Use of the PCR technique to diagnose meningitis in children admitted to Red Cross War Memorial Children's Hospital.

By

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Declaration

I Phindile Mteshana, hereby declare, that the submitted thesis in partial fulfillment of the MMed degree, entitled **Use of Real-time polymerase chain reaction to diagnose meningitis in children admitted to Red Cross War Memorial Children's Hospital** is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

The submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

I further declare that the electronic version of the submitted thesis is congruent with the printed version both in content and format.

Signature: Signed by candidate

Date: 07 January 2019

List of Abbreviations

BM	Bacterial Meningitis
BMS	Bacterial Meningitis Score
CNS	Central Nervous System
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CT	Computed Tomography
GCS	Glasgow Coma Scale
Hib	*Haemophilus influenzae type b*
HIV	Human Immunodeficiency Virus
HSV	Herpes simplex virus
IMCI	Integrated Management of Childhood Illnesses
LOS	Length of hospital stay
LP	Lumbar puncture
MEU	Medical Emergency Unit
MOPD	Medical outpatient department
NHLS:	National Health Laboratory Services
PCR	Polymerase Chain Reaction
RCWMCH	Red Cross War Memorial Children's Hospital
TB	Tuberculosis
UCT	University of Cape Town
VM	Viral Meningitis
WHO	World Health Organisation

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Summary of the article

The purpose of this paper was to discuss meningitis in children with respect to epidemiology, how the introduction of vaccines affected the incidence of bacterial meningitis. It then focused on challenges faced by clinicians in distinguishing between bacterial and viral meningitis.

It was a retrospective study which started with a review of **89** articles followed by a review of patient folders with a diagnosis of meningitis during the study period. The main question was whether there is a better way of differentiating between bacterial and viral meningitis. We looked at different methods that have been used in other countries and found PCR to be the most relevant and practical one for our population especially those children who get antibiotics before lumbar puncture is done.

We collaborated with the department of virology at the University of Cape Town which ran PCRs on the cerebrospinal fluid (CSF) specimens for the patients whose folders were reviewed. Clinical and laboratory features were compared between different groups to see whether there are other ways of telling the difference between VM and BM in resource poor settings where PCR is not available. We also calculated the sensitivity and specificity of PCR compared to routine microscopy and culture.

We concluded that PCR is a better test for patients who have a LP after administration of antibiotics however it should be used in conjunction with microscopy and culture in order to get the bacterial sensitivity results in cases of bacterial meningitis. Financial evaluation would be required before implementing the use of PCR as a test of choice.

Chapter 1: Introduction and Literature review

Abstract

Introduction: Viral meningitis (VM) is more common than bacterial meningitis (BM) and is a self-limiting disease. Clinicians still tend to admit patients with VM and treat them for BM because they fear the morbidity and mortality that is associated with a delay in treating or not treating BM. Unnecessary admissions have huge cost implications and they separate children from their parents while exposing them to painful procedures and unnecessary antibiotics.

Methods: A structured literature review was undertaken to see whether clinical manifestation and examination findings and laboratory findings including viral PCR of cerebrospinal fluid can help to diagnose viral meningitis and avoid unnecessary admissions.

Results: Viral and bacterial meningitis have similar clinical findings. CSF examination is crucial in confirming the diagnosis of meningitis. Microscopy and culture remain the gold standard in making the diagnosis. The introduction of the *Haemophilus influenzae type b* and pneumococcal vaccines into the South African Expanded Programme on immunization (EPI) markedly reduced the incidence of invasive *Haemophilus influenzae type b* and pneumococcal disease in children under 5 years-of-age as in other countries where they are used. Viral meningitis is the leading cause of childhood meningitis however the clinical and CSF findings in viral meningitis and bacterial meningitis overlap. The sensitivity of CSF culture has been shown to be around 81.3%, but is very much affected by prior antibiotics. Traumatic/bloody CSF taps also make it difficult to interpret

results. Inflammatory markers have been used in conjunction with CSF results in differentiating between BM and VM however, the use of polymerase chain reaction technique in the diagnostic methodology improves the sensitivity to more than 95%.

Conclusion: Viral meningitis is common worldwide. Real-time multiplex PCR offers value in accurately detecting common viral and bacterial pathogens thus allowing for appropriate patient management. In order to avoid the risk of not identifying organisms not included in the PCR assay and further not being able to do susceptibility testing on those organisms, it important to realize that PCR testing would have to be done in addition to culture, and not as a replacement.

