Improving early diagnosis of tuberculous meningitis in children

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For Gailyn, Nicolas, Celine,
and my parents

"It always seems impossible till it's done"
- Nelson Rolihlahla Mandela 1918-2013

TABLE OF CONTENTS

1 Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis (M.tuberculosis), a small aerobic bacillus, with a unique high lipid-content cell wall that plays an important role in its pathogenicity [1]. The organism affects the lungs predominantly, but extrapulmonary involvement is particularly common in young children and immune-compromised individuals [2]. Most people infected with M.tuberculosis have latent disease and are asymptomatic [3]. However, the smaller proportion of people who develop active disease, have significant morbidity and mortality. TB is essentially a preventable disease. Tuberculosis is unique in that it is transmitted almost exclusively through aerosolized droplets formed by coughing [4]. Factors affecting transmission of M.tuberculosis include sputum bacillary load and the presence of lung cavitation in the source. Effective anti-tuberculous treatment, isolation of sputum-individuals and the wearing of masks provide simple opportunities for prevention of mycobacterial transmission [5]. Adequate, well-ventilated, housing and the prevention of overcrowding are as effective in preventing transmission but are, due to resource limitation, much more difficult to achieve.

In 2013, there was an estimated 9.0 million new TB cases worldwide [6]. From 1990 to 2013 the incidence of new TB cases in South Africa increased from 301 to 860/100,000 population. Currently the incidence of TB with human immunodeficiency virus (HIV) co-infection is 520/100,000 population, revealing the additional strain of the HIV pandemic on the TB burden [6]. Central nervous system (CNS) involvement, mostly tuberculous meningitis (TBM), accounts for approximately 1% of all cases [7]. In the Western Cape Province of South Africa, a TB endemic area with routine access to Haemophilus influenza type-B and pneumococcal vaccination, TBM was shown to be the most common form of bacterial meningitis in children [8].

In the late nineteenth century, it was thought that TBM resulted from haematogenous spread to the meninges, due to the frequent finding of TBM and miliary TB occurring in the same patient [9]. In 1933, Rich and McCordock, published their findings that in the majority of TBM cases that came to autopsy, a single caseous focus (Rich's focus) could be found from which, when ruptured, bacilli could spread to the subarachnoid

space. In addition they found that the Rich focus was histologically more developed ("older") than the granulomas found in concurrently occurring miliary TB [10]. This formed the basis of understanding of the pathogenesis of TBM. However, this model does not fully explain the frequency of simultaneously occurring miliary TB and TBM [11,12], or the mechanism whereby M.tuberculosis spreads from the lungs to the meninges and crosses the blood-brain barrier [13]. Animal models have been developed to improve understanding of the pathogenesis of CNS tuberculosis but findings are frustrated by the poor correlation between human and animal studies [13]. Magnetic resonance imaging has detected numerous concurrent leptomeningeal granulomas in children with miliary TB, further challenging Rich's pathogenetic model [13,14]. It is likely that early hematogenous spread to the brain occurs, before a T-cell mediated immune response is activated. This could explain the vulnerability to TBM when T-cell mediated immunity is sub-optimal as in patients with untreated HIV disease [15].

The protective role of lymphocytes is essential, including CD4+ and CD8+ T-cells, along with macrophages in isolating and engulfing M.tuberculosis to form a granuloma. Furthermore, disequilibrium of pro- and anti-inflammatory cytokines influence the severity and course of TBM [16,17]. Many of the signs, symptoms, and sequelae of TBM result from an immunologically-directed inflammatory response to the infection [16,17]. A better understanding of the entry of M.tuberculosis into the central nervous system and the immunological mechanisms allowing survival of the bacilli is crucial to improve prevention and treatment. Once spread to the brain occurs, TBM causes pathology by the formation of a dense basal exudate, initially containing erythrocytes, neutrophils and macrophages. When the dense exudate surrounds the circle of Willis, especially the perforators of the middle cerebral artery, cranial nerves and brainstem this can lead to infarction, cranial nerve palsy and direct brainstem parenchymal involvement, respectively. Obstruction of cerebrospinal fluid (CSF) flow can result in hydrocephalus [7].

While accurate prediction of outcome in childhood TBM is difficult due to the

diversity of underlying pathological mechanisms and variation in host immunological response and early detection of TBM is onerous, due to a non-specific clinical presentation with symptoms such as cough, loss of weight, fever, vomiting and malaise. With disease progression, meningism, focal neurological signs and depressed level of consciousness become manifest. TBM represents the most severe manifestation of TB and is a major cause of death in paediatric TB [18]. The moment of initiating treatment is the most critical factor affecting morbidity and mortality, highlighting the importance of early diagnosis of TBM [19,20].

The hypothesis tested is two-fold. Early diagnosis of childhood TBM can be improved by 1) applying and refining a uniform case definition 2) enhancing rapid identification of M.tuberculosis in CSF.

The clinical objective is to validate the uniform case definition for TBM in clinical practice and to test its utility a clinical tool to detect early childhood TBM, with high sensitivity and specificity. The laboratory-based objectives are:

1. To determine the role of CSF volume in obtaining a positive culture of M.tuberculosis.
2. To determine the diagnostic accuracy, and resistance detection, of the MTBDRplus® assay on fresh CSF samples and to compare these results with those obtained from cultured specimens.
3. To determine the diagnostic accuracy, and resistance detection, of the Xpert MTB/RIF® assay on fresh CSF samples and to compare these results with those obtained from cultured specimens.

With this thesis, the aim is to improve the early and/or more accurate diagnosis of childhood TBM by defining predictive clinical criteria, and evaluate the diagnostic accuracy of diagnostic methods.

My colleague, Dr Ronald van Toorn, and I were invited to submit an update on the diagnosis and management of TBM in children to a 2014 edition of Seminars in Pediatric Neurology focusing on key challenges specific to the African continent in

the management of children with neurologic diseases. This review, to which both authors contributed equally, highlights the importance of TBM as a cause of neurologic handicap in resource-poor countries (see chapter 2) [21]. In this review I discuss early diagnosis and treatment of TBM as the single most important factor determining outcome and prioritization of the development of affordable, accurate diagnostic tests for TBM in resource-poor settings.

A definite diagnosis of TBM in meningitis suspects is possible when acid-fast bacilli are seen on CSF microscopy, CSF M.tuberculosis culture is positive and/or when M tuberculosis antigen is detected in CSF by commercial nucleic acid amplification (NAA) tests. Because of the low sensitivity of these tests, diagnosis of TBM in clinical practice is based on a combination of clinical, laboratory and radiological findings. Typical cerebro-spinal fluid (CSF) features include a moderately increased white cell count with lymphocyte predominance, increased protein, and decreased glucose. A clinical prediction rule differentiating TBM from other forms of meningitis in adults found five useful parameters: young age (<36 yrs), sub-acute presentation (symptom duration >5 days), normal peripheral white blood cell count, moderately raised CSF white cell count (<500 cells/ml), and lymphocyte predominance (CSF neutrophil proportion <50%) [22]. A subsequent study that tested this prediction rule found that it had good diagnostic accuracy when using a composite clinical case-definition and bacteriologically-confirmed TBM as reference standards [23]. Although macroscopically clear CSF with lymphocyte predominance differentiated TBM from bacterial meningitis [24], many additional characteristics (sub-acute onset, focal neurological deficit, low CSF/serum glucose ratio, and elevated CSF protein) were required to differentiate TBM from viral meningitis in adult patients [25].

However data that compare diagnostic criteria of TBM and other causes of meningitis in children are limited. No studies describe clinical prediction rules derived from comparing childhood TBM to other forms of meningitis. Case definitions for TBM in the existing literature include definite and probable cases. There is general agreement that definite TBM includes meningitis suspects with acid-fast bacilli seen on CSF

microscopy, positive CSF M.tuberculosis culture and/or detection by commercial NAA tests. A large number of clinical, CSF, and radiological criteria have been used by different authors in different combinations for diagnosis of probable TBM. Clinical criteria for TBM included: 1) Prolonged symptoms 2) Recent poor weight gain 3) Headache and/or fever and/or night sweats and/or vomiting 4) Recent contact with an infectious TB source case or a positive tuberculin skin test 5) Neurological features including depressed level of consciousness, focal neurological signs, raised intracranial pressure, seizures, or meningism 6) Positive tuberculin skin test 7) Chest X-ray compatible with pulmonary TB 8) CT compatible with TBM 9) Other clinical specimens positive for acid-fast bacilli. CSF criteria for TBM included clear appearance, pleocytosis 10-500/µl, increased protein >1g/dl and decreased glucose defined as <2.2mmol/l or CSF to serum ratio of <50% [26-30].

Since most TBM cases are diagnosed on clinical grounds without bacteriological confirmation from CSF, research is impeded by the lack of reliable diagnostic criteria and standardized approaches [31,32]. Since attaining adequate sample sizes of only culture-confirmed TBM cases is difficult, most studies will include cases of probable and possible TBM. In order to improve standardization of clinical diagnosis of TBM for research purposes, a uniform research case definition for both adults and children was proposed by an international panel of experts. This case definition categorized patients as definite, probable, or possible TBM according to a composite score based on clinical, CSF, and neuroimaging findings [33]. Individual scoring criteria and score weighting were based on an extensive literature review and international expert consensus.

Part 2 of this thesis focuses on the diagnostic utility of the uniform research case definition criteria for TBM. In chapter 3.1 I aim to retrospectively evaluate the diagnostic performance of probable and possible TBM criteria in children with culture-confirmed TBM and culture-confirmed bacterial meningitis [34]. The culture-confirmed TBM cases were prospectively collected in different studies over a 20-year period at Tygerberg Children's Hospital, comprising the largest database of childhood

TBM in the world. Study questions were: The purpose of this retrospective study was to determine the diagnostic accuracy of the uniform research case definition for TBM when comparing culture-confirmed TBM to culture-confirmed bacterial meningitis. Another aim was to determine which of the individual criteria in the uniform research case definition could differentiate between culture-confirmed TBM and culture-confirmed bacterial meningitis?

In chapter 3.2 the uniform research case definition is prospectively applied in a study comparing culture-confirmed TBM cases with bacterial and viral meningitis control patients. Study questions included the following: What is the diagnostic accuracy of the uniform research case definition for differentiating culture-confirmed TBM from a control group comprising bacterial and viral meningitis? Which criteria in the cases definition most accurately differentiate microbiologically-confirmed TBM from bacterial and viral meningitis?

CSF findings are essential for early diagnosis of TBM. Leukocytosis with lymphocyte predominance, elevated protein and abnormally decreased CSF glucose are highly suggestive of TBM in endemic areas [30,35,36]. A CSF to serum glucose ratio below 0.4 and an absolute CSF glucose concentration less than 2.2 mmol/L was shown to be highly specific for bacterial meningitis [37]. No studies have yet explored CSF glucose cut-off values for children with TBM. A CSF protein cut-off of >1g/L (100mg/dL) differentiated between cases of TBM and both viral and bacterial meningitis [24,25]. Chapter 3.3 aimed to assess the diagnostic utility of CSF glucose levels, CSF to serum glucose ratio and CSF protein levels in differentiating TBM from other types of meningitis. Study questions were: What is the optimal cut-off value for CSF glucose in TBM? What is the optimal cut-off value for CSF protein in TBM? Can decreased CSF glucose and raised CSF protein differentiate between TBM and other forms of meningitis?

Chest X-ray is available even in resource-limited settings, and often is the first investigation that clinically confirms TB in a suspected case. Previous studies showed

that 30% to 65% of adults with TBM have chest X-ray findings consistent with active pulmonary TB [38-40]. In children the frequency is even higher ranging from 70% to 84% [41]. Chapter 3.4 aims to describe the frequency of radiological features suggestive of pulmonary TB in children with TBM. Study questions were: What is the frequency of a chest X-ray suggestive of active pulmonary TB in childhood TBM? How many children with TBM have evidence of miliary TB on chest X ray? Does age of the patient or severity of TBM disease influence chest X-ray findings?

Microbiological confirmation from CSF remains the gold-standard of diagnosis of TBM, but is challenging in young children due to the paucibacillary nature of disease and low CSF volumes available for diagnostic analysis [42]. Definite diagnosis of TBM requires visualization of acid-fast bacilli and/or a positive M.tuberculosis culture from CSF. Direct microscopy for acid-fast bacilli in CSF is fast but has low sensitivity (<20%) [43]. M.tuberculosis culture may take up to 42 days and has only slightly improved sensitivity (<50%) [30,44,45]. The bacterial yield of M.tuberculosis can be improved by obtaining larger volumes of CSF, optimizing centrifugation and increasing the amount of time taken with microscopy [46,47].

The introduction of NAA methods, including polymerase chain reaction (PCR) techniques, have increased the specific detection and identification of M.tuberculosis. Automated amplification assays have simplified PCR within the routine laboratory and have also made standardization possible [48]. A decade old meta-analysis of the diagnostic value of NAA tests in TBM (studies 1990-2002 comprising both adults and children were analysed) showed that heterogeneity affected the accuracy of in-house NAA tests while commercial NAA tests were a useful adjunct to clinical findings and conventional M.tuberculosis testing but could not confidently exclude a diagnosis of TBM [49].

Since the above study, several new commercially available NAA tests have been developed for the rapid diagnosis of TB, but no studies have yet explored the diagnostic accuracy of the newer NAA tests in TBM. Chapter 4.1 consists of a

systematic review of all studies published since 2003 that evaluated the use of NAA tests to diagnose TBM, with particular emphasis on commercial tests, including Xpert MTB/RIF® [50]. Study questions included: Is the diagnostic accuracy of newer commercial NAA tests improved? What is the diagnostic accuracy of the Xpert MTB/RIF® assay on CSF samples?

This last question is especially relevant since the WHO recommended in 2013 that Xpert MTB/RIF® be used for rapid diagnosis in preference to conventional microscopy and culture as the initial diagnostic test in all adults and children suspected of having TBM [51]. Xpert MTB/RIF® also detects susceptibility to rifampicin by amplification of the rpoB gene [52,53] The MTBDRplus® assay (Hain Lifescience GmbH, Nehren, Germany) version 2 is a line probe assay targeting the rpoB, katG and inhA genes, detecting M.tuberculosis, but with the additional advantage of detecting both rifampicin and isoniazid susceptibility. Although the MTBDRplus® assay version 2 has similar sensitivity and specificity to Xpert MTB/RIF® in smear microscopy-negative specimens, Xpert MTB/RIF® detects M.tuberculosis more rapidly (under 2 hours vs 5 hours), with easier handling and lower contamination rates [54-56]. In order to stop empiric treatment, a diagnostic test for TBM requires a negative predictive value >99%. As the negative predictive value of Xpert MTB/RIF® is lower than <99%, it is recommended for use in conjunction with clinical findings [57]. In chapter 4.2 the utility of MTBDRplus® and Xpert MTB/RIF® to diagnose TBM in a clinical setting, alone and/or in combination with established diagnostic methods is assessed [58]. Study questions were: What is the diagnostic accuracy of Xpert MTB/RIF® on CSF in childhood TBM? What is the diagnostic accuracy of MTBDRplus® on CSF in childhood TBM? What is the diagnostic accuracy of combined Xpert MTB/RIF® and MTBDRplus® on CSF in childhood TBM?

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Part I An overview of tuberculous meningitis in children

2 Update on the diagnosis and management of tuberculous meningitis in children

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ABSTRACT

Tuberculous meningitis (TBM), the most devastating manifestation of tuberculosis, is often missed or overlooked due to non-specific symptoms and difficulties in diagnosis. It continues to be an important cause of neurological handicap in resource-poor countries.

Due to the suboptimal performance of diagnostic tests of TBM, diagnosis relies on thorough history, clinical examination and relevant investigations. The development of affordable, accurate diagnostic tests for TBM in resource-poor settings remains a priority.

Short intensified treatment is safe and effective in both HIV-infected and HIV-uninfected children. Treatment of tuberculous hydrocephalus depends on the level of the cerebrospinal fluid (CSF) obstruction. Corticosteroids reduce risk of neurodisability and death in HIV-uninfected children. Thalidomide should be considered in children compromised by TB abscesses and tuberculous-related optochiasmic arachnoiditis. In resource-poor countries, home-based TBM treatment after initial in-hospital stabilization is feasible in carefully selected patients. Early diagnosis and treatment of TBM is the single most important factor determining outcome.

INTRODUCTION

Tuberculous meningitis (TBM) is the most devastating manifestation of tuberculosis and it continues to be an important cause of neurological handicap in resource-poor countries. A recent study in the Western Cape Province of South Africa found TBM to be the commonest cause of pediatric meningitis.[1] South Africa is one of the 22 high tuberculosis (TB) burden countries that account for 80% of the world TB cases. The estimated incidence of TB in SA is 1000 or more per 100 000 people. One of the Millennium Development Goals (MDG) targets are to halt and start to reverse the rising incidence of TB and to halve the 1990 prevalence and death rates by 2015.[2] Unfortunately, most African regions, including SA, are not on track to achieve this objective due to reasons such as resource constraints, conflict and instability and generalized HIV epidemics.

Bacille Calmette-Guerin (BCG) is currently the only available vaccine against TB, and is widely administered within the World Health Organization (WHO) Expanded Programme for Immunization. It provides protection against disseminated TB and TBM (73%; 95% confidence limits 67-79%) but has highly variable and often low efficacy against pulmonary TB in adults.[3] The impact of BCG vaccination on transmission of *Mycobacterium tuberculosis* (*M.tb*) is therefore limited. The variable efficacy of BCG vaccination together with the not inconsequential threat of multi-drug resistant TB highlights the neccessity of new vaccine development, but this is hindered by the lack of immune correlates, suboptimal animal models, and limited funding.[4]

CLINICAL MANIFESTATIONS

TBM may present at any age but is less common at the extremes of life. The peak incidence is in children between 2 to 4 years of age.[5] Early clinical diagnosis is notoriously difficult and often delayed, with disastrous consequences. Although delayed diagnosis of TBM is common, the very young infant, patients with another co-existing illness and those from non TB-endemic regions carry the highest risk for missed diagnosis. The classical presentation of TBM is as a subacute meningitic illness. The resulting dilemma is that the classical sign of meningitis, neck stiffness, is usually absent during the early disease stage in children and adults.[6] Early diagnosis and treatment of TBM has been long recognized as the single most important factor determining outcome.[5]

Although much effort has gone into improving diagnostic investigations, these may not be requested if the possibility of meningitis has not crossed the physician's

mind. This is applicable to health practitioners in resource-poor, as well as resource-equipped countries, where the increase in migrant populations could potentially lead to an increased incidence of TBM. It is therefore important to recognize TBM during the early stage, mainly characterized by non-specific symptoms of general ill health rather than specific, classical signs of meningitis. In young children these include poor weight gain, low-grade fever and listlessness. Most early symptoms relate to underlying pulmonary tuberculosis present in the vast majority of infants who develop TBM as a complication of primary infection. The only factor differentiating these symptoms of TBM from common illnesses such as influenza is their persistence[7]; however, this is often not recognized because care-givers may not return to the same health professional (especially if treatment failed) and often do not inform subsequent doctors of previous diagnoses and treatments of the current illness.[7] Thus early stage, fully curable TBM may progress to the final stages of coma, opisthotonus and death following this course of events.

In older children common non-specific symptoms of early TB meningitis are fever, headache and vomiting, closely representing a flu-like illness. Recent close contact with an infectious pulmonary TB patient is an important diagnostic clue. Once the classical neurological signs of advanced TBM (including meningeal irritation, coma, seizures, signs of raised intracranial pressure, cranial nerve palsies, hemiparesis, movement disorders) appear, the diagnosis is usually apparent but at a considerable cost to the patient. It should be noted, however, that the initial presentation of TBM may be acute and accompanied by any of the above-mentioned "late" signs and without a distinct prodromal period. Neither organism genotype, resistance patterns (MDR TB), co-infection with HIV or BCG immunization status consistently modify the disease presentation as described above.[6]

COMPLICATIONS OF TBM

Tuberculous hydrocephalus and raised intracranial pressure

Hydrocephalus occurs in up to 80% of TBM patients.[5] In 70% of cases the hydrocephalus is of a communicating nature. This occurs when the exudate that fills the basal cisterns causes a bottle-neck obstruction of the cerebrospinal fluid (CSF) pathways at the level of the tentorium. In 20% of cases, CSF obstruction occurs when the basal exudates obstructs the outflow foramina of the 4th ventricle leading to a non-communicating hydrocephalus. Other rare causes of non-communicating hydrocephalus are obstruction of the foramina of Munro or the aqueduct by strategically located tuberculomas.

Tuberculous hydrocephalus is often complicated by raised intracranial pressure (ICP).[5] Studies have shown that clinical diagnosis of the presence and degree of raised ICP is unreliable, especially in children with closed anterior fontanels.[8] The value of computed tomography (CT) is limited by the poor correlation that exists between the degree of hydrocephalus (ventricular size) and severity of ICP.[9] Signs of raised ICP may also mimic signs of brainstem dysfunction.[10] It is therefore often difficult to distinguish between raised ICP and brainstem ischemia in the deeply comatose child with stage III TBM.

Tuberculous cerebrovascular disease

Stroke is a common and most devastating complication of TBM. Vessel pathology appears to be a consequence of its immersion in the local inflammatory exudate.[11] The terminal segments of internal carotid artery (ICA) and proximal portions of middle (M1 portion of MCA) and anterior (ACA) cerebral arteries are most frequently involved. Anti-tuberculous chemotherapy is relatively ineffective in preventing the vascular complications, suggesting an immune mechanisms. This has led to clinical intervention studies aimed at halting the progressive nature of the vasculitis.[12]

TB-IRIS

Central nervous system TB Immune reconstitution inflammatory syndrome (IRIS) often manifests as a life-threatening condition and should be considered when new neurological symptoms or signs develop shortly after initiation of antiretroviral therapy (ART) in children.[13] Two clinical scenarios may occur; "unmasking" IRIS when subclinical, previously unrecognized TB infection flares up after starting ART while "paradoxical IRIS" is diagnosed when new or worsening symptoms of TB develop despite adherence to appropriate antituberculous treatment in a patient who initiated combination antiretroviral ART.[13] Neurological manifestations described, include neck stiffness, intracranial and spinal tuberculous mass lesions, radiculomyelitis, hydrocephalus, visual compromise and seizures.[13] Paradoxical TBM-IRIS tends to occur within 3 weeks of initiation of ART in children.[13]

The frequency and mortality of neurological TB-IRIS in children is not well documented; only 1 case series has been published.[14] In adults, TBM-IRIS complicates the course of treatment of HIV-associated TBM in 47% of cases, despite the use of adjunctive corticosteroid therapy.[15] Mortality is high (up to 30%) in those affected.[14]

As yet, no means exist to predict the syndrome. The optimal time to initiate ART in children or adults with HIV-associated TBM is unknown. A recent randomized double-blind placebo-controlled trial of immediate versus deferred ART in adult Vietnamese

patients with TBM showed that HIV-associated TBM in the study population had such a poor prognosis that the timing of ART made no appreciable difference regarding survival probability.[16] Early initiation of ART was not associated with an increased risk of IRIS. Corticosteroids are the mainstay of treatment for TBM-IRIS, with interruption of ART reserved for life-threatening complications. Other immune-modulatory agents that have been used to treat IRIS in a limited number of patients include thalidomide, chloroquine, mycophenolate mofetil and cyclosporine.[13]

TB mass lesions

Tuberculomas of the central nervous system may occur in isolation or in association with TBM. Intracranial tuberculomas are often silent and unsuspected, especially if no clinical evidence of TB is present. A focal seizure in an otherwise normal child is the most common mode of presentation in TB endemic populations. Tuberculomas may also manifest with focal neurological signs or raised intracranial pressure due to obstruction of cerebrospinal pathways. Diagnosis is dependant on neuroimaging as the cerebrospinal findings and culture is negative in most patients. Most tuberculomas will resolve uneventfully in response to antituberculous treatment. Corticosteroids (prednisone 2mg/kg/day) should be reserved for cases with paradoxically enlarging tuberculomas.

TB mass lesions (large tuberculomas or abscesses) are known to develop or enlarge despite appropriate anti-TB treatment. This phenomenon, the result of IRIS, is often more severe in the setting of HIV co-infection and may be life threatening.[13] Clinical manifestations depend on the size and location of the lesion(s) and include focal neurological signs, ataxia, spastic paraplegia and raised intracranial pressure due to obstruction of cerebrospinal fluid pathways. In our experience TB abscesses are responsive to thalidomide, a potent tumor necrosis factor alpha (TNF-α) inhibitor.[17]

DIAGNOSIS

Due to the suboptimal performance of diagnostic tests of TBM, the diagnosis in children relies on a thorough assessment of all the evidence derived from a careful history, clinical examination and relevant investigations. About 60% of children with TBM will have radiological evidence of pulmonary TB.[5]

There have been efforts to create clinical prediction rules to differentiate TBM from other forms of meningitis, especially in resource-poor settings. When comparing TBM and bacterial meningitis in adults, using a composite clinical reference standard,

sensitivities of 86-97% and specificities of 71-97% were obtained.[18,19] When using a microbiologically proven *M.tb* reference standard, sensitivities (86-96%) and specificities (71-79%) were similar.[18,20] However, a prediction rule performed less well in an area of high HIV seroprevalence (sensitivity 78% and specificity 43%).[21]

As CSF in both TBM and viral meningitis is clear and lymphocyte predominant, distinguishing between them is more difficult. A recent prediction rule, comprised of a diagnostic scoring system, performed well with sensitivity 92% and specificity 94%.[22] Despite numerous reports in the literature describing clinical prediction rules, standardized diagnostic criteria are lacking.[23]

The tuberculin skin test (TST) performed with a sensitivity of 61% in a large retrospective cohort of children with TBM.[5] However sensitivity decreases (34%) when HIV co-infection is present due to the high rate of false negative results.[24] In the young infant population (< 6 months) with BCG vaccination, specificity is decreased due to high false-positivity. Furthermore, a positive TST implies probable *M.tb* infection, but cannot delineate active TB disease.[25]

Although a 2011 meta-analysis of the use of interferon gamma release assays (IGRAs) in adults with pulmonary TB showed that there is no value for the diagnosis of active TB,[26] CSF IGRA showed sensitivity of 59-84% and specificity 73-89%.[27-30] However, the large CSF volumes needed in order to obtain enough cells to perform IGRA,[31] is a limiting factor in children where much smaller CSF volumes are obtained.

A 2003 systematic review evaluated the test accuracy of nucleic acid amplification tests (NAATs) in the diagnosis of TBM.[32] The studies with commercial NAATs revealed a pooled sensitivity and specificity of 56% and 98%, respectively. The review concluded that commercial NAATs provided valuable information when positive, but due to poor sensitivity a negative test did not exclude TBM.[32] The World Health Organization has recently endorsed the Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, CA, USA) for both smear microscopy-positive and -negative sputum specimens.[33] When using Xpert for CSF specimens, promising sensitivities of 67-85% and specificities 94-98% were obtained.[34,35]

Neuroimaging plays an important role in the diagnosis of TBM especially during the early stage of the disease and in cases of diagnostic uncertainty. Computed tomography (CT) is most often used in resource-poor countries and a combination of hyperdense exudates on pre-contrast CT, basal meningeal enhancement, infarctions and hydrocephalus is highly suggestive (Figure 1).[36] Bilateral basal ganglia infarcts are

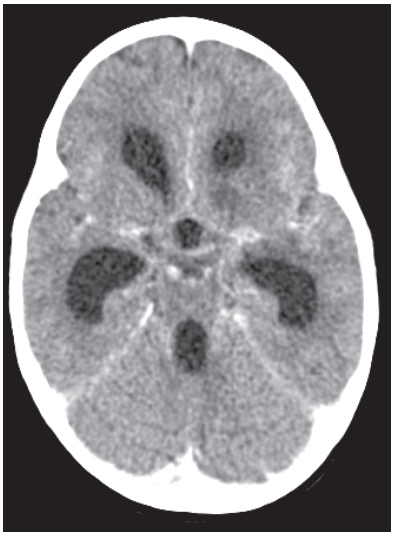

Figure 1. Contrasted computed tomography showing a combination of hydrocephalus, basal meningeal enhancement and infarction.

particularly characteristic of TBM and this finding on CT suggest a high likelihood of brainstem involvement. Approximately a third of children with stage 1 TBM disease will have a normal CT scan.[6]

Magnetic resonance imaging (MRI) is superior to CT for diagnosing TBM, by detecting basal enhancement and granulomas in more patients, and prognosis, by detecting many more infarcts in strategic locations such as the brainstem (Figure 2).[37] Gado-

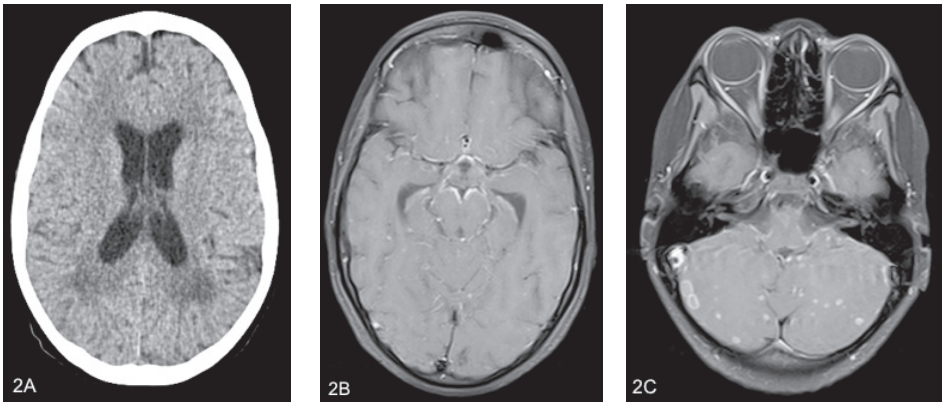

Figure 2A. Computed tomography of a 13-year old child presenting with disseminated TB and loss of consciousness. The initial CT only revealed bilateral periventricular hypodensities and hydrocephalus. Figure 2B-C. T1-weighted post-gadolinium MRI 2 days later revealed basal meningeal enhancement and multiple ring-enhancing lesions (miliary nodules) in the cerebellar hemispheres.

linium enhanced MRI allows detection of miliary leptomeningial tubercles which have been reported to be present in 88% of children with TBM.[38] MRI is also valuable for identification of optochiasmic arachnoiditis, which requires urgent intervention to reduce the risk of blindness.[39] Magnetic resonance angiography (MRA) is useful for assessment of vascular involvement. Vessels most commonly affected include the terminal portions of the internal carotid arteries, as well as the proximal parts of the middle and anterior cerebral arteries.

