CHILDHOOD TUBERCULOUS MENINGITIS: A THIRTY YEAR REVIEW OF CLINICAL AND CEREBROSPINAL FLUID FACTORS ASSOCIATED WITH BACTERIOLOGICAL CONFIRMATION

Michelle Roos

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Supervisors: **Prof. Ronald van Toorn**
Prof. Regan Solomons

DECLARATION

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ABSTRACT

Background: Tuberculous meningitis (TBM) is one of the most devastating complications of tuberculosis (TB)[3] and contributes significantly to the morbidity and mortality of children in high burden TB countries.[2] Early diagnosis of TBM is notoriously difficult due to its inconsistent clinical presentation and lack of a rapid, sensitive and specific diagnostic test. Decision-making in resource-constrained countries is most often guided by use of simple cerebrospinal fluid (CSF) analysis. However, the classical CSF findings of lymphocyte predominance, low glucose and high protein,[5,7,16-20] are only simultaneously present in one third of cases[5], whilst bacteriological confirmation only occurs in 15-40% of cases.[17,18,21,23] Neutrophil predominance,[22,24,26] high protein concentrations and Human Immunodeficiency virus (HIV) infection[24] is thought to increase likelihood of microbiological culture.
Defining the CSF parameters that are associated with confirmation of *Mycobacterium Tuberculosis* will allow for an increased suspicion, and hopefully earlier diagnosis and treatment; especially if those parameters identified are associated with a traditionally atypical TBM CSF picture, higher bacillary load and is influenced by HIV status.

Aim: To describe the CSF parameters that affect the sensitivity of bacteriological confirmation of TBM in 491 CSF samples of children diagnosed with the disease in the past 30 years, at Tygerberg Children's Hospital (TCH) in the Western Cape Province of South Africa.

Methods: Retrospective analysis of 491 cases, meeting the diagnostic criteria of definite and probable TBM.

Conclusion: 46% of TBM cases do not display the characteristic CSF profile traditionally associated with TBM. Clinico-diagnostic features including neutrophil predominance is not predictive of bacteriological confirmation. However neutrophil predominance was associated with earlier disease manifestations.

OPSOMMING

Agtergrond: Tuberkuleuse breinvliesontsteking is een van die mees verwoestende komplikasies van tuberkulose, en dra betekenisvol tot die morbiditeit en mortaliteit van kinders in hoë TB-belaste nasies by.[2] Die vroeë diagnose van tuberkuleuse breinvliesontsteking is berug daarvoor om moeilik te wees, dit is grootendeels as gevolg van die onkonsekwente manier waarop dit klinies voordoen, asook die gebrek aan ‘n spoedige, sensitiewe en spesifieke diagnostiese toets. Besluitneming in lande met beperkte hulpbronne word meestal gelei deur die gebruik van eenvoudige serebrospinale vog (SSV) ontleding.
Die klassieke SSV bevindinge van oorheersende limfosiete, lae glukose en ‘n hoë proteïen[5,7,16-20]is egter net gelyktydig teenwoordig in ‘n derde van gevalle,[5] en tuberkuleuse breinvliesontsteking word bakterieël bevesting in slegs sowat 15-40% van gevalle.[17,18,21,23]Oorheersende neutrofiele,[22,24,26] hoë proteïen konsentrasies en HIV positiwiteit[24] is geidentifiseer as faktore wat die waarskynlikheid van mikrobiologiese kweking kan verhoog.
Deur die SSV parameters te identifiseer wat geassosieer word met bevestigte *Mycobacterium Tuberculosis* infeksie, sal dit toelaat vir verhoogde suspisie, en hopelik vroeër diagnose an behandeling; veral as daardie geidentifiseerde parameters geassosieer word met ‘n tradisioneel atipiese tuberkuleuse breinvliesontsteking SSV beeld, hoër basillêre lading en deur HIV beïnvloed word.

Doelstelling: Om die SSV parameters te beskryf wat die sensitiwiteit van bakteriologiese bevestiging van tuberkuleuse breinvliesontsteking affekteer, in 491 SSV monsters van kinders wat met diè siekte gediagnoseer is in die afgelope 30 jaar, by die Tygerbergse Kinder Hospitaal in die Wes-Kaap Provinsie van Suid Afrika.

Metodes: Retrospektiewe ontleding van 491 gevalle wat aan die diagnostiese kriteria voldoen vir definitiewe en waarskynlike tuberkuleuse breinvliesontsteking.

