2165.63	2273.49	2414.41		
Min	250.17	0.01	0.01			873	328.69	1254.55		
Max	3207.74	819.52	0.01			4696.52	3272.34	3062.44		
p25	1010.29	43.9	0.01			1793	1149.74	1538.76		
p75	1852.98	358.33	0.01			2779.23	2560.79	2816.48		
p95			0.01				3272.34	3062.44		
Week 1	n=29	n=9				n=26				
Median	819.18	109.88				2155.46				
Min	130.51	0.01				127.94				
Max	4067.42	3269.84				3651.44				
p25	429.08	0.01				1274.39				
p75	1589.94	415.05				2859.11				
Week 2	n=30	n=6				n=23				
Median	696.18	179.1				2385.49				
Min	62.24	0.01				1030.95				
Max	2194.12	502.87				4252.57				
p25	320.24	38.72				1909.51				
p75	1267.34	290.01				3237.48				
Week 3	n=21	n=5				n=14				
Median	508.08	107.09				2530.25				
Min	0.01	0.01				956.73				
Max	2898.23	180.76				5093.25				
p25	348.11	73.41				1834.52				
p75	1351.99	160.21				2984.26				
Week 4	n=11					n=7				
Median	931.36					2609.84				
Min	77.43					1334.27				
Max	2425.63					3242.75				
p25	277.25					1597.04				
p75	1785.63					2948.99				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	IL-10 Cases (pg/mL)		IL-10 controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	IL-10 Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.83	p=0.07
Median	420.32	97.22	0.01			8.54	8.01	19.31		
Min	107.18	41.61	0.01			0.01	0.01	4.08		
Max	1209.5	536.41	0.01			78.76	31.98	44.67		
p25	252.45	61.66	0.01			1.78	3.4	7.58		
p75	653.04	255.17	0.01			17.86	18.93	32.26		
p95			0.01				31.98	44.67		
Week 1	n=29	n=9				n=26				
Median	140.29	50.24				14.04				
Min	46.45	11.47				0.01				
Max	873.74	603.21				85.51				
p25	108.71	29.09				5.47				
p75	234.29	153.16				27.43				
Week 2	n=30	n=6				n=23				
Median	156.3	55.89				16.69				
Min	9.73	34.79				0.01				
Max	439.5	605.45				132.23				
p25	84.18	35.62				4.92				
p75	251.34	518.97				36.53				
Week 3	n=21	n=5				n=14				
Median	156.41	108.99				5.87				
Min	22.22	27.07				0.01				
Max	291.39	267.55				159.74				
p25	95.38	64.57				0.01				
p75	215.3	154.01				25.74				
Week 4	n=11					n=7				
Median	216.77					16.88				
Min	74.58					8.41				
Max	275.68					68.74				
p25	125.43					12.38				
p75	254.48					38.48				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

