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REVIEW

Recent Developments in Tuberculous Meningitis Pathogenesis and Diagnostics [version 1; peer review: 1 approved with reservations]

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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. Whilst in Indonesia, CSF 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

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report

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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,



This article is included in the [Tuberculous Meningitis International Research Consortium](#) collection.

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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 traditional 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 that 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. This bloodborne spread highlights the possibility of coincident miliary TB in cases of TBM, especially common in children and people living with HIV (PLWHIV)^{8,9}. The contemporaneous nature of TBM and miliary TB refutes 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)¹⁰.

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;² 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 induced in TBM^{11,12}. Disequilibrium of pro- and anti-inflammatory cytokines influence the severity and course of TBM, [Figure 1](#)^{2,13}.

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 cytokine 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 noted¹⁴. 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. A separate study of 120 Vietnamese adults with TBM from a randomized controlled trial of adjunctive aspirin treatment 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 pathogenesis¹⁵.

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; (95% CI 1.03–1.10), respectively) were associated with mortality¹⁶. 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 CSF¹⁷. 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 compartments¹⁷.

Host genetic and metabolic factors.

Leukotriene A4 hydroxylase (LTA4H) catalyzes the final step in the synthesis of leukotriene B4 (LTB4), a potent chemoattractant and proinflammatory eicosanoid. In Vietnamese adults, a single-nucleotide polymorphism rs17525495 in the *LTA4H* gene results in either a hyper- or hypo-inflammatory state, correlating with TBM severity, corticosteroid responsiveness and survival in HIV-negative adults, [Figure 2](#)¹⁴. Individuals with the TT genotype have improved survival versus those with CC or CT genotype possibly due to inadequate inflammatory response, there is

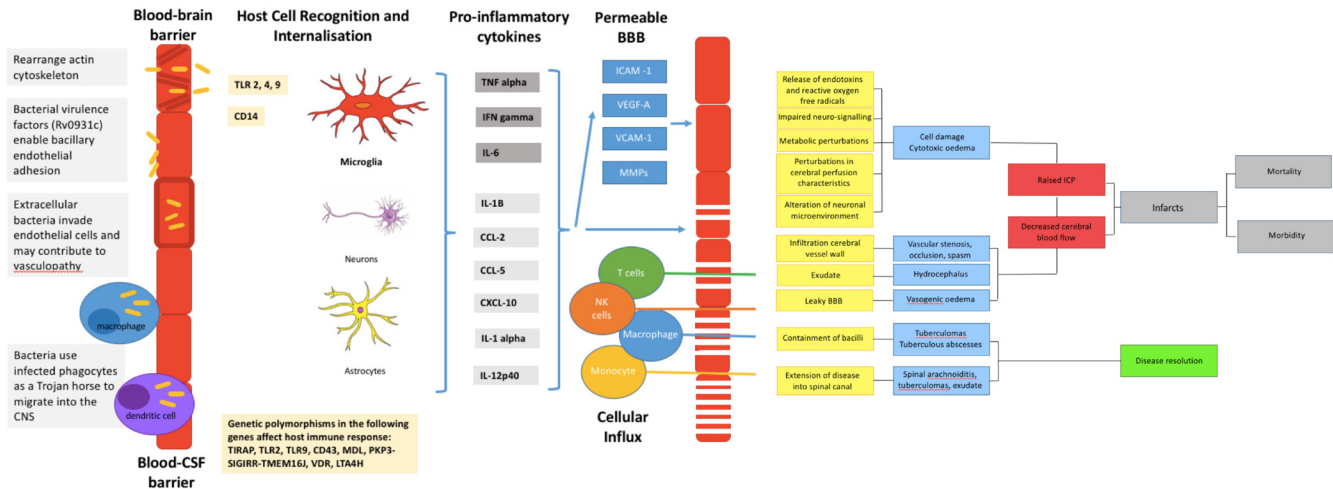


Figure 1. Illustrative summary of the pathogenesis of tuberculous meningitis (TBM). Reproduced with permission from author and Journal of Leukocyte Biology². A: *Mycobacterium tuberculosis* bacilli (*M.tb*) disseminate from the primary site of infection in the lung to seed the brain. The bacilli traverse 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.