Aims and objectives of the research project

- To determine the proportion of meningitis due to common viral pathogens in children over two months of age using PCR testing.
- To determine the number of cases of meningitis due to common viral and bacterial causes in children admitted to the Red Cross War Memorial Children's Hospital (RCWMCH), Cape Town.
    - Viruses tested for were: *enteroviruses, herpes simplex virus and mumps virus.*
    - Bacteria tested for were: *Neisseria meningitidis, Haemophilus influenzae type b* and *Streptococcus pneumoniae.*
- To correlate the clinical diagnosis of meningitis with laboratory findings of viral meningitis.
- To determine the association of abnormal cerebrospinal fluid

(CSF) findings and confirmed diagnosis of HIV infection.

- To describe the proportion of viral-PCR positive and negative patients treated as bacterial meningitis.

1.2 Aims and objectives of literature review

Our review of the literature was focused on meningitis in children over the age of two months with respect to the following:

- The epidemiology of meningitis in South Africa.
- Correlation between clinical diagnosis of meningitis with laboratory findings of viral meningitis.
- Diagnostic challenges resulting from bloody CSF tap and prior exposure to antibiotics.
- The value of laboratory markers of sepsis in making the diagnosis of meningitis.

Methodology of the literature review

A structured literature review was done on PUBMED (www.ncbi.nlm.nih.gov) using both text and MeSH terms. In addition, textbooks and articles identified in reference lists of individual papers were selected if considered appropriate. An independent literature search was also conducted using Google Scholar. The following terms and search filters were used.

Our search inclusion criteria included human studies reported in English and age limitations were customized for children aged 0-months – 18-years. We initially looked only at articles published between 2003 and 2013, which is 10 years prior to

215 the current study but for the epidemiology analysis, we did not apply any
216 restrictions as we wanted to look at the prevalence of meningitis before and after the
217 introduction of *Haemophilus influenzae type b* (Hib) conjugate vaccine into the
218 Expanded Programme on Immunisation (1999), and the 7-valent pneumococcal
219 conjugate vaccine (PCV) in April 2009 and 13-valent PCV in April 2011.
220 References that focused on meningitis caused by pathogens other than our tested
221 pathogens, and mycobacterium tuberculosis (TB) were excluded.

Table 1: Results of the literature review

Search terms	MeSH Term	Results
Viral meningitis in Children	("virology"[MeSH Terms] OR "virology"[All Fields] OR "viral"[All Fields]) AND "meningitis in children"[All Fields] AND (("2003/01/01"[PDAT] : "2013/12/31"[PDAT]) AND "humans"[MeSH Terms] AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms]))	**122 overall, 45 in past 10 years**
viral meningitis AND prevalence	("meningitis, viral"[MeSH Terms] OR ("meningitis"[All Fields] AND "viral"[All Fields]) OR "viral meningitis"[All Fields] OR ("viral"[All Fields] AND "meningitis"[All Fields])) AND ("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "prevalence"[All Fields] OR "prevalence"[MeSH Terms]) AND (("2003/01/01"[PDAT] : "2013/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang] AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms	**240**
Viral meningitis in Children AND South Africa	("virology"[MeSH Terms] OR "virology"[All Fields] OR "viral"[All Fields]) AND "meningitis in children"[All Fields] AND ("South Africa"[MeSH Terms] OR ("south"[All Fields] AND "africa"[All Fields]) OR "south africa"[All Fields])	**5 articles- 2 deemed relevant**
Viral meningitis AND PCR	(("meningitis"[MeSH Terms] OR "meningitis"[All Fields]) AND ("diagnosis"[Subheading] OR "diagnosis"[All Fields] OR "diagnosis"[MeSH Terms])) AND PCR[All Fields] AND (("2003/01/01"[PDAT] : "2013/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang] AND jsubsetaim[text] AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms]))	**125**
Meningitis AND HIV	("meningitis"[MeSH Terms] OR "meningitis"[All Fields]) AND ("HIV"[MeSH Terms] OR "hiv"[All Fields]) AND (("2003/01/01"[PDAT] : "2013/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang] AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms]))	**248**
Meningitis and CRP	(("meningitis"[MeSH Terms] OR "meningitis"[All Fields]) AND CRP[All Fields]) AND ("humans"[MeSH Terms] AND English[lang] AND jsubsetaim[text] AND ("infant"[MeSH Terms] OR "child"[MeSH Terms] OR "adolescent"[MeSH Terms]))	**39**

Literature review findings

The search yielded 779 articles as presented in **Table 1.** After scanning abstracts and topics a total of 699 were deemed relevant but only **89** were suitable for our review. The South African articles identified which mirrored the aim of our research project were old. The latest South African articles found focused mainly on tuberculous meningitis (TBM).