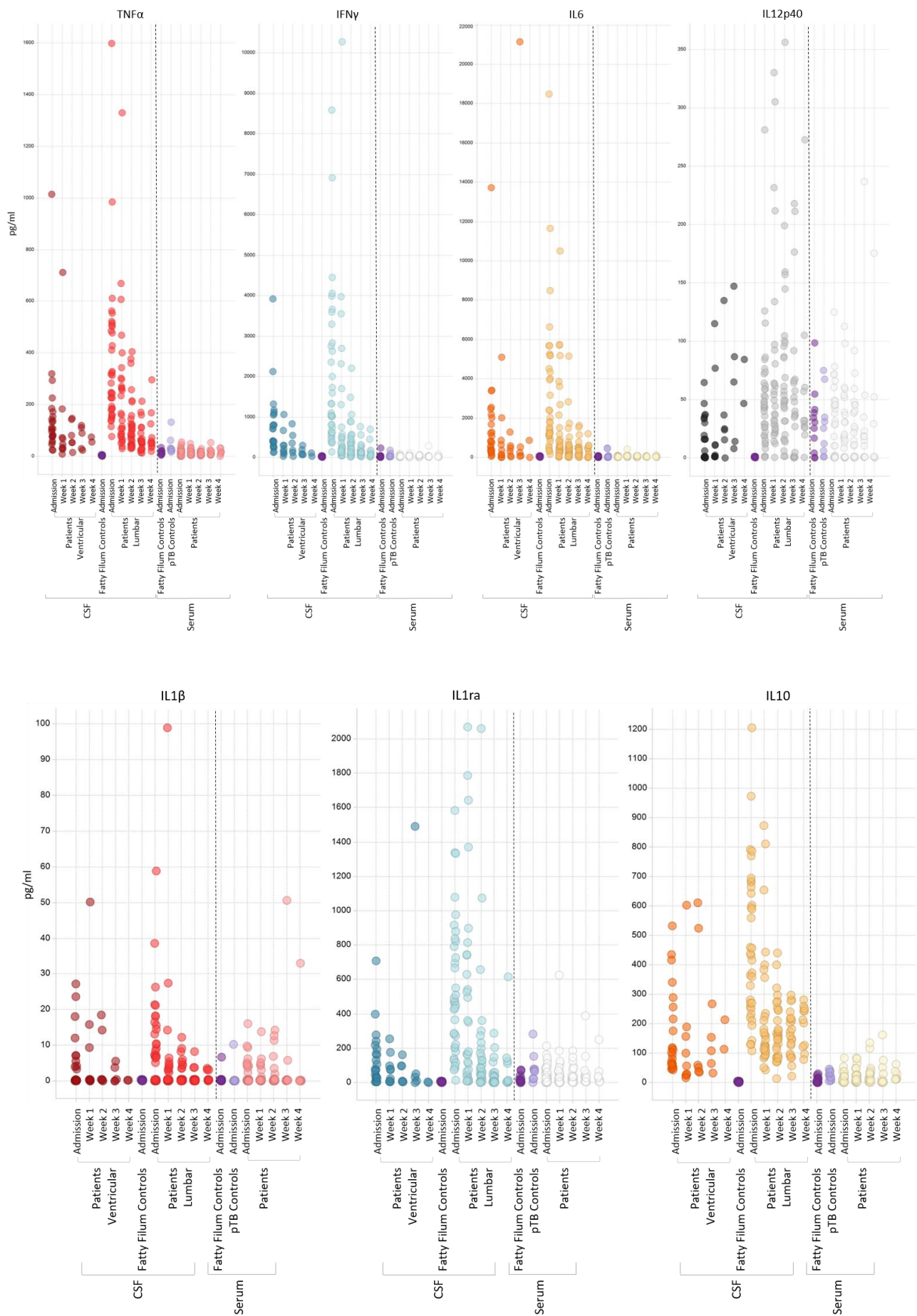
Marker	VEGF Cases (pg/mL)		VEGF controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	VEGF Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p<0.01*	p<0.01*	n=36	n=11	n=9	p=0.59	p=0.27
Median	145.33	136.15	0.01			220.27	327.99	383.57		
Min	0.01	0.01	0.01			0.01	0.01	0.01		
Max	836.7	424.78	0.01			1442.94	816.74	1950.31		
p25	108	0.01	0.01			152.5	90.24	220.27		
p75	269.13	220.27	0.01			382.75	546.56	681.05		
p95			0.01				816.74	1950.31		
Week 1	n=29	n=9				n=26				
Median	141.88	67.17				236.19				
Min	0.01	0.01				0.01				
Max	558.81	376.55				729.24				
p25	97.28	0.01				130.44				
p75	194.76	166.22				358.31				
Week 2	n=30	n=6				n=23				
Median	139.7	142.49				247.01				
Min	0.01	0.01				0.01				
Max	504.41	399.71				973.74				
p25	0.01	56.6				162.47				
p75	206.25	309				383.57				
Week 3	n=21	n=5				n=14				
Median	0.01 ^a	238.68				180.6				
Min	0.01	97.28				0.01				
Max	558.81	327.99				819.39				
p25	0.01	221.98				150.43				
p75	184.11	271.79				265.75				
Week 4	n=11					n=7				
Median	90.24					166.22				
Min	0.01					0.01				
Max	399.71					970.41				
p25	0.01					135.06				
p75	127.84					534.92				

