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. We 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 (chapter 3.1) [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**

The 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 (chapter 3.2). 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 were 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 our 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 investigations. 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. Our study on the diagnostic value of CSF chemistry in the diagnosis of childhood TBM (chapter 3.3) 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 study 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]. We 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 into account that only viral meningitis and non-meningitis were considered as alternative diagnoses in the 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].

Our 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]. Our findings differ from these in that we 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 (chapter 3.4). 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]. 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.

**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 [31]. The necessity of early, accurate and rapid TBM diagnosis is undisputable [32]. 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 [31]. Since 2003, newer commercial NAA tests have emerged, with attention focused on Xpert MTB/RIF®. Our recent meta-analysis of commercial NAA tests found an improved summary sensitivity of 69% and confirmed the previous excellent specificity of 98% (chapter 4.1) [31,33]. 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 [34]. Our 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% [32,33,35-38]. 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.

Our 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 [39].
Comparing different NAA tests prospectively

In a follow-up study we found that more than one NAA test incrementally increased diagnostic accuracy on CSF in childhood TBM (chapter 4.2) [40]. 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 [40]. 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 [36].

Other investigations in childhood TBM

In our update on the diagnosis and management of TBM in children we 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 [41]. Further limitations of TST is high false-positivity in young infants
that have received BCG vaccination, and failure to delineate active TB disease [42]. 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 [43]. MRI is an invaluable tool in children with TBM and acquired blindness due to optochiasmatic arachnoiditis, and can guide urgent intervention leading to improvement of vision [44].

**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 [45]. 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 [46]. 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 [47].
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 [48]. The same applies to the presence of a persistent non-remitting cough for longer than 2 weeks [49]. It should be highlighted that in our 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 [50]. 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 our 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 our studies were performed in a clinical setting, and had superior diagnostic performance to existing diagnostics. We 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. We 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 our 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 [34][51], underlines the need for more research in this area. We showed that Xpert MTB/RIF® performed with lower sensitivity, 26%, when compared to MTBDRplus® assay, 33%, and Xpert MTB/RIF® and MTBDRplus® combined, 49% [40]. My own 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.
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