Introduction

Infectious diseases have contributed to most of the under-five deaths in developing countries and this led to the introduction of antibiotics to treat patients. Since the 1940s antibiotics have decreased morbidity and mortality due to infectious diseases but the problem we have now is the unnecessary use of them which has contributed to antibiotic resistance (1, 2). According to the Medical Research Council (MRC) report from the year 2000 bacterial meningitis was found to be the 10^{th} most common cause of under- five mortality in South Africa(3).

It is a serious life-threatening illness responsible for an estimated 200 000 deaths of children outside the neonatal period each year (4). Early initiation of antibiotics has been shown to decrease morbidity and mortality from meningitis.

The South African Group for Enteric Respiratory and Meningeal disease Surveillance (GERMS-SA) for the year 2016 confirmed that the introduction of the Hib conjugate

vaccine into the Expanded Programme on Immunisation (EPI) for South Africa in 1999 resulted in a reduction of *Haemophilus influenzae (Hib)* cases. The report indicated that most cases of invasive Hib in 2016 occurred in children <15years-old who were unvaccinated, the main affected group were infants. The same report also established that the introduction of 7-valent pneumococcal conjugate vaccine (PCV) in April 2009 and 13-valent PCV in April 2011 also resulted in a substantial reduction in the incidence of pneumococcal meningitis in children <5 years of age from76% to 58%; prematurity and HIV disease considered important risk factors (5). von Gottberg et.al reported a 65% decrease in number of Hib cases in children under the age of one (from 55 cases in 1999–2000 to 19 cases in 2003–04). This shows a decrease in Hib disease burden among South African children following conjugate vaccine introduction (6).

Although there is no current vaccine for *Neisseria meningitidis* in our current South African EPI, GERMS-SA 2016 report also revealed that a total of 131 cases of laboratory-confirmed meningococcal disease were identified by the surveillance system. Compared to the 2015 report, the incidence of meningococcal meningitis was slightly lower (0.23 vs 0.28 cases per 100 000 population). According to the report, it is unclear whether these changes are a true reflection of changes in the incidence. It could also be due to changes in the ability to confirm disease in the laboratory or changes in reporting to the surveillance network (1, 5). In Latin America, Argentina and the Middle East; countries which have similar immunisation schedules to ours have confirmed that VM is the most common cause of meningitis outside the neonatal period with *enterovirus* being the most common identified pathogen (7, 8).

VM remains an important cause of morbidity and financial burden and merits efforts to improve diagnostic, treatment, and prevention options (9). Financial burden comes mainly from incorrect diagnosis, patient admission and administration of antibiotics because of fear of missing bacterial meningitis.

Bacterial meningitis (BM) on the other hand is a severe infection and can rapidly progress to multi-organ failure and death. Numerous sequelae include seizure disorders, focal neurologic deficits, hearing/vision loss, and impaired cognitive functioning. Approximately 25% of survivors in the United States have moderate or severe sequelae (10).

The other problem with our current laboratory methods is that they are inadequate. Administration of antibiotics before collecting the cerebrospinal fluid (CSF) can results in negative culture results even in the presence of bacterial meningitis (11). Secondly, the turnaround time for both microscopy and culture, including Gram stain, is affected by whether the on-site laboratory is able to provide this service and culture may take up to 48hours to provide definitive identification and sensitivity results.

BM usually presents with a polymorph predominance in the CSF with a high protein and low glucose, sometimes positive microscopy and culture results whereas VM would have lymphocytes predominance and normal chemistry. Studies have also shown that early viral meningitis can also present with a predominance of polymorphs(12).

