



REVIEW

REVIEWED Recent Developments in Tuberculous Meningitis

Pathogenesis and Diagnostics [version 3; peer review: 3 approved]

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Abstract

The pathogenesis of Tuberculous meningitis (TBM) is poorly understood, but contemporary molecular biology technologies have allowed for recent improvements in our understanding of TBM. For instance, neutrophils appear to play a significant role in the immunopathogenesis of TBM, and either a paucity or an excess of inflammation can be detrimental in TBM. Further, severity of HIV-associated immunosuppression is an important determinant of inflammatory response; patients with the advanced immunosuppression (CD4+ T-cell count of <150 cells/μL) having higher CSF neutrophils, greater CSF cytokine concentrations and higher mortality than those with CD4+ T-cell counts > 150 cells/μL. Host genetics may also influence outcomes with LT4AH genotype predicting inflammatory phenotype, steroid responsiveness and survival in Vietnamese adults with TBM. Whist in Indonesia, CSF

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tryptophan level was a predictor of survival, suggesting tryptophan metabolism may be important in TBM pathogenesis. These varying responses mean that we must consider whether a “one-size-fits-all” approach to anti-bacillary or immunomodulatory treatment in TBM is truly the best way forward. Of course, to allow for proper treatment, early and rapid diagnosis of TBM must occur. Diagnosis has always been a challenge but the field of TB diagnosis is evolving, with sensitivities of at least 70% now possible in less than two hours with GeneXpert MTB/Rif Ultra. In addition, advanced molecular techniques such as CRISPR-MTB and metagenomic next generation sequencing may hold promise for TBM diagnosis. Host-based biomarkers and signatures are being further evaluated in childhood and adult TBM as adjunctive biomarkers as even with improved molecular assays, cases are still missed. A better grasp of host and pathogen behaviour may lead to improved diagnostics, targeted immunotherapy, and possibly biomarker-based, patient-specific treatment regimens.

Keywords



Tuberculous meningitis, TBM, TB, HIV, pathogenesis, diagnostics,



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REVISED Amendments from Version 2

In light of comments from the reviewers, we have added a little more detail in relation to the CSF biomarkers in children, RNA signatures and added a table summarising CSF assays for TBM.

Any further responses from the reviewers can be found at the end of the article

Introduction

The pathogenesis of Tuberculous meningitis (TBM) is poorly understood. Mechanisms by which *Mycobacteria* disseminate from lung to the brain, key factors driving a dysregulated host response, and the pathogen specific factors influencing presentation and severity, compared to other forms of TB, are not well described. In recent years application of contemporary molecular biology ‘omics’ techniques to clinical samples, greater availability of advanced neuroradiology, emphasis on immune-mediated contributions to pathology, and use of refined experimental models of TBM have better illuminated its pathogenesis. A better grasp of these processes may also lead to improved diagnostics, targeted immunotherapy as well as a biomarker-based, patient-specific approach to personalized treatment. Diagnosis has been traditionally insensitive (AFB smear) and slow (culture). This has improved with the addition of GeneXpert MTB/Rif (Xpert) which gave sensitivities similar to culture in 2 hours (versus 2–4 weeks with culture). Subsequently, GeneXpert MTB/Rif Ultra (Ultra), a re-engineered version, has shown better sensitivities than culture in some settings. Yet, none of these technologies has adequate negative predictive value to ‘rule-out’ TBM. In this article we review important recently published studies that have informed our current understanding of TBM pathogenesis and diagnostics. We do not seek to present a comprehensive review of the history of TBM pathogenesis and diagnostics as a number of detailed papers have addressed this recently^{1–3}. Rather we provide a commentary of key studies published within the last 5 years and summarise knowledge gaps and future considerations to enable progress in the field.

TBM pathogenesis

Dissemination to the central nervous system

Understanding of the microbial and immune processes that allow *M. tuberculosis* to disseminate from the respiratory epithelium to reach the meninges remains incomplete^{2,4}. The foundations of what is known were laid through natural history and autopsy studies in the pre-chemotherapy era. The necessary steps to develop TBM include the pathogen surviving its initial encounter with the innate immune system at the respiratory epithelium and establishment of primary infection in the lung parenchyma with characteristic granulomatous inflammation^{5–7}. Spread beyond the lungs likely occurs through the blood and may be preceded by local invasion to the lymphatic system. Donald and Schoeman have highlighted the possibility of coincident miliary TB in cases of TBM, particularly in young children, where tubercles of different sizes and ages have been described on the meninges and confirmed by magnetic resonance imaging (MRI)^{8,9}. In children, miliary TB and TBM

develop most often within 3 months of primary infection, when fresh anatomical changes are still found in the primary lung focus¹⁰. In addition to children, people living with HIV (PLWHIV) are another vulnerable group who may be unable to control the infection in the lungs and therefore at risk of coincident miliary TB and TBM secondary to haematogenous dissemination of *M.tb*^{8,11}. The contemporaneous nature of TBM and miliary TB illustrates that the “Rich focus” model (of a single meningeal/sub-cortical granuloma rupturing years after initial haematogenous dissemination discharging acid-fast bacilli into the sub-arachnoid space)¹² does not apply to all TBM cases and there may be more than one pathway to the development of meningitis following *M. tuberculosis* infection.

Host immune response to TB infection in the CNS

The host immune response to TB bacilli in the sub-arachnoid space gives rise to a granulomatous inflammation predominantly affecting the basal meninges. Inflammatory exudates may obstruct the passage of cerebrospinal fluid (CSF), leading to hydrocephalus. Small and medium-sized intracerebral arteries can become inflamed and occluded, leading to cerebral infarcts. The majority of TBM pathology is believed to result from the host inflammatory response, which has been reviewed in depth elsewhere²; several pro- and anti-inflammatory cytokines such as tumour necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin (IL) 1 β , IL-6, IL-8, and IL-10 are shown to be induced in TBM^{13,14}. Disequilibrium of pro- and anti-inflammatory cytokines influence the severity and course of TBM. Current understanding of key established mechanisms known to play a role in host immune response in TBM are summarised in [Figure 1](#)^{2,15}.

In the recent literature, the long-standing belief that excessive inflammation is the cause of death in TBM was brought into question by a recent immunopathogenesis study in Vietnam. In HIV-negative adults, associations between death and both lower CSF cytokines concentrations and lower CSF leucocyte counts (median 59×10^3 cells/mL (IQR 13–240 $\times 10^3$ cells/mL) in those who died versus 135×10^3 cells/mL (IQR, 48–298 $\times 10^3$ cells/mL) in survivors) were noted¹⁶. These data support the notion that poor outcome from TBM, in the context of immunosuppressive treatment (adjunctive corticosteroids), is associated with an inadequate pretreatment inflammatory response in HIV-negative individuals. In a study of 120 Vietnamese adults with TBM included in a trial of adjunctive aspirin treatment, it was shown that there was an aspirin dose-dependent inhibition of thromboxane A₂ and upregulation of pro-resolving CSF protectins, resulting in potential reduction in new infarcts and deaths by day 60 of treatment in microbiologically confirmed TBM patients¹⁷. A further study investigated concentrations of host protective lipid mediators (specialized proresolving mediators, SPMs) in CSF. Prostaglandins and cysteinyl leukotrienes were found to be reduced in more severe cases, while the lipoxygenase 5-derived 13-series resolvin (RvT)2, RvT4, and 15-epi-lipoxin B4, were significantly increased in survivors. These data suggest SPMs may play an important role in TBM pathogenesis¹⁸.

Among 608 Indonesian adults with suspected TBM, higher CSF and blood neutrophil counts (HR 1.10 (95%CI 1.04–1.16) per 10% increase and HR 1.06 per 10⁹ neutrophils/L increase;

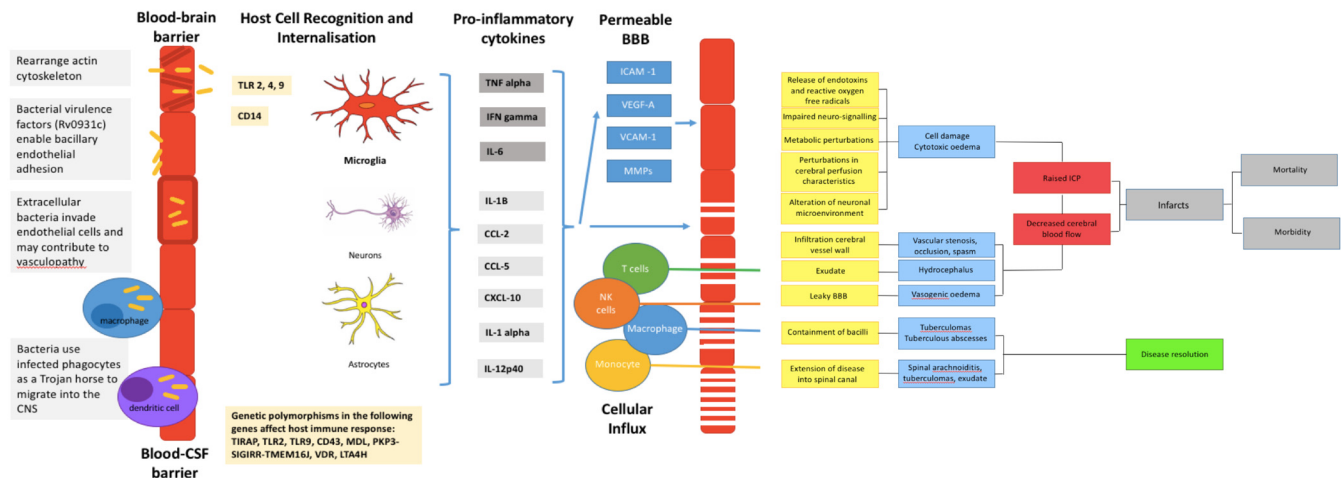


Figure 1. Illustrative summary of the pathogenesis of tuberculous meningitis (TBM). Reproduced with permission from author and Journal of Leukocyte Biology². A: *Mycobacterium tuberculosis* bacilli (*M.tb*) disseminate from the primary site of infection in the lung to seed the brain. The bacilli traverse the blood brain barrier (BBB) and blood cerebrospinal fluid barrier (BCSFB) through various virulence factors that enable the invasion of and migration through cerebral vascular endothelial cells, or are carried into the CNS by infected peripheral innate immune cells. B: In the CNS antigen recognition and internalization by microglia, neurons and astrocytes occurs, mediated by numerous host genetic factors. C: The resulting immune response stimulates the release of proinflammatory cytokines and chemokines and other immune mediators that contribute to the breakdown of the BBB and the influx of innate and adaptive immune cells from the periphery. D: A prolific inflammatory response ensues. The inflammatory exudate in the basal cisterns contributes to cerebral vascular pathology and the development of hydrocephalus and raised intracranial pressure. Vasogenic edema due to an influx of proteins through the leaky BBB, and cytotoxic edema as a result of cellular damage contribute to the raised pressure. The overall decrease in cerebral blood flow puts the brain at risk of ischemia, infarction and poor patient outcomes. In some cases the infection is controlled in discrete tuberculomas or abscesses, which may resolve with treatment and time.