TREATMENT

Fluid management

Hyponatremia occurs in up to 85% of children with TBM and is thought to be secondary to either syndrome of inappropriate anti-diuretic hormone (SIADH) or cerebral salt wasting. Fluid restriction has traditionally been recommended to counter the presumed threat of SIADH and reduce the risk of cerebral edema. There is however no evidence that indicates that fluid restriction is beneficial in children with meningitis. It may precipitate hypovolemia, which should be avoided at all costs as maintenance of adequate cerebral perfusion is of critical importance in TBM patients. TBM is known to induce a hypercoagulable state, which would then increase the risk of venous thrombosis and infarction in the setting of inadequate cerebral perfusion.[40] A safer option is to partially correct symptomatic hyponatremia (associated with seizures) by slow infusion of 5% hypertonic saline.

Antimicrobial therapy

There is limited evidence regarding the most appropriate treatment regimen for TBM or optimal duration of treatment.[41] The WHO recommends 12-months treatment (2RHZE/10RH) for children with suspected or confirmed TBM. Short, intensified anti-TBM therapy is advocated by several groups as similar completion and relapse rates have been reported when 6-months therapy was compared to 1-year.[42] High dose intravenous rifampicin may also be associated with a survival benefit in adult patients with severe disease.

Local experience is that short, intensified therapy (6RHZEth for HIV-uninfected and 9RHZEth for HIV-infected children) is safe and effective in children with drug susceptible TBM.[42] This regimen was prospectively evaluated in 184 consecutive TBM children and resulted in a good outcome in 80% of cases and mortality of 3.8%.[42] The incidence of antituberculous drug-induced hepatotoxicity in the study was low (5%) and in all cases the original regimen was restarted without recurrence.[42] The

rational for using ethionamide as the 4th drug is that it has good CSF penetration and less adverse effects compared to streptomycin and ethambutol. Another advantage is that isoniazid mono-resistant TBM may be overcome when ethionamide and pyrazinamide are used continuously for a 6-month period.[43]

In resource-poor countries, lengthy in-hospital treatment of TBM is often not a realistic option. Local experience is that home-based TBM treatment after initial in-hospital stabilization is feasible in carefully selected patients under close supervision.[44]

Multidrug-resistant TBM should be considered in cases where there is deterioration despite compliance with adequate antituberculous treatment. In such cases it is vitally important to obtain cultures from source contacts. Newer NAAT allows resistance to rifampicin and/or isoniazid to be detected. Second-line agents for MDR TBM include levofloxacin, amikacin, terizadone and para-aminosalicylic acid (PAS).

Treatment of tuberculous hydrocephalus

Treatment of tuberculous hydrocephalus depends on the level of CSF obstruction. Air-encephalography is the most reliable way of determining the level of CSF obstruction (Figure 3).[45] CT is not a useful tool as panventricular dilatation occurs in both communicating and non-communicating types of hydrocephalus.[45] Communicating hydrocephalus can be successfully treated with medical therapy consisting of acetazolamide 50 mg/kg/day and furosemide 1 mg/kg/day in 3 divided daily doses) for a

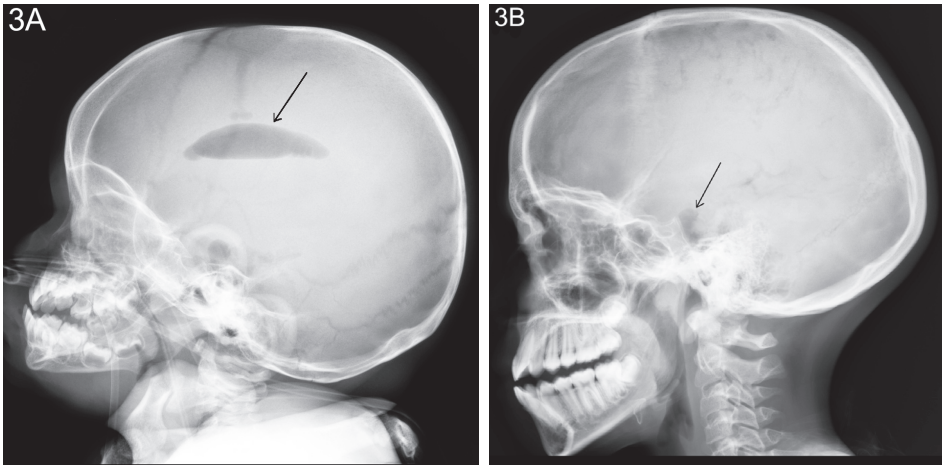

Figure 3A. The lateral skull X-ray shows air in the basal cistern and lateral ventricles. This indicates communicating hydrocephalus (arrow) due to basal cistern obstruction to the flow of cerebrospinal fluid. **3B.** The lateral skull X-ray shows only air at the level of the basal cistern (arrow). This indicates non-communicating hydrocephalus due to obstruction of the 4th ventricle outlet foramen.

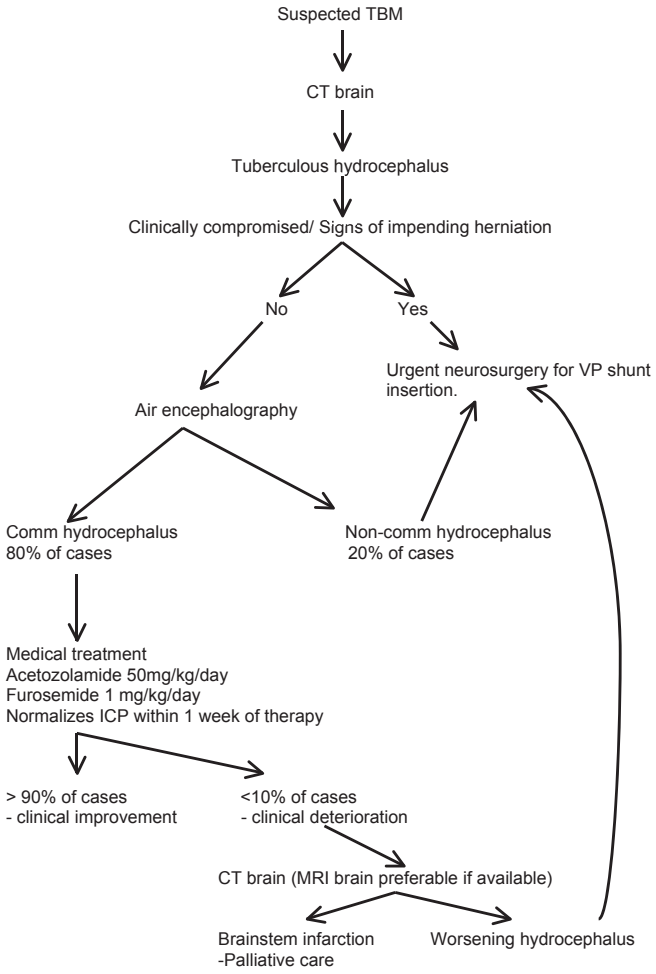

Figure 4. Tygerberg Children's Hospital treatment algorithm for tuberculous hydrocephalus.

period of 4 weeks.[9] This drug combination reduces CSF production by blocking carbonic anhydrase activity and reduces ICP by decreasing the rate of CSF production. It is our experience that normalization of intracranial pressure occurs within days in more than 90% of children. Figure 4 illustrates our suggested treatment algorithm for children with tuberculous hydrocephalus.

Adjunctive anti-inflammatory therapy

Corticosteroids

A 2008 Cochrane systematic review of 7 clinical trials involving 1140 participants found that corticosteroids reduce the risk of death (RR 0.78, 95% CI 0.67-0.91) or

disabling neurological deficit (RR 0.82, 95% CI 0.70-0.97) in HIV uninfected TBM patients.[46] The benefit of corticosteroids in HIV infected patients has not been demonstrated. There are also no controlled trials comparing corticosteroid regimens. Local preference is to prescribe prednisone 2 mg/kg/day (maximum 60 mg/day) for the first month of treatment and then to gradually wean over the next 2-weeks.

Aspirin

The value of aspirin's antithrombotic, anti-ischemic and anti-inflammatory properties in TBM was explored in two studies. An adult TBM study reported a significant reduction in mortality at 3 months (p=0.02).[47] In contrast, a childhood TBM study found no significant benefit in morbidity (hemiparesis and developmental outcome) or mortality at 6 months.[40]

Thalidomide

TB abscesses are notoriously resistant to therapy and require total surgical excision for cure.[48] Surgical excision is often not achievable due to the proximity of the abscesses to vital brain structures and the lack of neurosurgical care in resource poor countries. TB abscesses often teem with tubercle bacilli, which induce a strong cytokine response. The most important cytokine implicated is tumour necrosis factor alpha (TNF-α). Insufficient TNF-α production delays granuloma formation, which is required for control of bacillary growth whilst excessive production leads to extensive liquefaction necrosis as is evident in TB abscesses. Thalidomide 3-5 mg/kg/day, given orally, is our drug of choice in children who develop life-threatening TB mass lesions (IRIS) despite corticosteroids.[12] The use of Thalidomide should also be considered in children with visual compromise due to tuberculous optochiasmic arachnoiditis.[39]

Outcome in childhood TBM

Prognosis in TBM largely depends on the stage the disease has reached at the time of treatment intervention. Children with stage I TBM disease are likely to have a normal outcome, whereas children with stage III disease have a high risk of mortality.[42] Multidrug-resistant TBM in children has a poor clinical outcome and is often associated with death.[43] Inpatient mortality rates are generally similar between HIV-infected and uninfected children with TBM.[42] However, mortality after hospital discharge is substantially worse in HIV-infected TBM children due to HIV-related illnesses. Long-term behavioural complications of TBM survivors include general behavioural disinhibitions and internalized emotional disorders.[49]

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Part II

Testing the utility of a uniform research case definition for tuberculous meningitis in children

3.1 Uniform research case definition criteria differentiate tuberculous and bacterial meningitis in children

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ABSTRACT

BACKGROUND: Tuberculous meningitis (TBM) research is hampered by low numbers of microbiologically-confirmed TBM cases and the fact that they may represent a select part of the disease spectrum. A uniform TBM research case definition was developed to address these limitations, but its ability to differentiate TBM from bacterial meningitis has not been evaluated.

METHODS: We assessed all children treated for TBM from 1985 to 2005 at Tygerberg Children's Hospital, Cape Town, South Africa. For comparative purposes a group of children with culture-confirmed bacterial meningitis, diagnosed between 2003 and 2009, was identified from the National Health Laboratory Service database. The performance of the proposed case definition was evaluated in culture-confirmed TBM and bacterial meningitis cases.

RESULTS: Of 554 children treated for TBM, 66 (11.9%) were classified as "definite TBM", 408 (73.6%) as "probable TBM" and 72 (13.0%) as "possible TBM". "Probable TBM" criteria identified culture-confirmed TBM with sensitivity 86% and specificity 100%; sensitivity was increased but specificity reduced when using "possible TBM" criteria (sensitivity 100%, specificity 56%).

CONCLUSION: "Probable TBM" criteria accurately differentiated TBM from bacterial meningitis and could be considered for use in clinical trials; reduced sensitivity in children with early TBM (stage I disease) remains a concern.

INTRODUCTION

Tuberculosis (TB) continues to pose a major global health challenge with a high morbidity and mortality, despite effective anti-tuberculosis medication, in TB endemic areas [1,2]. Tuberculous meningitis (TBM) is the most devastating manifestation of TB and is generally associated with poor outcomes. Diagnosis is often delayed due to the non-specific nature of early symptoms such as cough, weight loss, fever, vomiting and lethargy. With disease progression, a more definitive clinical picture becomes manifest [3], however, early treatment initiation is critical to optimize outcome [4,5].

Identification of *Mycobacterium tuberculosis* (*M. tuberculosis*) in cerebrospinal fluid (CSF) provides bacteriological confirmation of TBM. Unfortunately, due to the paucibacillary nature of TBM, CSF microscopy for acid-fast bacilli (AFB) [6] and CSF culture for *M. tuberculosis* has low sensitivity (<20% and <50% respectively) [3,7,8] compared to clinical criteria. Although culture provides the accepted "gold standard" it has little clinical utility, since it takes up to 42 days to confirm a positive result. A meta-analysis of the accuracy of commercial nucleic acid amplification tests (NAATs) for the diagnosis of TBM, mostly adult studies, showed a sensitivity of 64% and specificity of 98% compared to culture [9]. Thus it may serve as a rapid "rule-in" test, but does not provide a reliable 'rule out' test.

TBM research is greatly hampered by the absence of reliable diagnostic criteria and standardized approaches [10]. A recent review found that existing trials assessing anti-tuberculosis treatment for TBM had limited power, poor methodology and used varying treatment regimens with conflicting results [11]. Answering critical research questions will require multi-centre randomized controlled trials using standardized diagnostic and staging criteria, with sufficient patient numbers to demonstrate differences in outcome [11]. As it is difficult to obtain adequate sample sizes using only culture-confirmed TBM cases, inclusion of probable and possible TBM cases require consideration.

A uniform research case definition for TBM in adults and children was developed to improve standardization of diagnosis for research purposes [12]. Classification as definite, probable or possible TBM is based on a composite score of clinical findings and special investigations; summarized in Table 1. Individual criteria and their relative weights were assigned based on an extensive literature review together with international expert consensus [12-17]. The accuracy of proposed criteria for probable and possible TBM has not been assessed. Our study aimed to evaluate the diagnostic performance of probable and possible TBM criteria in children with culture-confirmed TBM and culture-confirmed bacterial meningitis.

PATIENTS AND METHODS

We conducted a retrospective review of all children (<13 years of age) treated for TBM between January 1985 and April 2005 at Tygerberg Children's Hospital, Cape Town, South Africa. Clinical data were prospectively collected in all TBM patients as part of ongoing studies conducted at the time. For comparative purposes we identified a group of children (<13years of age) with culture-confirmed bacterial meningitis, diagnosed between January 2003 and November 2009. Bacterial meningitis cases were identified from the National Health Laboratory Service database at Tygerberg Children's Hospital; relevant clinical data were retrieved from patient files. The study was approved by the Human Research Ethics Committee of Stellenbosch University (HREC reference number N10/11/367).

CASE DEFINITIONS

Tuberculous meningitis

Patients were classified as "definite TBM" if the CSF culture was positive for *M. tuberculosis*; smear microscopy for acid-fast bacilli and/or commercial NAAT testing was not performed at the time.

Identification of probable and possible TBM was based on pre-defined diagnostic scoring criteria (Table 1) [12]. Points were allocated in the following categories: 1) clinical findings 2) CSF results 3) neuroimaging findings 4) evidence for TB outside the central nervous system and 5) additional laboratory criteria. A total score of at least 12 was compatible with probable TBM (when neuroimaging was unavailable, the total score was reduced to at least 10), while a total score of 6-11 equated to a possible TBM diagnosis (when neuroimaging was unavailable the total score required was reduced to 6-9), with a minimum number of points coming from CSF or neuroimaging criteria.

Potential alternative causes had to be excluded by performing at least 1) CSF Gram stain and bacterial culture 2) CSF India ink stain, cryptococcal antigen latex agglutination test and/or cryptococcal culture. These investigations were routinely performed. Additional investigations such as 3) viral culture and/or polymerase chain reaction (PCR) and tests to exclude 4) neurosyphilis and 5) cerebral malaria should be conducted if clinically or epidemiologically indicated; these investigations were not routinely performed in our cohort. Due to the retrospective nature of the study, the following imaging criteria could not be evaluated: pre-contrast basal hyperdensity

Table 1. Diagnostic criteria in the uniform TBM research case definition [12]

	Diagnostic score
Clinical criteria (Maximum category score=6)	
Symptom duration of more than 5 days	4
Systemic TB symptoms (1 or more of): weight loss/(poor weight gain in children), night sweats or persistent cough > 2 weeks	2
History of recent close contact with an individual with pulmonary TB or a positive TST/IGRA in a child <10 years	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
CSF criteria (Maximum category score=4)	
Clear appearance	1
Cells: 10–500 per µl	1
Lymphocytic predominance (>50%)	1
Protein concentration greater than 1 g/L	1
CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2.2mmol/L	1
Cerebral imaging criteria (Maximum category score=6)	
Hydrocephalus (CT and/or MRI)	1
Basal meningeal enhancement (CT and/or MRI)	2
Tuberculoma (CT and/or MRI)	2
Infarct (CT and/or MRI)	1
Pre-contrast basal hyperdensity (CT)	2
Evidence of tuberculosis elsewhere (Maximum category score=4)	
Chest radiograph suggestive of active TB (excludes miliary TB)	2
Chest radiograph suggestive of miliary TB	4
CT/ MRI/ US evidence for TB outside the CNS	2
AFB identified or *M. tuberculosis* cultured from another source i.e., sputum, lymph node, gastric washing, urine, blood culture	4
Exclusion of alternative diagnoses- An alternative diagnosis must be confirmed microbiologically, serologically or histopathologically	
Definite TBM = AFB seen on CSF microscopy, positive CSF *M. tuberculosis* culture or CSF *M. tuberculosis* commercial NAAT with symptoms/signs suggestive of meningitis; or AFB seen in the context of histology consistent with TB brain or spinal cord together with suggestive symptoms/signs and CSF changes, or visible meningitis (on autopsy).	
Probable TBM = total score of ≥12 when neuroimaging available = total score of ≥10 when neuroimaging unavailable	
Possible TBM = total score of 6-11 when neuroimaging available = total score of 6-9 when neuroimaging unavailable	

TBM=tuberculous meningitis, TB=tuberculosis, TST=tuberculin skin test, IGRA=interferon gamma-release assay, CSF=cerebrospinal fluid, CT=computed tomography, MRI=magnetic resonance imaging, US=ultrasound, AFB=acid-fast bacilli, NAAT=nucleic acid amplification test

on computed tomography (CT), imaging of TB outside the CNS (apart from standard postero-anterior and lateral chest radiographs). TBM disease staging was determined as follows: Stage I) Glasgow Coma Scale (GCS) of 15 and no focal neurological signs, Stage IIa) GCS of 15 plus focal neurological signs, Stage IIb) GCS of 11-14 with focal neurological signs and Stage III) GCS <11 [17,18].

Bacterial meningitis

A diagnosis of definite bacterial meningitis was made if bacterial pathogens were identified by Gram stain and/or culture from CSF, and patients were treated for bacterial meningitis.

Statistical analysis

Statistical analysis was done using SPSS version 20 (SPSS Inc, Chicago, IL, USA) and SAS version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). The Fishers-exact test and odds ratios were used in univariable analyses comparing "definite TBM" and "definite bacterial meningitis"; using a two-tailed test for statistical significance.

RESULTS

In total, 554 children were treated for TBM during the study period; 66 (11.9%) were classified as definite TBM, 408 (73.6%) as probable TBM, 72 (13.0%) as possible TBM and 8 had insufficient information for reliable classification [12]. Table 2 provides an overview of TBM cases, including staging and diagnostic criteria. Diagnostic criteria are also reflected for the 32 cases with definite bacterial meningitis identified. When applying the uniform research case definition for TBM to bacterial meningitis cases, 14 scored as possible TBM and 18 as 'no TBM'. None of the bacterial meningitis cases tested positive for human immunodeficiency virus (HIV). Among TBM patients, 8 were HIV-infected, 205 HIV-uninfected and the HIV status was unknown in 341 patients. HIV prevalence was low during the earlier part of the 20-year study period when HIV testing was not routinely done. In the possible TBM group, 25% of patients had stage III disease, compared to 42% and 41% in the probable and definite TBM groups respectively.

When scoring children with definite TBM independent of their *M. tuberculosis* culture results, 57/66 (86%) scored as probable TBM and 9/66 (14%) as possible TBM. Of the nine culture-confirmed TBM cases that scored as 'possible' TBM, three had limited clinical symptoms (one TBM stage I and IIa) and six had minimal signs on neuroimaging. Seven culture-confirmed TBM cases were within 2 points of the cut-off

Table 2. Overview of scoring criteria in children with definite, probable and possible TBM compared to bacterial meningitis

	Definite TBM n/N (%)	Probable TBM n/N (%)	Possible TBM n/N (%)	Definite BM n/N (%)
Total number	**66**	**408**	**72**	**32**
Stage I	2 (3)	5 (1)	4 (6)	n/a
Stage IIa	35 (53)	214 (52)	47 (65)	n/a
Stage IIb	2 (3)	16 (4)	1 (1)	n/a
Stage III	27 (41)	172 (42)	18 (25)	n/a
Unknown stage	0	1	2	n/a
Symptom duration > 5 days	47/66 (71)	366/408 (90)	40/72 (56)	6/32 (19)
≥1 of: weight loss/poor weight gain, night sweats or persistent cough >2 weeks	31/66 (47)	204/408 (50)	25/72 (35)	1/32 (3)
History of recent TB contact or positive TST or IGRA	33/65 (52)	291/374 (78)	36/66 (55)	2/32 (6)
Focal neurological deficit	33/66 (50)	286/408 (70)	27/72 (38)	2/32 (6)
Cranial nerve palsy	18/66 (27)	110/408 (27)	17/72 (24)	2/32 (6)
Altered consciousness	46/66 (70)	398/408 (98)	65/72 (90)	19/32 (59)
CSF clear	66/66 (100)	408/408 (100)	72/72 (100)	18/32 (56)
CSF cells: 10–500 per µl	55/63 (87)	361/396 (91)	53/54 (98)	20/32 (63)
CSF lymphocyte predominance (>50%)	51/63 (81)	326/396 (82)	53/54 (98)	19/32 (59)
CSF protein concentration >1 g/L	49/61 (80)	292/387 (72)	42/52 (81)	15/32 (47)
CSF:serum gluc ratio <50% and/or CSF gluc concentration <2.2mmol/L	47/59 (80)	263/361 (73)	34/48 (71)	17/32 (53)
Hydrocephalus (CT and/or MRI)	60/62 (97)	353/391 (90)	51/62 (82)	9/21 (43)
Basal meningeal enhancement (CT and/or MRI)	48/62 (77)	318/391 (81)	19/62 (31)	1/21 (5)
Tuberculoma (CT and/or MRI)	5/62 (8)	55/391 (14)	5/62 (8)	1/21 (5)
Infarct (CT and/or MRI)	15/62 (24)	142/391 (36)	3/62 (5)	3/21 (14)
Pre-contrast basal hyperdensity (CT)	No data	No data	No data	0
CXR suggestive of active TB	26/66 (39)	206/398 (52)	17/66 (26)	3/32 (9)
CXR suggestive miliary TB	11/66 (17)	53/398 (13)	1/66 (2)	0
Extraneural radiological TB	0	0	0	0
Extraneural *M. tuberculosis* confirmation	19/66 (29)	100/408 (25)	2 (3)	0

TBM=tuberculous meningitis, BM=bacterial meningitis, TB=tuberculosis, TST=tuberculin skin test, IGRA=interferon gamma-release assay, CSF=cerebrospinal fluid, gluc=glucose, CT=computed tomography, MRI=magnetic resonance imaging, CXR=chest radiograph

for a 'probable' TBM score; the remaining 2 patients had low scores for both clinical and neuroimaging criteria.

Among the definite bacterial meningitis group, 18/32 (56%) scored as unlikely TBM and 14/32 (44%) as possible TBM. Comparing patients with definite TBM and definite bacterial meningitis, a probable TBM score provided good diagnostic accuracy; sensitivity 86% and specificity 100%. A possible TBM score was more sensitive but very non-specific; sensitivity 100% and specificity 56%.

Table 3 summarizes findings for specific criteria on univariate analysis. Comparing definite TBM to definite bacterial meningitis the most informative clinical criteria were either weight loss or persistent cough longer than 2 weeks (OR 27.5; 95% Confidence interval (CI) 3.5-213.1), recent close TB contact or positive tuberculin skin test (OR 15.0; 95% CI 3.3-67.9), and focal neurological deficit (OR 15.0; 95% CI 3.3-67.9). Macroscopically clear CSF was highly indicative (OR 50.6; 95% CI 6.2-410.7) as were hydrocephalus (OR 53.7; 95% CI 13.4-215.7) and basal meningovascular enhancement (OR 82.7; 95% CI 10.5-651.0) on CT scan.

Table 3. Findings on univariate analysis comparing definite TBM to definite bacterial meningitis

	OR (95% CI)	**p value**
Symptom duration > 5 days	10.7 (3.8-30.2)	<0.01
Weight loss or persistent cough > 2 weeks	27.5 (3.5-213.1)	<0.01
Recent close TB contact or positive TST/IGRA	15.0 (3.3-67.9)	<0.01
Focal neurological deficit	15.0 (3.3-67.9)	<0.01
Cranial nerve palsy	5.6 (1.2-26.0)	0.03
Altered level of consciousness	1.6 (0.7-3.8)	0.31
CSF clear	50.6 (6.2-410.7)	<0.01
CSF cells 10-500/µl	3.0 (1.1-7.9)	0.26
CSF lymphocyte predominance >50%	1.6 (0.6-4.0)	0.37
CSF raised protein >1g/L	3.3 (1.4-7.9)	<0.01
CSF:serum glucose ratio <50%	2.2 (0.9-5.2)	0.08
Hydrocephalus (CT and/or MRI)	53.7 (13.4-215.7)	<0.01
Basal meningovascular enhancement (CT and/or MRI)	82.7 (10.5-651.0)	<0.01
Tuberculoma (CT and/or MRI)	2.5 (0.3-22.7)	0.40
Infarct (CT and/or MRI)	2.8 (0.8-10.7)	0.12
CXR suggestive of active TB	6.3 (1.7-22.8)	<0.01
CXR suggestive miliary TB	6.2 (0.8-50.3)	0.09
Extraneural *M. tuberculosis* confirmation	12.5 (1.6-98.5)	0.02

OR= odds ratio, CI= confidence interval, TB= tuberculosis, TST=tuberculin skin test, IGRA=interferon gamma-release assay, CSF= cerebrospinal fluid, CT= computed tomography, MRI= magnetic resonance imaging, CXR= chest radiograph

DISCUSSION

In this retrospective assessment, the proposed uniform research case definition [12], provided excellent diagnostic accuracy compared to culture-confirmation of TBM, if a 'probable' TBM score was used. With a 'possible' TBM score not a single TBM case would have been missed, but clinical utility was minimal given the low specificity achieved. It is important to try and strike an optimal balance between improved sensitivity and adequate specificity given the need to initiate anti-tuberculous therapy as early as possible in all TBM cases.

TBM often presents with non-specific symptoms in stage I disease, which complicates early diagnosis. Morbidity and mortality increases exponentially with disease progression. Therefore rapid diagnosis is needed to initiate treatment at the earliest opportunity. Unfortunately it is difficult to achieve a bacteriologically confirmed diagnosis at this early stage.