Resultate: 46% van die tuberkuluese breinvliesontsteking gevalle vertoon nie die klassieke SSV bevindinge wat tradisioneel daarmee geassosieer word nie. Kliniese en diagnostiese voordoening, insluitend ‘n beeld van oorheersende neutrofiele op SSV is nie aanduidend van ‘n verhoogde kans op mikrobiologiese bevestiging nie. Oorheersende neutrofiele is egter gessosieer met vroeë siekte.

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INTRODUCTION AND LITERATURE REVIEW

The first literary reference to tuberculous meningitis (TBM) was made in the Lancet of 1836[1]. Then termed "acute hydrocephalus", the disease was uniformly fatal, and diagnosis could only be confirmed by post mortem examination.

175 years later, the burden of the disease has significantly increased, with an estimated 9 million new tuberculosis (TB) cases diagnosed globally in 2013, of which 550 000 occurred in children[2]. Although the 2015 Millennium Development Goal of halting and reversing TB incidence has been achieved globally, an estimated 1.5million people still died from this preventable and treatable disease in 2013.

TBM is one of the most devastating complications of TB[3], and continues to contribute significantly to the morbidity and mortality of the paediatric population, especially in such a high burden TB country as South Africa. [4]

One of the most important determinants of the outcome of TBM is the early and rapid diagnosis of the disease. [5-7] However, diagnosis is especially difficult in children, due to the paucibacillary nature of disease and suboptimal diagnostic testing. [8-11] As a result, decision making is often guided by history, clinical presentation, as well as the cerebrospinal fluid (CSF) findings.

CSF findings in childhood TBM

CSF features highly suggestive of TBM in TB endemic areas include:[12-15]

A. Moderately increased leukocytosis (10-500 cells/μL with >50% lymphocyte predominance. [5,7,16-20]

B. Elevated protein (>1g/L) [5,16-20]

C. An abnormally decreased CSF to serum glucose ratio <0.5 or an absolute CSF glucose concentration less than 2.2 mmol/L. [5, 16-21]

Although these classically described findings may be characteristic of TBM, various studies[5,16,18,21-27] have shown that it lacks specificity as a diagnostic tool.

In a 2004 study by van den Bos et al[16], the CSF of 104 children with TBM were analysed, polymorphonuclear leucocytes were predominant in the CSF of 17%; in 16% the CSF glucose was >2.2mmol/l; and in 26% the CSF protein was <0.8g/l. Similarly, in a Turkish study of 214 children[18], diagnosed with TBM on clinical and microbiological criteria, 15% had a polymorphonuclear predominance; 17% had a CSF glucose of >2.2mmol/l; and 23% a CSF protein lower than 1g/dl.

VB Patel in their 2008 South African study[5], found the characteristic CSF findings in only 34% of their 99 patients with TBM. This correlates with another South African study[21] by PR

Donald et al, that found a polymorphonuclear predominance in 9%, a CSF glucose of >2.2mmol/l in 40% and a CSF protein of <0.8g/l in 18% of their 99 children with TBM.

Therefore, the characteristic CSF parameters used in most case definitions for TBM, may miss up to two thirds of children with the disease, adding to the disease morbidity and mortality, given the poor outcome with delayed initiation of anti-tuberculous therapy.

Bacteriological confirmation

The bacteriological confirmation of Mycobacterium tuberculosis (M.tuberculosis) by identification of acid fast bacilli on CSF microscopy, positive CSF M.tuberculosis culture and positive commercial nucleic-acid amplification test (NAAT) confirms the diagnosis of TBM.[16,22,28] Many studies use microbiological evidence as the reference standard when analysing CSF parameters in patients with TBM.[16,22] Yet, research has shown that most TBM cases will not be proven microbiologically. Less than half of patients who satisfied clinical diagnostic criteria for TBM, had a positive CSF culture of the organism. Therefore, using CSF culture positivity as a diagnostic criteria will fail to identify more than half of all TBM cases.[17,18,21,29]

Likelihood of TBM bacteriological confirmation

Certain CSF parameters increase the likelihood of bacteriological confirmation of M.tuberculosis. It is proposed that CSF neutrophil predominance is associated with the discharge of a large amount of tuberculoprotein into the CSF, typically occurring in the initial and acute phase of the infection. Therefore, a polymorphonuclear pleocytosis increases the likelihood of a positive culture for M.tuberculosis. [22,26,30] Elevated CSF protein concentration, as well as co-infection with the human immunodeficiency virus (HIV), have been associated with increased detection of M.tuberculosis on CSF culture.[30]

Defining parameters that are associated with confirmation of M.tuberculosis will allow for an increased suspicion, and hopefully earlier diagnosis and treatment; especially if those parameters identified are associated with a better outcome.