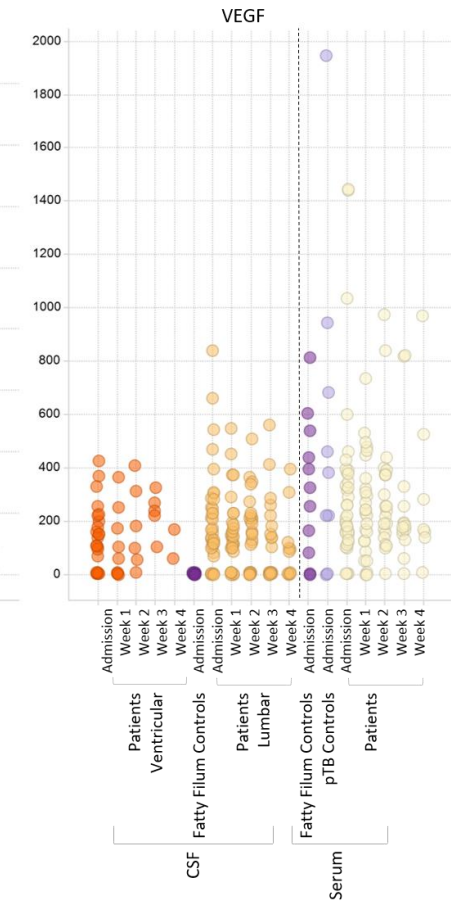
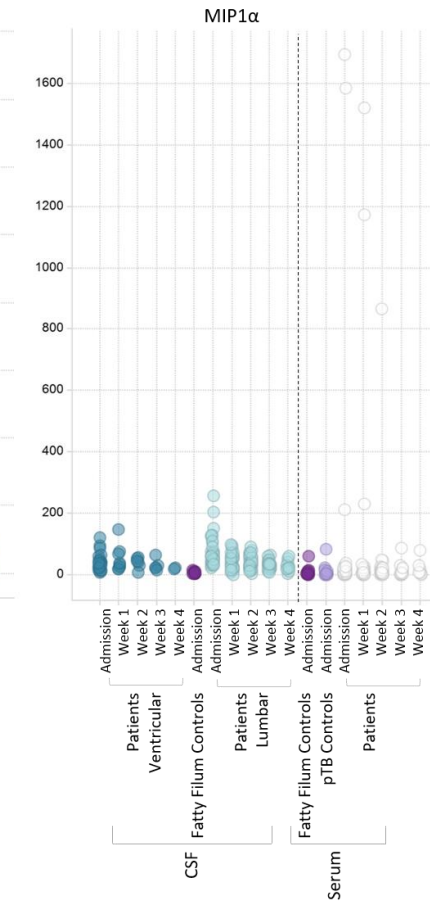
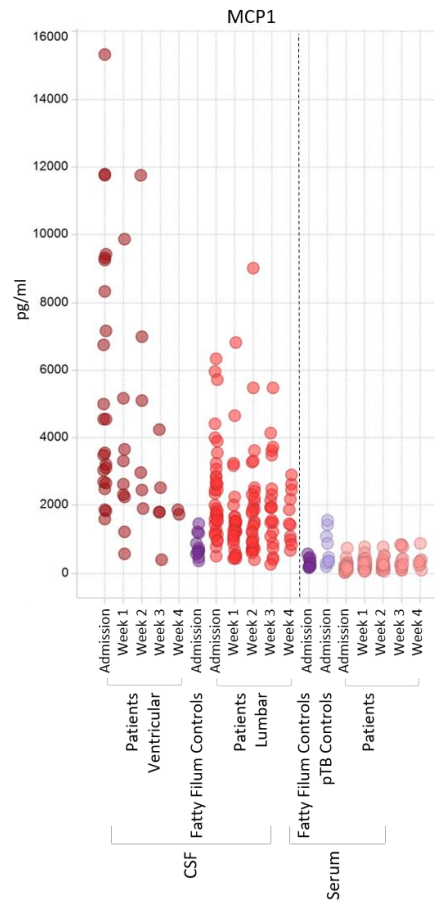
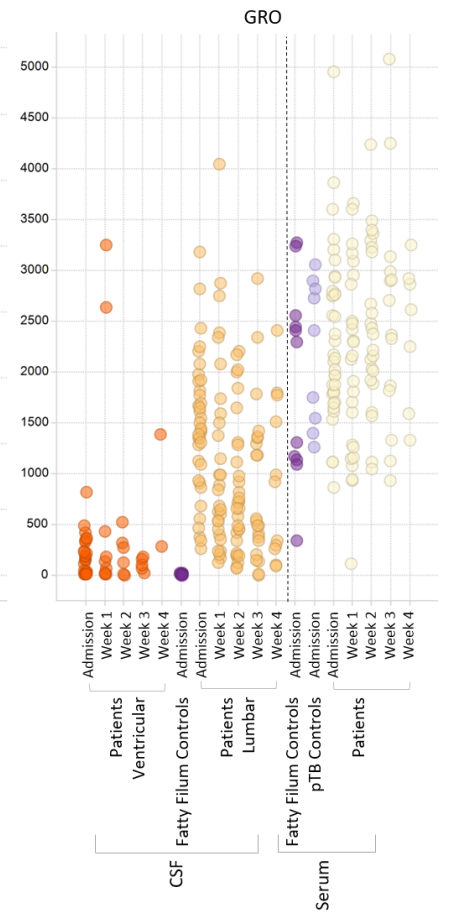
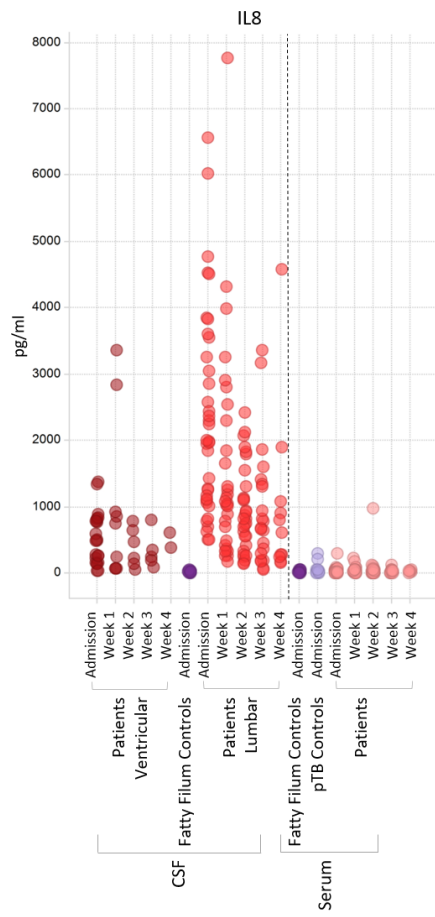
Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, ^aThe LLOD for VEGF was 80, therefore values <80 were assigned 0.01; therefore the median in week 3 appears much lower than the week 2 and week 4 medians, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Marker	RANTES Cases (pg/mL)		RANTES controls (pg/mL)	Cases vs controls Lumbar	Cases vs controls Vent	RANTES Cases (pg/mL)	Fatty filum controls (pg/mL)	pTB controls (pg/mL)	Cases vs fatty filum controls	Cases vs pTB controls
Sample Type	Lumbar	Vent	Lumbar	p-value	p-value	Serum	Serum	Serum	p-value	p-value
Admission	n=36	n=23	n=11	p=0.1		n=36	n=11	n=9	p=0.1	p<0.01
Median	0.01	0.01	0.01			4375.69	7270.56	9843.51		