concern for potential harm caused by adjunctive dexamethasone in these cases. 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 2¹⁶. A clinical trial is currently underway in Vietnam (NCT03100786) to evaluate LTA4H genotype-directed corticosteroid therapy, an exciting example of personalised medicine in TBM¹⁸. Others factors associated with death in a Cox proportional hazards regression model of the large Vietnamese clinical trials cohort were higher MRC disease severity grade, older age, previous TB, focal neurology and not receiving adjunctive steroids¹⁹.

Although prior studies have considered sodium, glucose and lactate as related to TBM pathogenesis, recent interest has focused on tryptophan. 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 survival²⁰. 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 TBM²⁰.

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)¹⁹. 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; $P=0.08$)²¹ 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 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%), cytokine concentrations and 9-month mortality (44%) than those with a CD4+ T-cell count ≥ 150 cells/ μ L (neutrophils 10%; $P=.021$, mortality 13%) and patients without HIV infection (neutrophils

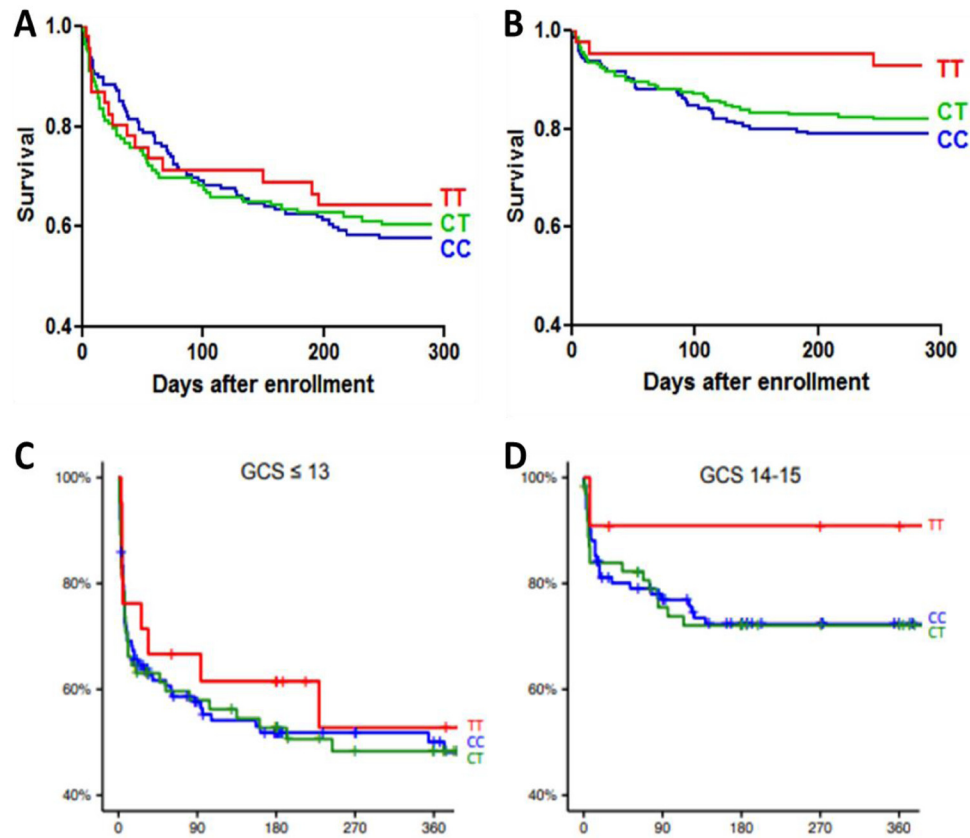


Figure 2. Kaplan-Meier survival curves stratified by LTA4H genotype. Figures **A** and **B** show survival in 763 patients with tuberculous meningitis in Vietnam¹⁴, with human immunodeficiency virus (HIV) infection (**A**), and without HIV infection (**B**). In HIV-infected patients, case-fatality rates 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 Indonesia¹⁶. These patients are HIV-uninfected with severe (GCS \leq 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.