Maintaining a high index of suspicion and using a combination of clinical, laboratory and neuroimaging criteria for early identification of TBM suspects, remains essential for early treatment initiation. In two previous studies (one comprising both adults and children and the other comprising only adult patients) comparing TBM and bacterial meningitis features suggestive of TBM included: young age, sub-acute onset, headache, peripheral white blood cell count, clear CSF appearance, total CSF white cell count, low CSF neutrophil proportion, and elevated CSF protein [13,16]. Good sensitivities and specificities were obtained using both a composite clinical reference standard, and when applied to a microbiologically proven *M. tuberculosis* reference standard [14].

Our study was limited by absence of data regarding pre-contrast basal hyperdensity, suggestive CT, MRI or ultrasound imaging of TB outside the CNS and positive PCR evidence of extra neural TB. Pre-contrast basal hyperdensity was described only in 2004 and was therefore not available [19]. Univariate analysis showed that the majority of criteria were associated with a diagnosis of TBM, which warrants inclusion in the proposed research algorithm [12]. The uniform research TBM case definition needs to be tested robustly in future prospective studies including bacterial meningitis, viral meningitis and normal CSF control groups, and in both adult and pediatric populations. It should be noted that while some of the TBM data were collected prospectively, all bacterial meningitis data were collected retrospectively.

It is important to appreciate that the proposed research case definition criteria were not designed for use in clinical practice. The normal clinical examination findings in stage I TBM are a limitation of the diagnostic criteria. It would therefore be less likely to score as probable TBM at an earlier stage of disease. The uniform case definition holds promise for research purposes, also in resource-constrained settings with limited availability of more sophisticated diagnostic methods until better rapid diagnostic testing becomes available. Larger prospective studies are required where the uniform case definition is robustly tested.

CONCLUSION

The proposed uniform TBM research case definition performed well when using a 'probable' TBM score to differentiate childhood TBM and bacterial meningitis. Rigorous data collection is essential to obtain the necessary information in all criterion categories and should assist comparability of future prospective studies. However, caution is needed when applying the uniform research case definition to clinical diagnosis of children with early TBM.

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CONFLICT OF INTEREST

None of the authors had to declare a conflict of interest.

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3.2 Diagnostic accuracy of a uniform tuberculous meningitis research case definition in children

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ABSTRACT

BACKGROUND: Bacteriological confirmation of tuberculous meningitis (TBM) is problematic and rarely guides clinical management. A uniform TBM case-definition has been proposed for research purposes, but its diagnostic accuracy in a clinical setting has not been evaluated.

METHODS: We prospectively enrolled patients with meningitis confirmed by cerebrospinal fluid analysis aged 3 months to 13 years at Tygerberg Children's Hospital, Cape Town, South Africa. Participants were investigated for TBM, bacterial and viral meningitis. Criteria that differentiated TBM from other causes were explored and the accuracy of a probable TBM score assessed by comparing bacteriologically-confirmed cases to "non-TBM" controls.

RESULTS: Of 139 meningitis suspects, 79 were diagnosed with TBM (35 bacteriologically-confirmed), 10 with bacterial meningitis and 50 with viral meningitis. Among bacteriologically-confirmed TBM, 15 were *M. tuberculosis* culture positive, 20 were culture negative but positive on GenoType MTBDR*plus*® and Xpert MTB/RIF®, 18 were positive on only a single commercial nucleic acid amplification test. A probable TBM score provided good diagnostic accuracy; sensitivity 74% (95% confidence interval (57-88%) and specificity 97% (95% confidence interval 86-99%) compared to bacteriologically-confirmed TBM.

CONCLUSION: A probable TBM score demonstrated excellent specificity compared to bacteriological confirmation, justifying consideration as a "rule-in" test. However, accurate diagnosis of early TBM remains a challenge.

INTRODUCTION

In 2013, there was an estimated 9.0 million new tuberculosis (TB) cases world-wide.[1] Central nervous system involvement, mostly tuberculous meningitis (TBM), is considered to account for approximately 1% of all cases.[2] Although TB is predominantly a pulmonary disease, extrapulmonary involvement is particularly common in young children and immune-compromised individuals.[3] In TB endemic areas with access to routine *Haemophilus influenza* type-B and pneumococcal vaccination, such as Cape Town (South Africa), TBM has surpassed other causes of bacterial meningitis.[4] In clinical practice, diagnosis is based on a combination of clinical, laboratory, and neuroimaging findings.

The early clinical presentation of TBM is often non-specific. With delayed treatment initiation TBM outcome is often poor, emphasizing the importance of early and accurate diagnosis.[5,6] TBM is a paucibacillary disease and the sensitivity of cerebrospinal fluid (CSF) microscopy is low.[7] The sensitivity of CSF culture is increased compared to microscopy, but remains low and the result rarely influences clinical management due to delays of up to 6 weeks.[8-10] Nucleic-acid amplification tests (NAATs) offer the prospect of rapid and specific diagnosis, but sensitivity remains suboptimal. A recent meta-analysis assessing the accuracy of commercial NAATs for the diagnosis of TBM showed a sensitivity of 64% and specificity of 98%, compared to CSF culture.[11]

In clinical practice, TBM is diagnosed based on a combination of clinical, laboratory, and neuroimaging findings. Previous studies identified key characteristics suggestive of TBM. Initial symptoms and signs are non-specific but persistent, and include poor weight gain or weight loss, fever, and lethargy. More specific signs have a sub-acute onset and include meningeal irritation, reduced consciousness, focal neurological deficits, raised intracranial pressure, brainstem dysfunction, and cranial nerve palsies.[6] Typical CSF features include a moderately increased white cell count with lymphocyte predominance, increased protein, and decreased glucose. Apart from hyponatremia,[12] no peripheral blood test result has consistently been associated with TBM. On neuroimaging, hydrocephalus, basal ganglia infarctions, and basal meningeal enhancement are common features.[6]

Clinical prediction rules that differentiate TBM from other forms of meningitis in adults include five parameters: young age (<36 yrs), sub-acute presentation (symptom duration >5 days), normal peripheral white blood cell count, moderately raised CSF white cell count (<500 cells/ml), and lymphocyte predominance (CSF neutrophil proportion <50%).[13] Good diagnostic accuracy was obtained using both a composite clinical

case-definition and bacteriologically-confirmed TBM as reference standards,[14] but was reduced in human immunodeficiency virus (HIV)-infected patients.[15] Although macroscopically clear CSF with lymphocyte predominance may differentiate TBM from bacterial meningitis,[16] many additional characteristics (sub-acute onset, focal neurological deficit, low CSF/serum glucose ratio, and elevated CSF protein) were required to differentiate TBM from viral meningitis in adult patients.[17] Data that compare TBM to other causes of meningitis in children are limited.

The absence of standardized TBM diagnostic criteria hampers progress, since diagnostic accuracy measures are often reported against variable composite clinical reference standards. For this reason a uniform research case definition was proposed by an international panel of experts, categorizing patients as definite, probable, or possible TBM according to clinical, CSF, and neuroimaging findings (Table 1).[18] The diagnostic accuracy and potential clinical utility of the proposed case definition has not been prospectively evaluated. We aimed to describe the clinical features of children with suspected meningitis, identify criteria that differentiate bacteriologically-confirmed TBM from other forms of meningitis, and assess the diagnostic accuracy of the proposed TBM research case definition in clinical practice.

METHODOLOGY

This prospective study was conducted at Tygerberg Children's Hospital, a tertiary referral centre in Cape Town, South Africa. All children with meningitis confirmed by CSF analysis aged 3 months to 13 years were prospectively enrolled between January 2010 and June 2013.

Signs and symptoms

Patients underwent comprehensive clinical evaluation by a pediatric neurologist. A TB contact was determined as household contact within the previous 12 months with an adult receiving treatment for pulmonary TB. The following signs and symptoms were collected; fever (axillary temperature >38.5°C); seizures (generalized or partial); recent weight loss, or poor weight gain (documented in the Road to Health booklet); meningeal irritation; focal motor deficit (monoparesis, hemiparesis, quadriparesis); cranial nerve palsy (deficit in any of the cranial nerves, especially III and VI); extrapyramidal signs (dystonia and/or chorea, athetosis, ballismus); raised intracranial pressure (bulging fontanelle, setting sun sign, acute onset strabismus in infancy, papilledema in the older child).

Table 1. Diagnostic criteria in the uniform TBM research case definition[18]

	Diagnostic score
Clinical criteria (Maximum category score=6)	
Symptom duration of more than 5 days	4
Systemic symptoms suggestive of TB (1 or more of): weight loss/ (poor weight gain in children), night sweats or persistent cough > 2 weeks	2
History of recent close contact with an individual with pulmonary TB or a positive TST/IGRA in a child <10 years	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
CSF criteria (Maximum category score=4)	
Clear appearance	1
Cells: 10–500 per µl	1
Lymphocytic predominance (>50%)	1
Protein concentration greater than 1 g/L	1
CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2.2mmol/L	1
Cerebral imaging criteria (Maximum category score=6)	
Hydrocephalus	1
Basal meningeal enhancement	2
Tuberculoma	2
Infarct	1
Pre-contrast basal hyperdensity	2
Evidence of tuberculosis elsewhere (Maximum category score=4)	
Chest X-ray suggestive of active TB (excluding miliary TB)	2
Chest X-ray suggestive of miliary TB	4
CT/ MRI/ US evidence for TB outside the central nervous system	2
AFB identified or *M.tuberculosis* cultured from another source i.e. lymph node, gastric washing, urine, blood culture	4
Exclusion of alternative diagnoses- An alternative diagnosis must be confirmed microbiologically, serologically or histopathologically	
Definite TBM = AFB seen on CSF microscopy, positive CSF *M.tuberculosis* culture, or positive CSF *M.tuberculosis* commercial NAAT in the setting of symptoms/signs suggestive of meningitis; or AFB seen in the context of histological changes consistent with TB brain or spinal cord together with suggestive symptoms/signs and CSF changes, or visible meningitis (on autopsy).	
Probable TBM = total score of ≥12 when neuroimaging available = total score of ≥10 when neuroimaging unavailable	
Possible TBM = total score of 6-11 when neuroimaging available = total score of 6-9 when neuroimaging unavailable	

TBM- tuberculous meningitis, TB- tuberculosis, TST- tuberculin skin test, IGRA- interferon gamma-release assay, CSF- cerebrospinal fluid, CT- computed tomography, MRI- magnetic resonance imaging, US- ultrasound, AFB- acid-fast bacilli, NAAT- nucleic acid amplification test

Clinical procedures

Routine special investigations included peripheral blood for full blood count and differential, renal function tests, liver transaminases, tuberculin skin testing, sputum or gastric washing microscopy and culture for *Mycobacterium tuberculosis* (*M.tuberculosis*), blood culture, and chest radiography. HIV screening included HIV enzyme-linked immunosorbent assay in children >18 months and <18 months without maternal HIV exposure (ARCHITECT® HIV Ag/Ab Combo; Abbott Laboratories, Abbott Park, IL, USA). HIV DNA polymerase chain reaction (PCR) was performed in children <18 months exposed to maternal HIV or where the HIV enzyme-linked immunosorbent assay screening test was positive (Roche CAP/CTM HIV-1 assay; Roche Molecular Systems, Branchburg, NJ, USA). Neuroimaging, including brain computed tomography (CT), and magnetic resonance imaging, was performed if clinically indicated (routine in TBM, but not in uncomplicated viral and bacterial meningitis).

CSF, by lumbar puncture, was evaluated in all patients including macroscopic appearance, total and differential cell count, protein, glucose, chloride, Gram stain, India ink examination, auramine "O" fluorescence microscopy, culture for pyogenic bacteria, culture for *M.tuberculosis*, GenoType MTBDR*plus*® and GeneXpert MTB/RIF® assays. When viral meningitis was suspected, a CSF PCR panel for cytomegalovirus, Epstein-Barr virus, enteroviruses, human herpesvirus-6, herpes simplex 1 & 2 and varicella zoster was performed.

For *M.tuberculosis* culture, 0.5 ml of CSF was directly inoculated into a Mycobateria Growth Indicator Tube (MGIT; Becton Dickinson, Sparks, MD, USA) and incubated at 37°C. The presence of acid-fast bacilli was verified by Ziehl-Neelsen staining and microscopy. The Genotype MTBDR*plus*® assay analyzed a CSF volume of 0.5ml, with a 160 colony forming unit/ml limit of detection. Improvements in the DNA extraction from sonication and heat (version 1) to a chemical method (version 2), enabled its usage on smear microscopy-positive and -negative samples. For the *Xpert MTB/RIF*® assay, an aliquot of 1 ml CSF was mixed with 2 ml Xpert Sample Reagent (Cepheid) and incubated at 37°C. All further processing happened automatically (GeneXpert Dx 4.0, Cepheid). Bacterial load was semi-quantitatively reported, with rifampicin resistance indicated separately. The limit of detection was 100 colony forming units/ml.

Descriptive case definitions

For descriptive purposes children were categorized as TBM and non-TBM. TBM was clinically diagnosed when CSF changes were suggestive of TBM (clear appearance and pleocytosis 10-500/µl and/or increased protein >1g/dl, and/or decreased glucose defined as <2.2mmol/l or CSF to serum ratio of <50%) and at least two of the follow-

ing criteria were met: 1) recent contact with an infectious TB source case or a positive tuberculin skin test, 2) a chest x-ray suggestive of TB, 3) CT or magnetic resonance imaging demonstrating features of TBM (hydrocephalus, meningovascular enhancement, infarction, and/or granuloma/s).[19] TBM was staged according to revised British Medical Research Council criteria as: Stage I) Glasgow Coma Scale (GCS) of 15 and no focal neurology, Stage IIa) GCS of 15 plus focal neurology, Stage IIb) GCS of 11-14 with focal neurology and Stage III) GCS <11.[20,21]

Non-TBM included bacterial and viral meningitis. Bacterial meningitis was identified by either 1) microscopy and/or culture confirmation of a bacterial pathogen on CSF or 2) culture confirmation of a bacterial pathogen on blood and purulent CSF.[22] Viral meningitis was diagnosed when a viral pathogen was identified by CSF PCR, or the clinical outcome was favourable with only supportive care and other causes of meningitis excluded.[17]

Comparative case definitions

For comparative purposes, children with bacteriologically-confirmed (definite) TBM were used as the reference standard. "Definite TBM" included meningitis suspects with acid-fast bacilli seen on CSF microscopy, positive CSF *M.tuberculosis* culture and/or detection by commercial NAAT. "Definite TBM" cases were differentiated into probable and possible TBM cases (not taking into account bacteriological confirmation) in order to evaluate the proposed research case definition criteria,[18] with points allocated for 1) clinical presentation, 2) CSF findings, 3) neuroimaging, 4) evidence of extraneural TB and 5) additional laboratory criteria (Table 1). "Probable TBM" required a score >9 if neuroimaging was not performed and >11 if neuroimaging was performed, while "possible TBM" required a score 6-9 if neuroimaging was not performed and 6-11 if neuroimaging was performed.

STATISTICAL ANALYSIS

Data analysis was performed using Statistical Package for the Social Sciences version 21 (SPSS Inc, Chicago, IL, USA). For descriptive purposes, frequencies were determined for categorical variables, with median and interquartile range reflected for continuous variables. The X^2 and Fisher's exact tests were used to assess differences between continuous and categorical variables respectively. The level of significance was set at $p < 0.05$ (2-sided). For diagnostic accuracy assessment a multivariable logistic regression model was constructed to identify criteria that were independently associated with bacteriologically-confirmed TBM, compared to "non-TBM". Three

criteria that were identified by both forward and backward stepwise selection at the 15% level were then used to construct a receiver operating characteristic curve. The diagnostic accuracy between the multivariable model and a probable TBM score using criteria from the proposed uniform research case definition was compared.

The study was approved by the Human Research Ethics Committee of Stellenbosch University, South Africa (study nr. N11/01/006).

RESULTS

We identified 139 children with suspected meningitis: 79 were bacteriologically or clinically diagnosed with TBM, 10 with bacterial meningitis and 50 with viral meningitis.

Table 2 reflects the relevant demographic and clinical characteristics. Few TBM patients presented with early (stage I) disease; 85% had stage II or III disease. Four bacterial meningitis cases had positive CSF cultures; 2 *Streptococcus pneumoniae* and 2 *Neisseria meningitides*. Twenty children with viral meningitis had viral pathogens detected by PCR in their CSF, including cytomegalovirus (3), Epstein-Barr virus (2), herpes simplex type-2 virus (1), human herpes virus-6 (2) and human enterovirus (12). Among those clinically diagnosed with TBM, 35/79 (44%) had bacteriological confirmation; 3/35 (9%) by microscopy, 15/35 (43%) by culture, and 29/35 (83%) by commercial NAAT.

Table 3 shows the bacteriological yield achieved with various methods. Among commercial NAATs, the sensitivity of GenoType MTBDR*plus*® was (20/35; 57%) and Xpert MTB/RIF® (14/35; 40%). With one exception, all cases with a positive

Table 2. Demographics, symptoms and signs in children with TB, bacterial and viral meningitis

	TB meningitis*	Bacterial meningitis	Viral meningitis
DEMOGRAPHICS			
		Median (IQR)	
Age (in months)	31 (21-54)	29 (20-81)	62 (22-92)
Monthly income (US $)	150 (50-250)	120 (28-200)	173 (73-300)
		n (%)	
Gender (male)	41 (52)	5 (50)	38 (76)
Informal housing	29 (37)	3 (30)	13 (26)
Clinic visits	2 (2-3)	2 (2-3)	2 (2-3)
TB contact	30 (38)	4 (40)	23 (46)

Table 2 (continued)

	TB meningitis*	Bacterial meningitis	Viral meningitis
SYMPTOMS			
Fever	62 (79)	9 (90)	44 (88)
Vomiting	51 (65)	7 (70)	35 (70)
Seizures	24 (30)	2 (20)	7 (14)
Weight loss	20 (25)	1 (10)	10 (20)
Coughing	26 (33)	4 (40)	14 (28)
Poor feeding	49 (62)	5 (50)	27 (54)
Headache	27 (34)	3 (30)	32 (64)
>5 days duration	45 (57)	3 (30)	10 (20)
SIGNS			
GCS <11	27 (34)	4 (40)	4 (8)
11-14	31 (39)	3 (30)	1 (2)
Normal	21 (27)	3 (30)	45 (90)
Meningism	22 (28)	4 (40)	19 (38)
Focal motor deficit	28 (35)	3 (30)	0 (0)
Cranial nerve palsy	25 (32)	1 (10)	0 (0)
Extrapyramidal signs	6 (8)	0 (0)	0 (0)
Raised ICP	11 (14)	3 (30)	3 (6)
Brainstem dysfunction	9 (11)	2 (20)	2 (4)
TOTAL	**79**	**10**	**50**

*As per descriptive case definition defined in methods; 15% TBM stage I, 15% stage IIa, 35% stage IIb, 35% stage III
TB – tuberculosis; GCS – Glasgow Coma Scale; ICP – intracranial pressure

NAAT test on CSF had clinical and radiological signs suggestive of TBM. A single case diagnosed as stage I TBM on account of a positive GenoType MTBDR, presented with non-specific symptoms and CSF pleocytosis but had no other clinical, CSF, or imaging findings typical of TBM. Of the 29 cases diagnosed by NAAT, 21 only tested

Table 3. Overview of bacteriological confirmation achieved in 35 children with "definite" TB meningitis.

Bacteriological confirmation from CSF	Smear microscopy	MGIT® culture	Xpert MTB/RIF®	GenoType MTBDR*plus*®
Smear microscopy	**3** (0)*	2	0	1
MGIT® culture	2	**15** (1)*	5	7
Xpert MTB/RIF®	0	5	**14** (4)*	5
GenoType MTBDR*plus*®	1	7	5	**20** (7)*

CSF- cerebrospinal fluid
*The number in brackets reflects the patients who tested positive with a particular method only

positive for one of the two NAAT tests used in this study. CSF volume recorded in 52 patients (42 TBM and 10 non-TBM) was relatively small (mean 2.1ml: range 0.5 ml to 5.0 ml), with no clear correlation between CSF volume and diagnostic yield.

Table 4 summarizes laboratory and radiological features of children diagnosed with TBM compared to those diagnosed with bacterial and viral meningitis. Overall the

Table 4. Special investigations and imaging findings in children with TB, bacterial and viral meningitis

	Bacteriologically confirmed TBM	Not bacteriologically confirmed TBM	Bacterial meningitis	Viral meningitis
		n (%)		
HIV-infected	3 (9)	3 (7)	2 (20)	2 (4)
TST ≥10mm	7 (20)	14 (32)	0 (0)	3 (6)
Hyponatremia*	12 (34)	12 (27)	1 (10)	4 (8)
CSF				
		n (%)		
Clear	33 (94)	38 (86)	6 (60)	45 (90)
Leucocytes 10-500 cells/L	28 (80)	32 (73)	8 (80)	42 (84)
Lymphocytes > 50%	31 (89)	37 (84)	6 (60)	34 (68)
Protein >1g/L	24 (69)	24 (55)	5 (50)	7 (14)
Glucose <2.2mmol/L	16 (46)	19 (43)	6 (60)	5 (10)
		Median (IQR)		
Leucocytes (cells/uL)	129 (63-268)	36 (11-133)	279 (72-558)	70 (36-174)
Neutrophils (cells/uL)	12 (4-31)	1 (0-7)	46 (6-369)	11 (0-54)
Lymphocytes (cells/uL)	122 (50-215)	35 (9-114)	127 (28-401)	40 (23-134)
Protein (g/L)	1.9 (1.0-2.0)	1.2 (0.8-2.0)	1.6 (0.8-2.2)	0.4 (0.2-0.8)
BRAIN CT				
		n (%)		
Precontrast basal hyperdensity	5 (14)	10 (23)	1 (10)	0 (0)
Hydrocephalus	24 (69)	38 (86)	4 (40)	1 (2)
Infarctions	4 (11)	18 (41)	1 (10)	1 (2)
Basal meningeal enhancement	24 (69)	35 (80)	3 (30)	2 (4)
Tuberculoma(s)	5 (14)	9 (20)	0 (0)	0 (0)
Extraneural TB				
CXR suggestive of TB	14 (40)	19 (43)	1 (10)	7 (14)
***M.tuberculosis* isolated**	11 (31)	11 (25)	0 (0)	1 (2)
TOTAL	**35**	**44**	**10**	**50**

* Serum sodium <130mmol/L

TBM – tuberculous meningitis; TST- tuberculin skin test; CSF- cerebrospinal fluid; CT- computed tomography

proportion of HIV-infected children was low (9%) and not increased among TBM cases. The vast majority of TBM patients had neuroimaging abnormalities with hydrocephalus and basal meningeal enhancement the most common findings.

Table 5 includes uni- and multivariate analyses assessing the diagnostic value of various clinical, laboratory and CT criteria in TBM and non-TBM cases; the multivariable

Table 5. Analysis of clinical, laboratory and radiological criteria in children with bacteriologically-confirmed TB meningitis compared to "non-TB meningitis"

Criteria	OR (95% CI)	p value
HIV-infected	1.08 (0.27-5.15)	0.93
Sub-acute onset	3.10 (1.27-7.58)	0.01
Seizures	2.27 (0.82-6.29)	0.11
Weight loss	1.54 (0.57-4.20)	0.40
TB contact	0.82 (0.35-1.90)	0.64
GCS <15	6.77 (2.66-17.21)	<0.01
Focal deficit	6.58 (1.64-26.31)	<0.01
Cranial nerve palsy	23.60 (2.87-194.30)	<0.01
Raised ICP	1.50 (0.42-5.33)	0.53
TST ≥10mm	4.75 (1.14-19.77)	0.02
Serum Na <130mmol/L	4.66 (1.41-15.41)	<0.01
CSF clear	2.91 (0.59-14.33)	0.17
CSF 10-500 cells/uL	0.80 (0.27-2.34)	0.68
CSF lymphocytes >50%	3.88 (1.20-12.50)	0.02
CSF protein >1g/L	10.22 (3.78-27.66)	<0.01
CSF glucose <2.2mmol/L	4.99 (1.89-13.18)	<0.01
CT hydrocephalus	7.86 (2.32-26.63)	<0.01
CT infarctions	1.36 (0.23-8.08)	0.74
CT basal meningeal enhancement	7.86 (2.32-26.63)	<0.01
CT tuberculoma(s)	*0 non-TBM pts	0.06
Chest X-ray suggestive of TB	2.33 (0.81-6.75)	0.11
***M.tuberculosis* from extraneural source**	9.43 (1.06-84.04)	0.02
Criteria that differentiated TBM from non-TBM on multivariable analysis*		
Cranial nerve palsy	9.94 (0.89-110.86)	0.06
CSF protein >1g/L	11.55 (3.72-35.87)	<0.01
TST ≥10mm	3.89 (0.65-23.33)	0.14

OR - odds ratio, CI - confidence interval; TST - tuberculin skin test; GCS - Glasgow Coma Scale; ICP - intracranial pressure, CSF - cerebrospinal fluid; CT – computed tomography; TB – tuberculosis; OR-odds ratio; CI - confidence interval

*Neuro-imaging criteria had to be excluded since these were performed on a minority of non-TBM patients

logistic regression analysis excluded neuroimaging findings, since this was performed in a minority (62/157; 39%) of non-TBM cases. Three variables were significantly associated with TBM on multivariable analysis; cranial nerve palsy, TST ≥10mm and CSF protein >1g/L. However these three criteria had sensitivity of only 0.50, 95% confidence interval (CI) 0.12-0.88, in stage I disease compared to 0.88 (95% CI 0.70-0.98) in stage II and II disease. A receiver operating characteristic curve using the three-variable predictive model delivered an area under the curve of 0.82 (95% CI 0.73-0.92); sensitivity 0.79 (95% CI 0.61-0.91) and specificity 0.78 (95% CI 0.65-0.88) (Figure 1).

Of 79 children clinically diagnosed with TBM, 66 scored as "probable TBM", 12 as "possible TBM", and one as "not TBM" using the proposed research case definitions. Using clinically diagnosed TBM cases as the reference standard, a probable TBM score had sensitivity of 0.84 (95% CI 0.74-0.91) and specificity of 0.95 (95% CI 0.86-0.99). The ability to detect stage I TBM was sub-optimal with a sensitivity of only 42% (5/12). Among 35 bacteriologically-confirmed TBM cases ("definite TBM"), 26 had a probable TBM score; sensitivity 0.74 (95% CI 0.57-0.88) and specificity 0.95 (95% CI 0.86-0.99). A possible TBM score had greater sensitivity 0.97 (95% CI 0.85-1.00), but reduced specificity 0.48 (95% CI 0.35-0.62).

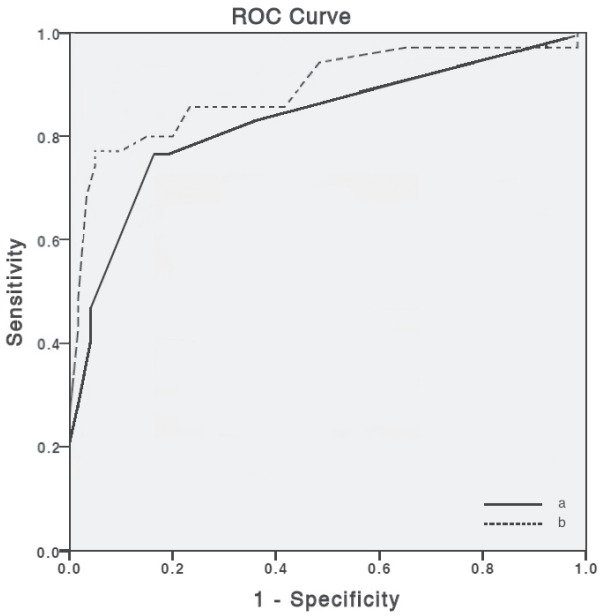

Figure 1. ROC curves of a) a "probable TBM" score and b) the three criteria identified on multivariable analysis*; using bacteriologically-confirmed TBM" as the reference standard
*Cranial nerve palsy, elevated CSF protein and a TST ≥10mm (Table 5)

DISCUSSION

This study represents the first prospective evaluation of the diagnostic accuracy of the recently proposed uniform TBM research case definition. A probable TBM score demonstrated reasonable sensitivity, 84% in clinically diagnosed and 74% in bacteriologically-confirmed TBM cases, with excellent specificity. The clinical importance of a reliable clinical case definition for TBM is emphasized by the fact that bacteriological confirmation was achieved in less than half (44%) of the children clinically diagnosed with TBM, despite exhaustive evaluation. The high specificity of a probable TBM score justifies its use as an alternative reference standard to bacteriological confirmation in future studies, although the diagnosis and study inclusion of early stage 1 disease remains problematic.