(95% CI 1.03–1.10), respectively) were associated with mortality¹⁹. Flow-cytometry on blood in a subset of 160 HIV-negative adults with TBM showed lower $\alpha\beta$ T and $\gamma\delta$ T cells, NK cells and MAIT cells in TBM subjects compared to 26 pulmonary TB adults (2.4 to 4-fold, all $p < 0.05$) and 27 healthy controls (2.7–7.6-fold, $p < 0.001$), but higher neutrophils and classical monocytes (2.3–3.0-fold, $p < 0.001$). CSF flow cytometry of TBM patients showed a predominance of $\alpha\beta$ T and NK cells, associated with better survival, as well as the presence of MAIT cells, previously undescribed in CSF²⁰. Indonesian HIV-negative TBM patients showed a strong myeloid blood response and a remarkably broad lymphoid CSF response including innate lymphocytes, however there was little correlation between blood and CSF compartments²⁰.

These recent studies in Vietnamese and Indonesian adults with TBM, aimed at gaining insights into mechanisms of the inflammatory response in disease pathogenesis, used novel and high-resolution methods to look at lipid mediator profiles and immune cell populations. Data indicated specific lipid mediator signatures and cell populations that are associated with disease severity before treatment and mortality; these should be considered for host-directed therapy of TBM.

Host genetic and metabolic factors

More efficient and cost-effective genomics platforms have enabled of late better understanding of variable host responses in TBM through the study of host genetics. Polymorphisms in CD43 encoding a surface glycoprotein involved in *M.tb* adhesion

and proinflammatory cytokine induction and PKP3-SIGIRR-TMEM16J gene region encoding a negative regulator of TLR/IL-1R signalling have both been linked to survival in TBM^{21,22}. However, the greatest interest has been around the role of leukotriene A4 hydrolase (LTA4H). LTA4H catalyzes the final step in the synthesis of leukotriene B4 (LTB4), a potent chemoattractant and pro-inflammatory eicosanoid. A common functional promoter variant rs17525495 in the LTA4H gene can predict survival and dexamethasone responsiveness in HIV-uninfected adults with TBM^{16,23}. This human candidate gene association study was guided by findings in a zebra fish model where LTA4H was found to determine the balance of pro-inflammatory and anti-inflammatory eicosanoids in response to mycobacterial infection^{16,24}. In a retrospective study in Vietnamese HIV-uninfected adults with TBM, while LTA4H rs17525495 TT and CC genotypes were both associated with susceptibility to mycobacterial infection, the associations involved opposing inflammatory states: high inflammation for the TT genotype and low inflammation for the CC genotype. CT genotype had an intermediate inflammatory response and were more likely to survive TBM. Dexamethasone treatment improved survival in TT genotype patients with hyper-inflammatory response but was possibly harmful to CC patients with hypo-inflammatory response²³. A later prospective study in Vietnam reported that in TBM HIV-uninfected adults, LTA4H genotype influences cytokine inflammatory response and correlates with TBM severity, Figure 2¹⁶. More importantly, this study confirmed that the LTA4H genotype determined corticosteroid responsiveness and survival.

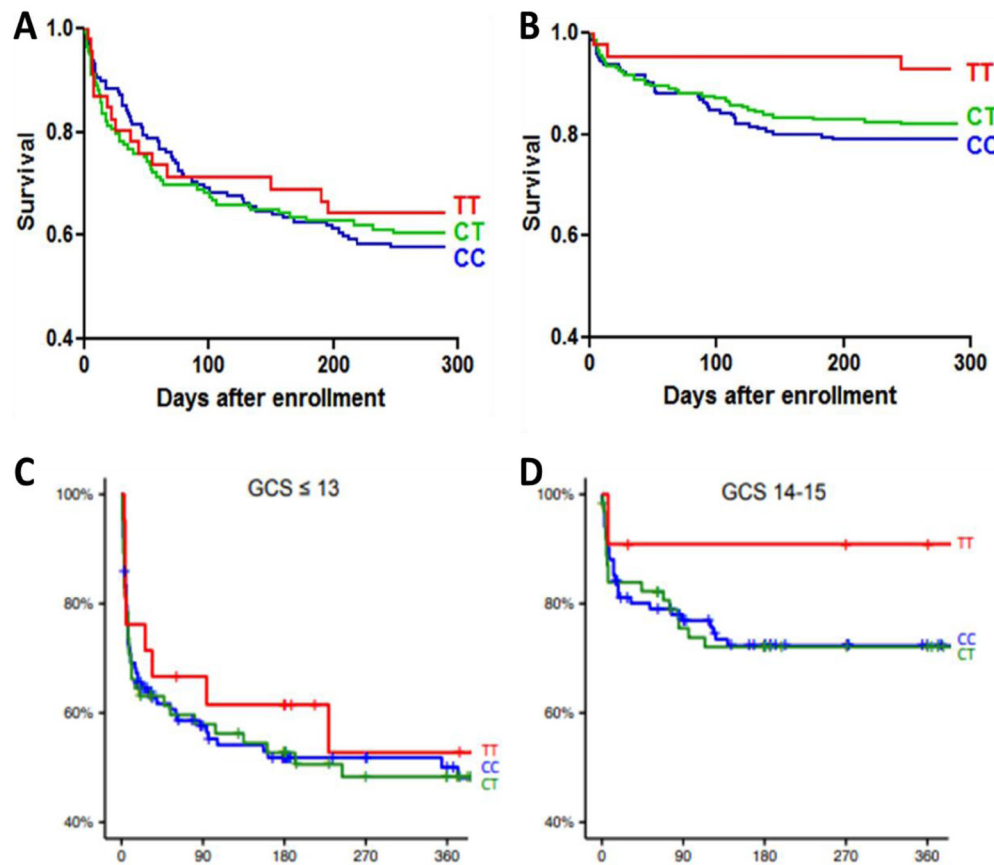


Figure 2. Kaplan-Meier survival curves stratified by LTA4H genotype. Figures **A** and **B** show survival in 763 patients with tuberculous meningitis in Vietnam¹⁶, with human immunodeficiency virus (HIV) infection (**A**), and without HIV infection (**B**). In HIV-infected patients, case-fatality rates were 34.8% (16 of 46) in those with TT genotype, 42.1% (61 of 145) in CT genotype, and 38.8% (52 of 134) in CC genotype. In HIV-uninfected patients, case-fatality rates were 7.1% (3 of 42) in TT, 21.4% (40 of 187) in CT, and 18.7% (39 of 209) in CC. Figures **C** and **D** show survival in 375 patients with tuberculous meningitis in Indonesia¹⁹. These patients are HIV-uninfected with severe (GCS ≤ 13) (**C**) or milder (GCS 14–15) disease (**D**). In a recessive model, TT genotype versus CT/TT combined had HR 0.81 (95% CI 0.41–1.62, $p = .550$) in severe and 0.31 (95% CI 0.04–2.25, $p = .156$) in milder disease.

Interestingly, LTA4H genotype did not predict outcomes in Indonesian adults with TBM, but there was a trend towards improved survival with TT genotype compared to CC or CT genotype, Figure 2¹⁹. A clinical trial is currently underway in Vietnam (NCT03100786) to evaluate LTA4H genotype-directed corticosteroid therapy, an exciting example of personalised medicine in TBM²⁵.

Although prior studies have considered sodium, glucose and lactate as related to TBM pathogenesis, recent developments in the application of targeted metabolomics have provided greater insight in the role of tryptophan, a potential key metabolite in TBM. This amino acid required for protein biosynthesis is a precursor to serotonin and melatonin (serotonin pathway) and kynurenine and quinolinic acid (kynurenine pathway). The latter is stimulated at the expense of the former by pro-inflammatory cytokine such as IL-6, TNF- α and IFN- γ via indoleamine 2, 3-dioxygenase. In a recent study of serum and CSF metabolites, low levels of tryptophan were associated with survival²⁶. One theory regarding this association could be the neuroprotective

effects of the associated kynurenine pathway downstream metabolites. Either this pathway, or the 11 genetic foci related to CSF tryptophan metabolism could have novel clinical implications for TBM²⁶.