5%; $P < .0001$, mortality 18%)¹⁴. These findings, amongst others, suggest a role for neutrophils in the immunopathogenesis of HIV-associated TBM²².

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^{23,24}. 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.

Pathogenesis of TBM in childhood

The protective role of CD4+ and CD8+ T lymphocytes is essential, along with macrophages, to isolate and engulf mycobacteria to form a granuloma. This could explain 1) the vulnerability to TBM of young children and PLWHIV when T-cell mediated immunity is sub-optimal 2) the protective effect of the BCG-primed T-cell response in childhood TBM meningitis and 3) the association between TBM and genetic polymorphisms influencing the early, innate immune response²⁵.

Donald and Schoeman have highlighted that miliary TB and TBM are closely related, particularly in young children, where tubercles of different sizes and ages have been described on the meninges and confirmed by magnetic resonance imaging (MRI)^{8,26}. In children, miliary TB and TBM develop most often within 3 months of primary infection, when fresh anatomic

changes are still found in the primary lung focus²⁷. Miliary TB, a severe form of symptomatic haematogenous dissemination arising in people who lack the ability to control the infection in the lungs (frequently children or adults with advanced HIV disease or other risk factors), may therefore be the precursor for TBM by increasing the likelihood of *M.tb* seeding the meninges, cerebral cortex or choroid plexus which subsequently precipitates the development of TBM.

Brain injury markers.

In lumbar CSF of children with TBM, high levels of proteins S100B and neuron-specific enolase (NSE) and their increase over time (along with an increase in glial fibrillary acidic protein (GFAP)) were associated with poor outcomes²⁸. Neuromarker concentrations increased over time in those who died (whilst inflammatory markers decreased), and were overall highest in those with cerebral infarction. In ventricular CSF of children with TBM, transcriptomic signatures showed neuro-excitotoxicity driven by glutamate release and NMDA binding and receptor uptake as well as upregulation of genes associated with nitric oxide, cytochrome c, myelin basic protein, tau, and amyloid. These mechanisms have been described in neurodegenerative conditions such as Alzheimer's and Huntington's disease and traumatic brain injury and may occur following ischaemia in TBM²⁹.

Neuroimaging in pathogenesis studies

Technical advances and increasing availability of imaging modalities has 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-8²⁸. 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 performance³⁰. 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 patients³¹. 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 gyrus³¹. 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.

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 lesions³². 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^{33–35}. 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^{36,37}.

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 burden³⁸. Over 50% of diagnosed cases are microbiologically undetectable and defined as 'probable' or 'possible' TBM which obviously limits this approach³⁹. 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 patients⁴⁰. 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 death³⁸.

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)⁴¹. 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 interaction⁴².

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 people⁴³. 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^{44,45}. 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^{46–48}. 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^{14,38,49}. The current one-size-fits-all approach to TBM treatment fails to recognize divergent pathologies and may explain the poor outcomes in