METHODOLOGY

1. AIMS AND OBJECTIVES:

a. Research question:

Null hypothesis: There is no difference in the clinical presentation, radiological imaging and CSF findings of children diagnosed with probable and definite (culture confirmed) TBM.

b. Aim:

The primary aim of this study is to determine whether there is a difference in the clinical presentation, radiological imaging and CSF findings of children diagnosed with probable and definite TBM over the past 30 years at Tygerberg Children's Hospital (TCH).
The secondary aim is to identify cases of definite and probable TBM with a neutrophil predominance in the CSF, and determine whether it is associated with bacteriological confirmation, advanced TBM stage, radiological changes and differences in clinical presentation.

c. Objectives:

Probable and definite TBM cases will be analysed to determine the difference in:

a. Demographics and baseline characteristics
b. Clinical disease characteristcs at presentation
c. Results of radiological, laboratory and cerebrospinal fluid investigations

Secondly, probable and definite TBM cases with neutrophil predominance will be analysed, to determine the association with:

a. Bacteriological confirmation
b. Severity of TBM disease
c. Clinical presentation
d. Radiological changes

2. STUDY DESIGN

A retrospective, observational study, using a cross-sectional design, comparing the CSF samples of paediatric patients with definite and probable TBM. The independent variable is

bacteriological confirmation of *M. tuberculosis* on CSF (definite TBM), versus probable TBM (inability to demonstrate *M. tuberculosis* from CSF. The dependent variable will be the demographic, clinical, and diagnostic parameters assessed.

3. METHODS

The study was conducted at Tygerberg Children's Hospital (TCH), a tertiary level referral hospital in Cape Town, South Africa, serving a population of over 2.6 million people in the Western Cape Province. The hospital's drainage area includes the Northern Metro subdistricts, Khayelitsha, Eastern Tygerberg, West Coast, Cape Winelands and Overberg rural districts. The hospital has 1899 beds, of which 319 are allocated to paediatrics.

The Western Cape province of South Africa is a region where TB occurs endemically and is considered a high burden TB region. In 2005 children aged less than 13 years comprised 13.7% of the total disease burden, and had a reported incidence of 400/100 000 per year.[31]

A patient presenting to health care services with features suggestive of TBM, would be referred from primary health care clinics and hospitals to secondary and/ or tertiary hospitals, as the diagnosis becomes evident. Upon arrival to TCH, the patient and available results (usually a basic screening test for TB (Mantoux/ PPD), basic radiography like a chest X-ray, cerebrospinal fluid cell counts and chemistry if a lumbar puncture was not contraindicated, and basic serological results including a full blood count, C-reactive protein and human immunodeficiency virus (HIV) test) would be evaluated. If warranted, further tests would include a computerised tomography (CT) scan of the brain, an airencephalogram, and further serological and cerebrospinal fluid tests. A patient would then be classified as possible, probable or definite TBM, according to the uniform case definition (see Appendix 1). The severity would be staged according to the British Medical Research Council (BMRC) staging system (see below). Treatment is initiated as soon as the diagnosis is suspected, and not delayed until confirmatory test results are available. Once a decision has been made to continue treatment, a course of anti-tuberculous chemotherapy, with adjunctive corticosteroids, is given for 6 months: Rifampicin 20mg/kg, Isoniazid 20mg/kg, Pyrazinamide 40mg/kg and Ethionamide 20mg/kg. Prednisone 4mg/kg weaned over 4 weeks after a month on treatment. Appropriate referrals are made to neurosurgery, physiotherapy, occupational therapy and social services. Care during and after treatment can be continued at our institution, one of the TB hospitals in the province or supervised by specialised home-based care programmes.

A database containing information about all suspected, possible, probable and definite TBM cases, occurring in children aged 3 months to 13 years, between the 1st January 1985 and 31st December 2015 was utilized to extract relevant data from. This database has been utilized in previous TBM studies. (HREC reference numbers N10/11/367, N11/01/006)[38,39]

The data from children admitted with suspected, possible, probable and definite TBM were transcribed onto a case recording form, allocated a study number and then added to the anonymised database.