HIV co-infection and immune reconstitution inflammatory syndrome

HIV infection is a strong independent predictor of death from TBM (hazard ratio, 3.94; 95% confidence interval (CI), 2.79–5.56)²⁷. The role of adjunctive corticosteroids in HIV-associated TBM is inconclusive (relative risk of death with adjunctive steroids, 0.78; 95% CI, 0.59 to 1.04; $P=0.08$)²⁸ and a randomized placebo-controlled trial is underway (NCT03092817) to address the use of steroids in HIV-associated TBM. Pathogenesis studies in PLWHIV are required to identify the unique pathogenic determinants of poor prognosis. Thuong *et al.* compared the pretreatment CSF cells and cytokine profiles of 764 HIV-positive and HIV-negative participants in Vietnamese TBM clinical trials. HIV-positive individuals had higher mean CSF neutrophil percentage (17% vs 5%; $P < .0001$)

and global cytokine expression (aside from IL-10 which inhibits its response to *M. tuberculosis*) than their HIV-negative counterparts. PLWHIV with CD4+ T-cell counts <150 cells/ μ L showed higher median CSF neutrophil percentage (25%), than those with a count \geq 150 cells/ μ L (neutrophils 10%; $P=0.021$) and patients without HIV infection (neutrophils 5%; $P<0.0001$). Of patients with a CD4+ T-cell count of <150 cells/ μ L, 44% (105 of 238) died, compared with 13% (5 of 39) with a count of \geq 150 cells/ μ L and 19% (83 of 439) without HIV infection¹⁶. These findings, amongst others, suggest a role for neutrophils in the immunopathogenesis of HIV-associated TBM²⁹.

Marais *et al.* conducted longitudinal analyses of paired blood and CSF samples in South Africans with HIV-associated TBM, describing the relationships between the development of immune reconstitution inflammatory syndrome (IRIS) and CSF leucocytes, the concentrations of >30 blood and CSF inflammatory mediators, and blood transcriptional profiles. They found TBM-associated CNS IRIS to have an inflammatory signature characterized by neutrophil and inflammasome-mediated proinflammatory responses^{30,31}. The neutrophil-dependent inflammatory activation could be detected in peripheral blood before the start of TB treatment and therefore has potential to predict who will develop IRIS.

Brain Injury Markers

The study of neurodegenerative-associated proteins to describe extent and type of brain injury post TBM has recently been explored through omics analysis, approaches which strive to understand genetic or molecular profiles of humans, particularly in paediatric TBM. In lumbar CSF of children with TBM, S100B and NSE (structural proteins of the CNS, and biomarkers of CNS tissue damage) at disease onset were associated with poor outcome, as was highest concentration overall and an increasing profile over time in S100B, NSE, and GFAP neuro-marker concentrations increased over time in those who died (whilst inflammatory markers decreased), and were overall highest in those with cerebral infarction³². It is of interest that despite markers of inflammation reducing, proteins traditionally associated with neurodegenerative processes continued to rise.

In ventricular CSF of children with TBM, transcriptome analysis has revealed significant enrichment of transcripts associated with neuro-excitotoxicity predominantly driven by glutamate release and NMDA binding and receptor uptake³³. Upregulation of genes associated with nitric oxide, cytochrome c, brain injury proteins like myelin basic protein, and proteins including tau, amyloid-beta and apo- lipoprotein were also seen³³; many of which have also been described in neurodegenerative conditions such as Alzheimer's disease and traumatic brain injury³⁴.

These findings raise the possibility of ongoing brain injury which in TBM seem to occur following ischaemic injury, despite resolving acute inflammation³². Further studies, including those which investigate the longer-term pathogenic processes in TBM are required to validate these results and understand further neurological sequelae including those which may indicate a post-infectious process in TBM.

Neuroimaging in pathogenesis studies

Technical advances and increasing availability of imaging modalities have recently enabled research in which imaging is used to assess pathogenic mechanisms in TBM *in vivo* in animal and human subjects. In a blood and CSF biomarker study of childhood TBM tuberculomas, magnetic resonance imaging has been used to note an association between tuberculomas and elevated interleukin (IL) 12p40, interferon-inducible protein 10, and monocyte chemoattractant protein 1 concentrations, whereas infarcts were associated with elevated TNF- α , macrophage inflammatory protein 1 α , IL-6, and IL-8³². Specific sequences can also be used to describe morphology of structural damage and correlate this to meaningful clinical measures. For instance poorer Diffusion Tensor Imaging (DTI) parameters of white matter integrity in the anterior cingulate gyrus, parahippocampal gyrus and globus pallidus are associated with worse neuropsychological performance³⁵. A further study by the same group used Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) voxel-based morphometry (VBM) to assess the integrity of grey matter in these same TBM patients³⁶. Patients with TBM performed significantly poorer on the digit symbol, similarities, block design, matrix reasoning, and letter-number sequencing subtests of the Wechsler Adult Intelligence Scale compared to healthy adults. These changes correlated with smaller grey matter volumes in the right thalamus, right superior temporal gyrus, right precuneus, right middle temporal gyrus, left putamen, right caudate nucleus, and right middle temporal gyrus³⁶. These studies suggest that structural damage can be cortical as well as subcortical which may in turn be related to degree of long-term impairment. This has implications for understanding long term outcomes particularly neurocognitive impairment in TBM, which in light of these findings may share features with other forms of dementia (including vascular and HIV associated neurocognitive impairment) where a subcortical pattern of neurocognitive impairment (including frontal and executive functions) can be observed.

A rabbit model study of childhood TBM, utilized ionized calcium binding adapter molecule (Iba-1) to approximate microglial activation with fludeoxyglucose-positron emission tomography (FDG-PET) and demonstrated the presence of activated microglia and macrophages localized to TB lesions³⁷. In humans, case reports and a prospective study have advocated the use of FDG-PET as a diagnostic tool, as it has been effective in detecting extra-cranial evidence supportive of a TBM diagnosis^{38–40}. The role of FDG-PET in unravelling time course of inflammation in TBM remains to be seen, although it has played a role in understanding Alzheimer's, a disease in which, similar to TBM, inflammation plays a key pathogenic role^{41,42}.

Pathogen factors: bacillary load, pathogen strain and virulence factors

TBM patients generally have low bacterial loads in CSF which causes difficulties in both diagnosis and ability to study bacterial load evolution-related pathophysiology. The time-to-positivity of a culture and cycle threshold (Ct) of nucleic acid amplification tests such as GeneXpert MTB/Rif (Xpert) can provide an indication of likely bacterial burden⁴³. Over 50% of diagnosed cases are microbiologically undetectable and defined as

‘probable’ or ‘possible’ TBM which obviously limits this approach⁴⁴. Marais *et al.* showed that in patients where *M.tb* was cultured from CSF taken before and after two weeks of anti-tuberculosis treatment, there was a 9.3-fold increased risk of subsequently developing TBM-IRIS, although the sample size is small with 15 TBM-IRIS patients compared with 6 non-TBM-IRIS patients⁴⁵. Thuong *et al.* found that among 692 Vietnamese adults with TBM, pre-treatment CSF *M.tb* load (by Xpert Ct) was correlated with increased CSF neutrophil counts, increased cytokine production, and new neurological events after treatment initiation, but not death⁴³.

In addition, epidemiological trends of *M.tb* lineage from TBM (n=73) and pulmonary TB (n=220) patients in Thailand showed that the Indo-Oceanic lineage is more frequently found in TBM patients (41% versus 13% in PTB)⁴⁶. This association did not hold true in Indonesia, though specific genetic variations were identified which were associated with TB phenotype, including one (Rv0218) whose encoded protein may play a role in host-pathogen interaction⁴⁷.

Host-pathogen interactions

It is estimated that the global burden of latent TB infection (LTBI) is approximately 23.0% (95% CI 20.4%–26.4%), amounting to approximately 1.7 billion people⁴⁸. Innate immune

responses are critical to control TB infection yet also contribute to tissue damage. This delicate balance is illustrated in the damage response framework which provides a theory of microbial pathogenesis that incorporates the contributions of both host and microbe to host damage that stems from host-microbe interaction^{49,50}. This framework likely applies to TBM based on evidence of both failed immunity and excessive inflammation being linked to increased TBM pathology, see Figure 3^{23,51,52}. Both the microbe and the host contribute to host damage and where an individual patient’s immune response lies on the continuum of the damage response framework parabola determines the nature of the disease process^{16,43,53}. Evidence from recent studies shows *LTA4H* genotype, CSF cytokines and CSF immune cells such as neutrophils are determinants of inflammatory state, which impacts both bacterial growth and host damage and thus leads to different outcomes. The current one-size-fits-all approach to TBM treatment fails to recognize divergent pathologies and may explain the poor outcomes in certain populations. Being able to identify where on the parabola an individual lies and tailoring therapy to achieve the optimal milieu is an approach that warrants further investigation. *LTA4H* genotype is an example of using host genotype to predict inflammatory response and to tailor treatment by host directed therapy. Omics technology are now being used to identify additional host genetic markers and treatment targets in TBM.