Min	0.01	0.01	0.01			1356.4	3511.32	4984.65		
Max	1022.21	0.01	0.01			13288.76	12311.36	12977.91		
p25	0.01	0.01	0.01			3680.88	4008.91	7407.29		
p75	0.01	0.01	0.01			7155.71	9864.55	11179.14		
p95			0.01				12311.36	12977.91		
Week 1	n=29	n=9				n=26				
Median	0.01	0.01				4970.54				
Min	0.01	0.01				0.01				
Max	412.04	0.01				12984.16				
p25	0.01	0.01				4164.56				
p75	0.01	0.01				8418.42				
Week 2	n=30	n=6				n=23				
Median	0.01	0.01				5026.29				
Min	0.01	0.01				862.17				
Max	314.6	0.01				11374.73				
p25	0.01	0.01				3800.18				
p75	0.01	0.01				8559.62				
Week 3	n=21	n=5				n=14				
Median	0.01	0.01				4851.89				
Min	0.01	0.01				1615.88				
Max	0.01	0.01				11787.36				
p25	0.01	0.01				4037.31				
p75	0.01	0.01				7747.56				
Week 4	n=11					n=7				
Median	0.01					4403.37				
Min	0.01					2381.16				
Max	0.01					13632.33				
p25	0.01					3670.28				
p75	0.01					9796.18				