Another huge diagnostic problem is that of differentiating between TBM and viral meningitis because they both have lymphocyte predominance in the CSF(12). While viral meningitis and TBM do share a lymphocyte pleocytosis, the CSF protein levels are usually markedly different; GeneXpert on CSF also offers some option to differentiate TBM from viral meningitis.

Swingler et al. did a retrospective review of children who were presumed to have bacterial meningitis, and found antibiotic treatment to be unjustified in 35 (81.4%) of the 43 patients treated for longer than 2 days (13). The issue with inappropriate use of antibiotics is that it may enhance resistance or cause adverse effects. Inappropriately admitting children can also have emotional disturbance later in life (14).

A rapid diagnosis is essential to rule out other causes of meningitis. Our study aimed to determine the proportion of meningitis due to common viral pathogens in children over two months of age using molecular diagnostic techniques.

Definition of meningitis and encephalitis

Meningitis is defined as inflammation of the meninges which manifests by an increased number of white cells in the cerebrospinal fluid (7). It can be differentiated into bacterial and aseptic meningitis. Common bacterial causes also include *Neisseria meningitidis, Haemophilus influenzae type b, Streptococcus pneumoniae and Mycobacterium tuberculosis* whereas aseptic meningitis includes viral, fungal and other causes.

VM constitutes a large number of aseptic meningitis hence the term is commonly used

to describe aseptic meningitis as it is the most common cause of raised white cell count in the majority of cases. By definition, viral meningitis is a type of meningitis due to a viral infection (15). It is a clinical syndrome of meningeal inflammation by viruses with negative CSF cultures for routine bacterial pathogens in a patient who did not receive antibiotics before lumbar puncture(7). The common viral causes of meningitis that we see are enteroviruses, herpes simplex virus and mumps virus. Sometimes it is difficult to differentiate between meningitis and encephalitis in our patients.

The term encephalitis refers to the presence of an inflammatory process in the central nervous system and is usually accompanied by clinical evidence of neurological dysfunction such as encephalopathy and seizures. When the condition is affecting the meninges it is then referred to as meningoencephalitis. Both encephalitis and meningoencephalitis are grouped together as they share same epidemiology, aetiology and management (16).

Causes of meningitis

The described commonest causes of meningitis in ages between two months and 13 years are summarised in table 2.

Table 2: Major causes of meningitis in children aged 2months and 13 years

Organism	Common	uncommon
Bacteria	*Neisseria meningitidis, Haemophilus influenzae type b, Streptococcus pneumoniae*	
Viruses	Enteroviruses Herpes Simplex Virus	Mumps HIV
Fungi		*Cryptococcus neoformans*
Other causes	Parasites, Drugs, Malignancy, Auto-immune	

Adapted from: *Sawyer MH, Rotbart HA. Aseptic and viral meningitis. In: Principles and Practice of Pediatric Infectious Diseases, 3rd ed, Long SS, Pickering LK, Prober CG (Eds), Churchill Livingstone, New York 2008. Copyright © 2008 Elsevier*.

Epidemiology of meningitis

Viral meningitis has been recognised for years both internationally and locally however there has been a huge change in causative organism. Before the 1960s, mumps and measles were the most common causes of viral meningitis that were reported. In 1963 measles vaccine was introduced followed by mumps vaccine in 1967. Since then, the enteroviruses have taken a lead in viral causes of meningitis (17, 18). Researchers in the United States have also shown similar results to South Africa where they documented most cases of viral meningitis and infant febrile syndromes to be due to enteroviruses (19-21).

Enteroviruses can be associated with outbreaks especially during hot months (21). Outbreaks of echovirus (EV family) meningitis were also reported from Johannesburg and from Worcester. In 1957, in a Johannesburg Children's Home, 58 of 121 children became ill in the first 3months. Some children had clinical features of viral meningitis. Virus studies were carried out in 18 children of whom 10 had confirmed viral infection , isolated from the stool in 3 and cerebrospinal fluid in 7 children (22). Echo virus Type 4 was also isolated in children and staff members from a school for the deaf and blind at Worcester in the Cape Province during June 1960. A total of 204 out of 482 cases were reported for the period 6 - 19 June (23).

Viral meningitis is self-limiting and is not the cause of any great mortality or

morbidity (12). In a study done by Beghi et al. in Minnesota, recovery from VM was reported at the end of the acute phase in 95% of patients and there were no deaths (24). Potter et al. reported similar findings where they noted very low morbidity and death to be extremely rare (12).