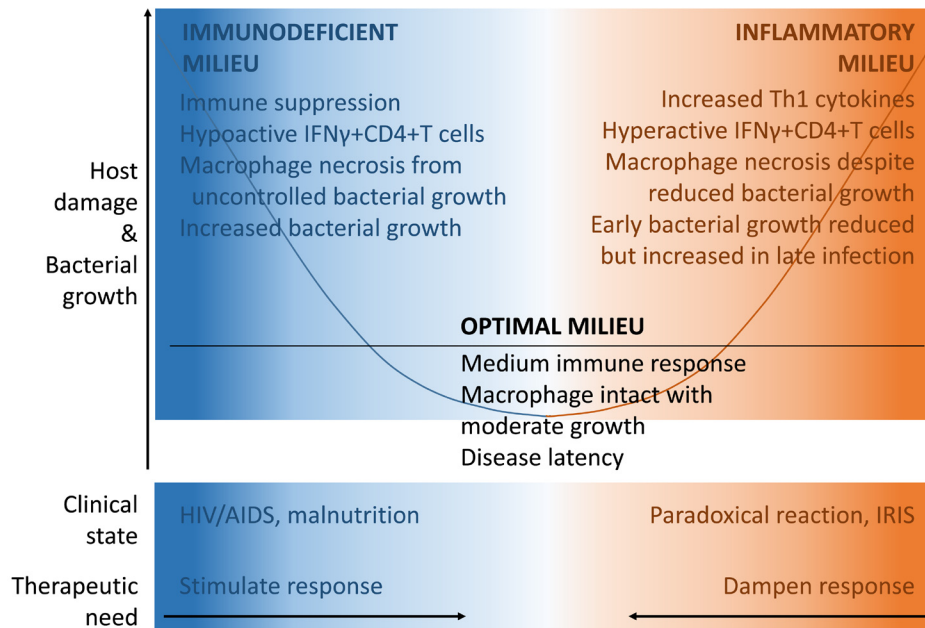


Figure 3. Outcomes of the host – *M. tuberculosis* interaction depicted by the basic parabola of the damage-response framework. On the left side of the parabola, shaded in blue, the immune system fails to limit mycobacterial growth and invasion which results in host damage. On the right side, shaded 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.

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.

TBM diagnostics

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, 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 TBM⁵⁰. 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%)⁵⁰. 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 ^{51,52}. 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 inadequate⁵². 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 meningitis⁵².

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 study⁵³. 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 performance⁵⁴. 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)⁵⁵. Use of

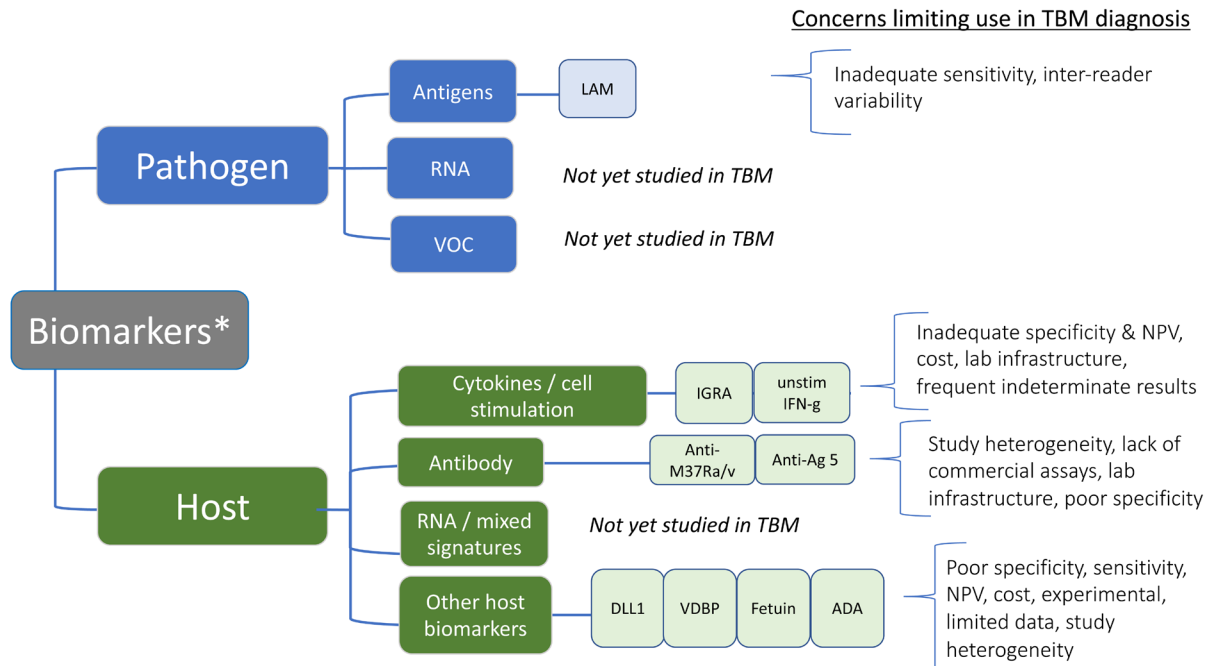


Figure 4. Novel Host and Pathogen biomarkers for diagnosis of tuberculous meningitis^{50–56}. LAM = lipoarabinomannan, RNA = ribonucleic acid, VOC = volatile organic chemicals, IGRA = interferon gamma release assay, DLL1 = delta-like ligand 1, VDBP = vitamin d binding protein, ADA = adenosine deaminase. * 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.