Lumbar = lumbar CSF, Vent = ventricular CSF, p= percentile, min = minimum, max = maximum, pTB = pulmonary TB controls, * = statistically significant at $p<0.05$, lumbar 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, only 2 patients had ventricular CSF samples taken in week 4, due the low sample size biomarker concentrations are not presented

Supplement 5: Inflammatory marker temporal profile plots



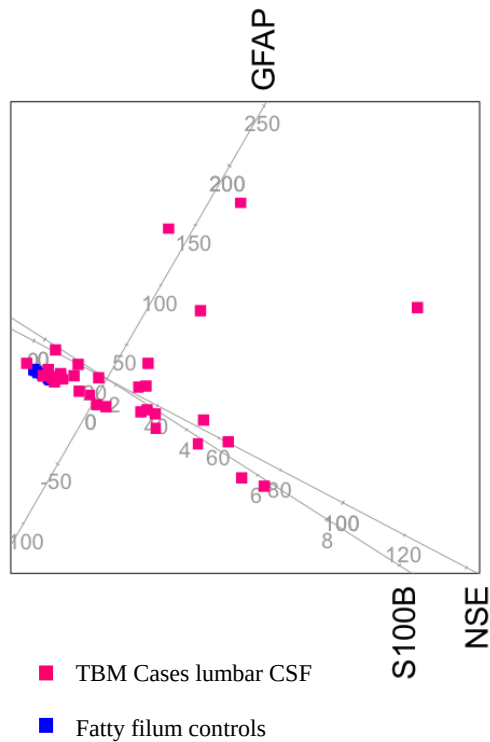


Supplement 6: PCA bi-plots

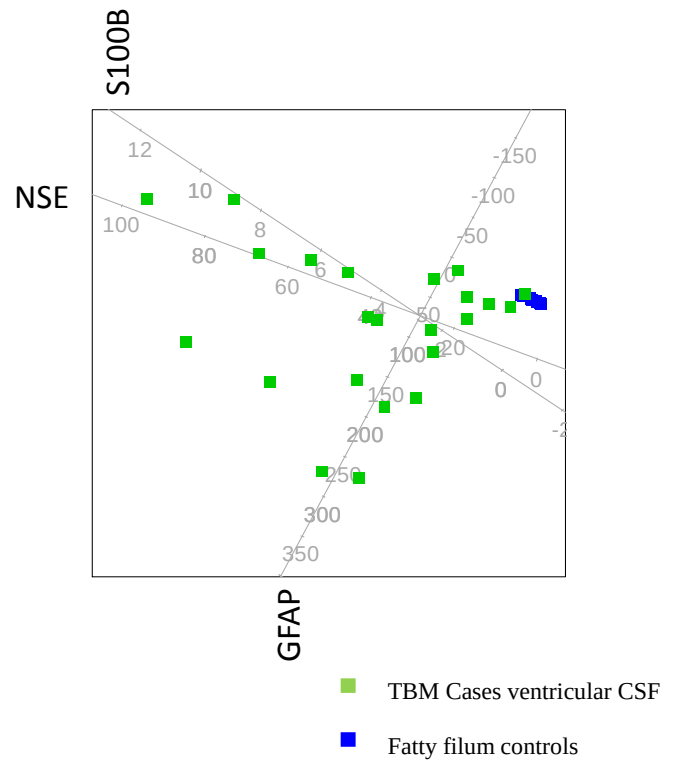
Principal component analysis biplots demonstrated neuro- and inflammatory marker concentrations for cases and controls across sample types.