The case definition[20] was utilized to identify probable and definite cases of TBM from the database. Samples from the above group with neutrophil predominance were also selected for further analysis.

Inclusion criteria that was utilised were definite and probable TBM cases, presenting for diagnosis of TBM or initiation or continuation of TBM treatment with a documented cerebrospinal fluid (CSF) lymphocyte and neutrophil count.

Samples were excluded if they did not satisfy the diagnostic and clinical criteria for probable and definite TBM (i.e. suspected and possible TBM), and if caregivers did not give permission to participate in the research project.

4. DEFINITIONS

a. Tuberculous meningitis (TBM) case definitions:
The uniform research case definition for TBM[20] was used to classify patients as definite or probable TBM. (Appendix 1)

Definite TBM
A diagnosis of 'definite' TBM was made if acid-fast bacilli were present in the CSF, *M.tuberculosis* was cultured from CSF, a commercial nucleic acid amplification test of CSF was positive or there was histopathological evidence of *M.tuberculosis* from a central nervous system site.

Probable TBM
The diagnosis was made if two or more of the following criteria were present in the setting of a characteristic history and CSF changes associated with TBM: a positive history of contact with an adult TB case, a positive tuberculin skin test, a chest x-ray suggestive of pulmonary tuberculosis (hilar lymphadenopathy, miliary tuberculosis or cavitation), CT or magnetic resonance imaging (MRI) demonstrating the characteristic features of TBM (ventricular dilatation, meningovascular enhancement and/or granuloma/s), poor weight gain/ weight crossing percentiles documented on the Road to Health Card or positive microbiological identification of acid fast bacilli from gastric washings.

b. Severity of TBM at diagnosis was classified according to:
The refined British Medical Research Council (BMRC) staging system[17] 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;

Stage III: GCS <11.

c. Meningeal irritation

Meningeal irritation was considered to be present when nuchal rigidity, a positive Kernig or positive Brudzinski sign was demonstrable.

d. Brain stem dysfunction

Included all cases presenting with dysfunction of the spinothalamic, posterior column and corticospinal tract; as well as cranial nerve III to XII fallout. Clinical features include, but were not limited to: pupillary abnormalities, diplopia, weakness, vertigo, dysphagia, dysphonia, ataxia, breathing abnormalities, abnormal posture and diminished or absent brainstem reflexes.

e. Hemiparesis

A hemiparesis was present if muscular weakness or partial paralysis occurred unilaterally.

f. Human immunodeficiency virus (HIV) infection:

Cases were considered HIV infected if the diagnosis was established using the HIV enzyme-linked immunosorbent assay (ELISA) in patients older than 18 months, or the HIV polymerase chain reaction (PCR) test in those younger than 18 months. An unconfirmed rapid (point of care) HIV test was not included in the criteria.

g. Radiological signs of pulmonary Tuberculosis on chest X-ray:

A chest X-ray was considered suggestive of pulmonary Tuberculosis if it displayed the following characteristics: segmental or lobar airspace consolidation with ipsilateral hilar and mediastinal lymphadenopathy and/or pleural effusion and/or cavitatory changes. Haematogenous dissemination of Tuberculosis, visible as a miliary picture on chest X-ray, was also considered suggestive of pulmonary Tuberculosis.

h. Cerebrospinal fluid parameters:

Four CSF features suggestive of TBM were assessed:

1. Clear macroscopic appearance
2. Moderately increased leucocytosis (10-500 cells/μL or >50% lymphocyte predominance.
3. Elevated protein (>1g/L)
4. An abnormally decreased CSF to serum glucose ratio or an absolute CSF glucose of <2.2mmol/L.

CSF was considered typical of TBM when all four the above was present.

i. Neutrophil predominance

CSF samples with more than 50% neutrophils on cell count were considered to be neutrophil predominant.

5. STATISTICAL METHODS

Statistical analysis was carried out using SPSS version 21 (SPSS Inc, Chicago, IL, USA). Numerical variables were summarised using means, standard deviations and ranges, if the data was normally distributed. In the case where it was not normally distributed; medians, interquartile ranges, minimums and maximums was used. Categorical variables were tabulated using proportions and percentages.

For bivariate associations the t-test was used in the case of numerical variables. The Chi-square test was used for associations between categorical variables. The measures of associations depended on whether or not the data was normally distributed. These included the Pearson correlation coefficient, odds ratio, relative risk and the Mann Whitney test. Significance was set at the 5% level or p-value of less than 0.05.