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.

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 tests 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 findings⁵⁷. Even so, there are often multiple missed opportunities prior to a diagnosis of childhood TBM⁵⁸. 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 TBM⁵⁹. Recent technological advances have made it possible to screen for many biomarkers in as little as 3 μ l of sample using the Luminex multiplex cytokine beaded arrays, albeit in research context currently, rather than routine clinical practice.

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 respectively¹³. 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 respectively⁶⁰. In a study investigating potentially useful host biomarkers in CSF for childhood TBM (23 children with TBM and 24 controls), 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, respectively⁶⁰.

Despite the potential of CSF-based biosignatures, collection of CSF is invasive, 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, A β 42 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, A β 42 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 exploration⁶¹.

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 definition⁵⁷. This case definition was derived by expert consensus rather than being data-driven and may therefore 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. It must also be noted that the potential to score points is affected by the ability to comprehensively investigate patients with brain, chest and abdominal imaging as well as microbiological sampling from outside the CNS; in resource constrained settings the ability to score a high number of points may therefore be compromised. We advocate 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 TBM³⁹. 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 standard⁶². 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 TBM⁶³, 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%, respectively⁶⁴. 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 unknown⁶⁵. Individual studies have also found inferior performance for Xpert compared to multiplex PCR⁶⁶ or Amplicor assay⁶⁷ 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)⁶⁸. Ultra has 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^{69,70}. Ultra is endorsed by the WHO as the best initial test for TBM and is being rolled out currently, superseding Xpert⁷¹. The number of copies of IS1081 and IS6110 genes varies by *M.tb* strain and lineage, therefore the performance of the assay could potentially vary based on local strain patterns, highlighting the importance of local evaluation of diagnostic performance as the assay is rolled out. Importantly, negative predictive value does not appear to be adequate to rule-out TBM with Ultra. Another commercial NAAT, the MTBDRplus assay, has been evaluated only in few cases of TBM and needs further validation⁷². 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^{66,73}. Ultra uses melt curve analysis to improve detection of rifampicin resistance but both are about 95% sensitive^{74,75}. Ultra will not be able to adequately define rifampin resistance in samples with a low quantity of bacilli (trace category positive)⁷⁶. 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 diagnosis⁷⁷. Ultra is an important step in the right direction but the result should be considered in the context of the clinical probability of TBM⁷⁸.

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'. 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 μ l 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 standards⁷⁹.

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 test⁸⁰. 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)⁸¹. 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 occurs⁸². Combining FLASH and mNGS technologies could improve detection of TB DNA and associated antimicrobial resistance mutations⁸³. 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 culture⁸⁴. 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/ml⁵⁶. 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^{85,86}, along with a larger Zambian study which examined culture positive TBM in Zambia (TB-LAM sensitivity 22% (23/105))^{85,87}. 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 hour⁸⁸. The Fujifilm LAM was recently tested on 968 urine samples (600 with definite pulmonary TB) in South Africa and showed a sensitivity of 70% (95% CI 53 - 83%) compared to 42% (95% CI 32 - 52%) for the Alere TB-LAM against a microbiological reference standard⁸⁸. There is no data on this assay for diagnosis of TBM published to date.

Clinical prediction rules.

Work is underway to develop a more accurate multivariable clinical prediction rule derived from large international cohorts using individual patient data⁸⁹. 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/μl) 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^{65,90}, 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^{91,92}. 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^{93,94} 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 response⁹⁵. 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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James E. Scriven

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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 military 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.

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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: 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.