The low incidence of stage I TBM in our study is consistent with other studies from TB endemic areas and underlines the difficulty in diagnosing TBM early.[6,23] The poor sensitivity of a probable TBM score to diagnose stage I TBM versus stage II and II TBM was disappointing but not unexpected, since scoring is based on clinical, CSF and radiological findings which all tend to become more pronounced as the disease progresses. The diagnosis of early stage I TBM remains a major clinical dilemma. Since the early presentation of TBM is so non-specific, diagnosis is essentially reliant on a very high clinical index of suspicion in vulnerable young children with recent TB exposure and persistent non-specific signs despite treatment for other possible causes. Early diagnosis and treatment of TBM is vital to ensure an optimal outcome and this poses a major clinical dilemma.[6,23]

The majority of CSF specimens tested positive by means of a single bacteriological-confirmation method, highlighting the need for multiple diagnostic tests in children suspected with TBM and optimizing bacteriological confirmation in paucibacillary CSF specimens; especially with the collection of low CSF volumes. It has been suggested that high volumes of CSF should be collected and concentrated to improve diagnostic yields, as well as provide complete drug susceptibility information.[24,25] The relatively low CSF volumes collected in this study prevented specimen concentration, however, the volumes obtained are consistent with what is achieved in routine pediatric practice. The GenoType MTBDR*plus*® assay was found to be the most sensitive single test. There are concerns about the possibility of cross-contamination with the GenoType MTBDR*plus*® assay, but this was not a major concern in the current study; 19/20 patients had multiple clinical-radiological signs consistent with TBM and a single one had CSF pleocytosis only.

A reliable clinical reference standard should be more representative of the disease spectrum seen in clinical practice and be more feasible than bacteriological confirmation in TB endemic areas. The overall performance of a probable TBM score was better than the three-variable predictive model, but the use of three simple criteria (cranial nerve palsy, TST ≥10mm and elevated CSF protein) may offer better clinical utility in areas with limited access to neuro-imaging. However, its diagnostic accuracy is likely to have been over-estimated, given that the analysis was optimized for the study cohort and the non-TBM arm included relatively few children with bacterial, fungal or complicated viral meningitis.

A number of clinical prediction rules to differentiate TBM from bacterial meningitis in adults from resource-poor settings have been proposed.[13,14,16] However, most of these were based on case-control studies and failed to include all meningitis suspects. Our study focused on a pediatric population and is strengthened by the inclusion of bacterial and viral meningitis, as encountered in every-day clinical practice. We were unable to determine the role of HIV co-infection, because of the relatively low prevalence of HIV co-infection among TBM patients in our cohort (8%). Karande et al. also found that only 6.5% of 123 children with clinically diagnosed TBM had HIV co-infection.[26] TBM seems to be proportionally less common than other TB manifestations among HIV-infected children; an intriguing finding which may reflect the contribution of an appropriate immune response to TBM pathology.[27] Immune reconstitution inflammatory syndrome, a well-recognized complication in HIV-infected patients with TBM who are initiated on antiretroviral therapy provides further support for the role of immunity in the pathogenesis of TBM.[28-30]

Our study is limited by the fact that the study cohort included few cases of bacterial meningitis. *Haemophilus influenza* type-B and pneumococcal vaccination are provided free of cost to all children in South Africa. Neuroimaging was performed in a minority of non-TBM cases, which prevented its inclusion in multivariable analysis. However, neuroimaging is not routinely performed in non-TBM suspects and ethical justification would have been problematic. The relatively low proportion of bacteriologically-confirmed TBM cases reflects the paucibacillary nature of the disease, sub-optimal sensitivity of available diagnostic tests, and low CSF volumes obtained.[5,8-11] The study was performed at a single hospital which may limit generalization of the study findings to other settings, but Tygerberg Children's Hospital serves a population that share a similar disease burden and health challenges experienced in other TB endemic areas.

In conclusion, a probable TBM score demonstrated good diagnostic accuracy in this prospective study,[18] both in children with clinically diagnosed and bacteriologically-confirmed TBM. The excellent specificity achieved support its use as an alternative reference standard in research studies, at least in settings with a similar disease spectrum among non-TBM patients, although its clinical utility is limited in resource-limited settings.

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CONFLICT OF INTEREST

None of the authors had to declare a conflict of interest.

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3.3 Chest radiograph findings in children with tuberculous meningitis

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ABSTRACT

BACKGROUND: Tuberculous meningitis (TBM) is diagnosed based on a combination of clinical, laboratory, and radiological findings, including signs suggestive of tuberculosis (TB) on a standard chest radiograph.

METHODS: We describe the radiological features suggestive of intra-thoracic TB in children diagnosed with TBM during a prospective evaluation of meningitis suspects seen at Tygerberg Children's Hospital, Cape Town, South Africa.

RESULTS: Of 84 children treated for TBM, 31 (37%) had 'definite' TBM, 45 (55%) 'probable' TBM and 8 (9%) 'possible' TBM. In total, 37 (44%) TBM patients had chest radiograph findings suggestive of TB; 9 (11%) with disseminated (miliary) TB. Only 1 in 4.39 children ≤3 years of age with TBM had suggestive chest radiograph findings. The presence of complicated intra-thoracic lymph node disease was significantly higher in children ≤3 years of age, odds ratio 21.69 (95% confidence interval 2.73-172.67); p <0.01. Among 6 HIV-infected children, 3 (50%) had intrathoracic lymphadenopathy.

CONCLUSION: The majority of children with TBM, including the very young, did not have signs suggestive of TB on chest radiograph.

INTRODUCTION

Globally there were an estimated 8.6 million new cases and 1.3 million deaths from tuberculosis (TB) in 2012[1]. TB is predominantly a pulmonary disease, but extrapulmonary involvement is particularly common in young children and in immunocompromised individuals[2]. Central nervous system (CNS) involvement, mostly tuberculous meningitis (TBM), accounts for approximately 1% of all TB cases[3]. TBM is the most devastating manifestation of TB and early treatment initiation is critical to achieve optimal outcomes[4]. In clinical practice, diagnosis is hampered by non-specific clinical features and sub-optimal accuracy of existing diagnostic methods[5-9], therefore TBM is mainly diagnosed based on a combination of clinical, laboratory, and radiological findings.

A uniform research case definition was proposed by an international panel of experts, categorizing patients as definite, probable, or possible TBM (Table 1)[10]. Chest radiograph findings are included within the scoring criteria. Previous observations suggest that chest radiograph findings consistent with active pulmonary TB are observed in 30% to 65% of adults with central nervous system TB[11-13]. Chest radiograph evidence of TB in children with TBM is usually considered to be more frequent, ranging from 70% of HIV-uninfected to 84% of HIV-infected children diagnosed with TBM[14]. In the group of children with TBM and HIV co-infection, hilar lymphadenopathy, pleural effusion and cavity formation was significantly increased compared to HIV uninfected children[14].

We aimed to describe the radiological features and frequency of chest radiograph signs suggestive of TB in children with TBM.

METHODOLOGY

This prospective descriptive cross-sectional study was conducted at Tygerberg Children's Hospital, South Africa, a major referral centre for Cape Town and surrounding areas. Children were enrolled between January 2010 and January 2014. Inclusion criteria were 1) age 3 months to 13 years 2) clinical suspicion of TBM 3) CSF evaluation 4) chest radiograph performed at admission 5) written consent from the caregiver and assent if the child was older than 7 years and competent to do so. The study was approved by the Human Research Ethics Committee of Stellenbosch University, South Africa (study nr. N11/01/006).

Clinical procedures

All patients underwent a comprehensive clinical evaluation. Routine investigations included full blood count, basic biochemistry, human immunodeficiency virus (HIV) screening, tuberculin skin test (TST), microbiological analysis of sputum or gastric washings, bacterial blood culture, chest radiography and neuroimaging (if clinically indicated). Chest radiographs were independently interpreted by a pediatrician and an experienced pediatric pulmonologist, using a standard reporting form. Findings were categorized as certain TB, uncertain TB or not TB. Intra-thoracic lymph node disease was classified as either uncomplicated or complicated, accordingly to a radiological classification of childhood intrathoracic tuberculosis, using a structured approach to interpretation and recording chest radiograph findings[15]. Airway compression was defined as either compression of the trachea, left main bronchus or bronchus intermedius. Parenchymal changes were defined as either consolidation (including expansile pneumonia) or miliary.

Case definition of tuberculous meningitis

A diagnosis of TBM was based on the proposed uniform research case definition (Table 1)[10]. TBM was classified as 'definite' when CSF demonstrated acid-fast bacilli, positive *M. tuberculosis* culture and/or positive *M. tuberculosis* commercial nucleic acid amplification test (NAAT). TBM was classified as 'probable' when patients scored ≥ 12 when neuroimaging was available and ≥10 when neuroimaging was unavailable. TBM was classified as 'possible' when a patient had a diagnostic score of 6–11 when neuroimaging was available and 6–9 when neuroimaging was unavailable[10]. TBM was staged according to revised British MRC criteria as: Stage I) Glasgow Coma Scale (GCS) of 15 and no focal neurology, Stage IIa) GCS of 15 plus focal neurology, Stage IIb) GCS of 11-14 with focal neurology and Stage III) GCS <11[16,17]. All patients diagnosed with TBM were treated with a standard short-course regimen[18].

Statistical analysis

Data analysis was performed using SAS (Statistical analysis system) version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). Frequencies were obtained for chest radiograph findings, stratified for TBM stage. An unweighted kappa statistic was used to assess inter-observer agreement. Comparison was made, using the X^2 test with a p-value <0.05 considered statistically significant. Chest radiograph criteria were further compared between children ≤3 years and age >3 years and odds ratios determined. A need to treat calculation was used to reflect the number of TBM patients ≤3 years of age with "certain TB" on chest radiograph evaluation.

Table 1. Diagnostic criteria in the uniform TBM research case definition[10]

	Diagnostic score
Clinical criteria (Maximum category score=6)	
Symptom duration of more than 5 days	4
Systemic symptoms suggestive of TB (1 or more of): weight loss/(poor weight gain in children), night sweats or persistent cough > 2 weeks	2
History of recent close contact with an individual with pulmonary TB or a positive TST/IGRA in a child <10 years	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
CSF criteria (Maximum category score=4)	
Clear appearance	1
Cells: 10–500 per µl	1
Lymphocytic predominance (>50%)	1
Protein concentration greater than 1 g/L	1
CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2·2mmol/L	1
Cerebral imaging criteria (Maximum category score=6)	
Hydrocephalus	1
Basal meningeal enhancement	2
Tuberculoma	2
Infarct	1
Pre-contrast basal hyperdensity	2
Evidence of tuberculosis elsewhere (Maximum category score=4)	
Chest X-ray suggestive of active TB (excluding miliary TB)	2
Chest X-ray suggestive of miliary TB	4
CT/ MRI/ US evidence for TB outside the CNS	2
AFB identified or *M.tuberculosis* cultured from another source i.e. lymph node, gastric washing, urine, blood culture	4
Exclusion of alternative diagnoses- An alternative diagnosis must be confirmed microbiologically, serologically or histopathologically	
Definite TBM = AFB seen on CSF microscopy, positive CSF *M.tuberculosis* culture, or positive CSF *M.tuberculosis* commercial NAAT in the setting of symptoms/signs suggestive of meningitis; or AFB seen in the context of histological changes consistent with TB brain or spinal cord together with suggestive symptoms/signs and CSF changes, or visible meningitis (on autopsy).	
Probable TBM = total score of ≥12 when neuroimaging available = total score of ≥10 when neuroimaging unavailable	
Possible TBM = total score of 6-11 when neuroimaging available = total score of 6-9 when neuroimaging unavailable	

TBM- tuberculous meningitis, TB- tuberculosis, TST- tuberculin skin test, IGRA- interferon gamma-release assay, CSF- cerebrospinal fluid, CT- computed tomography, MRI- magnetic resonance imaging, US- ultrasound, AFB- acid-fast bacilli, NAAT- nucleic acid amplification test

RESULTS

In total 84 children met the inclusion criteria; 31 (37%) had 'definite/microbiologically-confirmed' TBM, 45 (55%) had 'probable' TBM and 8 (9%) had 'possible' TBM[10]. According to revised British MRC TBM staging criteria, 12 (14.3%) had stage I, 13 (15.5%) stage IIa, 30 (35.7%) stage IIb and 29 (34.5%) stage III disease. Tuberculin skin testing was positive in 24 (28.6%) TBM patients, of whom 11/37 (29.7%) had certain pulmonary TB on chest radiograph, 10/39 (25.6%) had no visible abnormality, and 3 had inconclusive signs. HIV co-infection was identified in 6 patients. Of these, 3 had no radiographic evidence of pulmonary TB. Of the three HIV-infected patients with abnormal chest radiograph, one had isolated lymph node involvement, one lymph node involvement plus lobar pneumonia and the third lymph node involvement plus a miliary picture.

A summary of chest radiograph findings is reflected in table 2. Inter-reviewer variability between the pediatrician and pediatric pulmonologist was minimal (unweighted kappa statistic 0.62 95% confidence interval (CI) 0.46-0.79); differences were resolved by consensus. The proportion of 'certain TB' and miliary TB on chest

Table 2. Demographics and chest radiograph findings in 84 children with childhood TBM

Demographics	**n/N (%)**
Male	43/84 (51)
Age group	
≤3 years	46/84 (55)
>3 years	38/84 (45)
Chest radiograph findings	
Normal CXR	39/84 (46)
Abnormal CXR (not TB)	5/84 (6)
Abnormal CXR (Uncertain TB)	3/84 (4)
Abnormal CXR (certain TB)	37/84 (44)
Miliary TB	9/84 (11)
Parenchymal consolidation	15/84 (18)
Intrathoracic lymphadenopathy	32/84 (38)
Paratracheal	16/84 (19)
Hilar	24/84 (29)
Complicated lymph node disease	18/84 (21)
Airway compression	16/84 (19)
Bronchus intermedius	8/84 (10)
Left main bronchus	8/84 (10)

TBM= tuberculous meningitis, CXR= chest radiograph, TB= tuberculosis

radiograph was 44% (37/84) and 11% (9/84) when including all TBM categories; 39% (12/31) and 13% (4/31) respectively in those with microbiologically-confirmed TBM. Among those with microbiological TBM confirmation, 1/31 (3%) had acid-fast bacilli on microscopy, 13/31 (42%) were *M. tuberculosis* culture positive, and 27/31 (87%) confirmed by commercial NAAT (Either GenoType MTBDR*plus*® assay and/or GeneXpert MTB/RIF® assay). *M. tuberculosis* was cultured in gastric washings from 27 patients; 10 with microbiologically-confirmed and 17 with 'probable' TBM. No significant differences were observed when comparing chest radiograph findings in children with different stages of TBM.

Chest radiographs were more frequently indicative of TB in very young children (≤3 years of age) compared to older children (>3 yrs); 25/46 (54%) versus 12/38 (32%) (odds ratio (OR) 2.58 (95 % CI 1.05-6.33; p=0.04 (Table 3). Chest radiograph findings in children ≤3 years of age were more likely to include complicated intra-thoracic lymph node disease (OR 21.69; 95% CI 2.73-172.67), and the presence of airway compression (OR 17.90; 95% CI 2.24-143.27) than in older children Despite the fact that chest radiographs were most informative in young children, only 1 in 4.39 children ≤3 years of age had "certain TB" on chest radiograph evaluation.

Table 3. Chest radiograph findings in children investigated for TBM, comparing very young (≤3 years) to older children

	≤3 years (n=46)	>3 years (n=38)	Odds ratio (95% CI)	p-value
Normal CXR	17	22	0.43 (0.18-1.03)	0.06
Abnormal CXR (not TB)	3	2		
Abnormal CXR (Uncertain TB)	1	2		
Abnormal CXR (certain TB)	25	12	2.58 (1.05-6.33)	0.04
Miliary TB	5	4	1.04 (0.26-4.17)	0.96
Parenchymal consolidation	12	4	3.00 (0.88-10.24)	0.07
Intra-thoracic lymphadenopathy	23	9	3.22 (1.25-8.29)	0.02
Complicated lymph node disease	17	1	21.69 (2.73-172.67)	<0.01
Airway compression (any)	15	1	17.90 (2.24-143.27)	<0.01

TBM= tuberculous meningitis, CI= confidence interval, CXR= chest radiograph, TB= tuberculosis, LAD= lymphadenopathy

DISCUSSION

The main finding in this study is the lower percentage of chest radiograph findings suggestive of certain pulmonary TB in children with TBM, compared to the study by van Weert et al (44% vs 70%)[14], with a need to treat calculation showing that only

1 in 4.39 of children ≤3 years of age with TBM are likely to have certain TB on chest radiograph. The lower proportion of certain pulmonary TB in our study compared to van Weert et al could potentially be explained by our 2 reviewers reaching consensus on chest radiograph findings thereby minimizing the possibility of over-reporting of chest radiograph findings.[14] Another possible reason could be the difference in the study cohort, with 28% of our TBM group having positive tuberculin skin testing compared to 62% in the HIV-uninfected TBM group of the study by van Weert et al.[14] A normal chest radiograph was found in almost half (46%) of children clinically diagnosed with TBM, and in 52% of cases with microbiologically-confirmed TBM. This less than expected diagnostic sensitivity of the chest radiograph in TBM may impact the scoring of future diagnostic algorithms for the disease.

The most common radiological findings in young children (<5 years) with pulmonary TB is hilar or paratracheal lymph nodes[19]. This age group also has a higher risk for developing lympho-bronchial TB due to small airway size[15]. Our findings on chest radiograph of visible lymph nodes, complicated lymph node disease and airway compression were significant in children ≤3 years, confirming that this is the predominant radiological finding in young children. TBM stage did not affect the radiographic picture.

Sixteen of eight-four (19%) patients had radiological evidence of airway compression highlighting the need for pulmonary assessment, including flexible bronchoscopy[20]. The smaller percentage of patients with airway compression, compared to a reported figure of 41-63%[20], could possibly be explained by differences in the immune response between pulmonary TB in isolation versus pulmonary TB in the setting of CNS involvement. A better understanding of immunology in CNS TB is warranted[3].

Inter-observer variability is a well-recognized problem in the interpretation of chest radiographs in children with pulmonary TB. Swingler et al have reported difficulty in distinguishing lymphadenopathy from a normal thymus and were not able to distinguish normal from pathological nodes[21]. The areas most reliable for lymphadenopathy were the right hilum and the sites around the carina[22].

The uniform research case definition for TBM by Marais et al. uses chest radiograph findings as part of the scoring criteria[10]. The score weighting of a miliary pattern is higher than that of active TB on chest radiograph (excluding miliary TB). Our finding that hilar lympadenopathy is the more common finding of certain TB on chest radiograph, in both suspected and definite TBM, suggests that its weighting may have to be reconsidered.

A limitation of our study was the small number of HIV co-infected patients (7%), which did not allow separate statistical analysis of this group. This low percentage is consistent with a previous study that found that only 7% of 123 children with clinically diagnosed TBM had HIV co-infection[23]. The low number of microbiologically-confirmed TBM cases, (31/84), is another limitation but not unexpected in a cohort of this age group[5-9]. CSF NAATs offer improved sensitivity (27/84; 32.1%), compared to CSF culture (13/84; 15.5%), and a potential for same day diagnosis. A further limitation is that all the chest radiographs were from patients with TBM. A sample that includes other forms of meningitis could allow improved analysis of disseminated TB (miliary pattern on chest radiograph).

The main finding of this study is that about half of children with suspected and proven TBM have a normal chest radiograph. Significant chest radiograph findings in children ≤3 years of age were the presence of certain TB, complicated lymph node disease and the presence of airway compression. However, only 1 in 4.39 of children ≤3 years of age with TBM were likely to have certain TB on chest radiograph. Apart from a lower proportion of airway compression, the radiological findings of pulmonary TB in the setting of TBM, irrespective of stage of disease, did not differ from those reported for pulmonary TB in isolation. Definite evidence of TB on a chest radiograph remains valuable supportive evidence for TBM in a patient suspected of having the disease. However, in cases with a normal chest radiograph, diagnosis of TBM depends even more on microbiological confirmation of the disease.

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CONFLICT OF INTEREST

None of the authors declared a conflict of interest.

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3.4 The diagnostic value of cerebrospinal fluid chemistry results in childhood tuberculous meningitis

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ABSTRACT

PURPOSE: Cerebrospinal fluid (CSF) hypoglycorrhachia and elevated protein is well-described in bacterial meningitis, but evidence for its differential diagnostic value in tuberculous meningitis (TBM) is lacking. We aimed to assess the diagnostic utility of CSF glucose, CSF to serum glucose ratio and CSF protein in children with suspected TBM.

METHODS: We describe CSF glucose and protein values, as well as CSF to serum glucose ratios in a prospective evaluation of TBM suspects seen at Tygerberg Children's Hospital, Cape Town, South Africa from January 1985 to January 2014.

RESULTS: Of 615 TBM suspects, 88 (14%) had microbiologically-confirmed TBM, 381 (62%) 'probable' TBM and 146 (24%) 'no-TBM'. Mean absolute CSF glucose concentration was significantly lower in the microbiologically-confirmed (1.87 +/-1.15 mmol/L, and 'probable' TBM (1.82 +/-1.19 mmol/L) groups compared to non-TBM (3.66 +/-0.88 mmol/L). A CSF glucose concentration of <2.2 mmol/L diagnosed TBM with sensitivity 0.68 and specificity 0.96. Sensitivity using a CSF:serum glucose ratio of <0.5 was 0.90. Mean CSF protein was significantly elevated in the microbiologically-confirmed TBM (1.91 +/-1.44 g/L), and 'probable' TBM (2.01 +/-1.49 g/L) groups compared to the non-TBM (0.31 +/-0.31 g/L). A CSF protein >1g/L diagnosed TBM with sensitivity 0.78 and specificity 0.94.

CONCLUSION: Absolute CSF glucose values of <2.2 mmol/L and protein values of >1g/L differentiated between TBM and non-bacterial meningitis with good specificity, although sensitivity was poor. A CSF:serum glucose ratio is more informative than the absolute value.

INTRODUCTION

In 2012 there were an estimated 8.6 million new cases and 1.3 million deaths from tuberculosis (TB), worldwide [1]. Although progress has been made, Africa as well as Europe are not on track in achieving the reduced prevalence and mortality targets suggested by the World Health Organization [1]. TB affects predominantly the respiratory system, and central nervous system (CNS) involvement, mostly tuberculous meningitis (TBM), accounts for approximately 1% of all TB cases [2]; none the less TBM is a major cause of TB mortality and morbidity amongst children. Diagnosis of TBM is often delayed by the non-specific clinical presentation and poor diagnostic accuracy of available investigative methods [3-6]. TBM is therefore frequently diagnosed based on a combination of clinical, laboratory, and radiological findings.

Cerebrospinal fluid (CSF) findings are integral to the diagnosis of TBM. Typical CSF findings consist of leukocytosis with lymphocyte predominance, elevated protein, abnormally decreased CSF glucose (hypoglycorrhachia) [7,8]. These findings are highly suggestive of TBM in TB endemic areas [9]. Hypoglycorrhachia is reflected as either CSF to plasma glucose ratio [7-9] or decreased CSF glucose [7,10], and is not affected by HIV co-infection [11]. Despite the determination of CSF glucose being one of the commoner investigations undertaken in pediatric practice, the diagnostic cut-off values for the diagnosis of bacterial meningitis vary considerably in different reference textbooks [12-15].

As a result of the interplay between serum and CSF glucose, exact determination of a cut-off value for CSF glucose is difficult to elucidate [16]. Other studies on meningitis suggest lower limits of absolute CSF glucose concentrations from 2.20 to 2.78 mmol/L (40-50 mg/dl) and that of CSF to serum glucose ratios range from 0.40 to 0.66 [12-15,17]. For bacterial meningitis, a CSF to serum glucose ratio below 0.4 and an absolute CSF glucose concentration less than 2.2 mmol/L was shown to be highly specific [17]. Similar evidence for values of hypoglycorrhachia in TBM is lacking. A CSF protein cut-off of >1g/L (100mg/dL) differentiated between cases of TBM and bacterial meningitis [10], as well as viral meningitis [18].

A uniform research case definition, categorizing patients as definite, probable or possible TBM, was proposed by an international panel of experts [19]. Scoring criteria to aid in the diagnosis of TBM include cerebrospinal fluid (CSF) to serum glucose ratio less than 0.5 or an absolute CSF glucose concentration less than 2.2 mmol/L and elevated CSF protein >1g/L. We aimed to describe CSF glucose, CSF to serum glucose ratio and CSF protein in children with suspected meningitis, and assess their diagnostic utility in the diagnosis of TBM.

METHODOLOGY

This study was conducted at Tygerberg Children's Hospital, Cape Town, a major referral centre for Cape Town and surrounding areas. Between January 1985 and April 2005 and between January 2010 and January 2014 children were prospectively enrolled in several TBM research studies and CSF and serum glucose prospectively determined. Inclusion criteria were 1) age 3 months to 13 years 2) clinical suspicion of TBM 3) CSF glucose and/or CSF glucose:serum glucose ratio 4) written consent from the caregiver and assent if the child was older than 7 years and competent to do so. Patients with bacterial meningitis were excluded.

Clinical procedures

All patients had comprehensive clinical assessment. Routine investigations, including full blood count, basic biochemistry, tuberculin skin test (TST), mycobacterial analysis of sputum, gastric washings and CSF, bacterial blood culture, HIV screening, chest radiography and neuroimaging, when clinically indicated. CSF was evaluated in all patients including macroscopic appearance, total and differential cell count, protein, glucose, chloride, Gram stain, India ink examination, auramine "O" fluorescence microscopy and culture for *M. tuberculosis*. CSF glucose was determined by the glucose oxidase method and CSF protein by the biuret method (currently both Beckman Synchron CX7 Clinical Systems, Beckman, Miami, FL, USA). CSF lactate was not routinely performed in meningitis suspects.

Descriptive case definitions

A diagnosis of TBM was based on the uniform research case definition by Marais et al [19]. TBM was classified as 'definite' when CSF demonstrated acid-fast bacilli and/or positive *M. tuberculosis* culture and/or positive commercial nucleic-acid amplification test for *M. tuberculosis* in a patient with symptoms or signs suggestive of the disease. Probable and possible TBM cases were identified according to the uniform research case definition criteria, with points allocated for 1) clinical presentation, 2) CSF findings, 3) neuroimaging, 4) evidence of extraneural TB and 5) additional laboratory criteria. For this study, only 'definite' and 'probable' TBM were included, as 'possible' TBM criteria had low sensitivity in the identification of TBM [20]. TBM was staged according to revised British MRC criteria as: Stage I) Glasgow Coma Scale (GCS) of 15 and no focal neurology, Stage IIa) GCS of 15 plus focal neurology, Stage IIb) GCS of 11-14 with focal neurology and Stage III) GCS <11 [21,22]. All patients diagnosed with TBM were treated with a standard short-course regimen [23].

Non-TBM included viral or no meningitis. Viral meningitis was diagnosed when a viral pathogen was identified by CSF PCR, or the clinical outcome was favourable with only supportive care and other causes of meningitis were excluded [18]. A diagnosis of "no meningitis" was made when an alternative cause could be identified that explained the clinical findings. Cases of proven bacterial meningitis were excluded, since the study focused on the value of CSF chemistry to differentiate TBM from non-bacterial meningitis.

STATISTICAL ANALYSIS

Data analysis was performed using Statistical Package for the Social Sciences version 21 (SPSS Inc, Chicago, IL, USA). For descriptive purposes, frequencies were determined for categorical variables. For continuous variables, mean and standard deviation were reflected with a normal distribution; median and interquartile range reflected for a skewed distribution. Logistic regression was used to determine odds ratios for categorical variables. The level of significance was set at $p < 0.05$. Using receiver operating characteristic (ROC) curves, optimal sensitivities and specificities were determined for different cut-off values of CSF glucose and CSF protein by comparing area under the curve (AUC). AUC of 0.7-0.8 is deemed adequate, 0.8-0.9 good and >0.9 excellent. Box plots were used to illustrate absolute CSF glucose concentration and mean CSF protein in both TBM and non-TBM.

The study was approved by the Human Research Ethics Committee of Stellenbosch University, South Africa (study nr. N11/01/006).

RESULTS

In total 615 children with suspected TBM were included; 469 TBM and 146 non-TBM. Of the TBM group, 88 patients had 'definite' TBM and 381 patients had 'probable' TBM [19]. Among non-TBM patients, there were 50 viral meningitis and 96 did not have meningitis. Twenty children with viral meningitis had viral pathogens detected by PCR in their CSF.