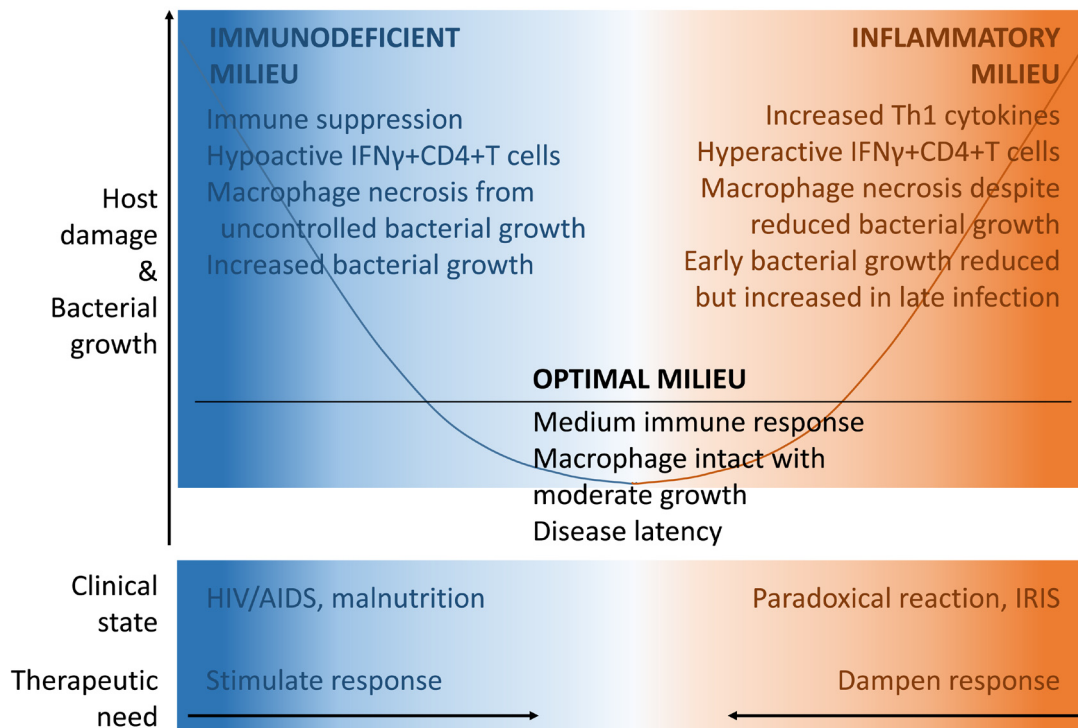


Figure 3. Outcomes of the host – *M. tuberculosis* interaction depicted by the basic parabola of the damage-response framework. On the left side of the parabola, shaded in blue, the immune system fails to limit mycobacterial growth and invasion which results in host damage. On the right side, shaded in red, the immune response is excessive and the resultant inflammation and host-damage. The proportion of the parabola lying below the black line represents disease latency, which is not associated with clinically evident host damage. On the blue side therapeutic interventions could be targeted at stimulating an immune response, whilst on the red side therapeutic interventions could aim to dampen immune response.

TBM diagnostics

The field of TBM diagnostics has evolved rapidly in recent years with both complex and low-tech assays being explored in a variety of populations. Whilst progress has been made, no single assay can be used as a rule out test. The characteristics of CSF tests studied to date are summarized in [Table 1](#).

Host-based diagnostic biomarkers

Traditional diagnostic techniques for TBM include CSF smear microscopy for acid fast bacilli (rapid and cheap but insensitive in most settings, 10–15%) and CSF culture (improved sensitivity of 50–60% but results in 2–6 weeks with a biosafety lab level three requirement)³. Given the limitations of traditional,

Table 1. Summary of TBM CSF Diagnostic Test Performance and Limitations.

Test	Sensitivity*	Specificity*	Time to Results	Strengths	Limitations
AFB Smear	10–34%	95–100%	Hours	Rapid, cheap, widely available, specificity	Poor sensitivity in most settings
Culture	48–60%	100%	2–6 weeks	Sensitivity, excellent specificity, antimicrobial resistance testing	Slow, lab infrastructure, costly, inadequate NPV
Adenosine deaminase	89%	91%	Days	Good sensitivity, low CSF volume requirement	Cost, lab infrastructure, false positives, study heterogeneity, variable test performance
IGRA	77%	88%	Days	Sensitivity	Cost, lab infrastructure, false positives, indeterminate results, varied study designs and cut-points
Antibodies	75–91%	91–98%	Days	Sensitivity	Variable study design, lack of commercial assays, false positives, numerous different targets
IL-13, VEGF, cathelicidin LL-37^a	52%	95%	Days	Sensitivity, low CSF volume requirement	Cost, lab infrastructure, requires validation, technical expertise
IFNγ, MPO, VEGF^a	91%	100%	Days	Sensitivity, low CSF volume requirement	Cost, lab infrastructure, requires validation, technical expertise
Traditional NAAT	68–82%	100%	Days	Sensitivity, specificity	Cost, lab infrastructure, many are 'in-house' tests, variable study design, variable targets, stringent operational conditions, technical expertise
Xpert MTB/Rif	40–70%	98–100%	Hours	Sensitivity, specificity, rapid, ease of use, detects rifampin resistance, widely distributed platform	Cost, inadequate NPV, variable study design and performance
Xpert Ultra	47–95%	100%	Hours	Sensitivity, specificity, rapid, ease of use, detects rifampin resistance, widely distributed platform	Cost, inadequate NPV, variable study design and performance
CRISPR-MTB	73%	98%	Hours	Low CSF volume, sensitivity, specificity, isothermal	Cost, lab infrastructure, stringent operational use, technical expertise, requires validation
mNGS	67%	98–100%	Days-Weeks	Can detect alternative pathogens, sensitivity, specificity	Cost, lab infrastructure, very stringent operational use, technical expertise, requires validation
Alere TB LAM	22–24%	95%	Minutes	Rapid, cost, heat stability, limited lab expertise or infrastructure requirements	Sensitivity, intra-operative variability, inadequate NPV
Fujifilm SILVAMP TB LAM	52–74%	98%	1 hour	Rapid, sensitivity, heat stability, limited lab expertise or infrastructure requirements	Cost, intra-operative variability, inadequate NPV

*All sensitivity and specificity values are approximate, based on current literature with the understanding that variability occurs between studies and with local disease prevalence.

^a: Reported values are for paediatric patients only

TBM: Tuberculous meningitis; AFB: acid-fast bacilli; NPV: negative predictive value; IGRA: interferon gamma release assay; IL: interleukin; VEGF: vasoactive endothelial growth factor; IFN γ : interferon gamma; MPO: myeloperoxidase; NAAT: nucleic acid amplification tests; Xpert: GeneXpert MTB/Rif; Xpert Ultra: GeneXpert MTB/Rif Ultra; CRISPR-MTB: Clustered regularly interspaced palindromic repeat associated proteins – *Mycobacteria tuberculosis*; mNGS: metagenomic next generation sequencing; LAM: lipoarabinomannan;

diagnostic tests for TBM that focus on bacillary detection, there is interest in the utilization of host-based diagnostic biomarkers for diagnosis of TBM, Figure 4. Adenosine deaminase (ADA), produced by lymphocytes, is an important regulator of follicular helper T-cells. ADA is commonly used for diagnosis of TB from other, typically extra-pulmonary locations and numerous studies have considered ADA for diagnosis of TBM⁵⁴. One 2017 meta-analysis found ADA to have a pooled sensitivity of 89% (95% CI 84–92%) with pooled specificity 91% (95% CI, 87–93%)⁵⁴. Yet ADA use for TBM diagnosis has been limited by the high cost of the test, required sophisticated lab infrastructure, study heterogeneity, inadequate negative predictive values, and variable test performance.

A number of studies have considered unstimulated CSF interferon gamma (IFN- γ) levels as a diagnostic test, in general, a high number of false positive results has limited the utility of CSF IFN- γ ^{55,56}. For instance, in one study of 39 controls (n=12 viral, n=16 purulent, n=11 cryptococcal meningitis) and 30 subjects with TBM while median IFN- γ levels were higher amongst subjects with TBM, diagnostic accuracy was inadequate⁵⁶. At the strongest cut-point (81pg/mL) determined by receiver operator curve analysis, positive predictive value was only 81% with positive results occurring in 2/12 (17%) with viral meningitis, 3/16 (19%) with purulent meningitis, and 1/11(9%) with cryptococcal meningitis⁵⁶.

Interferon gamma release assays (IGRAs) are commonly used to infer LTBI. A 2016 meta-analysis of six studies performing CSF IGRA's found a pooled sensitivity and specificity of 77% (95% CI 69%-84%) and 88% (95% CI 74%-95%), respectively, for TB meningitis, though reference standards varied by study⁵⁷. Limitations of IGRA include high cost, the need for advanced lab infrastructure, frequent “indeterminate” results, and false positives associated with other causes of meningitis. Additional host biomarkers including delta-like ligand 1, vitamin D binding protein, and fetuin have been evaluated in CSF though none were found to have satisfactory performance⁵⁸. Numerous CSF antibodies to *M.tb* in CSF has also been evaluated. Huang and colleagues found pooled sensitivities of 91% (95% CI 71–98%) for anti-M37Ra across five studies, 84% (95% CI 71–92%) for anti-antigen-5 across eight studies, and 84% (95% CI 71–92%) across 12 studies for anti-M37Rv, again using a variety of reference standards (making the pooled estimates somewhat flawed)⁵⁹. Use of blood antibody assays are discouraged for the diagnosis of TB, and their utility in CSF is limited by heterogeneity and the lack of a uniform reference standard across research studies as well as a lack of commercial assays.