6. ETHICAL CONCERNS AND CONSIDERATIONS

The following ethical principles were adhered to during the course of this study:

a. Social value:
TBM is a major public health problem, affecting a very vulnerable group of patients, especially in a high burden disease country as South Africa. By evaluating the CSF parameters that influence bacteriological confirmation, we aimed to relieve the diagnostic burden imposed upon health care staff. This translates into earlier diagnosis and initiation of effective treatment of this devastating disease.

b. Respect for persons
Only retrospectively collected data was analysed in this study. There was no direct contact between the researcher and patients.

c. Privacy and confidentiality
All information/data collected was kept in a separate password protected file and this was only known to, and accessed by the researchers. There were no risks for parents, patients or medical personnel involved in the study.

d. Independent review
The protocol was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University, with ethics approval number S15/07/152.

e. Informed consent
A waiver of individual informed consent was granted by the HREC as there was no contact between the researcher and subjects, data had already been collected retrospectively, there were minimal risks to the subjects involved and the study did not adversely affect the right and welfare of the subjects.

f. Collaborative partnerships

The researchers worked closely with their supervisors, advisors and stakeholders. Ethical principles, especially privacy and confidentiality, were strictly adhered to.

RESULTS

A total of 491 cases complied with the inclusion criteria as set out above. Of those, 98 had definite TBM, whilst 393 cases satisfied the criteria for probable TBM.

Demographic profile and baseline characteristics

Table 1 (see Appendix 2) illustrates the demographic differences between the definite and probable TBM cases. There was no statistically significant difference in age, gender and ethnicity between the two groups.

Clinical presentation

Table 2 (see Appendix 2) compared the differences in clinical presentation between definite and probable TBM cases. There were no statistically significant differences regarding stage of disease on presentation. The only presenting symptom that was significantly different was vomiting, occurring more in definite TBM cases ($p=0.013$). Definite TBM cases were also more likely to have a BCG scar ($p=0.006$), whilst probable TBM cases were more likely to exhibit meningeal irritation on admission ($p<0.001$), present with an acute hemiparesis ($p<0.001$) and have a longer duration of illness ($p<0.001$).

Laboratory, CSF and radiological investigations

Table 3 (see Appendix 2) illustrates the differences in laboratory, CSF and radiological investigations between probable and definite TBM cases. The prevalence of HIV infection was low (3%) in both groups. There were no statistically significant differences in CSF biochemistry and cell count, with 54% of samples in both groups displaying the typical TBM CSF picture. Patients with definite TBM tended to have a higher prevalence of Tuberculin reactivity (Mantoux) ($p=0.03$), whilst children with probable TBM were more likely to exhibit chest radiography changes suggestive of PTB ($p=0.002$). Those in the probable TBM group were also more likely to have cerebral infarcts on CT scan ($p=0.002$), as well as features suggestive of hydrocephalus ($p=0.033$).

Neutrophil predominant samples

Table 4 (see Appendix 2), shows the differences between neutrophil predominant and lymphocyte predominant CSF samples in all the TBM patients. Patients with CSF neutrophil predominance were less likely to have advanced TBM disease ($p=0.019$), but more likely to have a miliary picture on chest X-ray (CXR) (0.005).

DISCUSSION

In this study we compared the differences in clinical presentation, radiological imaging and cerebrospinal fluid (CSF) findings of children diagnosed with probable and definite tuberculous meningitis (TBM) in a leading tertiary hospital in South Africa.

There were no statistically significant differences in the demographics of the two groups, and the ethnic distribution reflects the epidemiology of TB in South Africa.

The majority of patients presented with advanced stage disease. The delay in presentation to the referral centre may reflect the broad differential diagnosis for initial presenting symptoms, the paucibacillary nature of the disease in childhood and a complex referral pathway from primary and district services. Most of the available literature agrees that duration of symptoms before admission is an important predictor of TBM diagnosis[4,17,18]Unexpectedly, culture positive cases were more likely to present with less advanced stage disease and have a shorter duration of illness. The difficulty in culturing the bacilli especially in the pediatric population, due to its paucibacillary nature and the availability of GeneXpert molecular DNA testing from 2012 onwards, while cases in the study population are included from 1985, are cited as possible explanations.

