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Improving early diagnosis of tuberculous meningitis in children

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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.

In 2013, there was an estimated 9.0 million new TB cases worldwide [4]. 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 [4]. Central nervous system (CNS) involvement, mostly tuberculous meningitis (TBM), accounts for approximately 1% of all cases [5]. In the Western Cape Province of South Africa, a TB endemic area with routine access to *Haemophilus influenzae* type-B and pneumococcal vaccination, TBM was shown to be the most common form of bacterial meningitis [6].

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 [7]. 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 sub-arachnoid space. In addition they found that the Rich focus was histologically more developed ("older") than the granulomas found in concurrently occurring miliary TB [8]. 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 [9,10], or the mechanism whereby *M.tuberculosis* spreads from the lungs to the meninges and crosses the blood-brain barrier [11]. 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 [11]. Magnetic resonance imaging has detected numerous concurrent leptomeningeal granulomas in children with miliary TB, further challenging Rich's pathogenetic model [11,12]. 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 [13].

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. Many of the signs, symptoms, and sequelae of TBM result from an immunologically-directed inflammatory response to the infection [14,15]. 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.

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 [16]. The moment of initiating treatment is the most critical factor affecting morbidity and mortality, highlighting the importance of early diagnosis of TBM [17,18].

With this thesis, the aim is to improve the early and/or more accurate diagnosis of childhood TBM by defining predictive clinical criteria, and evaluating existing and newer 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) [19]. 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%) [20]. 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 [21]. Although macroscopically clear CSF with lymphocyte predominance differentiated TBM from bacterial meningitis [22], 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 [23].

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/ μ l, increased protein >1g/dl and decreased glucose defined as <2.2mmol/l or CSF to serum ratio of <50% [24-28].

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 [29,30]. Trials assessing TBM therapy had limited power, poor methodology and various treatment regimens were used with discordant results [30]. 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 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 [31]. 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 [32]. 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 [28,33,34]. 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 [35]. 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 [22,23]. 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 [36-38]. In children the frequency is even higher ranging from 70% to 84% [39]. 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 [40]. 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%) [41]. *M.tuberculosis* culture may take up to 42 days and has only slightly improved sensitivity (<50%) [28,42,43]. 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 [44,45].

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 [46]. A decade old meta-analysis of the diagnostic value of NAA tests in TBM (studies 1990-2002 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 [47].

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® [48]. 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 [49]. Xpert MTB/RIF® also detects susceptibility to rifampicin by amplification of the *rpoB* gene [50,51]. The MTBDR*plus*® 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 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* more rapidly (under 2 hours vs 5 hours), with easier handling and lower contamination rates [52-54]. 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 [55]. In chapter 4.2 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 is assessed [56]. Study questions were: What is the diagnostic accuracy of Xpert MTB/RIF® on CSF in childhood TBM? What is the diagnostic accuracy of MTBDR*plus*® on CSF in childhood TBM? What is the diagnostic accuracy of combined Xpert MTB/RIF® and MTBDR*plus*® on CSF in childhood TBM?

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