Neuro-markers concentrations: cases versus controls

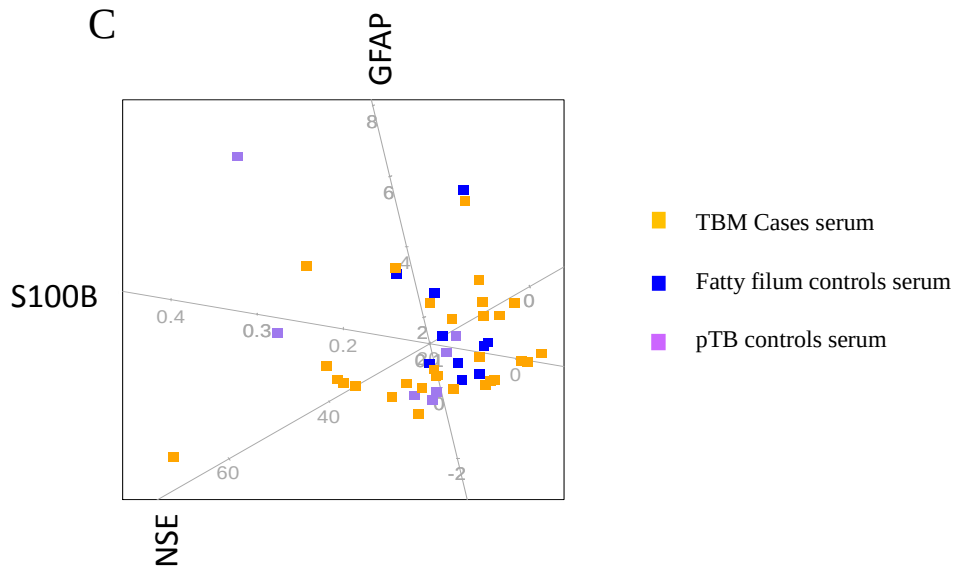
A



B



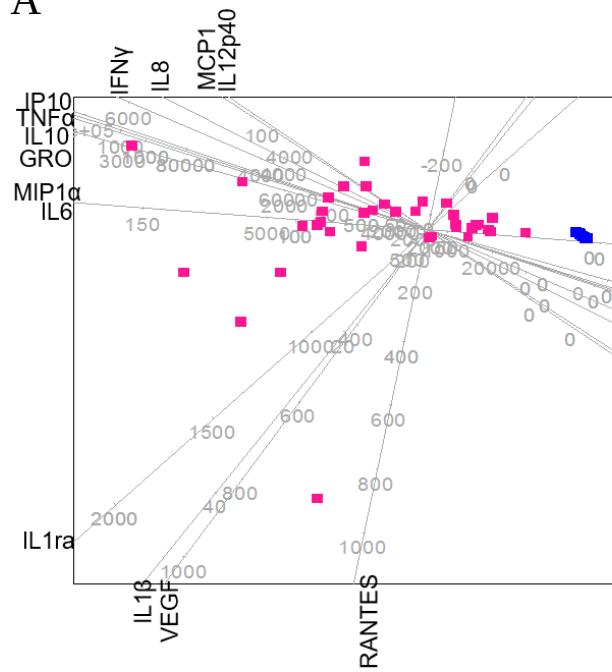
C



These PCA bi-plots demonstrate admission S100B, NSE and GFAP concentrations simultaneously. Markers on the graphs represent individual patients/controls and their concentration (ng/ml) of S100B, NSE and GFAP is indicated by their distance from the 3 axes. **A:** Lumbar CSF concentrations of neuromarkers for TBM cases (pink) and fatty filum controls (blue). Cases had higher concentrations for all neuromarkers. **B:** Ventricular CSF concentrations of neuromarkers for TBM cases (green) and controls (blue). Cases had higher concentrations for all neuromarkers. **C:** Serum concentrations for TBM cases (orange), fatty filum controls (dark blue), and pulmonary TB (pTB) controls (light purple markers). Serum concentrations of all 3 neuromarkers in controls and TBM cases overlapped, indicating that TBM serum neuromarker concentrations were not significantly different from control concentrations.

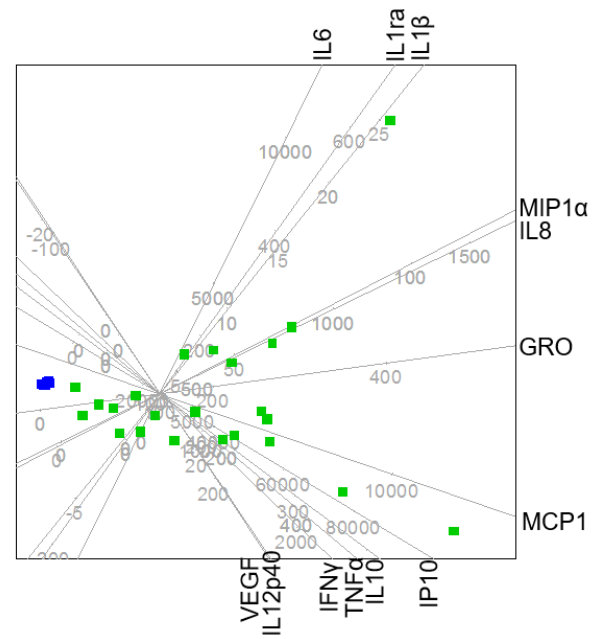
Inflammatory marker concentrations: cases versus controls