TBM stage, age, gender, HIV status and CSF findings are shown in table 1, 15 (3%) patients were stage I, 245 (52%) patients stage IIa, 137 (29%) patients stage IIb and 71 (15%) patients stage III. Besides CSF volume, all CSF criteria differed significantly between non-TBM and TBM groups. The most distinctive findings in the TBM group

Table 1. Clinical and CSF findings of children with suspected meningitis

Clinical characteristics		Non-TBM* (n=146)	TBM** (n=469)	p-value
TBM stage	**Stage I (n/N/%)**	n/a	15/468 (3)	
(1 unknown)	**Stage IIa (n/N/%)**	n/a	245/468 (52)	
	Stage IIb (n/N/%)	n/a	137/468 (29)	
	Stage III (n/N/%)	n/a	71/468 (15)	
Age in months- mean (SD) /N		47.70 (40.33) /146	36.063 (29.11) /463	<0.01
Male gender (n/N/%)		97/146 (66)	244/468 (52)	<0.01
Positive HIV (n/N/%)		15/146 (10)	9/469 (2)	<0.01
Macroscopically clear CSF (n/N/%)		136/145 (94)	462/467 (99)	<0.01
CSF volume - mean (SD) /N		2.23 (1.03) /11	2.20 (1.32) /33	0.94
CSF leucocytes (cells/µL) - median (IQR) /N		2.00 (0-43.00) /144	123.00 (54.00-243.00) /458	<0.01
CSF neutrophils (cells/µL) - median (IQR) /N		0 (0-3.75) /144	10.00 (2.00-38.75) /458	<0.01
CSF lymphocytes (cells/µL) - median (IQR) /N		1.50 (0-29.75) /144	94.50 (40.0-180.75) /458	<0.01
CSF protein (g/L) - mean (SD) /N		0.33 (0.31) /140	1.98 (1.48) /442	<0.01
CSF glucose (mmol/L) - mean (SD) /N		3.66 (0.88) /146	1.85 (1.18) /469	<0.01
Serum glucose (mmol/L) - mean (SD) /N		4.50 (1.29) /4	6.71 (2.21) /152	***
CSF:serum glucose ratio - mean (SD) /N		0.86 (0.62) /4	0.28 (0.17) /152	***

TBM= tuberculous meningitis, n/a= not applicable, IQR= interquartile range, HIV= Human Immunodeficiency Virus, CSF= cerebrospinal fluid

*Non-TBM: 50 viral meningitis and 96 No meningitis. Cases of bacterial meningitis were excluded

**TBM: Micobiologically-confirmed (definite) and probable TBM according to the uniform research case definition [19]

***Due to only 4 patients in the non-TBM group with recorded serum glucose, p-values were not determine

were high CSF protein and low CSF glucose. Human immunodeficiency virus (HIV) co-infection was identified in 24 patients; 9 TBM and 15 non-TBM. There was no significant difference in mean CSF glucose concentration (p=0.37) or mean CSF protein (p=0.56) between HIV-infected and -uninfected TBM patients.

The mean CSF glucose in microbiologically-confirmed TBM was 1.87 (standard deviation +/-1.15 mmol/L), that in the TBM group (comprising 'definite' and 'probable' TBM), 1.85 +/-1.18 mmol/L and 3.66 +/-0.88 mmol/L in the non-TBM group. In both microbiologically-confirmed and case-defined TBM groups, the mean CSF glucose differed significantly from the non-TBM group (both p<0.01). Mean CSF glucose did not differ between microbiologically-defined and case-defined TBM (p=0.88). Serum glucose was measured in only 4 out of 146 non-TBM patients, and in 152 out of 469 TBM patients. This precluded comparison. In the microbiologically-defined TBM group the mean serum glucose was 6.31 +/-1.27 mmol/L and the CSF:serum glucose ratio was 0.27 +/-0.14, and in the case-defined TBM group the mean serum

glucose was 6.71 +/-2.21 mmol/L and the CSF:serum glucose ratio was 0.28 +/-0.17. Again, mean serum glucose did not differ between microbiologically-defined and case-defined TBM (p=0.37).

The mean CSF protein in the microbiologically-confirmed TBM group was 1.91 +/-1.44 g/L), that in the TBM group (comprising 'definite' and 'probable' TBM), 1.98 +/-1.48 g/L) and 0.33 +/-0.31 g/L) in the non-TBM group. In both microbiologically-confirmed and case-defined TBM groups, the mean CSF protein differed significantly from the non-TBM group (both p<0.01). Mean CSF protein did not differ between microbiologically-defined and case-defined TBM (p=0.68).

Box plots illustrate absolute CSF glucose concentration and mean CSF protein in TBM and non-TBM groups (figure 1). TBM outliers with increased CSF glucose concentration numbered 14/469 patients. Age range was 6-180 months. Mean CSF lymphocyte and neutrophil counts were 162 cells/μL and 20 cells/μL; protein concentration was 2.78 g/L. Mean serum glucose was increased in these patients, 12.42 mmol/L. There were no TBM outliers with low CSF protein. Non-TBM outliers with decreased CSF glucose concentration numbered 5/146 patients. Four patients had a diagnosis of viral meningitis, 2/4 had CSF PCR confirmation of a viral pathogen, both cytomegalovirus; 1 patient did not have meningitis. Age range was 4-142 months. Mean CSF lymphocyte and neutrophil counts were 127 cells/μL and 10 cells/μL. Mean serum glucose was decreased in these patients, 2.24 mmol/L. Non-TBM outliers with raised CSF protein numbered 10/146 patients; age range 4-165 months. Mean CSF lymphocyte and neutrophil counts were 97 cells/μL and 10 cells/μL. Mean CSF glucose concentration was decreased; 1.25 mmol/L. Seven patients had a diagnosis of viral meningitis, 2/7 had CSF PCR confirmation of a viral pathogen, 1 cytomegalovirus and 1 Epstein- Barr virus; 3 patients did not have meningitis.

As CSF glucose concentration and mean CSF protein did not differ between microbiologically-confirmed and case-defined TBM, for comparative purposes only case-defined TBM was used. A CSF glucose concentration cut-off of <1.0 mmol/L had 100% specificity, but resulted in sensitivity of only 0.22. The best combination of good (0.8-0.9) AUC (indicating optimal sensitivity and specificity) and highest odds ratio was achieved with a CSF glucose cut-off of <2.2 mmol/L (table 2). This cut-off point resulted in an odds ratio of 48.9 (95% confidence interval (CI) 21.18-112.77); p<0.01 when comparing non-TBM to TBM. Although specificity could not be determined for CSF:serum glucose ratio, the sensitivity in clinically-defined TBM for a ratio <0.4 was 0.80 and for a ratio of <0.5 was 0.90. For an CSF glucose cut-off of <2.2

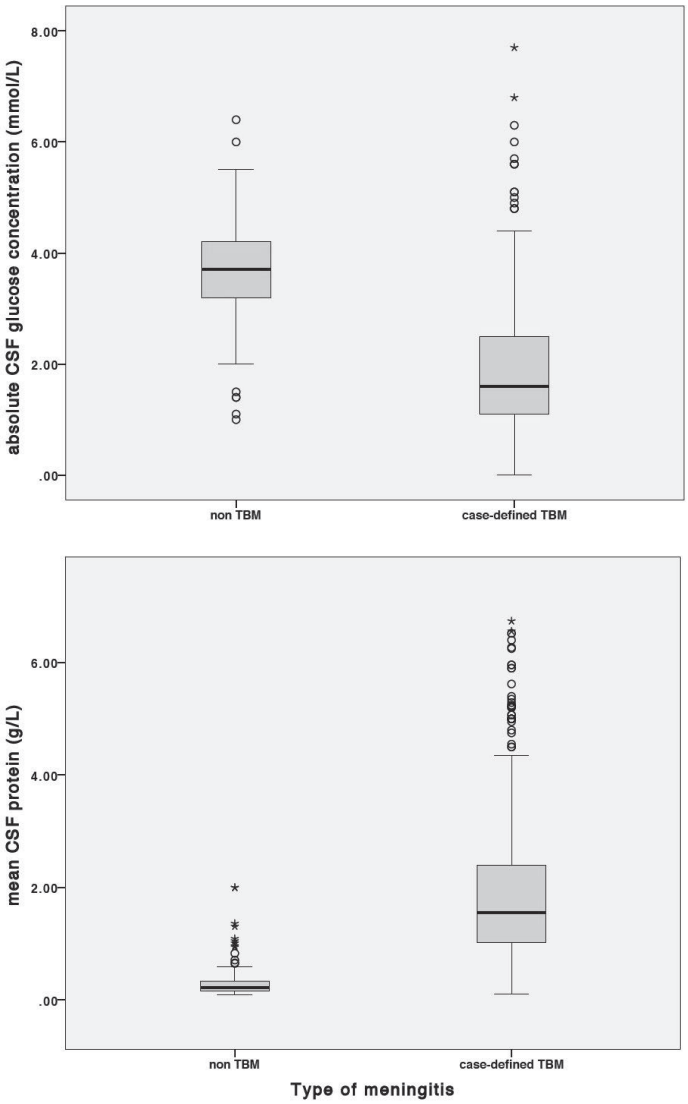

Figure 1. Box plots illustrating absolute CSF glucose concentration and mean CSF protein in non-TBM and TBM groups.

mmol/L 32% of clinically-defined TBM would have been missed compared to 10% for a CSF:serum glucose ratio of <0.50.

For mean CSF protein level, the best combination of excellent (>0.9) AUC (indicating optimal sensitivity and specificity) and highest odds ratio was achieved with a CSF protein cut-off of >0.6 g/L (table 2). This cut-off point resulted in an odds ratio of 167.4 95% CI 84.6-331.33; p<0.01 when comparing non-TBM to TBM.

Table 2. Sensitivities and specificities of different cut-off points for CSF levels of absolute glucose concentration and mean protein in clinically-defined TBM**

CSF glucose (mmol/L)	Sensitivity	Specificity	Area under ROC curve	OR	95% CI
<1.8	0.54	0.97	0.75	32.45	13.10-80.39
<2.0	0.60	0.97	0.78	42.87	17.29-106.29
<2.2	0.68	0.96	0.82	48.87	21.18-112.77
<2.4	0.72	0.93	0.82	30.99	16.32-58.87
<2.6	0.76	0.92	0.84	35.91	18.75-64.99
<2.8	0.80	0.90	0.85	38.31	21.26-69.06
CSF protein (g/L)					
>0.4	0.97	0.81	0.89	118.12	62.89-221.84
>0.6	0.95	0.91	0.93	167.42	84.6-331.33
>0.8	0.85	0.93	0.89	75.81	38.13-150.70
>1.0	0.76	0.95	0.86	60.32	27.49-132.37
>1.2	0.70	0.97	0.82	68.78	25.03-188.99

TBM= tuberculous meningitis, CSF= cerebrospinal fluid, ROC= receiver operating characteristic, OR= odds ratio, CI= confidence interval

**TBM: Micobiologically-confirmed (definite) and probable TBM according to the uniform research case definition [19]

DISCUSSION

The optimal lower limit of CSF glucose concentration as a diagnostic aid for TBM, 2.2 mmol/L (40 mg/dl), is similar to that of bacterial meningitis [17]. This is problematic, but illustrates the importance of considering the macroscopic appearance and cell count in combination with CSF chemistry. The finding that less patients with TBM would have been missed using a CSF:glucose ratio <0.5 (10%) compared to an absolute CSF glucose concentration of <2.2 mmol/L (32%), highlights the value of determining both a serum and CSF glucose at the same time. Unfortunately, this is rarely done in clinical practice. Use of an absolute CSF glucose <2.2 mmol/L or CSF:serum glucose ratio <0.50, as suggested by expert consensus opinion when criteria for a uniform TBM research case definition was devised [19], is confirmed by this study.

The optimal CSF protein cut-off to differentiate TBM from bacterial meningitis [10], and viral meningitis [18], is >1g/L (100mg/dL). Our finding of an optimal CSF protein cut-off of >0.6 g/L only considered viral meningitis and non-meningitis as alternative diagnoses. Consideration of a higher CSF protein cut-off (>1g/L), as suggested by the uniform TBM research case definition [19], as well as cell counts are required to assist the distinction between TBM and bacterial meningitis. A macroscopically clear

CSF and total CSF cell count 10-500 cells/μL with >50% lymphocyte predominance points to a diagnosis of TBM [19].

Although CSF lactate levels were not measured in our cohort, there is potential for its use in children with TBM. CSF lactate has been shown to be a good biomarker to distinguish bacterial from aseptic meningitis [24]. CSF lactate remains unaffected by serum lactate concentration, reflecting the severity of cerebral hypoxia, and therefore the overall prognosis [25]. In a study by Thwaites et al., CSF lactate levels were significantly higher in adult TBM patients that subsequently demised [25].

Many other CSF biomarkers have been considered in TBM cases, but diagnostic utility is rarely described [26]. A 2011 meta-analysis on the use of interferon gamma release assays (IGRAs) in adult pulmonary TB showed limited value for active TB diagnosis [27]. Studies evaluating CSF IGRA showed good sensitivity and specificity [28-31], but low CSF volumes are a limitation in children when requiring sufficient cells to perform IGRA (typically 5-10ml CSF is required) [32, 33]. A study of the host immune response to *M. tuberculosis* showed the potential value of CSF interleukin-13, vascular endothelial growth factor and cathelicidin LL-37 as biomarkers when differentiating TBM from other forms of meningitis [34]. HIV-infected patients with TBM immune reconstitution inflammatory syndrome (IRIS) compared to TBM non-IRIS showed a marked neutrophil driven immune response [35]. Understanding the host immune response is key to a better understanding of the pathophysiology, clinical presentation of TBM and treatment of TBM.

The study was limited by the small number of patients with serum glucose concentrations, and HIV co-infection (4%). Our incidence of HIV co-infection in TBM is in agreement with the study of Karande et al. who reported an incidence of only 7% in a similar cohort of 123 children [36]. There was no difference in absolute CSF glucose concentration or CSF protein between TBM with and without HIV co-infection, but the small numbers precluded statistical comparison.

In conclusion, a 2.2 mmol/L cut-off point for absolute CSF glucose differentiated "non-bacterial non-TBM "from TBM cases with reasonable specificity. Poor sensitivity (0.68) indicates that an absolute CSF glucose concentration cannot be used as a 'rule-out' test. A CSF:serum glucose ratio as an adjunct to CSF glucose concentration provides additional information. Although CSF protein >0.6g/L distinguished TBM from non-TBM with sensitivity 0.95 and specificity 0.91, the non-TBM group excluded bacterial meningitis cases. According to the literature a CSF protein cut-off of >1g/L improves specificity when bacterial meningitis cases are included, but

sensitivity in our study dropped significantly (to 0.76). Using cut-offs of <2.2 mmol/L for CSF glucose and >1g/L for CSF protein seems justified as optimal CSF chemistry criteria in the uniform TBM research case definition.

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CONFLICT OF INTEREST

None of the authors declared a conflict of interest.

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Part III Nucleic acid amplification testing in childhood tuberculous meningitis

4.1 Commercial nucleic acid amplification tests in tuberculous meningitis - a meta-analysis

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ABSTRACT

INTRODUCTION: Although nucleic acid amplification tests (NAATs) promise a rapid, definitive diagnosis of tuberculous meningitis, the performance of first-generation NAATs were sub-optimal and variable.

METHODS: We conducted a meta-analysis of studies published between 2003 and 2013, using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool to evaluate methodological quality. The diagnostic accuracy of newer commercial NAATs was assessed.

RESULTS: Pooled estimates of diagnostic accuracy for commercial NAATs measured against a cerebrospinal fluid *Mycobacterium tuberculosis* culture positive gold standard were; sensitivity 0.64, specificity 0.98 and diagostic odds ratio 64.0. Heterogeneity was limited; p value=0·147 and I^2 =33·85%. The Xpert MTB/RIF® test was evaluated in one retrospective study and four prospective studies, with pooled sensitivity 0.70 and specificity 0.97. The QUADAS-2 tool revealed low risk of bias, as well as low concerns regarding applicability. Heterogeneity was pronounced among studies of in-house tests.

CONCLUSIONS: Commercial NAATs proved to be highly specific with greatly reduced heterogeneity compared to in-house tests. Sub-optimal sensitivity remains a limitation.

Keywords: central nervous system; tuberculosis; nucleic acid amplification tests; diagnostic accuracy

INTRODUCTION

In 1993, the World Health Organization (WHO) declared tuberculosis (TB) a global public health emergency, with an estimated 7-8 million cases and 1.3-1.6 million TB deaths per year. By 2012, the situation has improved in many areas, but absolute numbers remain virtually unchanged with an estimated 8.7 million new cases and 1.4 million TB deaths.[1] Central nervous system (CNS) involvement, mostly tuberculous meningitis (TBM), accounted for approximately 1% of all TB cases.[2] In fact, TBM has been reported as the most common form of meningitis diagnosed in children from TB endemic areas with access to expanded program of vaccination (EPI) vaccines, including *Haemophilus influenza* type-B and pneumococcal vaccination.[3] Delayed diagnosis of TBM is universally associated with poor treatment outcome.[4]

The early clinical presentation of TBM is often non-specific with symptoms such as cough, loss of weight, fever, vomiting and malaise. As the disease progresses, more specific features such as meningism, focal neurological signs, convulsions and depressed level of consciousness occur.[5] TBM outcome is often poor despite adequate anti-mycobacterial therapy, due to irreversible damage preceding delayed diagnosis and ongoing immune-mediated pathology on treatment. Early treatment initiation is critical to reduce TBM-associated morbidity, mortality and healthcare costs, emphasizing the importance of early and accurate diagnosis.[6,7]

Culture of *Mycobacterium tuberculosis* (*M.tb*) from cerebrospinal fluid (CSF) is regarded as the most definitive diagnosis, although this is rarely attained. TBM is a paucibacillary disease. This could explain that direct microscopy for acid-fast bacilli in CSF is rarely positive,[8] while mycobacterial culture may take up to 42 days and has limited sensitivity (<50%) compared to clinical criteria.[5,9,10] In clinical practice the diagnosis of TBM is usually based on a combination of clinical, laboratory and radiological findings. The use of uniform case definition categories has been proposed for research purposes[11] with "definite TBM" defined as a positive CSF *M.tb* culture and/or commercial nucleic acid amplification test (NAAT).

NAATs have been introduced to provide rapid TB diagnosis and enhanced sensitivity compared to smear microscopy.[4,12-15] Although primarily developed for the analysis of respiratory specimens, these methods are often used in non-respiratory specimens as well.[13,14,16-18] They are presumed to be highly specific,[11,19] since they detect *M.tb*-specific DNA sequences such as the IS6110 insertion element, MBP64, 65 kDa antigen, and the rpoB region.[20,21]

In 2003 a systematic review evaluated the test accuracy of NAATs in the diagnosis of TBM.[18] The authors included 49 studies published between 1990 and 2002; both commercial and in-house NAATs were evaluated. The 14 studies with commercial NAATs revealed a pooled sensitivity and specificity of 56% and 98%, respectively. Summary accuracy measures of 35 studies with in-house NAATs could not be determined due to heterogeneity of the tests. Reasons for heterogeneity included: 1) inadequate standardization of laboratory techniques, 2) use of highly variable reference standards, 3) and small patient numbers with limited statistical power.[4] The review concluded that commercial NAATs provided valuable information when positive, but due to poor sensitivity a negative test did not exclude TBM.[18] This finding motivated the inclusion of a positive commercial NAAT as a marker of "definite TBM" in a proposed uniform TBM case definition for use in clinical research.[11]

Since then, many additional studies evaluated the use of commercial NAATs in the diagnosis of TBM, but no updated meta-analysis has been performed. We performed a systematic review of all recent studies (published since 2003) that evaluated the use of NAATs to diagnose TBM, with particular emphasis on commercial tests including the Xpert MTB/RIF® test.

METHODS

We identified all studies published between January 2003 and April 2013 from the following online databases: PubMed (MedLine), Web of Knowledge, Scopus and LILACS. Search terms used were: "Tuberculosis, Central Nervous System", "Tuberculoma, Intracranial", "Tuberculosis", "Mycobacterium tuberculosis", "Extrapulmonary tuberculosis", "Tuberculous meningitis", "Tuberculous pachymeningitis", "Central nervous system" and/or "Kochs disease" and "Polymerase Chain Reaction", "Ligase chain reaction", "GeneXpert" and/or " Nucleic acid amplification testing". Only articles written in English were included. Case reports and review articles were excluded. Studies with less than 10 subjects were also excluded. References of selected articles were reviewed to identify additional eligible studies. Three reviewers (RS, SLvE and AMvF) independently evaluated study inclusion; differences were resolved by consensus.

Data extraction

Two reviewers (RS and SLvE) independently extracted data including number of cases, number of controls, reference standard used, type of NAAT evaluated. Diagnostic odds ratios were extracted or calculated from the data provided. Differences

were resolved by consensus. Methodological quality was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool.[23-25]

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences version 19 (SPSS Inc, Chicago, IL, USA), Comprehensive Meta Analysis version 2 (Biostat, Eaglewood, NJ, USA) and Meta-DiSc (Unit of Clinical Biostatistics, Ramón y Cajal Hospital, Madrid, Spain). Sensitivity, specificity and diagnostic odds ratio (DOR) were computed for each of the included studies. Pooled summary effect estimates were calculated, using a random effects model. Where both CSF culture and clinical criteria were analyzed separately as reference standards, only the studies with CSF culture as the reference standard were included. When articles evaluated more than one NAAT, or more than one quality measure, these were analyzed separately.

Receiver operating characteristic (ROC) curves based on either the regression of logit sensitivity on specificity, the regression of logit specificity on sensitivity, or an orthogonal regression line by minimizing the perpendicular distances were derived. These lines were transformed back to the original ROC scale to obtain a summary ROC (SROC) curve. Derived logit estimates of sensitivity, specificity and respective variances were used to construct a hierarchical SROC curve with these summary estimates. The area under the curve serves as a global measure of test performance; a value of 1 indicates perfect accuracy.[26] Heterogeneity was assessed by applying the X^2 homogeneity test to calculated odds ratios (as a single measure) and determining I^2, with values of more than 50% indicating heterogeneity.[26-28] Statistical significance was set at 0.05 for heterogeneity testing.

RESULTS

The study selection process is summarized in Figure 1. The literature search revealed 1125 potential articles, which was narrowed down to 69 articles after title screening. This was narrowed down further to 62 articles after abstract screening. Thirty-six articles were excluded after screening the text, and 4 articles added after cross referencing. Ten studies in 8 articles, describing commercial tests were selected; 40 studies in 22 articles describing in-house NAATs were tabulated seperately[4,8,14,15,19,21,28-48-53] (Supplementary table 1). Reference standards used in the ten studies evaluating commercial NAATs included a positive CSF *M.tb* culture in nine (90%) and clinical criteria in one (10%). To avoid misleading results, only the 9 commercial studies with positive CSF *M.tb* culture as the reference standard were analyzed. A variety of

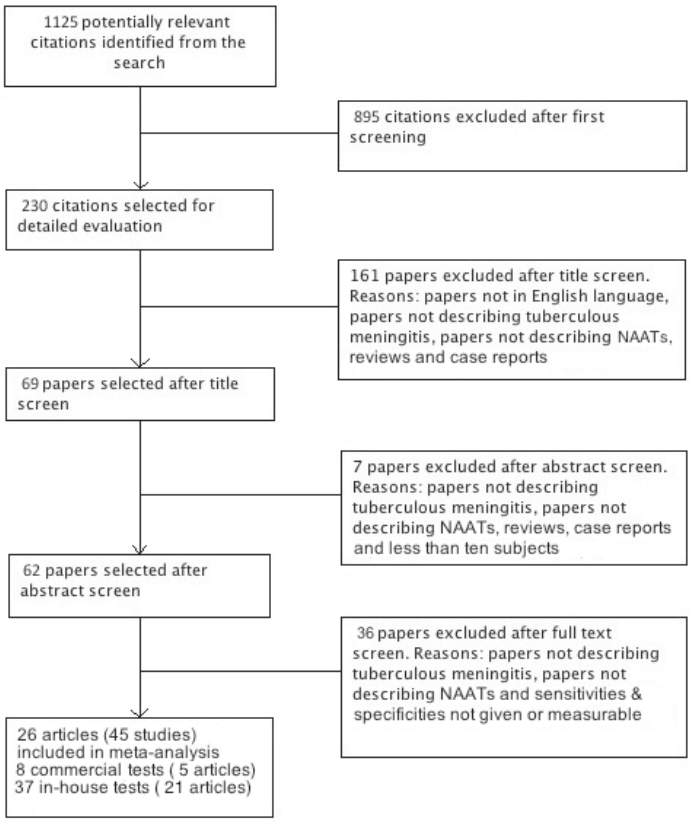

Figure 1. Flow diagram of all studies identified and those selected for meta-analysis

DNA extraction techniques and target sequences were used. Table 1 summarizes key characteristics of the commercial NAAT studies. Figure 2 reflects formal assessment of the four study domains evaluated by the QUADAS-2 tool; inter-reviewer variability using the tool was 10.6%.[24]

Summary test accuracy estimates for the nine commercial NAATS evaluated were; sensitivity 0.64 (95% CI 0.56-0.72), specificity 0.98 (95% CI 0.96-0.99), positive likelihood ratio 20.36 (95% CI 11.29-36.73), negative likelihood ratio 0.39 (95% CI 0.30-0.53) and DOR 64.0 (95% CI 26.9-152.1). Heterogeneity was limited; p value=0.147 and I^2 =33.85%. Table 2 shows heterogeneity testing after stratification of the commercial NAATs based on study design, prospective nature and Xpert MTB/RIF testing. Figure 3 provides an overview of sensitivities and specificities of commercial NAATs in forest plot format. Figure 4 presents the SROC curve for the commercial NAAT studies combined, with the respective studies presented as circles. The area under the curve (AUC) for all commercial tests combined was 0.92.

Table 1. Characteristics of commercial nucleic acid amplification test studies included in the meta-analysis

tAuthor	Study design	Reference standard	NAAT used	Sensitivity (95% CI)	Specificity (95% CI)
Johnsson[9]	Retrospective case-control	Clinical criteria	Cobas Amplicor	0·56(0·21-0·86)	0·97(0·93-0·99)
Johansen[29]	Prospective cross-sectional	CSF culture	standard BD ProbeTec ET	0·62(0·32-0·86)	0·99(0·94-1·00)
Johansen[29]	Prospective cross-sectional	CSF culture	modified BD ProbeTec ET	0·77(0·46-0·95)	0·99(0·94-1·00)
Thwaites[4]	Retrospective case-control	CSF culture	enhanced MTD	0·50(0·34-0·66)	0·95(0·88-0·99)
Causse[50]	Prospective cross-sectional	CSF culture	Xpert MTB/Rif	0·83(0·36-1·00)	1.00(0·92-1·00)
Causse[50]	Prospective cross-sectional	CSF culture	Cobas Taqman MTB	0·67(0·22-0·96)	0·98(0·88-1·00)
Malbruny[51]	Prospective cross-sectional	CSF culture	Xpert MTB/Rif	1·00(0·03-1·00)	1·00(0·77-1·00)
Vadwai[52]	Prospective cross-sectional	CSF culture	Xpert MTB/Rif	0·33(0·01-0·91)	0·95(0·74-1·00)
Tortoli[47]	Retrospective case-control	CSF culture	Xpert MTB/Rif	0.85(0·55-0·98)	0.98(0·94-1·00)
Patel[53]	Prospective cross-sectional	CSF culture	Xpert MTB/Rif	0·67(0·53-0·79)	0·94(0·85-0·98)

CI= confidence interval, CSF= cerebrospinal fluid

*NAAT used: Cobas amplicor (Roche Molecular Systems, Branchburg, NJ, USA), BD ProbeTec ET assay (Becton, Dickinson and Company, Sparks, MD, USA), MTD (Gen-Probe Inc, San Diego, Ca, USA), Genei Amplification kit (Bangalore Genei, Banglore, India), Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale, CA, USA)

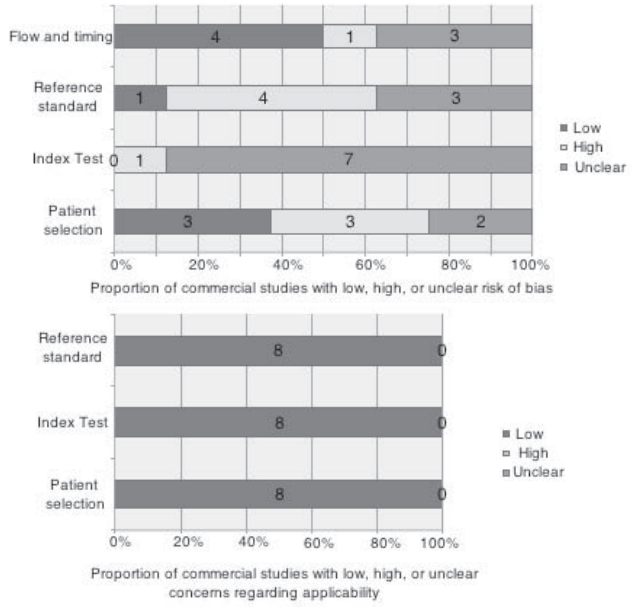

Figure 2. Bar graphs representing quality assessment by the QUADAS-2 tool[24]
The numbers in the bars represent the individual commercial NAATs

Table 2. Heterogeneity testing of commercial NAATs in stratified sub-groups

Subgroup	Number of studies	Summary DOR	95% CI	Test for heterogeneity p-value	I^2 (%)
Study design					
Case-control	2	68.5	4.3-1106.8	0.018	82.06
Cross-sectional	7	59.8	26.2-136.2	0.416	1.08
Prospective data collection					
Yes	7	59.8	26.2-136.2	0.416	1.08
No	2	68.5	4.3-1106.8	0.018	82.06
PCR type					
Xpert MTB/Rif	5	70.7	17.4-287.1	0.157	39.65

DOR= diagnostic odds ratio, CI= confidence interval, I^2 is a measure of heterogeneity (>50%= heterogeneity)

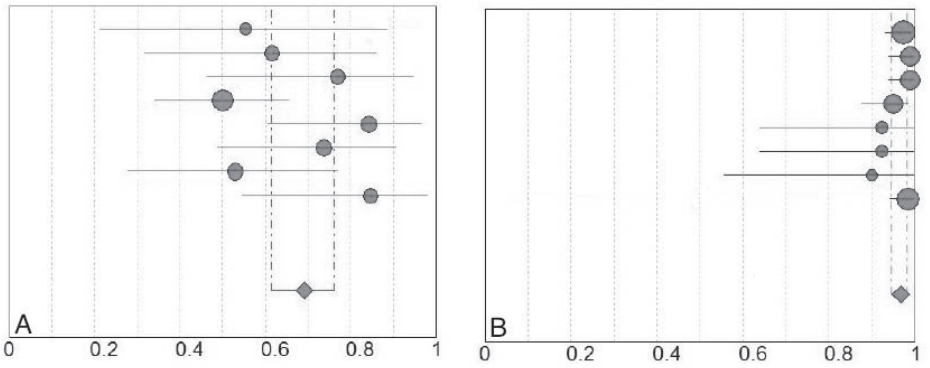

Figure 3. Forest plots of A) sensitivity and B) specificity of commercial NAATs
Each circle shows the point estimate of sensitivity and specificity from each included study. Error bars represent 95% confidence intervals.