The clinical presentation of TBM is highly variable and early clinical diagnosis notoriously difficult, especially when taking the non-specific signs and symptoms of the early stages into account. Vomiting was the only symptom to differ between the two groups, with more culture confirmed definite cases presenting with vomiting. TB associated hydrocephalus leads to raised intracranial pressure which in turn causes vomiting. It is therefore a manifestation of disease progression, and expected to occur more frequently in advanced stage disease.

The presence of hemiparesis is included in the staging criteria of TBM, and is a strong predictor of the prognosis of the disease process. Hemiparesis was present in more of the probable TBM cases. Previous studies[34,35] conducted on adult patients revealed either no difference, or a statistically insignificant difference in the prevalence of hemiparesis among their definite and probable cases.

Meningeal irritation is a reflection of duration of symptoms, severity of the disease stage and the immune status of the host. It is a clinical sign not typically present in stage I disease. The prevalence of meningism in the study population as a whole was 88%, which is not consistent with previous studies[17-18], but linked to prolonged duration of symptoms. Previous pediatric studies have documented the number of patients diagnosed with TBM who presented with nuchal rigidity, but failed to discern between probable and definite cases. Table 5, Appendix 2 is a summary of adult data differentiating between the clinical presentation of probable and definite TBM cases.

More patients with definite TBM had a previous BCG scar, in comparison to cases with probable TBM. Although the reason for difference between the two groups is unclear, it is worrying that almost 40% of the study participants had a scar present. The limited effect of BCG vaccination is well known[3], but the number is particularly disappointing as studies outside of South Africa[17,18] reported a much lower immunization rate in their TBM cases. It is however a much better figure than the worrying 83% of immunised children with TBM found in a 1995 study by PR Donald et al, also conducted at Tygerberg Hospital.[33]

More patients with definite TBM had a positive Mantoux skin test, and 41% of patients overall had a positive skin reaction. In both groups patients presented fairly late in the course of their illness, so one cannot postulate that they presented too early in their course to have mounted a type 4 immune reaction, and neither can improper technique or inter-observer variability be to blame for the significant difference in this large sample size. However, disseminated TB, malnutrition, HIV and an age less than 6 months can account for false negatives which could explain why more than half of patients did not mount an immune reaction. The prevalence of HIV in the sample was small, but the cutoff (≥5mm) for a positive Mantoux was adjusted for HIV positivity; moreover malnutrition was not assessed and could also have contributed.

TBM is a paucibacillary disease in children, with diagnosis often guided by simple CSF analysis. Studies have shown conflicting results in the number of patients whose initial CSF display the classically described findings suggestive of TBM. Data from both adults and children,[5,18,22,23]show typical CSF features in 27-80% of their patients.

Neutrophil predominance has traditionally been described early in the course of disease, and has been associated with an increased likelihood of bacteriological diagnosis.[26]We therefore expected a higher prevalence of neutrophil predominance in our definite TBM group, but found that an equal percentage of both groups had more than 50% polymorphonuclear cells in the CSF.

The prevalence of pulmonary changes on CXR was higher in the probable TBM group, and can be explained on the basis that the group as a whole had a longer duration of symptoms on presentation.

TBM was first termed “acute hydrocephalus”[1], called as such due to the inflammatory exudates that accumulate at the base of the brain after the rupture of a caseous granuloma in the subarachnoid space. Hydrocephalus is a common complication of TBM, and has been found in 42-99% of cases.[14,34] Non-communicating or obstructive hydrocephalus makes out about a quarter of these.[14] Hydrocephalus is a manifestation of stage II and III disease, and its prevalence was determined by both air-encephalography and computerised tomography (CT) scan of the brain. The majority of cases in both groups had hydrocephalus; and was classified as non-communicating in a third of cases. A previous Indian and South African study[32,40] also revealed a similar prevalence between their two groups.

Infarctions in TBM are mostly secondary to an on-going vasculitic process and commonly affect the basal ganglia. The presence of an infarction on neuroimaging is uniformly associated with a poor prognosis. Previous studies have shown a prevalence of 9-56% of infarcts in patients with TBM.[12,14,29,32,36] Twice as many of our probable cases had an infarct on CT scan, most likely linked to the longer duration of illness in that group.

It is thought that neutrophil predominance on cerebrospinal fluid is more likely to occur early in the disease process and be associated with an increased likelihood of culture positivity.[26] Neutrophil predominant samples were associated with less advanced disease, in comparison to lymphocyte predominant samples which were associated with an advanced stage of TBM. There was also no statistically significant difference in the number of patients with positive CSF cultures between the neutrophil predominant and lymphocyte predominant groups.