A

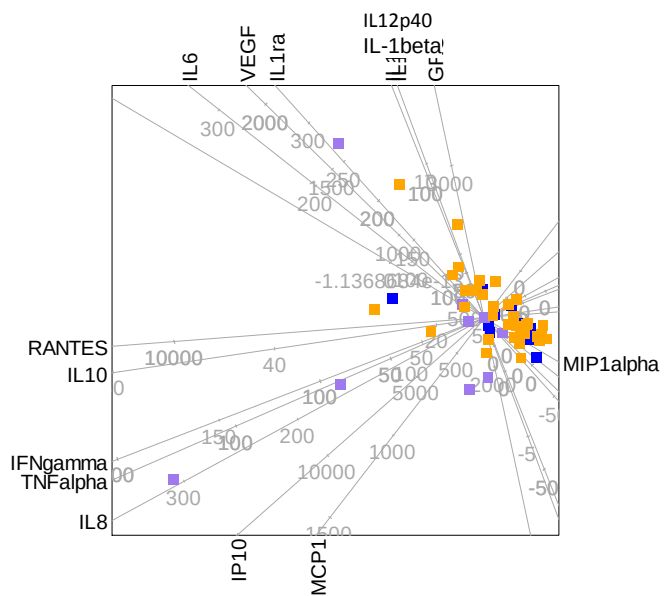


■ TBM Cases lumbar CSF ■ Fatty filum controls

B



■ TBM Cases ventricular CSF ■ Fatty filum controls

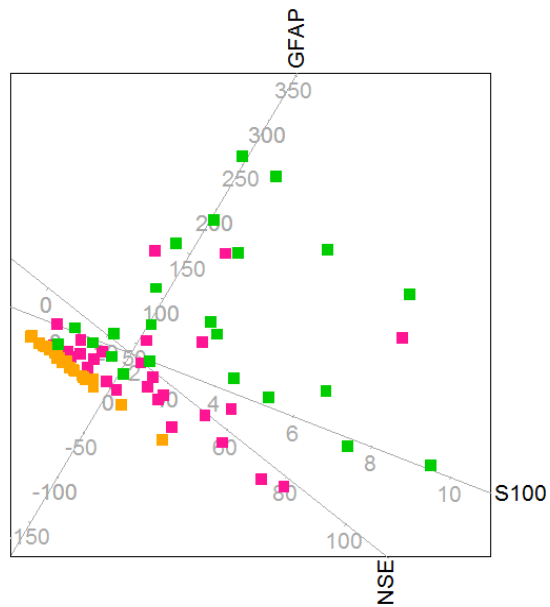


■ TBM Cases serum
■ Fatty filum controls serum
■ pTB controls serum

These PCA bi-plots demonstrate admission inflammatory marker concentrations simultaneously. Markers on the graphs represent individual patients/controls and their concentration (pg/mL) of inflammatory markers are indicated by the distance from the 3 axes. **A:** Lumbar CSF concentrations of inflammatory markers for TBM cases (pink) and fatty filum controls (blue). Cases had higher concentrations for all inflammatory markers. **B:** Ventricular CSF concentrations for TBM cases (green) and controls (blue). Cases had higher concentrations on all inflammatory markers. **C:** Serum concentrations for TBM cases (orange), fatty filum controls (dark blue), and