Though on the surface, many of these markers look to have promise, uptake has been limited. Heterogeneity in study design and widely variable study performance has limited the consensus regarding the utilities for most host-based tests. Further, many

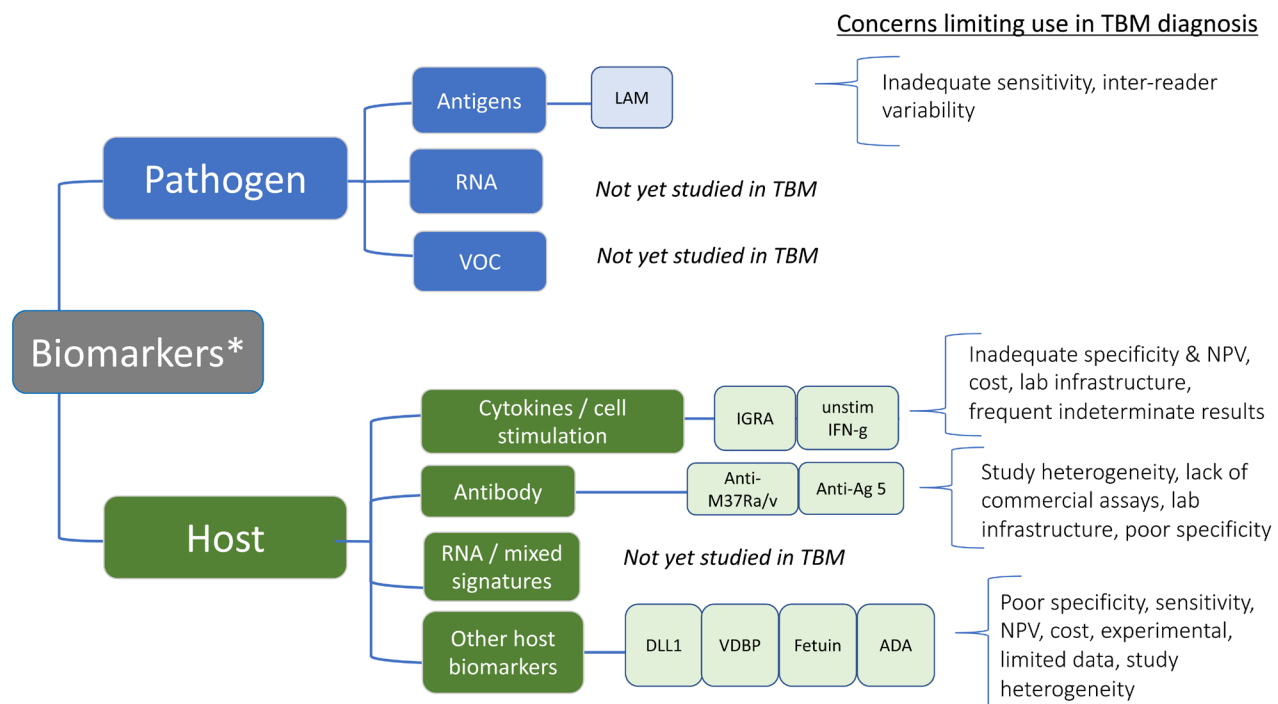


Figure 4. Novel Host and Pathogen biomarkers for diagnosis of tuberculous meningitis^{54–60}. LAM = lipoarabinomannan, RNA = ribonucleic acid, VOC = volatile organic chemicals, IGRA = interferon gamma release assay, DLL1 = delta-like ligand 1, VDBP = vitamin D binding protein, ADA = adenosine deaminase. * This image focuses only on novel host or TB biomarkers for diagnosis of TBM and does not incorporate traditional tools such as culture or AFB smear, or newer nucleic acid amplification tests in use commonly such as GeneXpert or GeneXpert Ultra, or experimental techniques such as metagenomic next generation sequencing.

of these tests require sophisticated laboratory infrastructure, are costly, and in some cases are not commercially available. None of these CSF tests are routinely used and as far as the authors are aware, none are actively being studied further.

In the field of pulmonary tuberculosis, host RNA transcriptomic signatures have been leveraged to predict incipient and active tuberculosis with moderate short-term (<3 month) sensitivity (41–81%)⁶¹. A whole blood 3-gene signature (GBP5, DUSP3, KLF2) has been shown to accurately differentiate active pulmonary tuberculosis from healthy controls (area under receiver operator curve, AUC 0.90), latent TB infection (AUC 0.88) and other diseases⁶² (AUC 0.84). Whether this 3-gene signature in whole blood has diagnostic value in TBM, whereby the inflammation can be compartmentalised to the CNS, remains to be investigated. An ongoing study in Uganda is adopting a machine learning approach to develop a classifier that categorises patients as TBM or not-TBM based on their CSF RNA signature. In CSF, genes predominantly predicted TBM (FTL, NFKBIA, SOD2, GBP5) and the classifier demonstrated good sensitivity and specificity (unpublished data).

Biomarkers in children

The often-dismal outcome of TBM is contributed to by delayed diagnosis and/or initiation of treatment, especially in high burden settings⁴. Currently available diagnostic test performance is especially poor in young children with TBM. Thus, diagnosis of childhood TBM is mostly based on a combination of clinical findings, CSF analysis and radiological findings⁶³. Even so, there are often multiple missed opportunities prior to a diagnosis of childhood TBM⁶⁴. Since it can be challenging to identify bacilli in paediatric extrapulmonary TB, the use of host or pathogen biomarkers to aid diagnosis is being explored. Host biomarker-based tests have shown promise in extrapulmonary TB outside of the CNS and therefore have potential applications in TBM⁶⁵. Recent technological advances have made it possible to screen for many biomarkers in as little as 3 µl of sample using the Luminex multiplex cytokine beaded arrays, albeit in research context currently, rather than routine clinical practice.

In a study evaluating disease-specific CSF biomarkers of paediatric TBM, a combination of 28 cytokines and soluble mediators were assessed; 27 host biomarkers by Luminex multiplex bead array technology (Bio-Rad Laboratories) and cathelicidin LL-37 concentration by using an enzyme-linked immunosorbent assay (ELISA) kit (USCN Life Science). A three-marker CSF biosignature comprising IL-13, VEGF and cathelicidin LL-37, diagnosed childhood TBM with a sensitivity of 52%, specificity of 95%, with positive and negative predictive values of 91% and 66% respectively. Cut-off values for VEGF, IL-13 and cathelicidin LL-37 were 42.92 pg/mL, 37.26 pg/mL and 3221.01 pg/mL respectively¹⁵. Further evaluation of this three-marker CSF biosignature in a different cohort revealed positive and negative predictive values of 90% and 59.5% respectively, however with different cut-off values for VEGF, IL-13 and cathelicidin LL-37 of 9.4 pg/ml, 524.9 pg/ml and optical density of 0.045 respectively⁶⁶. In this study investigating

69 potentially-useful host biomarkers for childhood TBM (23 children with TBM and 24 controls) comprising a combination of cytokines and soluble mediators in CSF (cathelicidin LL-37 by using an ELISA kit purchased from Elabscience Biotechnology Inc. (catalog #E-EL-H2438) and the rest by Luminex assay), 28 proteins including IFN-γ, TNF-α, MPO, MMP-8, MMP-9, MIP-4 and CXCL9 amongst others, when analysed individually, showed areas under the receiver-operating curve (AUC) ≥0.80. When combined, biomarkers IFN-γ, MPO and VEGF showed good accuracy (AUC = 0.97, up to 91.3% sensitivity and up to 100% specificity), as well as ICAM-1, MPO, CXCL8, and IFN-γ (AUC of 0.97, up to 87.0% sensitivity and up to 95.8% specificity). Cut-off values for VEGF, IFN-γ, MPO, ICAM-1 and CXCL8 were >9.4 pg/ml, >99.5 pg/ml, >25823.0 pg/ml, >1372.0 pg/ml and >394.8 pg/ml, respectively⁶⁶.

Limitations of the studies assessing CSF host biomarkers in childhood TBM include relatively small sample size, and therefore few children with confirmed TBM, confirmed meningitis due to other pathogens, and confirmed HIV co-infection. External validation is a necessity in order to generalize the clinical usefulness of the prediction model in an independent group of patients. The potential of CSF-based biosignatures, a further limitation is the invasive nature of CSF collection, and blood or urine-based inflammatory biosignatures require exploration. In a study evaluating serum biomarkers, the combination of CRP, IFN-γ, IP-10, CFH, Apo-A1 and SAA showed moderate diagnostic accuracy for clinically-defined TBM, including both 'definite' and 'probable' TBM (AUC of 0.75, sensitivity of 69.6% and specificity of 62.5%). A three-biomarker combination of adipsin, Aβ42 and IL-10 showed improved accuracy (AUC of 0.84, sensitivity of 82.6% and specificity of 75.0%). Cut-off values for CRP, IFN-γ, IP-10, CFH, Apo-A1, SAA, adipsin, Aβ42 and IL-10 were >80721.0 ng/ml, <61.5 pg/ml, <57.2 pg/ml, >350185.0 ng/ml, >287512.0 ng/ml, >59894.0 ng/ml, <2393.0 ng/ml, <278.4 pg/ml and <7.0 pg/ml, respectively. Although sample size was small, these biomarkers warrant further exploration⁶⁷.

Pathogen-based diagnostics

The absence of a perfect gold standard for use in TBM diagnostic studies means that the results must be interpreted with an awareness of the pros and cons of the reference standard used. The 2010 uniform TBM case definition which defines cases as 'definite', 'probable', 'possible' or 'not TBM' is the most standardised tool to use when defining a case definition⁶³. This case definition was derived by expert consensus rather than being data-driven and, although designed to be applicable to any age, HIV infection status or geographical setting, may perform better in some contexts than others. In HIV-negative populations a reference standard of 'definite, probable or possible' is often used, however in PLWHIV including 'possible' in the reference standard can be imprecise due to the wide variety of infectious and non-infectious aetiologies that can fall into this category. We do strongly advocate the use of the case definition to standardise results, allow for greater comparison between studies and meta-analysis of data; use of other standards must be interpreted with a degree of caution.

Nucleic-acid amplification tests

To address the limitations of conventional microscopy and culture techniques, NAATs have emerged as important tools for rapid and accurate diagnosis of TBM⁴⁴. A recent meta-analysis evaluating NAATs in TBM reported heterogeneity in results with a pooled sensitivity of 82% against culture and 68% against a clinical reference standard⁶⁸. This variability, especially in in-house NAATs, is subject to difference in volume of sample, method of extraction, choice of targets used, presence of inhibitors in the sample and lack of optimal reference standard. Traditional NAATs require expensive equipment, stringent operational conditions and technical expertise limiting their use in routine clinical practice in lower-resource, high endemic settings. To circumvent these challenges, loop mediated isothermal amplification (LAMP) assays were developed and can be conveniently carried out under isothermal conditions in an ordinary laboratory water bath or heating block within one hour. Though LAMP has outperformed PCR in an Indian study on TBM⁶⁹, the assay is still in its infancy and needs further validation. Another method to potentially reduce the overall cost of NAAT would be to utilize magnetic bead assay technology, thus obviating the need of gel electrophoresis system or expensive dyes.