The association between miliary TB and tuberculous meningitis is well known. It was initially thought that meningitis developed only after haematogenous spread of the bacilli, but later studies suggest that the establishment of a sub-cortical or meningeal Rich focus leads to an eventual caseation and development of TBM.[37] Miliary TB can therefore be considered as an antecedent to developing TBM, and explains why a quarter of the neutrophil predominant group had a miliary picture on chest radiography.

STRENGTHS AND LIMITATIONS

To date, this is the first and largest study comparing probable and definite TBM cases in the paediatric population. The study spans over 30 years and include 491 cases occurring in a TB endemic region. All data was analysed retrospectively, but collected prospectively. The uniform case definition for use in clinical research was applied to all cases, making future comparison with further studies much easier.

A higher volume of CSF is associated with an increased chance of bacteriological confirmation[7] due to increased sensitivity of diagnostic tests. Unfortunately the volume of CSF obtained for analysis was not recorded in our database. As the study spans over 30 years, the more sensitive GeneXpert DNA testing was not available as a routine analysis for the majority of the time period. The study population also had a very low HIV prevalence, comparable to that of a previous paediatric Indian study[41], and reflects the prevalence of HIV in the Western Cape.[42]

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APPENDICES

Appendix 1:

Diagnostic criteria in the uniform TBM research case definition[20]

	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

Appendix 2:

Table 1: Demographic differences in cases with definite and probable TBM

	Definite TBM (N=98) n/N(%) or 95% CI	Probable TBM (N=393) n/N(%) or 95% CI	p-value
Demographics			
Age in months	40.04 (34.48 to 45.60)	35.03 (32.23 to 37.83)	0.16
Age group (in years)			0.27
Less than 1	12/98 (12)	70/388 (18)	
1-2 years	20/98 (20)	98/388 (25)	
2-5 years	46/98 (47)	158/388 (41)	
>5 years	20/98 (20)	62/388 (16)	
Female gender	52/98 (53)	188/392 (48)	0.37
Ethnicity			0.48
African black	23/98 (23.5)	79/391 (20)	
Cape Coloured	75/98 (76.5)	312/391 (80)	

(95% CI = 95% confidence interval)

Table 2: Difference in clinical presentation between definite and probable TBM cases.

	Definite TBM n/N (%)	Probable TBM n/N (%)	p-value
Clinical profile			
TB contact			0.082
Known	44/98 (45)	215/393 (55)	
Stage of TBM			0.348
Stage I and IIa	33/98 (34)	113/392 (29)	
Stage IIb and III	65/98 (66)	279/392 (71)	
Duration of illness before admission			**<0.001**
5 days and more	68/97 (70)	340/383 (89)	
Less than 5 days	29/97 (30)	43/383 (11)	
BCG scar	44/93 (53)	131/354 (37)	**0.006**
Presenting symptoms:			
Vomiting	61/95 (64)	183/366 (50)	**0.013**
Fever	64/95 (67)	249/366 (68)	0.902
Malaise	27/61 (44)	165/322 (49)	0.317
Weight loss	39/95 (41)	160/366 (44)	0.641
Convulsions	38/95 (39)	173/366 (47)	0.205
Cough	25/95 (28)	119/366 (33)	0.245
Headache	31/95 (33)	86/366 (24)	0.068
Presenting signs:			
Hemiparesis	38/98 (39)	263/393 (67)	**<0.001**
Meningeal irritation	60/86 (70)	300/332 (90)	**<0.001**
Brainstem dysfunction	27/98 (28)	146/393 (37)	0.075
Cranial nerve palsy	28/97 (28)	126/384 (33)	0.457

BCG= Bacillus Calmette-Guerin, LOC= level of consciousness

Table 3: Differences in results of laboratory, cerebrospinal fluid and radiological investigations between cases with definite and probable TBM.