pulmonary TB (pTB) controls (light purple markers) - control and TBM case concentrations overlapped, indicating that TBM serum inflammatory marker concentrations were not significantly different from control concentrations.

Biomarker concentrations across the 3 sample types for TBM cases

A

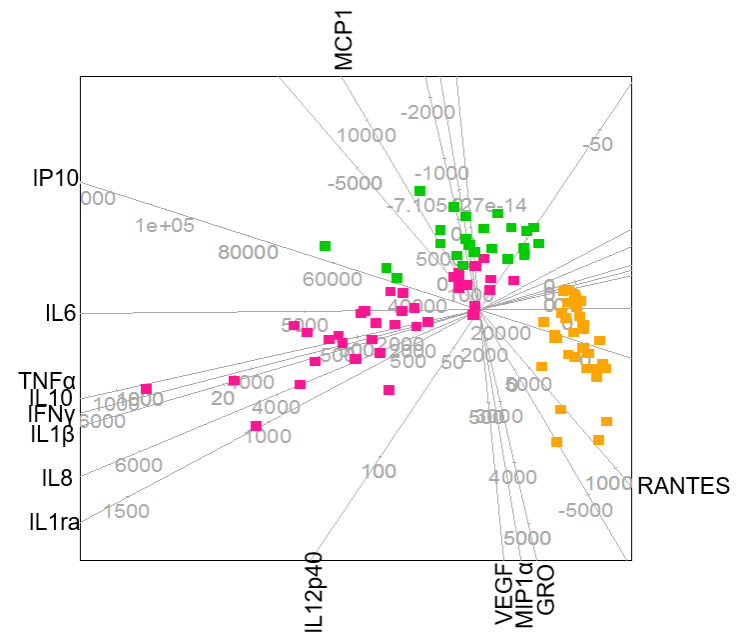


■ Serum

■ Lumbar CSF

■ Ventricular CSF

B



These PCA bi-plots demonstrate admission lumbar CSF (pink), ventricular CSF (green), and serum (orange) biomarker concentrations for TBM cases simultaneously as described for Figures 2-3. **A:** For all 3 neuromarkers ventricular CSF concentrations were greater than lumbar CSF which were greater than serum concentrations. **B:** lumbar and ventricular CSF concentrations exceeded serum concentrations for all 14 inflammatory markers, and lumbar CSF concentrations were greater than ventricular CSF for most inflammatory markers.