Summary test accuracy estimates for the 40 in-house tests revealed sensitivity of 0.73 (95% CI 0.71-0.75), specificity of 0.92 (95% CI 0.90-0.93), positive likelihood ratio of 9.56 (95% CI 6.61-13.84), negative likelihood ratio of 0.27 (95% CI 0.20-0.35) and DOR of 40.6 (95% CI 26.6-61.9). Heterogeneity was pronounced; p-value=0.001 and I^2 = 58·86%. Supplementary table 2 shows heterogeneity testing after stratification of the in-house NAATs based on study design, prospective nature, randomization, blinding, reference standard and type of PCR used. Forest plots of sensitivities and specificities of in-house NAATs and the relevant SROC plot are included in Supplementary figure 1. The AUC for all in-house tests combined was 0.94.

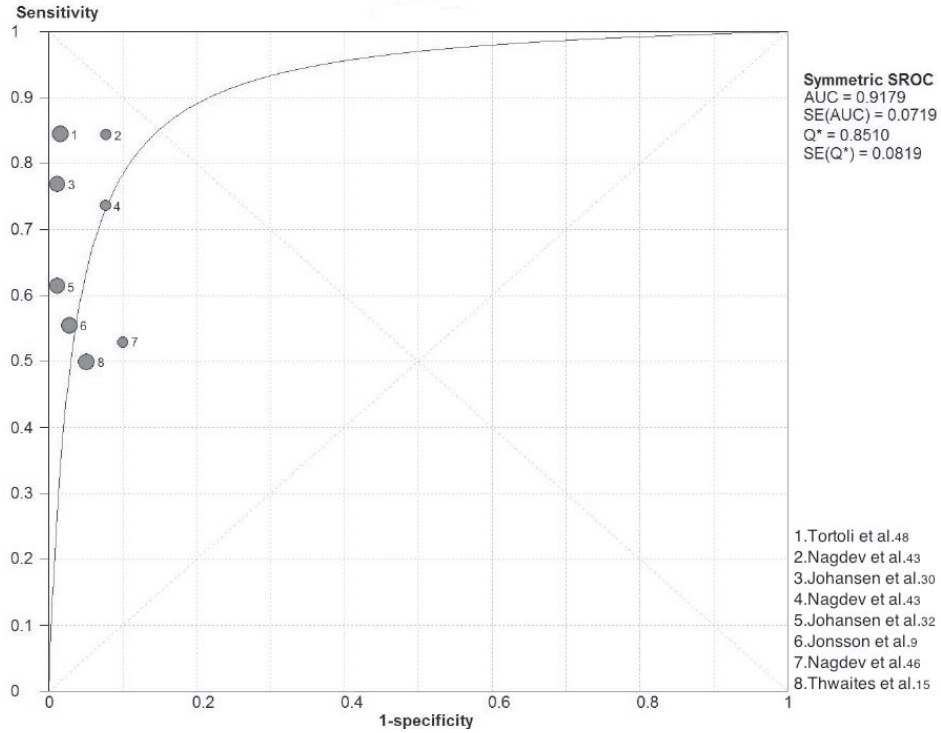

Figure 4. SROC curve for commercial NAATs
Each study is represented by a circle (the size of the circle reflecting study size) and the dark line shows the summary diagnostic accuracy.

DISCUSSION

The need for a test that can diagnose TBM rapidly and accurately, especially during its early phases, is self-evident.[54] Our systematic review and meta-analysis of commercial NAATs with a CSF *M.tb* culture-positive gold standard found a summary sensitivity estimate of 0.64. Unfortunately this remains suboptimal and is unlikely to greatly enhance early accurate diagnosis, since most study specimens were collected from patients with advanced TBM disease. In contrast, commercial NAATs exhibited excellent specificity of 0·98 and in the correct clinical context it may be regarded as a definitive test.[11] Similar to findings from the previous systematic review performed in 2003,[18] we found significant heterogeneity among in-house NAATs and consistent performance in the commercial group. When comparing our summary estimates to the previous meta-analysis, the sensitivity of more recent commercial NAATs shows improvement (0.64 vs 0.56) with a similar specificity (0.98). Our summary estimate of negative likelihood ratio is lower (0.39 vs 0.44), but still far from ideal when considering its use as a "rule-out" test.

Despite suboptimal sensitivity, the rapid turnaround time of NAATs compared to culture enhances its role in the early accurate diagnosis of TBM. However, most commercial NAATs are validated for pulmonary samples and are still not advised for routine diagnostic use.[47] The Xpert MTB/RIF assay (Cepheid, CA, USA) has been endorsed by the WHO for use on both smear positive and negative respiratory specimens. The findings of the Xpert MTB/RIF assay demonstrated rapid diagnosis in a large retrospective study of extrapulmonary specimens, including an encouraging sensitivity of 0.85 and specificity 0.98 for CSF samples.[47] When combined with the 4 prospective studies testing the Xpert MTB/RIF assay in CSF samples, a pooled sensitivity and specificity of 0.70 and 0.97 was obtained.[47,50-53] Provided that similar measures of sensitivity and specificity can be maintained in future studies using the Xpert MTB/RIF assay in CSF specimens, the goal of Xpert MTB/RIF assay as a "stand alone" test for diagnosis of TBM can be achieved.

The use of microscopy, culture and NAATs together with clinical features and neuroimaging in a pragmatic algorithm seems preferable to improve diagnostic accuracy. This updated meta-analysis supports the conclusion that a positive commercial NAAT result provides a definite TBM diagnosis in the right clinical context, as suggested in the proposed uniform research case definition for TBM in adults and children.[11] The rest of the components of the proposed uniform research case definition can compensate for commercial NAATs when excluding a diagnosis of TBM.

The 'gold standard' for the diagnosis of TBM is the identification of *M.tb* on CSF culture or identification of acid-fast bacilli on CSF microscopy. The low sensitivity of both these methods has prompted leading researchers to use alternate clinical reference standards. In our meta-analysis we attempted to avoid overestimating summary estimates of diagnostic accuracy by only analyzing commercial NAATs using an *M.tb* culture positive reference standard. The low heterogeneity observed when studies were prospective or cross-sectional was also encouraging (Table 2). Similar to previous findings, in-house NAAT studies demonstrated excessive heterogeneity with wide variability in methodological quality (Supplementary table 2).[18]

The quality and reporting of diagnostic accuracy studies on commercial tests for TB, malaria and HIV are problematic.[25] To minimize these concerns, screening and selection of articles were assessed by three independent reviewers followed by rigorous quality assessment using the QUADAS-2 tool.[24] This resulted in multiple study exclusions, but careful assessment of study accuracy and reliability strengthens the findings of our meta-analysis. Overall, the studies revealed a low risk of bias in the

categories of flow and timing, reference standard, index test and patient selection. There was little concern regarding the applicability of study findings.

In conclusion, commercial NAATs revealed good specificity and postive predictive values for the diagnosis of TBM on CSF samples in areas of high TB prevalence. However, sensitivity and negative predictive values remain suboptimal, hampering the ability to direct treatment, especially early in the disease process when the best treatment outcomes can be achieved.

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CONFLICT OF INTERESTS

None of the authors have any conflict of interests.

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Supplementary table 1. Characteristics of in-house studies included in the meta-analysis

Author	Study design	Reference standard	NAAT used	Sensitivity (95% CI)	Specificity (95% CI)
Rafi[15]	Retrospective case-control study	Clinical criteria	PCR 123 bp	0·86(0·68-0·96)	1·00(0·54-1·00)
Kulkarni[30]	Retrospective case-control study	Clinical criteria	PCR 340 bp So hybridization	0·90(0·73-0·98)	1·00(0·88-1·00)
Kulkarni[30]	Retrospective case-control study	Clinical criteria	PCR 340 bp eth. bromide	0·73(0·54-0·88)	1·00(0·88-1·00)
Desai[31]	Retrospective case-control study	Clinical criteria	PCR IS*6110* QIAmp	0·67(0·47-0·83)	1·00(0·87-1·00)
Desai[31]	Retrospective case-control study	Clinical criteria	PCR IS*6110* CTAB	0·50(0·31-0·69)	1·00(0·87-1·00)
Juan[32]	Retrospective case-control study	CSF micro/culture /clinical criteria	PCR IS*6110*	0·68(0·41-0·85)	0·99(0·94-1·00)
Quan[33]	Retrospective case-control study	Clinical criteria	PCR IS*6110*	0·75(0·53-0·90)	0·94(0·83-0·99)
Bhigjee[34]	Retrospective cross-sect study	CSF culture/ clinical criteria	PCR IS*6110*, MBP64, PT8/9	0·55(0·39-0·70)	0·88(0·68-0·97)
Bhigjee[34]	Retrospective cross-sect study	CSF culture/ clinical criteria	PCR (real-time) IS*6110*	0·70(0·55-0·83)	0·88(0·68-0·97)
Deshpande[35]	Retrospective case-control study	CSF culture	PCR IS*6110*	0·91(0·77-0·98)	0·76(0·56-0·90)
Rafi[36]	Retrospective case-control study	CSF culture	PCR IS*6110*	0·98(0·88-1·00)	1·00(0·95-1·00)
Rafi[36]	Retrospective case-control study	CSF culture	PCR MBP64	0·91(0·79-0·98)	0·91(0·82-0·96)
Rafi[36]	Retrospective case-control study	CSF culture	PCR 65 kDa	0·51(0·36-0·66)	0·92(0·83-0·97)
Rafi[20]	Prospective cohort study	CSF culture	PCR IS*6110*	1·00(0·97-1·00)	0·89(0·85-0·93)
Dora[37]	Prospective cross-sectional study	CSF culture	in-house PCR 65kDA nested	0·50(0·12-0·88)	0·99(0·95-1·00)
Takahashi[38]	Prospective cross-sectional study	Clinical criteria	PCR single MBP64	0·40(0·05-0·85)	1·00(0·48-1·00)
Takahashi[38]	Prospective cross-sectional study	Clinical criteria	PCR nested MBP64	1·00(0·48-1·00)	1·00(0·48-1·00)
Takahashi[38]	Prospective cross-sectional study	Clinical criteria	PCR OR-QNRT	1·00(0·48-1·00)	1·00(0·48-1·00)
Takahashi[38]	Prospective cross-sectional study	Clinical criteria	PCR WR-QNRT	1·00(0·48-1·00)	1·00(0·48-1·00)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	sediment PCR qRT	0·53(0·42-0·64)	0·92(0·84-0·97)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	sediment PCR devR	0·31(0·21-0·42)	0·94(0·87-0·98)

Author	Study design	Reference standard	NAAT used	Sensitivity (95% CI)	Specificity (95% CI)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	sediment PCR IS*6110*	0·40(0·29-0·51)	0·93(0·85-0·97)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	filtrate PCR qRT	0·88(0·78-0·94)	0·92(0·84-0·97)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	filtrate PCR devR	0·88(0·78-0·94)	0·87(0·78-0·93)
Haldar[39]	Retrospective case-control study	CSF culture/ clinical criteria	filtrate PCR IS*6110*	0·85(0·76-0·92)	0·84(0·74-0·91)
Huang[40]	Retrospective case-control study	Clinical criteria	single PCR rpoB	0·25(0·12-0·42)	1·00(0·86-1·00)
Huang[40]	Retrospective case-control study	Clinical criteria	nested PCR rpoB	0·86(0·71-0·95)	1·00(0·86-1·00)
Rana[41]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR IS*6110*	0·31(0·20-0·46)	0·92(0·78-0·98)
Nagdev[42]	Retrospective case-control	CSF culture or microscopy & clinical criteria	nested PCR IS*6110*- Genei Amplification kit Chelex protocol	0·84(0·60-0·97)	0·92(0·64-1·00)
Nagdev[42]	Retrospective case-control	CSF culture or microscopy & clinical criteria	nested PCR IS*6110*- Genei Amplification kit Phenol/ chloroform	0·74(0·49-0·91)	0·92(0·64-1·00)
Nagdev[43]	Retrospective case-control study	Sputum micro/ CSF culture/ clinical criteria	PCR IS*6110*	0·80(0·66-0·90)	0·84(0·77-0·90)
Sharma[44]	Retrospective case-control study	CSF culture/ clinical criteria	PCR protein b	0·83(0·72-0·91)	1·00(0·91-1·00)
Nagdev[45]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR IS*6110*	0·88(0·64-0·99)	0·80(0·44-0·97)
Nagdev[45]	Retrospective case-control	Clinical criteria	nested PCR IS*6110*- Genei Amplification kit	0·53(0·28-0·77)	0·90(0·55-1·00)
Kusum[22]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR IS*6110*	0·76(0·67-0·84)	1·00(0·96-1·00)
Kusum[22]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR protein b	0·81(0·72-0·88)	1·00(0·96-1·00)
Kusum[22]	Retrospective case-control study	CSF micro/ culture /clinical criteria	PCR MBP64	0·83(0·74-0·89)	1·00(0·96-1·00)
Iacob[46]	Prospective cohort study	CSF culture/ clinical criteria	PCR IS*6110*	0.87(0·81-0·93)	0.88(0·82-0·94)

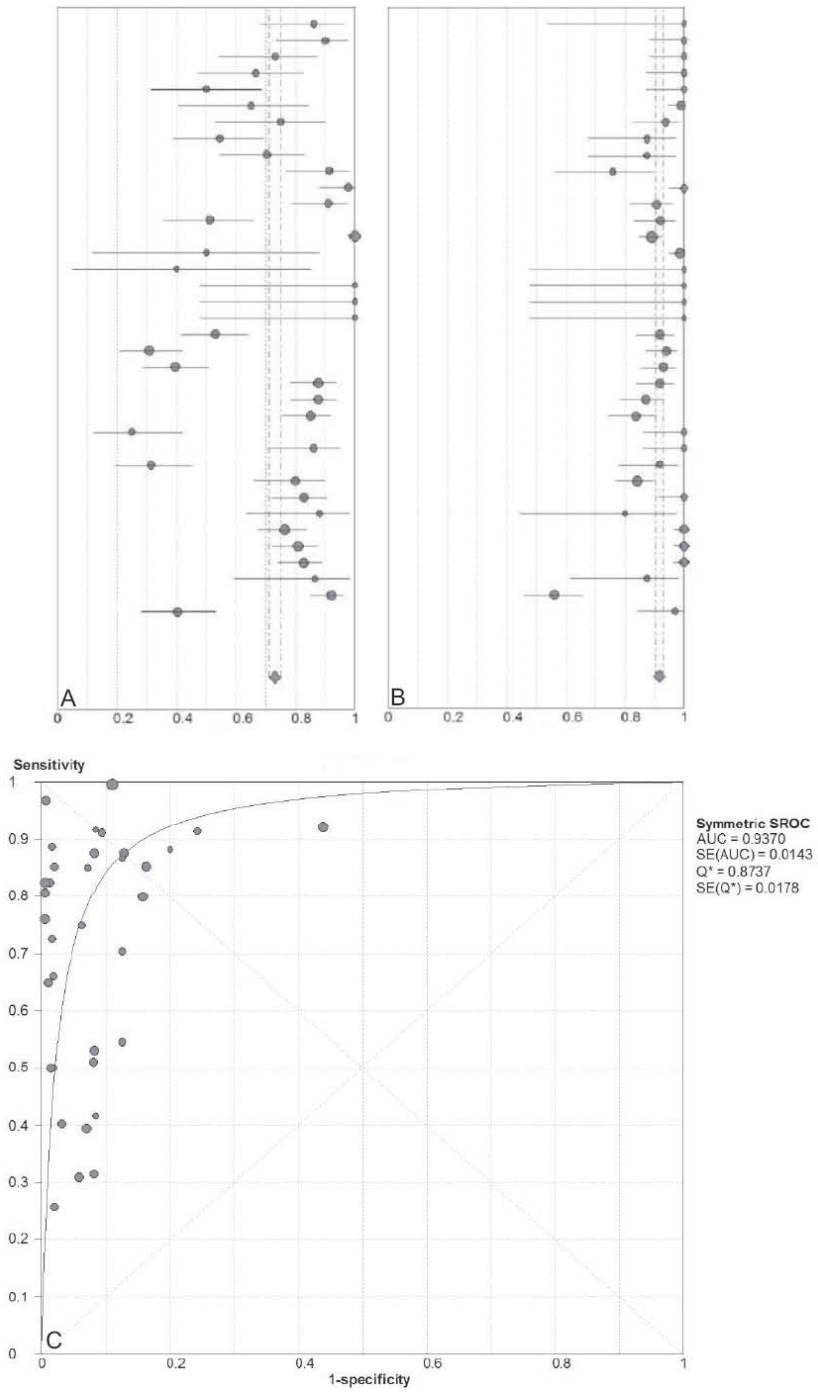
Author	Study design	Reference standard	NAAT used	Sensitivity (95% CI)	Specificity (95% CI)
Chaidir[49]	Prospective cohort study	CSF culture	PCR IS*6110*	0.92(0·90-0·94)	0.56(0·52-0·59)
Sastry[48]	Prospective cohort study	CSF culture/ clinical criteria	nested PCR IS*6110*	0.43(0·38-0·49)	0.97(0·95-0·99)

CI= confidence interval, CSF= cerebrospinal fluid, OR-QNRT= original quantitative nested real-time, WR-QNRT= wide-range quantitative nested real-time.

Supplementary table 2. Heterogeneity testing of in-house NAATs in stratified sub-groups

Subgroup	Number of studies	Summary DOR	95% CI	Test for heterogeneity p value	I^2(%)
Study design					
Case-control	29	44.7	27.1-73.8	0.000	63.43
Cross-sectional	7	19.3	8.7-42.8	0.479	0.00
Prospective data collection					
Yes	9	49.7	17.0-145.4	0.068	45.05
No	31	39.7	24.7-63.7	0.000	62.60
Randomization					
No	36	40.6	26.2-63.0	0.000	62.25
Blinding					
Single-blinded	10	106.4	27.1-417.6	0.002	66.34
Non-blinded	28	30.2	19.8-46.2	0.000	54.15
Reference standard					
Culture	7	72.2	21.3-245.5	0.000	78.65
Clinical criteria	13	57.7	26.9-123.5	0.743	0.00
CSF microscopy and/or culture and clinical criteria	20	31.0	18.1-53.2	0.000	64.39
PCR type					
IS*6110*	19	36.5	20.3-65.7	0.001	58.89
Other	21	46.2	24.6-86.7	0.000	60.19
Presence of nesting	7	42.0	16.8-104.8	0.648	0.00

DOR= Diagnostic odds ratio, CI= confidence interval, I^2 is a measure of heterogeneity (>50%= heterogeneity)



Supplementary Figure 1. Forest plots of A) sensitivity B) specificity and c) SROC curve of in-house NAATs

Each circle shows the point estimate of sensitivity and specificity from each included study.

4.2 Improved diagnosis of childhood tuberculous meningitis using more than one nucleic acid amplification test

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ABSTRACT

BACKGROUND: Early treatment is critical to reduce tuberculous meningitis (TBM)-related morbidity and mortality. Diagnosis based on cerebrospinal fluid (CSF) culture is impractical due to slow turn-around times, while microscopy has poor sensitivity. Enhanced detection methods are essential to guide early treatment initiation, especially in vulnerable young children.

METHODS: We assessed the diagnostic accuracy of Genotype MTBDR*plus*® and Xpert MTB/RIF® assays on CSF collected from pediatric meningitis suspects prospectively enrolled at Tygerberg Hospital, Cape Town, South Africa. Fluorescent auramine-O microscopy, liquid culture for *Mycobacterium tuberculosis*, MTBDR*plus*® and Xpert MTB/RIF® assays were performed on all CSF samples.

RESULTS: Of 101 meningitis suspects, 55 were diagnosed with TBM and 46 served as non-TBM controls. Using a pre-defined TBM case-definition as reference standard, sensitivities and specificities were 4% and 100% for fluorescent microscopy, 22% and 100% for culture, 33% and 98% for MTBDR*plus*®, 26% and 100% for Xpert MTB/RIF®, 22% and 100% for microscopy and/or culture combined and 49% and 98% for MTBDR*plus*® and Xpert MTB/RIF® combined. Culture, MTBDR*plus*® and Xpert MTB/RIF® combined performed best with 56% sensitivity and 98% specificity.

CONCLUSION: Commercial nucleic-acid amplification tests performed on CSF revealed incrementally-improved diagnostic accuracy, providing rapid microbiological confirmation but cannot serve as a rule-out test.

INTRODUCTION

In 1993, the World Health Organization declared tuberculosis (TB) a global public health emergency[1]. Although some progress has been made, patient numbers in 2012 are essentially unchanged with an estimated 8.6 million new cases and 1.3 million deaths from TB worldwide[2]. In South Africa, the TB incidence has risen to 1000 new cases/100,000 population in 2012[2], while large numbers of retreatment cases with a second or third episode of TB are not included in this figure[2,3].

Tuberculous meningitis (TBM) is the most devastating manifestation of TB and early treatment initiation is critical to optimize outcomes[4]. Confirmation of TBM diagnosis is challenging in young children due to the paucibacillary nature of disease and low cerebrospinal fluid (CSF) volumes available for diagnostic analysis[5]. Currently TBM confirmation requires visualization of acid-fast bacilli and/or a positive *Mycobacterium tuberculosis* (*M.tuberculosis*) culture from CSF. Direct microscopy for acid-fast bacilli in CSF is fast but has very low sensitivity (<20%)[6] whereas mycobacterial culture may take up to 42 days and has only slightly improved sensitivity[7-9].

Several commercially available nucleic acid amplification tests (NAATs) have been developed for the rapid diagnosis of TB. The World Health Organization has endorsed the Xpert MTB/RIF® assay (Cepheid, Sunnyvale, CA, USA) for both smear microscopy-positive and -negative sputum specimens. Xpert simultaneously detects *M.tuberculosis* and susceptibility to rifampicin by amplification of the *rpo*B gene[10,11]. It is usable for a variety of liquid clinical samples[12,13]. However, lower sensitivities attributed to low numbers of bacilli (59-62%), were obtained for CSF specimens[14,15].

The MTBDR*plus*® assay (Hain Lifescience GmbH, Nehren, Germany) version 1 is recommended for smear microscopy-positive specimens only[16,17], while version 2 of the assay can also be applied to smear microscopy-negative specimens, having similar sensitivity compared to Xpert MTB/RIF®[18,19]. The MTBDR*plus*® is a line probe assay targeting the *rpo*B, *kat*G and *inh*A genes, detecting *M.tuberculosis*, as well as rifampicin and isoniazid susceptibility. Although the MTBDR*plus*® assay version 2 has similar sensitivity and specificity to Xpert MTB/RIF® in smear microscopy-negative specimens, Xpert MTB/RIF® detects *M.tuberculosis* quicker (under 2 hours vs 5 hours) and is a closed-tube system, with easier handling and decreasing contamination rates[20].

In order to assess the utility of MTBDR*plus*® and Xpert MTB/RIF® to diagnose TBM in a clinical setting, alone and/or in combination with established diagnostic methods,

we collected CSF samples from children with suspected meningitis in a setting where TBM is common.

METHODS

We conducted a prospective hospital-based study of all children clinically suspected of having meningitis.

Study Population and Setting

This study was conducted at Tygerberg Hospital, Cape Town, a major tertiary referral centre for Cape Town and surrounding areas. TBM is a common diagnosis among children diagnosed with meningitis[21]. Children were enrolled between January 2010 and March 2013. Inclusion criteria were 1) age 3 months to 13 years 2) clinical suspicion of meningitis 3) CSF sample collected for fluorescent auramine-O microscopy, *M.tuberculosis* culture, MTBDR*plus*® and Xpert MTB/RIF® assays and 4) written consent from the caregiver and assent if the child was older than 7 years and competent to do so. The study was approved by the Human Research Ethics Committee of Stellenbosch University, Cape Town, Western Cape, South Africa.

Clinical procedures

All patients underwent a comprehensive clinical evaluation. Routine investigations, including full blood count, basic biochemistry, HIV-screening, tuberculin skin test (TST), microbiological analysis of sputum or gastric washing (fluorescence microscopy for acid-fast bacilli and *M.tuberculosis* culture), bacterial blood culture, chest radiography and if clinically indicated, neuroimaging. Children were categorized as TBM, and non-TBM.

CASE DEFINITIONS

Tuberculous meningitis (TBM)

A diagnosis of TBM was based on a uniform research case definition (Table 1)[22]. TBM was classified as 'definite' when CSF demonstrated acid-fast bacilli and/or positive *M.tuberculosis* culture in a patient with symptoms or signs suggestive of the disease. As MTBDR*plus*® and Xpert MTB/RIF® were tested, *M.tuberculosis* detected by commercial NAATs in CSF was not used as a criteria for 'definite' TBM. TBM was classified as 'probable' or 'possible' based on a scoring system[22]. All patients diagnosed with TBM were treated with a standard short-course regimen[23].

Table 1. Diagnostic criteria in the uniform TBM research case definition[22]

	Diagnostic score
Clinical criteria (Maximum category score=6)	
Symptom duration of more than 5 days	4
Systemic symptoms suggestive of TB (1 or more of): weight loss/(poor weight gain in children), night sweats or persistent cough > 2 weeks	2
History of recent close contact with an individual with pulmonary TB or a positive TST/IGRA in a child <10 years	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
CSF criteria (Maximum category score=4)	
Clear appearance	1
Cells: 10–500 per µl	1
Lymphocytic predominance (>50%)	1
Protein concentration greater than 1 g/L	1
CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2·2mmol/L	1
Cerebral imaging criteria (Maximum category score=6)	
Hydrocephalus	1
Basal meningeal enhancement	2
Tuberculoma	2
Infarct	1
Pre-contrast basal hyperdensity	2
Evidence of tuberculosis elsewhere (Maximum category score=4)	
Chest X-ray suggestive of active TB (excluding miliary TB)	2
Chest X-ray suggestive of miliary TB	4
CT/ MRI/ US evidence for TB outside the CNS	2
AFB identified or *M.tuberculosis* cultured from another source i.e. lymph node, gastric washing, urine, blood culture	4
Exclusion of alternative diagnoses- An alternative diagnosis must be confirmed microbiologically, serologically or histopathologically	
Definite TBM = AFB seen on CSF microscopy, positive CSF *M.tuberculosis* culture, or positive CSF *M.tuberculosis* commercial NAAT in the setting of symptoms/signs suggestive of meningitis; or AFB seen in the context of histological changes consistent with TB brain or spinal cord together with suggestive symptoms/signs and CSF changes, or visible meningitis (on autopsy).	
Probable TBM = total score of ≥12 when neuroimaging available = total score of ≥10 when neuroimaging unavailable	
Possible TBM = total score of 6-11 when neuroimaging available = total score of 6-9 when neuroimaging unavailable	

TBM- tuberculous meningitis, TB- tuberculosis, TST- tuberculin skin test, IGRA- interferon gamma-release assay, CSF- cerebrospinal fluid, CT- computed tomography, MRI- magnetic resonance imaging, US- ultrasound, AFB- acid-fast bacilli, NAAT- nucleic acid amplification test

Non-TBM

This included viral, fungal or bacterial meningitis (other than TBM) and cases without meningitis (normal CSF and/or confirmation of an alternative diagnosis). Viral meningitis was confirmed when a viral pathogen was identified in the CSF by polymerase chain reaction (PCR). Viral meningitis was considered probable with clinical evidence of acute meningitis and absence of any micro-organism on Gram stain of CSF and negative routine bacterial culture of CSF if antibiotics were not administered prior to the first lumbar puncture[24]. Bacterial or fungal meningitis was determined by the identification of a bacterial pathogen in the CSF using microscopy, culture or antigen detection methods. Probable bacterial meningitis was defined as clinical evidence of meningitis in addition to a suggestive CSF examination[25].