Xpert is a rapid (90 min run-time) fully-automated cartridge-based real-time PCR assay that detects the presence of *M.tb* complex DNA, as well as *rpoB* gene mutations responsible for rifampicin resistance. The pooled sensitivity and specificity of Xpert against culture in 33 studies on TBM, was 71.1% and 98%, respectively⁷⁰. Xpert has been shown to significantly increase microbiological confirmation of TBM in Uganda over a 6.5-year period but its impact on clinical outcomes is unknown⁷¹. Individual studies have also found inferior performance for Xpert compared to multiplex PCR⁷² or Amplicor assay⁷³ in diagnosing TBM although these results have not been confirmed. The next generation, GeneXpert MTB/Rif Ultra (Ultra) has an 8-fold lower limit of detection than Xpert (16 CFU/ml versus 113 CFU/ml) attributable to a larger chamber allowing double the volume of sample to reach the PCR reaction and two additional DNA probes (IS1081 and IS6110)⁷⁴. In a Ugandan study of Ultra using cryopreserved CSF, alongside MGIT culture and Xpert, Ultra demonstrated a sensitivity of 95% against a composite microbiological reference and 70% against probable/definite TBM in comparison to 45% and 43%, respectively for each Xpert and culture^{75,76}. In 2017, Ultra was endorsed by the WHO as the best initial test for TBM and is being rolled out currently worldwide, superseding Xpert⁷⁷.

In January 2020, two larger prospective studies evaluating Ultra were published. In the Ugandan study, 204 (96% HIV-positive) adults with suspected meningitis had CSF Xpert Ultra performed. Compared with a reference of definite/probable TBM, test sensitivities were 77% (95% CI 63 – 87%) for Ultra, 56% (95% CI 44 – 70%) for Xpert, and 61% (95% CI 45–76%) for mycobacterial culture⁷⁸. In this study ‘possible TBM’ cases were not included in the reference standard as this category is non-specific in HIV co-infection due to concomitant infectious and non-infectious brain pathologies associated with advanced immunosuppression. In the second study,

Donovan *et al.* employed a different study design and randomised 205 Vietnamese adults (15% HIV co-infected) with meningitis to either Ultra or Xpert testing. Against a reference standard of definite, probable, or possible TBM, test sensitivities were 47% (95%CI, 34 – 60%) for Ultra, 40% (95%CI, 28 – 53%) for Xpert, and 48% (95%CI, 38 – 58%) for mycobacterial culture⁷⁹. Specificity of Ultra for TBM diagnosis was high in both studies. The sensitivity of Ultra statistically superior to that of Xpert in Uganda but not in a Vietnamese predominantly HIV-negative population. How can we rationalise and interpret these differing results? Firstly, diagnostic tests cannot be expected to perform identically in all settings. Differences in tested CSF volume, CSF processing, HIV co-infection, genetics influencing host response to *M.tb*, and *M.tb* lineages (the number of copies of IS1081 and IS6110 genes varies by lineage) could all contribute to these different results, as could the differences in study design (e.g. head-to-head comparison versus randomizing samples) and smear microscopy sensitivity and reference standards used. Secondly, and most importantly, regardless of the differences in the exact performance of Ultra, the key point is that while Ultra demonstrates some improvement on the performance of Xpert, its negative predictive value is not sufficiently high to exclude TBM when the result is negative.

Another commercial NAAT, the MTBDRplus assay, has been evaluated only in few cases of TBM and needs further validation⁸⁰. Accurate and rapid detection of drug resistance is another challenge, rifampicin resistance detection by Xpert has imperfect sensitivity (93%) and where detected and ideally requires confirmation by sequencing or culture^{72,81}. Ultra uses melt curve analysis to improve detection of rifampicin resistance but both are about 95% sensitive^{82,83}. Ultra will not be able to adequately define rifampin resistance in samples with a low quantity of bacilli (trace category positive)⁷⁵. In summary NAATs, are a major diagnostic advance but they cannot yet fully replace culture methods. Ultra is too insensitive to rule out TBM, and like Xpert, should be considered as the first test and not the last in TBM diagnosis⁸⁴. Ultra is an important step in the right direction but the result should be considered in the context of the clinical probability of TBM⁸⁵.

CRISPR-MTB and metagenomic next generation sequencing.

Clustered regularly interspaced palindromic repeat (CRISPR) associated proteins (Cas) have the ability to cleave DNA at specific sites and are being used widely in gene-editing and more recently in infectious disease diagnostics. When combined with DNA amplification, the CRISPR system can detect nucleic acid molecules at extremely low abundance. There is one recent report of utilizing the CRISPR system for detection of *M.tb* (CRISPR-MTB). The study included 26 CSF specimens and found CRISPR-MTB to have a sensitivity of 73% compared to 54% for Xpert and 23% for culture against a reference standard of ‘clinical TBM’ as determined by the physician based on clinical presentation imaging and response to TB therapy. The specificity of the test was 98% when tested against 63 non-TB cases. CRISPR-MTB is isothermal and can be performed in under 2 hours using only 500 µl of CSF. CRISPR-MTB remains to

be tested against Ultra and requires a higher level of laboratory expertise, resources, and time than the Xpert platform but may be an advance in TB diagnostics if these findings can be confirmed in other settings with more standardized reference standards⁸⁶.

Metagenomic next generation sequencing (mNGS) is a rapidly developing technology that has proved useful in determining aetiologies for CNS infections that have evaded detection by conventional techniques. Further, mNGS, as opposed to organism-specific molecular tests has the ability to detect *any* low abundance infection with a single test⁸⁷. A recent small study applied mNGS to stored CSF samples from 23 TBM cases and found a sensitivity of 67% (8/12) against a reference standard of definite TBM, higher than AFB stain (33%, 4/12), PCR (25%, 3/12) and culture (8%, 1/12)⁸⁸. Paucibacillary conditions such as TBM where the bacillary load may fall below the LOD of commercial NAATs, or where mutations exist around specific PCR primer binding sites may find particular use for mNGS. Targeted enrichment of low abundance genes with Finding Low Abundance Sequences by Hybridization (FLASH), a novel CRISPR-Cas9 technology can increase DNA read abundance by up to 10⁵-fold before sequencing occurs⁸⁹. Combining FLASH and mNGS technologies could improve detection of TB DNA and associated antimicrobial resistance mutations⁹⁰. A first pilot of FLASH technology in TB demonstrated up to a 100-fold increase in TB read abundance, detection of 6/6 cases of TBM positive with Ultra and detection of an additional case of TBM that had been missed by Xpert, Ultra and MGIT culture⁹¹. Here again, large studies need to be performed to better understand this technology's performance and the cost, laboratory infrastructure, and degree of expertise will need to be improved upon to permit widespread usage.

Pathogen-based biomarkers. A urine lateral flow assay (LFA) that detects *M.tb* lipoarabinomannan (TB-LAM), a 17 kDa glycolipid found in the outer cell wall of MTB, has recently been recommended by the World Health Organization for the diagnosis of HIV-associated TB in HIV-positive inpatients (Alere Determine TB-LAM, Abbott, Chicago, USA). The unique characteristic of the test is that its sensitivity increases as CD4 T-cell count falls, with a sensitivity of 56% in those with CD4 <100 cells/ml⁶⁰. Yet, in CSF, despite some initial optimism related to an autopsy-based study in Uganda, the Alere TB-LAM has shown poor sensitivity on lumbar CSF in Uganda^{92,93}, along with a larger Zambian study which examined culture positive TBM in Zambia (TB-LAM sensitivity 22% (23/105))^{92,94,95}. The Alere TB-LAM is also limited by its susceptibility to individual reader interpretation of the darkness of the test line compared to the reference card (Figure 4). A novel LAM assay (Fujifilm SILVAMP TB-LAM, Fujifilm, Japan) is able to detect concentration of LAM at approximately 30-fold lower than Alere TB-LAM due to design differences, including a silver amplification step and gives a result in one hour⁹⁶. The Fujifilm LAM was recently tested on the CSF of 101 predominantly HIV-positive adults with suspected TBM. FujiLAM sensitivity was

74% (25/34, 95%CI 56-87%) versus definite TBM and 52% (30/58, 95%CI 38-65%) versus probable/definite TBM. The FujiLAM assay may play a role in reducing time to treatment where the Xpert Ultra platform is not immediately accessible or where clinical suspicion of TBM is high despite a negative CSF Ultra result. The sensitivity of FujiLAM was superior to Alere LAM (50% versus 14%, $p<0.01$ in those tested with both assays)⁹⁷.

Clinical prediction rules

Work is underway to develop a more accurate multivariable clinical prediction rule derived from large international cohorts using individual patient data⁹⁸. The hope is that a data-driven scoring system will be developed for use in a range of clinical settings by using common, readily available clinical or laboratory parameters to aide in clinical decision making.

Discussion

In the last five years, the pathogenesis of TBM has been better elucidated, in part thanks to detailed immunological studies on clinical samples preemptively stored during clinical trials. These advances highlight the importance of collecting and storing samples appropriately for future research to maximize scientific outputs, as highlighted in the paper on sampling strategies in this collection. HIV infection is a major predictor of mortality in TBM and advanced HIV infection (CD4 T cell count <150 cell/ μ l) appears to drive a dysregulated, hyperinflammatory phenotype with very poor outcomes. In HIV-endemic sub-Saharan African settings around 90% of all adult TBM occurs amongst HIV-positive individuals^{71,95}, often with either untreated advanced HIV or having recently initiated ART - both driving a hyperinflammatory response. In Vietnamese adults with TBM, LTA4H genotype is a strong predictor of mortality though this finding was not duplicated in Indonesia.