	Definite TBM n/N (%)	Probable TBM n/N (%)	p-value
Investigations			
HIV infected	3/98 (3)	9/393 (2)	0.658
Mantoux Positive (>15mm or >5mm if HIV+)	42/82 (51)	134/346 (39)	**0.039**
CSF investigations			
Ziehl Nielsen on CSF Positive	3/34 (9)	0/44 (0)	**0.044**
Early Hain PCR version 1 Positive	10/34 (29)	0/44 (0)	<**0.001**
Early Hain PCR version 2 Positive	9/34 (27)	0/44 (0)	<**0.001**
GeneXpert PCR on CSF Positive	13/34 (34)	0/44 (0)	<**0.001**
Any PCR positive	28/34 (82)	0/44 (0)	<**0.001**
Culture from CSF positive	78/98 (80)	0/393 (0)	<**0.001**
Culture outside CSF positive	31/98 (32)	101/393 (26)	0.236
Microbiological confirmation on CSF *any of Zn or culture or PCR	98/98 (100)	0/393 (0)	<**0.001**
Abnormality on CSF: Confined to one parameter Confined to two parameters Confined to three parameters Typical (all 4 parameters)	 96/98 (98) 88/98 (90) 73/98 (75) 53/98 (54)	 390/393 (99) 368/393 (94) 302/393 (75) 211/393 (54)	 0.260 0.155 0.623 0.944
Neutrophil predominance >50%	13/96 (14)	51/387 (13)	0.925

Radiological investigations			
Chest radiograph:			
Normal	49/98 (50)	136/383 (35.5)	**0.009**
Suggestive of PTB	34/98 (35)	199/383 (52)	**0.002**
Miliary TB	15/98 (15)	48/383 (12.5)	0.486
Airencephalogram			
Communicating hydrocephalus	58/88 (65.9)	260/380 (68)	0.771
Non- communicating	26 (29.5)	99/380 (26)	
No hydrocephalus	4 (4.5)	21/380 (6)	
CT scan:			
Infarcts	19/95 (20)	139/374 (37)	**0.002**
Basal enhancement	73/95 (77)	318/374 (85)	0.056
Hydrocephalus	85/98 (87)	361/387 (93)	0.033

Table 4: Differences between neutrophil predominant and lymphocyte predominant CSF samples in all TBM patients

	Neutrophil predominance in CSF n/N(%)	Lymphocyte predominance in CSF n/N(%)	p-value
Advanced TBM stage	36/63 (57)	302/419 (72)	**0.019**
HIV status	1/64 (2)	10/419 (2)	0.684
Any PCR on CSF positive	1/5 (20)	25/68 (37)	0.450
Culture from CSF positive	12/64 (19)	66/419 (16)	0.533
Miliary picture on CXR	15/62 (24)	47/411 (11)	**0.005**
Duration of symptoms ≥ 5 days	53/60 (88)	351/412(85)	0.518

Table5: Comparison between study results and previous studies discerning between definite and probable TBM cases.

	Definite TBM				Probable TBM			
	Roos et al	**Marais et al[40]**	**Sharma et al[35]**	**Jha et al[32]**	**Roos et al**	**Marais et al[40]**	**Sharma et al[35]**	**Jha et al[32]***
Stage of TBM								
I	7/98(7)	10/42(24)	12/44(27)	10/43(23)	11/392(3)	7/34(21)	15/80(19)	22/75(29.3)
II	49/98 (50)	29/42(69)	21/44(48)	11/43(26)	206/292 (52)	23/34(68)	28/80(35)	22/75(29.3)
III	42/98 (43)	3/42(7)	11/44(25)	22/43(51)	175/392 (45)	4/34(12)	37/80(46)	31/75(41.3)
Vomiting	61/95 (64)	15/42(36)	30/44(68)	38/43(88)	183/366 (50)	11/34(32)	53/80(66)	59/75(79)
Fever	64/95 (67)		38/44(86)	43/43(100)	249/366 (68)		70/80(88)	73/75(97)
Convulsions	38/95 (39)	7/42(17)	10/44(23)	8/43(18)	173/366 (47)	2/34(6)	14/80(18)	9/75(12)
Headache	31/95 (33)	26/42(62)	39/44(89)	43/43(100)	86/366 (24)	18/34(53)	73/80(91)	74/75(99)
Hemiparesis	38/98 (39)		7/44(16)	4/43(9)	263/393 (67)		13/80(16)	4/75(5)
Meningeal irritation	60/86 (70)	31/42(74)	31/44(71)	38/43(88)	300/332 (90)	23/34(68)	60/80(75)	58/75(77)
Cranial nerve palsy	28/97 (28)			6/43(14)	126/384 (33)			9/75(12)
Focal deficits		9/42(21)	12/44(27)			17/34(50)	24/80(30)	

*Probable and possible cases included