CSF COLLECTION AND TESTING

CSF was obtained by lumbar puncture from all children included and the following investigations performed: appearance and color determination, differential cell count determination by standard methods, protein, glucose and chloride determination by standard methods, centrifugation with Gram stain and India ink examination on the deposit and culture of the centrifuged deposit on blood agar plates for pyogenic bacteria. When viral meningitis was suspected, PCR for cytomegalovirus, Epstein-Barr virus, enteroviruses, human herpesvirus-6, herpes simplex 1 & 2 and varicella zoster, was performed on CSF. Fluorescence microscopy was conducted using standardized auramine-O staining methods[26].

M.tuberculosis culture

A volume of 0.5 ml of CSF was directly inoculated into a Mycobateria Growth Indicator Tube (MGIT; Becton Dickinson, Sparks, MD, USA) supplemented with 0.8 ml OADC (oleic acid, albumin, dextrose, catalase) containing PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin). The MGIT was placed into a BACTEC MGIT 960 instrument, incubated at 37°C. Flagged cultures were removed and the presence of acid-fast bacilli verified by Ziehl-Neelsen staining and microscopy. Bacterial contamination was excluded by placing one drop of culture on a blood agar plate with no growth after 48 hours incubation at 37°C. Specimens were determined negative if not flagged after 42 days of incubation.

GenoType MTBDRplus®

CSF samples were processed by the National Health Laboratory Service (NHLS) TB laboratory at Tygerberg Hospital. Samples were mixed by pipetting. The Genotype

MTBDR*plus*® assays were used according to the manufacturer's instructions. The CSF volume analyzed was 0.5ml, with a 160 colony forming unit (CFU)/ml limit of detection. Quality control included a negative and positive control. Improvements in the DNA extraction from sonication and heat (version 1) to a chemical method (version 2), enabled its usage on smear microscopy-positive and -negative samples; the laboratory adopted version 2 in July 2012.

Xpert MTB/RIF®

An aliquot of 1 ml specimen was mixed with 2 ml of Xpert Sample Reagent (Cepheid), inverted 10 times, and incubated at room temperature. The inversion was repeated after 8 minutes and the incubation continued until a total duration of 15 minutes. After this, the mixture was completely transferred into an Xpert MTB/RIF cartridge, which was loaded into the GeneXpert instrument. All further processing, measuring and analysis steps happened automatically (GeneXpert Dx 4.0, Cepheid). Bacterial load was semi-quantitatively reported as very low, low, medium or high positive, with the presence or absence of resistance against rifampicin indicated separately[27]. The limit of detection is 100 CFU/ml[28]. Invalid results were repeated or excluded.

Statistical analysis

The study reporting conforms to the STARD guidelines for diagnostic accuracy reporting (www.stard-statement.org). Data analysis was performed using Statistical Package for the Social Sciences version 20 (SPSS Inc, Chicago, IL, USA). Frequencies were obtained for categorical clinical variables. Median and interquartile range was determined for continuous variables. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic odds ratio (DOR) was calculated comparing non-TBM to TBM. Categorical variables were compared using Fisher's exact test and continuous variables were compared using the Mann-Whitney U test. A p-value <0.05 was considered statistically significant.

RESULTS

In total 101 children with suspected meningitis met the inclusion criteria; 55 TBM and 46 "non-TBM". Of the TBM group, 13 patients had 'definite' TBM, 32 patients had 'probable' TBM and 10 patients had 'possible' TBM[22]. Among "non-TBM" patients, 30 did not have meningitis. Non-TBM patients with meningitis included three cases of bacterial meningitis (1 with pneumococcal meningitis) and 13 cases of viral meningitis. Confirmed viral meningitis cases included human enterovirus (5), Epstein-Barr virus (2) and Herpes simplex type-2 virus (1). CSF volume was recorded in 45 patients

Table 2. Clinical and investigation findings of children enrolled with suspected meningitis

Clinical characteristics	TBM	Non-TBM	p-value
Age in months- median (IQR)	36.0 (21.0-54.0)	34.0 (17.0-56.3)	0.800
Male gender (n/N/%)	26/55 (47)	30/46 (65)	0.107
Positive HIV (n/N/%)	6/55 (11)	2/45 (4)	0.289
Positive TB contact* (n/N/%)	26/55 (47)	12/46 (26)	0.039
Poor weight gain** (n/N/%)	21/55 (38)	4/46 (9)	0.001
Hemiplegia (n/N/%)	17/55 (31)	2/46 (4)	0.001
Positive gastric washing culture (n/N/%)	25/39 (64)	11/33 (33)	0.017
Clear CSF macroscopic appearance (n/N/%)	51/55 (93)	45/45 (100)	0.125
CSF lymphocytes (cells/uL) - median (IQR)	54.0 (17.8-170.0)	1.0 (0.0-49.8)	0.515
CSF protein (g/L) - median (IQR)	1.49 (0.78-2.00)	0.25 (0.18-0.38)	0.000
CSF glucose (mmol/L) - median (IQR)	2.40 (1.10-3.40)	3.70 (3.00-4.20)	0.000
Positive AFB on CSF microscopy (n/N,%)	2/55 (4)	0/46 (0)	0.125
Positive CSF culture (n/N,%)	12/55 (22)	0/46 (0)	0.000
Positive Genotype MTBDRplus version 1 (n/N,%)	9/38 (24)	0/27 (0)	0.008
Positive Genotype MTBDRplus version 2 (n/N,%)	9/16 (56)	1/20 (5)	0.002
Positive Xpert (n/N,%)	14/55 (26)	0/46 (0)	0.000
CXR- suggestive PTB (n/N,%)	26/55 (47)	7/46 (15)	0.001
CT brain- suggestive TBM (n/N,%)	43/55 (78)	3/28 (11)	0.000

IQR= interquartile range, HIV= Human Immunodeficiency Virus, TB= tuberculosis, GCS= Glasgow coma score, CSF= cerebrospinal fluid, AFB= acid-fast bacilli, CXR= Chest X-ray, PTB= pulmonary tuberculosis, CT= computed tomography, TBM- tuberculous meningitis

*TB contact is defined as a history of recent close contact with a person with infectious tuberculosis within the past 1 year

**Poor weight gain is defined as weight loss, or slower weight gain compared to age and gender-matched controls on the WHO weight for age charts

(36 TBM and 9 non-TBM) with a mean of 2.19 ml (95% confidence interval 1.83-2.55ml). The odds ratio for CSF volume vs positive Genotype MTBDR*plus*® assay was 2.28 (95% confidence interval 1.20-4.36 p=0.012). There was no correlation between CSF volume and positive fluorescent microscopy, culture or Xpert MTB/RIF® assay. Clinical characteristics are summarized in Table 2.

Human immunodeficiency virus (HIV) co-infection was identified in 8 patients; 6 had neuroimaging suggestive of TBM. Of these, 5 had a positive TB contact within the last 12 months, 3 had a chest radiograph suggestive of pulmonary TB, 3 had bacteriologically-confirmed TBM and 1 had bacteriological confirmation of extra-neural TB. Of the 2 non-TBM patients with HIV, 1 had confirmed pneumococcal meningitis and 1 patient did not have meningitis.

The diagnostic accuracy of the CSF tests against a TBM case definition and culture-confirmed TBM is reflected in Table 3 and 4, respectively. When using a TBM case definition as the reference standard, both NAATs performed better than liquid culture and demonstrated some incremental value, although sensitivity remained sub-optimal. When using 'definite' TBM as the reference standard, the MTBDR*plus*® assay performed with 92% sensitivity and 98% specificity and Xpert MTB/RIF® assay performed with 39% sensitivity and 100% specificity in a small (n=13) group of children.

One "non-TBM" case tested positive with the Genotype MTBDR*plus*® assay version 2, but was negative on microscopy, culture and Xpert. The patient had a CSF picture suggestive of viral meningitis, and CSF PCR confirmation of Epstein-Barr virus, as well as normal brain computed tomography (CT). The patient improved clinically without any TB treatment and likely represented a false-positive test. Laboratory cross-contamination could not be ruled out with certainty, but no other cases of potential cross-contamination were detected.

Table 3. Sensitivity, specificity, predictive values and diagnostic odd ratios against a clinical TBM reference standard***

	TBM (n)	Non-TBM (n)	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Diagnostic odds ratio (95% CI)
Total no of subjects	55	46					
Fluorescence microscopy	2	0	0.04 (0.00-0.13)	1.00 (0.92-1.00)	1.00	0.46	4.35 (0.20-92.84)
MGIT	12	0	0.22 (0.12-0.35)	1.00 (0.92-1.00)	1.00	0.52	26.72 (1.54-465.15)
Fluorescence microscopy/MGIT	12	0	0.22 (0.12-0.35)	1.00 (0.92-1.00)	1.00	0.52	26.72 (1.54-465.15)
MTBDR*plus*	18	1	0.33 (0.21-0.47)	0.98 (0.89-1.00)	0.95	0.55	21.89 (2.79-171.78)
Xpert	14	0	0.26 (0.15-0.39)	1.00 (0.92-1.00)	1.00	0.53	32.49 (1.88-561.81)
MGIT/ MTBDR*plus*	25	1	0.46 (0.32-0.59)	0.98 (0.89-1.00)	0.96	0.60	37.50 (4.82-291.73)
MGIT/Xpert	21	0	0.38 (0.25-0.52)	1.00 (0.92-1.00)	1.00	0.58	57.96 (3.39-990.22)
MTBDR*plus*/Xpert combined	27	1	0.49 (0.35-0.63)	0.98 (0.89-1.00)	0.96	0.62	43.39 (5.58-337.39)
MTBDR*plus*/Xpert/ MGIT combined	31	1	0.56 (0.42-0.70)	0.98 (0.89-1.00)	0.97	0.65	58.13 (7.47-452.43)

TBM= tuberculous meningitis, MGIT= Mycobateria Growth Indicator Tube, PPV= positive predictive value, NPV= negative predictive value, CI= confidence interval

*** Uniform research case definition of Marais et al[22].

Table 4. Sensitivity, specificity, predictive values and diagnostic odd ratios against A) a bacteriologically-confirmed (definite) TBM reference standard B) a definite and 'probable' TBM reference standard

	TBM (n)	Non-TBM (n)	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Diagnostic odds ratio (95% CI)
A							
Total no of subjects	13	46					
MTBDR*plus*	12	1	0.92 (0.64-1.00)	0.98 (0.89-1.00)	0.92	0.98	540.00 (31.42-9279.80)
Xpert	5	0	0.39 (0.14-0.68)	1.00 (0.92-1.00)	1.00	0.85	60.18 (3.04-1191.80)
MTBDR*plus* /Xpert combined	12	1	0.92 (0.64-1.00)	0.98 (0.89-1.00)	0.92	0.98	540.00 (31.42-9279.80)
B							
Total no of subjects	45	46					
MTBDR*plus*	14	1	0.31 (0.18-0.47)	0.98 (0.89-1.00)	0.93	0.59	20.32 (2.54-162.62)
Xpert	14	0	0.31 (0.18-0.47)	1.00 (0.92-1.00)	1.00	0.60	42.81 (2.46-743.98)
MTBDR*plus* /Xpert combined	23	1	0.51 (0.35-0.66)	0.98 (0.89-1.00)	0.96	0.67	47.05 (5.96-371.35)

TBM= tuberculous meningitis, MGIT= Mycobateria Growth Indicator Tube, PPV= positive predictive value, NPV= negative predictive value, CI= confidence interval

An *inh*A mutation was detected in one patient, using the Genotype MTBDR*plus*® assay version 2, which is usually associated with low-level isoniazid resistance. This specific patient's treatment regimen included high dose isoniazid (20mg/kg), along with high dose rifampicin (20mg/kg), ethionamide (20mg/kg) and pyrazinamide (40mg/kg). As this patient defaulted treatment for 1 month, the total treatment period was 12 months. The patient was clinically followed up at 1-monthly intervals, with consistent weight-gain throughout. After 12 months of therapy the patient was considered cured, and discharged.

DISCUSSION

The main finding of this study is the incremental increase in diagnostic accuracy that can be achieved with commercial NAATs performed on CSF. Although both NAATs were superior to liquid culture, sensitivity remained low compared to a rigorous pre-defined clinical case definition. Combining any positive NAAT provided a sensitivity of 49%, which is insufficient to serve as a rule-out test and provides limited clinical guidance. However, a positive test provides useful microbiological confirmation with rapid turn-around times. When compared to culture-confirmed TBM, both NAATs

performed with better sensitivities (especially MTBDR*plus*® assay with sensitivity 92%), however patient numbers in this group were small.

A recent meta-analysis of the accuracy of commercial NAATs for the diagnosis of TBM revealed a pooled sensitivity and specificity of 64% and 98%. These studies used culture-confirmed TBM as the reference standard[29], a group where higher sensitivities would have been expected. A uniform research case definition proposed for adults and children state that a TBM diagnosis can be regarded as "definite" when *M.tuberculosis* is cultured from CSF and/or a commercial NAAT is positive for *M.tuberculosis*[22]. Our findings support this position, since only a single NAAT test was considered to be a false positive test; likely the result of laboratory contamination. This emphasizes the importance of ensuring optimal laboratory infection and contamination control standards.

The relatively poor correlation between NAATs and liquid culture may reflect the fact that NAATs detect DNA from viable and non-viable bacteria. Although every attempt was made to collect the CSF sample prior to the initiation of empiric therapy, some children were referred from outside centers and received initial treatment prior to CSF collection. This could explain the relatively low culture yields achieved, but it cannot explain why only a minority of cases with positive NAAT were both MTBDR*plus*® and Xpert MTB/RIF® positive. NAAT discrepancy may be due to random sampling variation in a pauci-bacillary CSF specimen. It has been suggested that at least 6 ml of CSF should be collected and concentrated to improve the diagnostic yield[30]. From our paediatric population we could only obtain a mean of 2.19 ml of CSF, and splitting these low volumes for four different tests could have resulted in false negative tests in instances where the bacterial load was below detection threshold. However, low CSF volumes are an unfortunate clinical reality in young children and in clinical practice all these tests will not have to be performed in parallel. Even with the low CSF volume obtained, the yield for the MTBDR*plus*® assay increased significantly with increased CSF volume.

The sensitivity of fluorescence microscopy (4%) was lower than that reported in the literature (10-20%)[6] and that of MGIT liquid culture was comparable (26% vs 22%)[31]. There are no studies describing the use of the MTBDR*plus*® assay in CSF samples of either adults or children. The sensitivity of 33% (98% specificity) against a TBM case definition and sensitivity of 98% (98% specificity) against microbiologically-confirmed TBM, is encouraging and compares favorably with the performance on smear microscopy-negative sputa (19%)[32]. Xpert was 26% sensitive (100% specificity) against a TBM case definition and 39% sensitive (100% specificity) against

microbiologically-confirmed TBM, but the use of this assay on CSF is not yet that well described. A pooled sensitivity of 70%; specificity of 97% for Xpert MTB/RIF® compared to liquid culture as a reference standard, was obtained in five studies in a recent meta-analysis[13,14,30,33-35]. Concentration steps could have helped to reach the Xpert MTB/RIF® assay's detection threshold of approximately 100 bacteria/ml[13].

Our clinical practice is to start anti-tuberculosis treatment on clinical suspicion prior to bacteriological confirmation. In settings with low TB incidence where experience with TBM is limited, treatment can be delayed with potentially dire consequences. In such settings NAATs offer improved CSF sensitivity, with good specificity, and a potential for same day diagnosis. The cost of the NAAT assays needs to be put into perspective to potential cost-savings by shorter hospital stay and better outcomes due to earlier initiation of treatment.

CONCLUSION

Commercial NAATs performed on CSF revealed incremental improvement in sensitivity, with specificity maintained. The best sensitivity was obtained with the combination of liquid culture and both NAATs, but there is not a massive gain when compared to both NAATs only. However, NAATs alone or in combination, cannot serve as a rule out test but can provide rapid microbiological confirmation. Cost-analysis needs to be performed comparing the expense of NAATs to the potential cost saving of early initiation of treatment and shorter hospital stay.

AUTHOR CONTRIBUTIONS

Each of the authors contributed equally.

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CONFLICT OF INTEREST

None of the authors declared a conflict of interest.

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PART IV GENERAL DISCUSSION AND SUMMARY

5 General discussion with overview of the studies, limitations and future perspectives

EARLY DIAGNOSIS

It is important to recognize TBM in the early stage of disease, as it is fully treatable. However, early stage TBM is characterized by non-specific symptoms of general ill health rather than features of meningitis. The only factor differentiating these symptoms of TBM from other common illnesses is their persistence, often >5 days duration [1]. Failure to recognize the threat of TBM by both caregivers and healthcare professionals can lead to neurological deterioration and death. Due to the suboptimal performance of definite diagnostic tests, the early identification of paediatric TBM relies on a thorough assessment of all the evidence derived from a careful history, clinical examination and relevant investigations. All the criteria for definite diagnosis of TBM depend on demonstrating the organism in the CSF. This fact underlines the difficulty in diagnosing stage 1 TBM which is defined by absence of neurological signs, including meningism. As a rule, lumbar puncture will only be done once meningitis is clinically suspected. This means that current criteria for definite diagnosis of TBM mainly apply to stage 2 and 3 TBM.

Previous studies (one comprising both adults and children and the other comprising only adult patients) have identified young age, sub-acute onset, headache, normal peripheral white blood cell count, clear cerebrospinal fluid (CSF) appearance, moderately raised total CSF white cell count (<900 cells/μL), low CSF neutrophil proportion, and elevated CSF protein as differentiating features between TBM and bacterial meningitis [2-4].

UNIFORM RESEARCH CASE DEFINITION FOR TBM

RETROSPECTIVE CLINICAL EVALUATION OF THE RESEARCH CASE DEFINITION

The uniform case definition for TBM was devised as an instrument to improve accuracy of clinical diagnosis in future interventional studies on TBM. It was compounded by a group of experts and based on existing knowledge of TBM as portrayed in the literature [5]. It has never before been tested clinically. Our existing database of hundreds of childhood TBM cases enabled us to test this proposed diagnostic tool retrospectively. I showed that the proposed clinical case definition had excellent diagnostic performance

in differentiating culture-confirmed TBM from culture-confirmed bacterial meningitis if the suggested 'probable' TBM score was used [6]. When the 'possible' TBM score was used not a single TBM case would have been missed, however clinical utility was minimal given the low specificity achieved. Univariable analysis showed that the majority of criteria were associated with a diagnosis of TBM, warranting inclusion in the uniform research algorithm [5].

Because it was performed retrospectively, the study was limited by absence of data required by the uniform case definition. This included pre-contrast basal hyperdensity as shown by computed tomography (CT), magnetic resonance imaging (MRI) or ultrasound imaging of TB outside the CNS and PCR evidence of extra neural TB. Pre-contrast basal hyperdensity on CT was described only in 2004 and was therefore not available retrospectively [7]. Another limitation of the clinical application of the uniform case definition, shown by the above study, was its inability to identify TBM in the early stage [8,9]. This can be explained by the absence of abnormal neurological findings as well as CSF and radiological features typical of TBM in stage 1 disease resulting in a low score when applying the uniform case definition. Consequently many of these cases will be diagnosed as 'possible' rather than 'probable' TBM. This is a significant limitation since both mortality as well as morbidity significantly increases when TBM progresses from stage 1 to stage 2. The diagnosis of early stage I TBM, therefore remains problematic and emphasizes the importance of a very high clinical index of suspicion in young children with recent TB exposure and persistent non-specific signs.

PROSPECTIVE CLINICAL EVALUATION OF THE RESEARCH CASE DEFINITION

My prospective evaluation of the uniform TBM research case definition was a first attempt to determine the diagnostic value of this research tool in clinical practice. The findings of this study confirmed those of the retrospective study in that excellent diagnostic accuracy was obtained for a diagnosis of TBM when compared to bacterial and viral meningitis controls. In this study a 'probable' TBM score demonstrated sensitivity of 84% and excellent specificity of 95% in clinically-diagnosed TBM, while in microbiologically-confirmed TBM cases a sensitivity of 74% and specificity of 95% was obtained. The high specificity of a 'probable' TBM score justifies its use as an alternative reference standard to microbiological confirmation in future studies.

However, univariable analysis showed that only half of criteria in the uniform case definition was associated with a diagnosis of microbiologically-confirmed TBM, warranting a re-look at inclusion criteria which were based on consensus opinion. The overall performance of a probable TBM score was better than a three-variable predictive model determined by multivariable analysis, but the use of three simple criteria (cranial nerve palsy, TST ≥10mm and elevated CSF protein) may offer better clinical utility in areas with limited access to neuro-imaging, taking into consideration that the analysis was optimized for my study cohort.

This study was limited by the small number of cases of bacterial meningitis. This can be explained by the fact that both Haemophilus influenza type-B and pneumococcal vaccination are part of the expanded national immunization program which is provided free of charge to all children. A further limitation is the low rate of neuroimaging in non-TBM cases, which is due to the fact that neuroimaging is not routinely performed in non-TBM suspects in the local setting. The relatively low proportion of microbiologically-confirmed TBM cases reflects the paucibacillary nature of the disease, sub-optimal sensitivity of available diagnostic tests, and low CSF volumes obtained [10-14]. The fact that the study was performed at a single site may theoretically limit generalization of the study findings to other settings. However, this is unlikely because our population shares a similar disease burden, health challenges and resource constraints with other TB endemic areas.

CEREBROSPINAL FLUID CHEMISTRY FINDINGS IN CHILDHOOD TBM

The clinical diagnosis of TBM relies on the triad of history, clinical examination and special investigtaions. The diagnosis of meningitis, including TBM, relies on a pattern of CSF findings, which also forms the basis for inclusion of CSF criteria in the uniform research case definition. My study on the diagnostic value of CSF chemistry in the diagnosis of childhood TBM found that the optimal lower limit of CSF glucose concentration as a diagnostic aid for TBM was 2.2 mmol/L (40 mg/dl). This is problematic, as it is similar to that proposed for bacterial meningitis [15]. This highlights the value of including other aspects of CSF chemistry such as macroscopic appearance, cell count and protein level in the final analysis. Absolute CSF glucose differentiated non-TBM from TBM cases with sensitivity of 68% and specificity of 96%, excluding its use as a 'rule-out' test. This sudy emphasized that fewer patients with

TBM would have been missed using a CSF:glucose ratio <0.5 (10%) compared to an absolute CSF glucose concentration of <2.2 mmol/L (32%). Unfortunately, this is rarely done in clinical practice as confirmed by the lack of retrospective data in this regard.

The optimal CSF protein cut-off differentiating TBM from bacterial meningitis and viral meningitis is >1g/L (100mg/dL) [2,16]. I found that an optimal CSF protein cut-off of >0.6 g/L performed with sensitivity and specificity of 95% and 91% respectively in differentiating TBM from viral meningitis. The suggested higher CSF protein cut-off (>1g/L) performed with less impressive diagnostic accuracy (sensitivity 76% and specificity 95%) but it must be taken in to account that only viral meningitis and non-meningitis were considered as alternative diagnoses in my study. The uniform TBM research case definition included CSF protein cut-off of >1g/L, clear CSF macroscopic appearance, moderately elevated leucocyte count and the presence of lymphocyte predominance to assist with the distinction between TBM and bacterial meningitis [6].

Many other CSF biomarkers have been identified in TBM, but diagnostic utility is rarely described [17]. Studies evaluating CSF interferon gamma release assays (IGRA) demonstrated good diagnostic accuracy [18-21], but difficulty in obtaining CSF volumes that will provide sufficient cells for analysis is a limitation in children (typically 5-10ml CSF is required) [22, 23]. A recent study from a co-worker using a CSF proteinomics approach reveals the potential value of CSF interleukin-13, vascular endothelial growth factor and cathelicidin LL-37 as biomarkers when differentiating TBM from other forms of meningitis [24]. There is potential for the use of CSF lactate in children with TBM. CSF lactate has been shown to be a good biomarker to distinguish bacterial from aseptic meningitis [25], and remains unaffected by serum lactate concentration, reflecting the severity of cerebral hypoxia [26].

My study on the value of CSF chemistry in the diagnosis of TBM was limited by the small number of patients with serum glucose determination. Low numbers of TBM and HIV co-infection (4%) precluded separate analysis of the value of CSF glucose and protein levels in this subgroup. This low incidence of HIV co-infection in TBM concurs with other reported studies on TBM [27].

CHEST X-RAY FINDINGS IN CHILDHOOD TBM

The finding of a chest X-ray suggestive of pulmonary TB in a child that presents with meningitis implies an increased likelihood of TBM [6]. Previous studies have confirmed evidence of active pulmonary TB on chest X-ray in 70-84% of children with TBM [28]. My findings differ from these in that I found chest X-ray findings highly suggestive of pulmonary TB in only 46% of children with TBM while 11% had evidence suggestive of miliary TB. The need to treat calculation showed that only 1 in 4.39 children ≤3 years of age with TBM are likely to have 'certain TB' on chest X-ray [29]. Possible explantions of the lower proportion of'certain' TB could be the minimization of over-reporting Chest X-ray findings by using two reviewers. Another possible reason could be our low proportion of children (less than one third) with a positive tuberculin skin test, reflecting immature cell-mediated immunity in childhood, manifesting as decreased radiographic evidence of pulmonary TB and under-reporting of the chest X-ray. Intra-thoracic lymph node disease was classified as either uncomplicated or complicated, according to a radiological classification of childhood intra-thoracic tuberculosis, using a structured approach to interpretation and recording chest X-ray findings [30]. Airway compression was defined as either compression of the trachea, left main bronchus or bronchus intermedius. Parenchymal changes were defined as either consolidation (including expansile pneumonia) or miliary.

The poor diagnostic sensitivity of chest radiography in children with TBM implies that it cannot be used as a rule-out test, even in combination with TST and may impact scoring in future diagnostic rules and algorithms. With a normal chest X-ray, the diagnosis of TBM is even more reliant on a combination of clinical features, CSF findings, neuroimaging and microbiological confirmation. As expected complicated lymph node disease and airway compression were significantly more common in children ≤3 years, confirming that this is the predominant radiological finding in young children. TBM stage did not affect the radiographic picture. Lung CT is more sensitive than chest X-ray in detecting TB lymphadenopathy, but less sensitive in detecting parenchymal involvement in children under 3 years old. CT is also more expensive and delivers a much higher dose of radiation, mitigating its role in routine lung imaging in childhood [31]. Due to excellent contrast resolution, MRI has equivalent diagnostic performance to high resolution CT for the detection of pulmonary TB [32], but again cost rules out its routine use in children.

NAA TESTING IN TBM

UPDATED META-ANALYSIS OF COMMERCIAL NAA TESTS

Definite diagnosis of TBM by CSF examination such as direct microscopy for acid-fast bacilli and M.tuberculosis culture are notoriously insensitive in the diagnosis of TBM [33]. The necessity of early, accurate and rapid TBM diagnosis is undisputable [34]. A 2003 systematic review on the diagnostic accuracy of NAA tests found that significant heterogeneity affected the interpretation of in-house NAA tests. The pooled sensitivity and specificity of commercial NAA tests was 56% and 98%, respectively, suggesting a role for commercial NAA tests in confirmation, but not exclusion, of TBM diagnosis [33]. Since 2003, newer commercial NAA tests have emerged, with attention focused on Xpert MTB/RIF®. My recent meta-analysis of commercial NAA tests found an improved summary sensitivity of 64% and confirmed the previous excellent specificity of 98% [33,35]. Summary sensitivity of commercial NAA tests remains suboptimal and is unlikely to greatly enhance early accurate diagnosis. Conversely the excellent specificity suggests that commercial NAA tests may be regarded as definitive in the correct clinical setting [6].