For years researchers focused on bacterial meningitis because of its huge burden of disease. The aim was to find ways to decrease morbidity and mortality that is caused by those organisms. Researchers in Bahrain assessed the trend in the incidence of bacterial meningitis from 1990 to 2013, before and after the introduction of new vaccines. They reported 1455 cases of meningitis during the study period of which 1051 (73.1%) were viral and 386 (26.9%) were bacterial in aetiology with *Mycobacterium tuberculosis* causing the highest number of BM cases -122 (8.5%). Between 1995 and 1996 they observed a peak in cases of bacterial meningitis. After the introduction of *H. influenzae* and *Neisseria meningitidis* vaccines in 1998 and 2001 there was a drop in cases of *H. influenzae* and *Neisseria meningitidis* meningitis; *Streptococcus pneumoniae* was noted to be the predominant organism after Mycobacterium tuberculosis (25) .

The available South African studies showed viral meningitis to be the leading cause of meningitis followed by tuberculous meningitis (TBM). A small proportion of patients with suspected viral meningitis may need to be screened for TBM. Whilst the lymphocytes are elevated in both, clinical presentation and CSF protein, and glucose, will also influence the diagnosis. There are other important factors that are taken into account when a diagnosis of TBM is being considered e.g. the history of a household contact with tuberculosis, the presence of growth-faltering, a longer

period of unwellness, evidence of tuberculosis infection elsewhere such as a chronic cough and chest radiograph that is suggestive of tuberculosis, the presence of a strongly reactive Mantoux test, and clinical features that would support stage two or three of TBM (26).

Between July 1981 and June 1984, 1223 cases of meningitis were seen in the Department of Paediatrics, Tygerberg Hospital. Aseptic meningitis was found to be the commonest form of meningitis in each population group contributing towards 739/1223 (60. 4%) of cases of meningitis of which 108/739(14.6%) cases were due to viruses. In the first two years enteroviruses were responsible for the cases of VM but then mumps became prominent afterwards. For confirmed bacterial meningitis the commonest cause was *Neisseria meningitidis* (140 cases; 11.5%) followed by tuberculosis which was responsible for 62 cases of meningitis (5%) and then *H. influenzae* (47 cases) and lastly *Streptococcus pneumoniae* (34 cases) (26) .

Between 1985 and 1993 before the introduction of Hib vaccine and PCV, a prospective survey was carried out of all cases of meningitis in children under the age of 13 years presenting to Tygerberg hospital in the Western Cape Province of South Africa. There were 2920 cases of meningitis identified whereby the most common form of bacterial meningitis was TBM diagnosed in 282 (9.7%) children followed by *N. meningitidis* in 220 (7.5%) children, *Haemophilus influenzae* in 156 (5.3%) children and *Streptococcus pneumoniae* in 106 (3.6%) respectively (27).

A follow up study at the same institution showed that TBM was more common despite the introduction of antiretroviral therapy (ART), followed by *N. meningitidis*, *Haemophilus influenzae* and lastly *S. pneumoniae* (27). A further follow up study was conducted between 2007-2009, after the introduction of the *Haemophilus influenzae type b* vaccine in July 1999 and pneumococcal vaccine in 2009. A decline in bacterial meningitis cases, excluding *S. pneumoniae* was noted. They reviewed a total of 557 cases of children older than 3 years of age. VM was still the leading cause followed by TBM which accounted for 126 (22%), *S. pneumoniae* in 23 (4%) cases, *Klebsiella pneumoniae* in 17 (3%) cases and lastly *Haemophilus influenzae in 3 (<1%)* cases reflecting the impact of Hib vaccine (8, 27).

Other similar studies that were conducted in South Africa, Cape Town showed a higher prevalence of TBM and an association with poor outcomes in certain population groups e.g. HIV infected and African population (28-29). Different South African studies have described a significant difference in prevalence of meningitis in communities of different socio-economic groups (26, 30). *N. meningitidis* was found to be common in coloured children, *H. influenzae* in white children and TBM in black children (26).