Recent insights have shown that neutrophils play a significant role in the immunopathogenesis of TBM, and that both a paucity and an excess of inflammation can be equally damaging in TBM. It has become increasingly clear that a 'one-size-fits-all' approach is too simplistic in TBM treatment, as in other infections such as pulmonary TB and sepsis^{99,100}. The damage-response framework may provide a useful structure for understanding host-pathogen interactions in TBM, illustrating how immune response could be exploited for therapeutic purposes. Additional anti-inflammatory therapy with aspirin^{17,101} or more targeted immunotherapy could have a role in persons with an excessive inflammatory response; whilst individuals with an inadequate response might do better without corticosteroid treatment or might even benefit from immunomodulating therapy to boost their immune response¹⁰². Future trials of novel specific host-directed therapies are needed and must include immune markers to allow for post-hoc identification of subgroups benefitting from the initiated therapy. Because of the lack of correlation between blood and CSF compartments we advocate inclusion of both blood and CSF markers when studying adjuvant therapies.

The field of TBM diagnosis is rapidly evolving with GeneXpert MTB/Rif Ultra being the most promising test to date for diagnosis of TBM. Ultra is rapid and has potential to confirm more cases of TBM at lower bacillary loads, though whether this will improve outcomes remains to be determined. Most importantly, Ultra does not appear to have adequate predictive value to 'rule-out' TBM and so it cannot meet the potential of an ideal TBM diagnostic test to avoid long, toxic TBM therapy in persons without TBM. Novel sequencing technologies hold potential to provide increased understanding of pathogen genomics and behavior and further illuminate host response, which may in turn lead to novel diagnostic and therapeutic targets. Sequencing technologies are increasingly available in TB endemic settings but will need further improvements in affordability and speed in addition to more data on accuracy to unlock their potential as diagnostic tools for TBM. It is now a realistic hope that a test (or set of tests) will one day be available that will be able to confirm or rule out TBM, provide *M.tb* resistance information, and direct clinicians to targeted, adjunctive host-directed therapy within hours.

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No additional comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neurology, infectious diseases, epidemiology, global health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 15 December 2020

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Michael J. Griffiths 

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Overall the manuscript represents an excellent review of recent developments in tuberculous meningitis pathogenesis and diagnostics. I agree with changes following the first reviewer's, Dr Scriven, suggestions. I add two relatively modest comments on the document.

Utilising host biomarkers for TBM diagnostics are an exciting development. In the section on 'Biomarkers in Children', the authors describe the results of two studies (by the same group) using different combinations of host CSF biomarkers among children [15,64]. The authors should state what type of host markers (i.e. a mixture of cytokine and soluble mediators) were measured in the studies (using ELISA and Luminex platform). In the follow-up study [64], a relatively small number of patients (n=47) were tested in un-blinded study design. The STARD diagram shows a series of markers (n=69) were measured before the samples were then split into two clinical groups (TBM [definite/probable] n=23 or non-TBM n=24) and later the accuracy of the best combination of markers reported. Given the small size and unblinded design, the results of these studies (particularly the latter) could be summarized further and technical study limitations stated (beyond using CSF).

In the section on TBM diagnostics- many different types of host biomarkers are now being measured and their diagnostic potential beginning to be assessed in TB, including host transcripts. There are a few publications reporting on host transcripts among TBM patients, including work by the authors (Rohlwink UK *et al.* 2019¹; Hemingway C *et al.* 2017²). While diagnostic accuracy of RNA signatures in TBM have not been assessed in these studies (as stated in Figure 4), there is an enlarging body of work in pulmonary TB. Reference to some of the larger host transcript studies assessing diagnostic accuracy may be worth mentioning, as these studies highlight the potential strengths/challenges ahead for this approach in TBM (Gupta RK *et al.* 2020³; Sweeney TE *et al.* 2016⁴).

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Is the topic of the review discussed comprehensively in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Is the review written in accessible language?

Yes

Are the conclusions drawn appropriate in the context of the current research literature?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatric neurology, CNS infections, diagnostics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 12 Jan 2021

Fiona Cresswell, London School of Hygiene and Tropical Medicine, London, UK

We would like to thank Dr Griffiths for this expert review.

In response to comment 1.

Thank you for raising the point requiring further information including the type of host biomarkers and assay methods, and study limitations. For the study (reference 15), we have added the following text: 'In a study evaluating disease-specific CSF biomarkers of paediatric TBM, a combination of 28 cytokines and soluble mediators were assessed; 27 host biomarkers by Luminex multiplex bead array technology (Bio-Rad Laboratories) and cathelicidin LL-37 concentration by using an enzyme-linked immunosorbent assay (ELISA) kit (USCN Life Science).'

For the follow-up study (reference 64), we have added the following text: 'In this study investigating 69 potentially-useful host biomarkers for childhood TBM (23 children with TBM and 24 controls) comprising a combination of cytokines and soluble mediators in CSF (cathelicidin LL-37 by using an ELISA kit purchased from Elabscience Biotechnology Inc. (catalog #E-EL-H2438) and the rest by Luminex assay).

Study limitations were addressed by adding the following text: 'Limitations of the studies assessing CSF host biomarkers in childhood TBM include relatively small sample size, and therefore few children with confirmed TBM, confirmed meningitis due to other pathogens, and confirmed HIV co-infection. External validation is a necessity in order to generalize the clinical usefulness of the prediction model in an independent group of patients. Despite the potential of CSF-based biosignatures, a further limitation is the invasive nature of CSF collection, and blood or urine-based inflammatory biosignatures require exploration.'

Comment 2.

Thank you for raising the point relating to the utility of RNA transcripts as a biomarker for TBM diagnosis. Indeed, a number of recent studies in adults and children have utilised the host transcriptome to better understand the pathogenesis of TBM. Work is underway to use the host transcriptome and develop a machine learning classifier to aid the diagnosis of TBM but this work is unpublished.

We have added the following text to the paper: "In the field of pulmonary tuberculosis, host RNA transcriptomic signatures have been leveraged to predict incipient and active tuberculosis with moderate short-term (<3 month) sensitivity (41-81%) [Gupta, Lancet Resp Med, 2020]. A whole blood 3-gene signature (GBP5, DUSP3, KLF2) has been shown to

accurately differentiate active pulmonary tuberculosis from healthy controls (area under receiver operator curve, AUC 0.90), latent TB infection (AUC 0.88) and other diseases (AUC 0.84) [Sweeney, Lancet Resp Med, 2016]. Whether this 3-gene signature in whole blood has diagnostic value in TBM, whereby the inflammation can be compartmentalised to the CNS, remains to be investigated. An ongoing study in Uganda is adopting a machine learning approach to develop a classifier that categorises patients as TBM or not-TBM based on their CSF RNA signature. The classifier. Four genes predominantly predicted TBM (FTL, NFKBIA, SOD2, GBP5) and the classifier has good sensitivity and specificity (unpublished data)."

Many thanks again for taking the time to improve the manuscript through these comments.

Competing Interests: No competing interests were disclosed.

Reviewer Report 14 December 2020

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Joseph Zunt

Department of Neurology, Harborview Medical Center, University of Washington, Seattle, WA, USA

This is a thorough review of current developments in the field of tuberculous meningitis. They included a very nice inclusion of promising new technology, such as CRISPR-MTB and metagenomics. Although not an obligatory recommended change, given the multiple diagnostic assays discussed, I think the reader would find useful a table that listed the various assays with their accompanying sensitivities, specificities and limitations.

Minor recommended edits:

- I agree with Reviewer 1 that the "Rich Focus" was not "refuted" and the editorial change to "potentially challenges" is perhaps not warranted, as there may be more than one pathway to development of meningitis – an event simultaneous with initial infection and a more-delayed event, such as that proposed by Rich and McCordock.
- On page 9, under Pathogen-based diagnostics, the authors start discussing the "potential to score points" without reference to any specific diagnostic criteria – please edit accordingly.
- Page 10; the authors discuss a study in Uganda – It would be helpful to know if the "reference of definite/probable TBM" was made by culture, biopsy or other. Later on this same page, they reference a study of CRISPR-MTB and Xpert that used a standard of "clinical TB" - It would be useful to know what this clinical diagnosis was based on – clinical + CSF findings + neuroimaging findings?

There were multiple minor grammatical errors that should be corrected:

- P 3. Second to last sentence in the Introduction – “that” should be removed.
- P 5. Second paragraph – “cytokine” should be “cytokines”.
- P6. First paragraph – please remove redundant sentence.
- First sentence under Neuroimaging in pathogenesis studies – “has” should be “have”.
- As mentioned by reviewer 1, comma should follow “anterior cingulate gyrus”.
- P8. Third paragraph – “where” should be “were”.
- P9. Third paragraph – “tests” should be “test”.
- P10. Sixth paragraph – “stanrdards” should be “standards”.
- P11. Second paragraph – “is susceptibility” should be “its susceptibility”.

Is the topic of the review discussed comprehensively in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Is the review written in accessible language?

Yes

Are the conclusions drawn appropriate in the context of the current research literature?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neurology, infectious diseases, epidemiology, global health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 12 Jan 2021

Fiona Cresswell, London School of Hygiene and Tropical Medicine, London, UK

We sincerely thank Dr Zunt for his expert review and suggestions.

In response to major comment:

We have added a table summarising the characteristics of CSF tests for TBM and added a

couple of lines about CSF FujiLAM following a recent publication relating to this assay in TBM.