In 2013, the WHO recommended Xpert MTB/RIF® as the preferential initial investigation in all adults and pediatric TBM suspects [36]. My meta-analysis of 5 studies reporting Xpert MTB/RIF® on CSF, 1 retrospective and 4 prospective, found summary sensitivity of 70% and specificity of 97% [34,35,37-40]. Despite sub-optimal sensitivity, the rapid turnaround time of NAA tests compared to culture enhances its role in the early accurate diagnosis of TBM. Future studies need to confirm excellent sensitivity, specificity and negative predictive value in order to justify the use of Xpert MTB/ RIF® as a "stand alone" test for diagnosis of TBM. A further benefit of NAA testing is the early detection of drug resistance.

My recent updated meta-analysis supports the use of a commercial NAA test as evidence of a 'definite' TBM diagnosis in the right clinical context, as suggested by the uniform research case definition for TBM [6]. This meta-analysis is limited by the inconsistent use of reference standards in the different studies, with the sub-optimal sensitivity of microscopy for acid-fast bacilli and CSF culture for M.tuberculosis prompting the use of alternate clinical reference standards. There were multiple study exclusions, but careful assessment of study accuracy and reliability using the

QUADAS-2 tool strengthened the findings of the meta-analysis [41].

COMPARING DIFFERENT NAA TESTS PROSPECTIVELY

In a follow-up study I found that more than one NAA test incrementally increased diagnostic accuracy on CSF in childhood TBM [42]. Although both the MTBDRplus® assay and Xpert MTB/RIF® were superior to liquid culture, sensitivity remained low compared to a rigorous predefined clinical case definition. The MTBDRplus® assay performed with an encouraging sensitivity of 33% (98% specificity) against a TBM case definition and sensitivity of 98% (98% specificity) against microbiologically-confirmed TBM. There are no other studies describing the use of the MTBDRplus® assay in CSF samples of either adults or children with TBM. Xpert MTB/RIF® was 26% sensitive (100% specificity) against a TBM case definition and 39% sensitive (100% specificity) against microbiologically-confirmed TBM. Combining these two NAA tests provided a sensitivity of 49% (98% specificity) against a TBM case definition, which is insufficient to serve as a rule-out test and provides only limited clinical guidance [42]. However, a positive test provides rapid microbiological confirmation providing further support for the use of a positive commercial NAA test as evidence of 'definite' TBM in the uniform research case definition for TBM [6]. Apart from improving the potential for same day diagnosis which prevents unnecessary treatment delay and potential life-threatening consequences, NAA tests has the added benefit of early recognition of drug resistance.

This study is limited by the relatively poor correlation between NAA tests and liquid culture. Although every attempt was made to collect the CSF sample prior to the initiation of empiric therapy, some children were referred from outside centers and received initial treatment prior to CSF collection. This could explain the relatively low culture yields achieved, but it cannot explain why only a minority of cases tested positive with both MTBDRplus® and Xpert MTB/RIF®. Discrepancy found in NAA test results may be partly due to random sampling variation in a condition known for its low bacilli count in CSF. Dividing the small mean CSF volume of 2.19 ml collected in this study into smaller volumes for four different tests could have resulted in false negative tests in instances where the bacterial load was below detection threshold. However, low CSF volumes are an unfortunate clinical reality in young children. Concentration steps could have helped to reach the Xpert MTB/RIF® assay's detection threshold of approximately 100 bacteria/ml [38].

OTHER INVESTIGATIONS IN CHILDHOOD TBM

In my update on the diagnosis and management of TBM in children I discuss other common investigations in the early diagnosis of TBM [23]. The tuberculin skin test (TST) performed with a sensitivity of 61% [10], however this is decreased in HIV co-infection [43]. Further limitations of TST is high false-positivity in young infants that have received BCG vaccination, and failure to delineate active TB disease [44]. Neuroimaging criteria are an important component of the uniform research case definition for TBM and helps clear up diagnostic uncertainty after clinical assessment and CSF analysis. Computed tomography (CT) is most often used in resource poor countries and a combination of hyperdense exudates on pre-contrast CT, basal meningeal enhancement, infarctions and hydrocephalus is highly suggestive of TBM [7]. Magnetic resonance imaging (MRI) is superior to CT for TBM diagnosis, by detecting basal enhancement and granulomas in more patients, and prognosis, by detecting many more infarcts in strategic locations such as the brainstem [45]. MRI is an invaluable tool in children with TBM and acquired blindness due to optochiasmic arachnoiditis, and can guide urgent intervention leading to improvement of vision [46].

STAGE 1 TBM

A consistent theme in the different chapters of this thesis is the small numbers of stage 1 TBM in most studies due to the difficulty of early clinical diagnosis of TBM. Ideally, in a thesis of this nature, early diagnosis of TBM should have been aimed at early identification of stage 1 TBM. However, this was unfortunately not possible mainly because my study was hospital-based and most cases of early, stage 1 TBM present at primary-care level. This is a major limitation of this study but opens the way for future studies in this regard. Early diagnosis of stage 1 childhood TBM, which is characterized by non-specific clinical features including the absence of meningism, is inseparably linked to improved surveillance of childhood TB. Since most of the clinical signs of stage 1 TBM relates to underlying pulmonary TB, a sound surveillance system could improve estimates and monitoring of both the TB and TBM burdens in young children [47]. In order to improve diagnosis of stage 1 TBM, awareness among health care workers must be improved especially at the primary-level healthcare setting, the point of first contact for many patients. Integrated Management of Childhood Illness (IMCI) a strategy developed by the World Health Organization (WHO) and the United Nations

Childrens Fund (UNICEF) is aimed at early detection of serious illnesses in young children under 5 in resource-limited settings. This allows early referral and thus a reduction in morbidity and mortality. IMCI is potentially a valuable tool in detecting early TB and TBM as it is practiced at primary healthcare level. A further strategy to improve the detection of childhood TB and TBM is household contact tracing which has the potential, when combined with preventive therapy, to reduce the burden of TB [48]. The failure to provide TB prophylaxis with isoniazid therapy to paediatric contacts is unacceptable as this can prevent the progression from infection with M.tuberculosis to active TB in a large proportion of children [49].

Because the early clinical presentation of TBM is non-specific, poor weight gain, or weight loss, reflected by crossing of weight centiles, should alert healthcare workers at first contact level with the patient to the possibility of TBM [50]. The same applies to the presence of a persistent non-remitting cough for longer than 2 weeks [51]. It should be highlighted that in my different studies not a single patient with TBM was white. If ancestry is used as a proxy for socioeconomic status and level of nutrition, the affinity of TBM to affect black and mixed ancestry children is glaring and an indication that socio-economic inequalities need to be addressed urgently and that available financial resources should be distributed more fairly.

FUTURE PERSPECTIVES

TBM is the most feared consequence of M.tuberculosis infection in young children. The epidemiology of TBM is poorly understood because of sub-optimal diagnosis and under-reporting of childhood TB [52]. Standardization of TBM diagnosis and treatment is problematic. In order to progress, researchers have to speak the same language as to what constitutes a diagnosis of TBM. The uniform research case definition of TBM was an attempt to reach expert consensus on diagnosis. Both my studies testing the utility of the uniform research case definition reveal excellent diagnostic accuracy and consideration of the uniform case definition as an alternative research reference standard in children. Having a conceptual reference standard is acceptable, but for those working in the field, research has to lead to meaningful improvement of clinical well-being. Both my studies were performed in a clinical setting, and had superior diagnostic performance to existing diagnostics. I envisage robust multi-centre prospective studies in both adults and children in order for the uniform case definition

to transcend research to clinical practice in TB endemic settings.

Prospective research should include validation of the components of the uniform case definition which were not addressed in this dissertation. I have shown that the CSF glucose and protein cut-off deserve inclusion in the uniform case definition. However, the diagnostic value of chest X-ray findings needs further investigation due to the lower proportion of children with TBM and radiological evidence of pulmonary TB in my study than previously reported. There are therefore research opportunities to evaluate the clinical, neuroimaging and supporting criteria in the uniform case definition. Provided that large numbers can be obtained, statistic modeling can be used to validate the uniform research case definition as a whole. Once supporting prospective research in both children and adults reveals validity and clinical applicability of the uniform research case definition, designing a smartphone application for identification of 'probable' or 'possible' TBM is a possibility. This is achievable as the rollout of cheaper smartphones and mobile networks is the fastest growing in resource-constrained settings.

The 2013 WHO recommendation that Xpert MTB/RIF® can be used as the preferential initial investigation in all adults and pediatric TBM suspects [36][53], underlines the need for more research in this area. I showed that Xpert MTB/RIF® performed with lower sensitivity, 26%, when compared to MTBDRplus® assay, 33%, and Xpert MTB/RIF® and MTBDRplus® combined, 49% [42]. My future research will include analyzing the performance of Xpert MTB/RIF® on bigger CSF samples, if feasible on a minimum volume of 5ml, with an additional concentration step in order to try and improve the diagnostic accuracy.

In conclusion, the proposed uniform research case definition provided excellent diagnostic accuracy compared to microbiologically-confirmed TBM, when tested both retrospectively and prospectively and is recommended as the 'standard' definition of TBM for research purposes. Clinical vigilance is advised in early (stage 1) TBM as the uniform research case definition performs with decreased sensitivity in this group. Similarly clinical vigilance is strongly advocated in the setting of a normal chest X-ray as less than half of the childhood TBM cases had chest X-ray findings highly suggestive of pulmonary TB. When differentiating childhood TBM from other forms of meningitis, the optimal lower limit of CSF glucose concentration is 2.2 mmol/L and upper limit of

CSF protein is 1g/L (100mg/dL). It is recommended that serum glucose concentration is determined at the same time as CSF glucose concentration, as the CSF:serum glucose ratio improves diagnostic sensitivity. Meta-analysis of newer commercial NAA tests, including Xpert MTB/RIF®, found a suboptimal summary sensitivity of 64% which is unlikely to enhance early accurate diagnosis. However, the excellent summary specificity of 98% suggests that commercial NAA tests may be regarded as definitive in the correct clinical setting. The low sensitivity of commercial NAA tests was confirmed when testing the MTBDRplus® and Xpert MTB/RIF® assays on fresh CSF smaples, individually and in combination. However, routine commercial NAA testing is strongly recommended, as rapid microbiological confirmation ensures early TBM diagnosis, preventing unnecessary treatment delay and potential life-threatening consequences, with the additional advantage of early detection of mycobacterial resistance.

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6 English & **Afrikaans summaries**

SUMMARY

Due to the sub-optimal performance of definite diagnostic tests, the early identification of paediatric TBM suspects relies on a thorough assessment of all the evidence derived from a careful history, clinical examination and relevant investigations. Rapid diagnosis is needed for early treatment initiation but microbiological confirmation is difficult at stage 1 disease because a lumbar puncture is generally only done once signs of meningitis have developed (stage 2 and 3 TBM). With this thesis, I aim to investigate mechanisms of improving the early and/or more accurate diagnosis of childhood TBM.

PART I

In chapter 2 I provide an update on the diagnosis and management of TBM in children, based on local experience, which can be transposed to similar settings. Highlights include firstly that short (6 months) intensified therapy in children with drug susceptible TBM is safe and effective, with a good outcome and low mortality. Secondly, home-based TBM treatment after initial in-hospital stabilization is feasible in carefully selected patients under close supervision. Thirdly, treatment of tuberculous hydrocephalus depends on the level of cerebrospinal fluid (CSF) obstruction. Communicating hydrocephalus can be successfully treated with medical therapy with normalization of intracranial pressure occurring within days in the majority children and non-communicating hydrocephalus requires neurosurgical intervention. Fourthly, thalidomide is the local drug of choice in children who develop life-threatening TB mass lesions (IRIS) despite corticosteroids.

PART II

In clinical practice, TBM diagnosis is most often based on a combination of clinical, laboratory and radiological findings. A uniform research case definition utilizing these criteria was proposed by an international panel of experts as a means of improving diagnostic standardization in order to answer critical research questions, categorizing patients as definite, probable, or possible TBM. Part 2 of my thesis focuses on the diagnostic utility of the uniform research case definition criteria for TBM. In chapter

3.1 I retrospectively evaluate the diagnostic performance of probable and possible TBM criteria in children with culture-confirmed TBM and culture-confirmed bacterial meningitis. The proposed uniform research case definition provided excellent diagnostic accuracy compared to microbiologically-confirmed TBM, when a 'probable' TBM score was used. When a 'possible' TBM score was used, not a single TBM case would have been missed, but clinical utility was minimal given the low specificity achieved. In order to strengthen my findings I prospectively assessed the diagnostic accuracy of the uniform TBM research case definition (see chapter 3.2). Excellent diagnostic accuracy was obtained for a diagnosis of TBM when compared to bacterial and viral meningitis controls. The high specificity of a probable TBM score justifies its use as an alternative reference standard to microbiological confirmation in future studies. In both studies poor sensitivity was obtained when a probable TBM score was used to diagnose early (stage 1) TBM, emphasizing a very high clinical index of suspicion of TBM in young children with recent TB exposure and persistent non-specific signs.

CSF findings are essential to early diagnosis of TBM. Cut-off values for CSF glucose in TBM lack evidence. A CSF protein cut-off of >1g/L (100mg/dL) differentiated between cases of TBM and other forms of meningitis. My study on the diagnostic value of cerebrospinal fluid chemistry results in childhood TBM found that the optimal lower limit of CSF glucose concentration as a diagnostic aid for TBM was 2.2 mmol/L (see chapter 3.3). Absolute CSF glucose differentiated non-TBM from TBM cases with sensitivity of 68% and specificity of 96%, excluding its use as a 'rule-out' test. Simultaneous determination of serum and CSF glucose was seldom performed but my findings suggest that the CSF:serum glucose ratio may further improve diagnostic sensitivity.. CSF protein cut-off of >1g/L as well as CSF macroscopic appearance, cell counts and the presence of lymphocyte predominance are required to assist the distinction between TBM and bacterial meningitis.

Previous studies suggest that chest X-ray findings consistent with active pulmonary TB are observed in 70% to 84% of children with TBM. In my study (chapter 3.4) only 46% of cases with TBM had chest radiograph findings highly suggestive of pulmonary

TB. A need to treat calculation showed that only 1 in 4.39 children ≤3 years of age with TBM are likely to have 'certain TB' on chest X-ray.

PART III

Microbiological confirmation of TBM remains the gold-standard of diagnosis, but is challenging in young children due to the paucibacillary nature of disease, low CSF volumes available for diagnostic analysis and sub-optimal sensitivity of direct microscopy for acid-fast bacilli and *M.tuberculosis* culture on CSF. Several new commercially available NAA tests have been developed for the rapid diagnosis of TB. In part III of the thesis my meta-analysis of newer commercial NAA tests found a summary sensitivity of 64% and specificity of 98%. Summary sensitivity of commercial NAA tests remains suboptimal and is unlikely to greatly enhance early accurate diagnosis. However, the excellent specificity suggests that commercial NAA tests may be regarded as definitive in the correct clinical setting. In 2013, the WHO recommended Xpert MTB/RIF® as the preferential initial investigation in all adults and pediatric TBM suspects. My sub-analysis of 5 studies reporting Xpert MTB/RIF® on CSF, found summary sensitivity of 70% and specificity of 97%.

In chapter 4.2 I aim to assess the utility of MTBDR*plus*® and Xpert MTB/RIF® to diagnose TBM in a clinical setting, alone and/or in combination. The main finding was the incremental increase in diagnostic accuracy achieved with combined use of these commercial NAA tests performed on CSF. Although both NAA tests were superior to liquid culture, sensitivity remained low compared to a rigorous predefined clinical case definition. The MTBDR*plus*® assay performed with sensitivity of 33% (98% specificity), Xpert MTB/RIF® was 26% sensitive (100% specificity) and combining positive results from both these tests provided a sensitivity of 49% (98% specificity) against a TBM case definition. This is insufficient to serve as a rule-out test and provides limited clinical guidance. However, microbiological confirmation provided by a positive test prevents unnecessary treatment delay and potential life-threatening consequences. The additional advantage of a positive NAA test is that of early detection of mycobacterial resistance.

A major limitation of this study was the failure to improve diagnosis of stage 1 childhood TBM, mainly because it was hospital-based. Good surveillance at primary healthcare level, identifying children with poor weight-gain (or weight loss) and persistent non-remitting cough for longer than 2 weeks, could improve the detection of both childhood TB and early TBM. IMCI is potentially a valuable tool to increase awareness of TBM among healthcare workers, and in detecting early TB and TBM, as it is practiced at the healthcare level of first contact. Household contact tracing and prophylaxis with isoniazid therapy, as well as more general measures such as improving nutrition, housing and poverty relief, are valuable measures in preventing TBM in young children.

OPSOMMING

Die vroeë identifisering van kinders met vermoedelike TBM berus op 'n deeglike geskiedenis, kliniese ondersoek en toepaslike spesiale ondersoeke weens die lae sensitiwiteit van definitiewe mikrobiologiese diagnose huidiglik. Vroeë diagnose is noodsaaklik vir spoedige inisiasie van behandeling. Mikrobiologiese bevestiging is selfs meer problematies in stadium 1 TBM omdat lumbale punksie gewoonlik slegs gedoen word in kinders met kliniese tekens van meningisme (stadium 2 en 3 TBM). Met hierdie tesis, beoog ek om meganismes te ondersoek wat vroeë en/of meer akkurate diagnose van pediatriese TBM kan verbeter.

DEEL I

In hoofstuk 2 word die plaaslike diagnose en behandeling van kinder TBM beskryf wat in soortgelyke omgewings toegepas kan word. Hoogtepunte sluit in, eerstens, dat kort (6 maande) intensiewe terapie in kinders met middel-sensitiewe TBM veilig en doeltreffend is, met goeie uitkoms en 'n lae sterftesyfer. Tweedens, tuis-gebaseerde TBM behandeling na aanvanklike binnepasient stabilisering is haalbaar in noukeurig geselekteerde pasiënte onder streng toesig. Derdens, die behandeling van tuberkuleuse hidrokefalus word bepaal deur die vlak van serebrospinale vog (SSV) obstruksie. Kommunikerende hidrokefalus kan suksesvol met mediese terapie behandel word, met normalisering van intrakraniale druk wat plaasvind binne dae in die meerderheid van kinders. Nie-kommunikerende hidrokefalus vereis neurochirurgiese intervensie. Vierdens, thalidomied is die plaaslike middel van keuse in kinders wat lewensgevaarlike TB massa letsels (IRIS) ontwikkel, ondanks kortikosteroïede.

DEEL II

In kliniese praktyk, word TBM dikwels gediagnoseer op grond van 'n kombinasie van kliniese, laboratorium en radiologiese bevindings. 'n Eenvormige gevalsdefinisie, gebaseer op die bogenoemde kriteria is voorgestel deur 'n internasionale paneel van kundiges om die diagnostiese standaard vir navorsingsdoeleindes te verbeter. Vermoedelike gevalle word gekategoriseer as definitiewe, waarskynlike, of moontlike

TBM. Deel 2 van my tesis fokus op die diagnostiese nut van hierdie voorgestelde eenvormige gevalsdefinisie kriteria vir TBM. In hoofstuk 3.1 evalueer ek retrospektief die diagnostiese akkuraatheid van waarskynlike en moontlike TBM kriteria in kinders met kultuur-bevestigde TBM en kultuur-bevestigde bakteriële meningitis. Die voorgestelde gevalsdefinisie het uitstekend vergelyk met mikrobiologies-bevestigde TBM wanneer 'n 'waarskynlike' TBM telling gebruik is. Wanneer 'n 'moontlike' TBM telling gebruik is, soos bepaal deur die gevalsdefinisie, was daar nie 'n enkele geval van TBM gemis nie, maar kliniese nut was minimaal gegewe die lae spesifisiteit. Om my bevindinge te versterk het ek ook die diagnostiese akkuraatheid van die voorgestelde TBM gevalsdefinisie prospektiewelik ondersoek (sien hoofstuk 3.2). Die gevalsdefinisie het diagnosties uitstekend vergelyk met bakteriële en virale meningitis kontroles. Die hoë spesifisiteit van 'n 'waarskynlike' TBM telling soos verkry met behulp van die gevalsdefinisie regverdig die gebruik daarvan as 'n alternatiewe verwysingsstandaard tot mikrobiologiese bevestiging in toekomstige studies. In beide studies (retrospektief en prospektief) was swak sensitiwiteit verkry wanneer 'n 'waarskynlike' TBM telling gebruik was vir vroeë (stadium 1) TBM diagnose. Hierdie bevinding bevestig weereens die belang van 'n hoe indeks van suspisie van TBM in jong kinders met onlangse blootstelling aan TB en persisterende non-spesifieke kliniese simptome en tekens. SSV bevindinge is noodsaaklik vir die vroeë diagnose van TBM, maar die bewyse vir afsnywaardes vir SSV glukose in TBM is gebrekkig. 'n SSV proteïen afsnywaarde van >1g/L (100mg/dL) is wel bewys om te onderskei tussen gevalle van TBM en ander vorme van meningitis. My studie wat die diagnostiese waarde van SSV chemie uitslae in kinder TBM beskryf het bevind dat die optimale ondergrens van SSV glukose konsentrasie as 'n diagnostiese hulpmiddel vir TBM 2.2 mmol/L was (sien hoofstuk 3.3). Absolute SSV glukose konsentrasie onderskei nie-TBM van TBM gevalle met sensitiwiteit van 68% en spesifisiteit van 96%, wat die gebruik daarvan as 'n eliminasie toets uitsluit. Gelyktydige bepaling van serum en SSV glukose was selde uitgevoer, maar ek het bevind dat die SSV tot serum glucose verhouding diagnostiese sensitiwiteit kan verbeter. 'n SSV proteïen afsnywaarde van >1g/L asook ander kenmerke van SSV soos makroskopiese voorkoms, seltelling en die seltipe (oorwegend limfosiete) is van waarde in die onderskeiding van TBM en bakteriële meningitis.

Vorige studies het bevind dat 70% tot 84% van kinders met TBM borskas X-straal bevindinge het van aktiewe long TB. In my studie (hoofstuk 3.4), het slegs 46%, van kinders met TBM borskas X-straal bevindinge hoogs suggestief van long TB getoon. 'n Behoefte tot behandeling berekening ('need to treat') toon dat slegs 1 in 4.39 kinders ≤3 jaar oud met TBM waarskynlik 'definitiewe long TB' op borskas X-straal sal hê.

DEEL III

Mikrobiologiese bevestiging van TBM bly die goud-standaard van diagnose, maar stel hoë eise in jong kinders as gevolg van die lae basillêre tellings wat kenmerkend van TBM is, lae SSV volumes beskikbaar vir diagnostiese analise en sub-optimale sensitiwiteit van direkte mikroskopie vir suur-vaste basille en M.tuberculosis kultuur op SSV. Verskeie nuwe kommersieël beskikbare nukleiensuur amplifikasie (NSA) toetse is ontwikkel vir die spoedige diagnose van TB. In deel III van die tesis, het 'n meta-analise van nuwe kommersiële NSA toetse 'n opsommings sensitiwiteit van 64% en spesifisiteit van 98% bevind. Sensitiwiteit van kommersiële NSA toetse bly dus suboptimaal en daarom 'n onwaarskynlike modaliteit vir verbetering van vroeë akkurate diagnose van TBM; die uitstekende spesifisiteit dui egter daarop dat 'n positiewe kommersiële NSA toets as definitief van TBM beskou kan word in die korrekte kliniese omstandighede. In 2013 het die WGO Xpert MTB/RIF® as die voorkeur aanvanklike ondersoek aanbeveel in alle volwassenes en kinders met verdagte TBM. Vyf studies wat Xpert MTB/RIF® op SSV beskryf het opsommings sensitiwiteit van 70% en spesifisiteit van 97% bevind.

In hoofstuk 4.2 stel ek my ten doel om die nut van MTBDRplus® en Xpert MTB/RIF®, onafhanklik en/of in kombinasie, vir die diagnose van TBM in 'n kliniese omgewing te evalueer. Die belangrikste bevinding van die studie was dat die gebruik van beide bogenoemde kommersiële NSA toetse op SSV die diagnostiese akkuraatheid verhoog het. Beide NSA toetse het beter sensitiwiteit getoon as SSV kultuur, maar was minder sensitief as 'n streng gedefinieërde kliniese gevalsdefinisie. Die onderskeie sensitiwiteit en spesifisiteit was 33% en 98% vir die MTBDRplus® toets, 26% en 100% vir die Xpert MTB/RIF® en 26% en 100% wanneer die gekombineërde toetse vergelyk is met

die TBM gevalsdefinisie. Dit is dus onvoldoende as 'n eliminasie toets en bied beperkte kliniese leiding, maar 'n positiewe toets bied vinnige mikrobiologiese bevestiging wat onnodige behandelingsvertraging en potensiële lewensgevaarlike nagevolge voorkom. ‘n Addisionele voordeel van ‘n positiewe NSA toets is die vroeë herkenning van mikrobakteriele weerstandigheid.

'n Beperking van hierdie tesis is die feit dat diagnose van stadium 1 TBM in kinders nie verbeter is nie. ‘n Hoë indeks van suspisie, veral in kinders met swak gewigstoename (of gewigsverlies) en kinders wat vir langer as 2 weke hoes, kan die diagnose van kinder TB en vroeë TBM verbeter. IMCI, wat op primêre sorg beoefen word, is potensieël 'n waardevolle hulpmiddel om gesondheidswerkers meer bewus te maak van TBM, en dus die diagnose van vroeë TB en TBM te verbeter. Die opsporing van huishoudelike TB kontakte sowel as profilakse met isoniasied, en meer algemene maatreëls soos die verbetering van behuising en die verligting van armoede, moet toenemend as 'n voorkomende strategie teen TBM aangewend word.

Addendum

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CURRICULUM VITAE

Regan Shane Solomons was born in Cape Town, South Africa, in 1975. He graduated MBChB at the University of Cape Town in 1998. From 2003 to 2006 he trained in paediatrics at Tygerberg Children's Hospital in Cape Town and obtained his MMed in paediatrics at Stellenbosch University in 2006. This was followed by a fellowship in paediatric neurology at Tygerberg Children's Hospital, where he completed his certificate in paediatric neurology in 2009. He worked as a general paediatric consultant from 2009 to 2012 at Tygerberg Children's Hospital, and in 2013 was appointed as a paediatric neurology consultant. In 2011 he received a Vrije University-NRF Desmond Tutu Phd Scholarship and commenced research towards a joint PhD at Stellenbosch University and Vrije Universteit Amsterdam. Regan is happily married to Gailyn. They have two children: Nicolas and Celine.

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ABBREVIATIONS

ACA	Anterior carotid artery
AFB	Acid-fast bacilli
ART	Antiretroviral therapy
AUC	Area under the curve
BI	Bronchus intermedius
BCG	Bacille Calmette-Guerin
CFU	Colony forming unit
CI	Confidence interval
CNS	Central nervous system
COMM	Communicating
CSF	Cerebrospinal fluid
CT	Computed tomography
CXR	Chest radiograph/ Chset X-ray
DOR	Diagnostic odds ratio
EPI	Expanded program of vaccination
GCS	Glasgow Coma Scale
HIV	Human immunodeficiency virus
ICA	Internal carotid artery
ICP	Intracranial pressure
IGRA	Interferon gamma release assay
IRIS	Immune reconstitution inflammatory syndrome
IQR	Interquartile range
LAD	Lymphadenopathy
LMB	Left main bronchus
MCA	Middle carotid artery
MDG	Millennium Development Goals
MGIT	Mycobateria Growth Indicator Tube
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
M.tb	*Mycobacterium tuberculosis*
M.tuberculosis	*Mycobacterium tuberculosis*
NAAT	Nucleic acid amplification test
NHLS	National Health Laboratory Service
NON-COMM	Non-communicating
NPV	Negative predictive value
OR	Odds ratio

OR-QNRT	Original quantitative nested real-time
PAS	Para-aminosalicylic acid
PCR	Polymerase chain reaction
PPV	Positive predictive value
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies-2
ROC	Receiver operating characteristic
SIADH	Syndrome of inappropriate anti-diuretic hormone
SROC	Summary receiver operating characteristic
TB	Tuberculosis
TBM	Tuberculous meningitis
TNF-α	Tumor necrosis factor alpha
TST	Tuberculin skin test
US	Ultrasound
VPS	Ventriculo-peritoneal shunt
WHO	World Health Organization
WR-QNRT	Wide-range quantitative nested real-time