Improving early diagnosis of tuberculous meningitis in children

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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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