In response to minor comments:

1. We have edited the text to reflect the fact that there may be more than one route to TBM disease.
2. We have removed this text about the points relating to the reference standard in the interest of brevity.
3. In the Ugandan study MGIT culture and Xpert were used alongside Ultra, this detail has been added to the paper. In the CRISPR study "clinical TB" was as determined by the physician based on clinical presentation imaging and response to TB therapy. This has been added to the paper.

We have rectified the multiple typos spotted.

Many thanks for your suggestions which have improved the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Report 02 September 2020

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James E. Scriven 

University of Birmingham, Birmingham, UK

Thank you for taking on board my comments and making the changes and clarifications detailed in your response. I am happy that the sections previously highlighted have now been explained fully.

The only think I have noticed on the re-reading is the second sentence in the **Brain Injury Markers** section (copied in below) doesn't quite make sense. Could the authors please review this. I think there may be some punctuation (and possibly words) missing?

".....In lumbar CSF of children with TBM, S100B and NSE (structural proteins of the CNS, and biomarkers of CNS tissue damage) at disease onset were associated with poor outcome, as was highest concentration overall and an increasing profile over time in S100B, NSE, and GFAP neuromarker concentrations increased over time in those who died (whilst inflammatory markers decreased), and were overall highest in those with cerebral infarction³²."

Is the topic of the review discussed comprehensively in the context of the current

literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Is the review written in accessible language?

Yes

Are the conclusions drawn appropriate in the context of the current research literature?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious Diseases; CNS infections; immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 13 January 2020

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James E. Scriven 

University of Birmingham, Birmingham, UK

Thank you for asking me to review this manuscript.

General comments

This is a comprehensive review of TB meningitis pathogenesis and diagnostics with the stated aim of concentrating on the most recent developments (last 5 years).

The authors should be commended on providing a detailed review. They have included the vast majority of recently published studies but should also include the Vietnamese study published in Lancet ID this month by Donovan et al that compares Xpert against XpertUltra in TBM (and compare it to their recently published study in HIV-TBM patients).¹

My main criticism of the review relates to the section on **TBM pathogenesis** which requires further work to improve understanding for the reader. There are several sections where the

authors have listed a series of findings from separate immunological studies but do not introduce the concept fully to the reader and do not draw the findings together at the end of each section to provide an overall interpretation or explanation for the reader. Some further explanation around these concepts would significantly improve the review.

This is particularly true for the section covering host genetic factors and the influence of LTA4H genotype on host response and outcome. This is a really important and fascinating piece of science but for readers not already familiar with this concept this section is confusing. The authors should provide further explanation, particularly regarding the differing inflammatory states associated with the three genotypes, and why it is important. They should consider including discussion and reference of the original paper by Tobin et al (Cell 2012).²

Specific comments:

Introduction

- The GeneXpert test performances should be referenced. I think there is a typo on line 7 ("traditional" should be "traditionally")?

TBM pathogenesis - Dissemination to the CNS

- The use of the word **refute** with regard to the Rich focus theory of TBM pathogenesis could be regarded as a bit strong. Do the authors think that the references provided fully support this statement?

TBM pathogenesis - Host immune response to TB infection in the CNS

- The first two sentences of the first paragraph in this section should probably contain a reference.
- In the second paragraph, the association between death and lower CSF leukocyte count should include the p=value.
- Further explanation/interpretation of the lipid mediator findings and flow cytometry findings would help improve understanding of this section.
- The authors should also consider discussing the Vietnamese phase II trial of Aspirin by Mai et al (Ref 94), further in this section. They very briefly mention it in the Discussion section but a more detailed discussion is probably warranted and it may be appropriate to do so here.

TBM pathogenesis - Host genetic and metabolic factors.

- As mentioned above, for readers not familiar with the original studies this section discussing LTA4H genotypes is difficult to understand as it is currently written. It would benefit from re-wording and further explanation, particularly a sentence or two to introduce the concept at the beginning of the paragraph and a precise explanation of the effects of LTA4H genotype on inflammation, steroid responsiveness and outcome (with reference to the original paper, Tobin et al. Cell. 2012).²
- The authors should also consider adding a couple sentences to the section on tryptophan to provide some additional information about this study and providing a more comprehensive

explanation for the association between low levels and better survival.

Figure 2.

- The figure should make it clear that the patients in A and B have all received steroids. It would also aid understanding if the figure legend also contained the p-values with respect to the differing outcomes quoted for graph A and B. The final sentence looks like it might contain a typo:

*"In a recessive model, **TT genotype versus CT/TT** combined had HR 0.81 (95% CI 0.41-1.62, $p = .550$) in severe and 0.31 (95% CI 0.04-2.25, $p = .156$) in milder disease."*

I think the comparison stated in the model should be be **TT vs CT/CC?**

TBM pathogenesis - HIV co-infection and IRIS

- In the first paragraph (line 9, copied below), a comparison in neutrophil counts and mortality is presented between patients with a CD4 count >150, CD4 <150 and HIV negative.

*"PLWHIV with CD4+ T-cell counts <150 cells/ μ L showed higher median CSF neutrophil percentage (25%), cytokine concentrations and 9-month mortality (44%) than those with a CD4+ T-cell count \geq 150 cells/ μ L (neutrophils 10%; **P=.021**, mortality 13%) and patients without HIV infection (neutrophils 5%; **P<.0001**, mortality 18%). These findings, amongst others, suggest a role for neutrophils in the immunopathogenesis of HIV-associated TBM."*

However, there are only two p values included and it is not clear whether they refer to neutrophils or mortality. Could the authors please alter this to make it a little clearer (and if appropriate add the two additional p-values).

TBM pathogenesis - Pathogenesis of TBM in childhood

- I don't think this section in its current form particularly adds to the review and there is no new data. I would suggest this section is either expanded (to further discuss the differences between adult and paediatric patients with TBM and the pathological processes underlying these differences) or dropped. If the authors do decide to drop it they could consider amalgamating the statements about Donald and Schoeman's TBM and milary TB findings into the earlier section: "Dissemination to the central nervous system".

TBM pathogenesis - Brain injury markers

- This paragraph would also benefit from further explanation so that the reader can appreciate the importance of the findings. Further detail about the brain injury markers S100B and NSE would be helpful (particularly whether they are used in other neurological conditions). Further text is also required to explain what the CSF transcriptomic signature means.

TBM pathogenesis - Neuroimaging in pathogenesis studies

- Is there a comma missing here?: "anterior cingulate gyrus parahippocampal gyrus and globus pallidus".
- The novel imaging studies reporting structural damage in cortical and subcortical areas are interesting. Perhaps the authors could expand slightly to suggest the potential implications

of this?

TBM pathogenesis - Host pathogen interactions.

- This section discusses the damage response framework concept and applies it to TBM. I am not sure why the first sentence includes data on global latent TB prevalence - it doesn't seem directly relevant to this section.
- It may improve the review if the authors expanded this section slightly and used it as a summary to draw together all the immunological findings discussed above. Particularly to draw attention to the importance of neutrophils.

TBM diagnostics

- This section provides a good summary of host-based and pathogen-based diagnostics and reads well.
- With regard to the host-based biomarkers, some of the pooled sensitivities appear promising but have drawbacks. It would be useful if the authors could provide a concluding comment at the end of the section discussing whether there is any future in these modalities (e.g. are any of those mentioned being taken forward into further trials? Are there any plans to introduce any of these tests?).
- Regarding the performance of XpertUltra in TBM. The authors have mentioned their prospective study examining its performance in Uganda (reference 70). They should emphasize that these results were in patients with HIV infection. They should also mention the similar study from Vietnam published alongside theirs this month in Lancet ID by Donovan et al which reports lower sensitivities and no significant improvement on Xpert. They should discuss why this might be.

References

1. Donovan J, Thu D, Phu N, Dung V, et al.: Xpert MTB/RIF Ultra versus Xpert MTB/RIF for the diagnosis of tuberculous meningitis: a prospective, randomised, diagnostic accuracy study. *The Lancet Infectious Diseases*. 2020. [Publisher Full Text](#)
2. Tobin DM, Roca FJ, Oh SF, McFarland R, et al.: Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. *Cell*. 2012; **148** (3): 434-46 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the topic of the review discussed comprehensively in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Is the review written in accessible language?

Yes

Are the conclusions drawn appropriate in the context of the current research literature?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious Diseases; CNS infections; immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 21 Jun 2020

Fiona Cresswell, London School of Hygiene and Tropical Medicine, London, UK

We thank the reviewer for taking the time to provide this very considered and detailed review. We have taken Dr Scriven's comments on board, modified the paper accordingly, and we hope the article has been strengthened as a result. In response to Dr Scriven's comments we have made the following changes:

- Added a paragraph relating to the two recent studies on Xpert MTB/RifUltra as suggested.
- We have added a reference to the introduction line 7 as suggested
- The statement regarding 'refuting' the Rich focus has been made less conclusive
- P value added to the paragraph about CSF leucocytes
- Further developed the sections relating to pathogenesis:
 - expanded section on aspirin study and lipid mediators
 - expanded background on LTA4H
 - expanded evidence around brain injury markers
- We have not added further information on tryptophan in the interest of word limit as there is already a full paragraph on this topic
- The section on pathogenesis of TBM in childhood has been removed as suggested. Donald and Schoeman's TBM and miliary TB findings are now included in the "Dissemination to the central nervous system" section
- We have added a summary statement on TBM biomarkers explaining that despite extensive research on this topic there are no currently biomarkers with adequate performance to be commercially viable in TBM diagnostics.
- Typos and grammatical errors have been corrected. Many thanks!

We are very grateful for Dr Scriven's expertise and hope he is satisfied with the updated article.

Competing Interests: No competing interests were disclosed.