Figure 1 summarises causes of meningitis in South Africa from 1957 to 1997. Not all studies looked at viral causes of meningitis as the focus was on BM. We can appreciate the high prevalence of *Haemophilus influenzae* meningitis and a drop after the introduction of the Hib vaccine. *S. pneumoniae* and *N. meningitidis* then became the leading causes afterwards. Current data shows a decline in cases of invasive pneumococcal disease but the HIV-infected population still gets more affected that the

HIV negative (31).

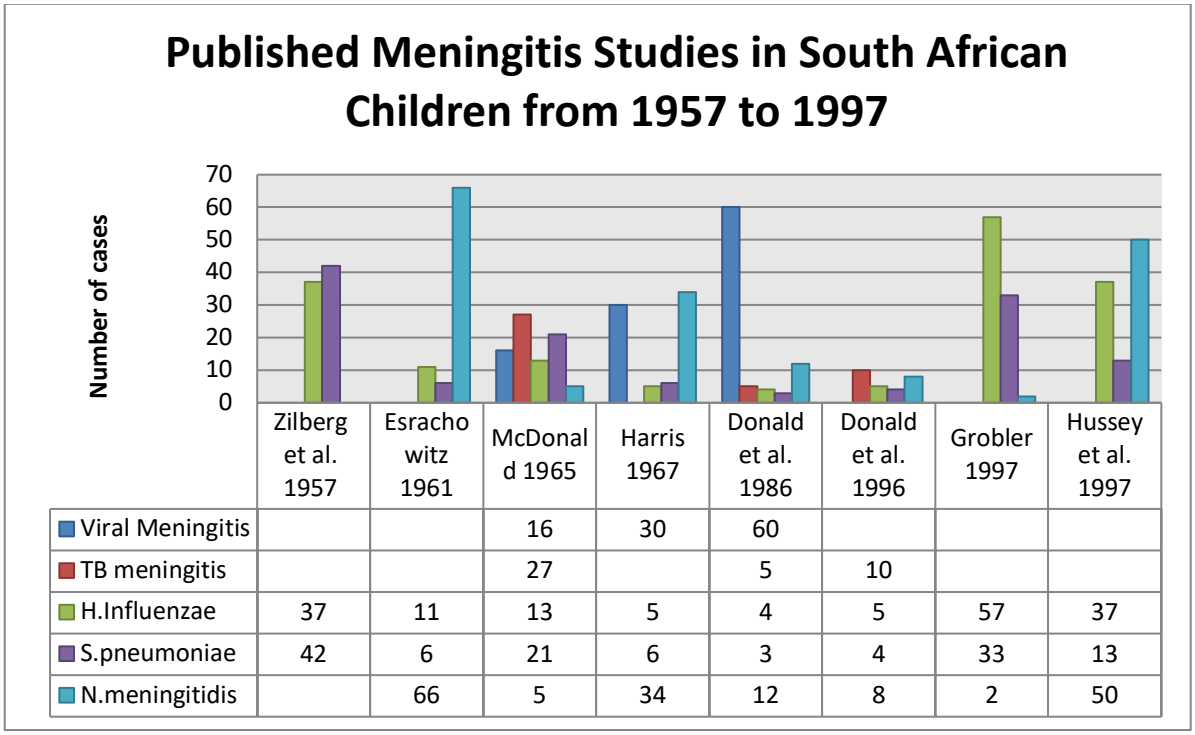

Figure 1: Causes of meningitis in South African children 1957-1997

Clinical picture of meningitis

The clinical presentation of VM is generally similar to that of BM, but is often less severe. VM may be suspected based on the epidemiologic data, clinical features and initial cerebrospinal fluid (CSF) studies (21).

Fever and vomiting are usually the most frequent reasons for consulting a physician but it can be a presenting sign for common viral illnesses, such as influenza. Other presenting signs and symptoms may include nausea, muscle and joint pain, breathing difficulties, and loss of appetite. Meningitis should be suspected in irritable or lethargic

febrile children despite the absence of neck rigidity (37).

Clinical manifestations may also vary according to the age, immune status of the patient as well as the aetiological agent(7). Older children may present with acute onset of fever, headache, nausea, vomiting, stiff neck, and photophobia. de Crom and Michos described the same clinical presentation, they also reported additional non-specific symptoms of malaise, irritability and sometimes abdominal pain (38, 39).

Physical findings in neonates and older children are variable. Neonates can either have normal clinical examination, have very non-specific signs like irritability, apnoea or they can have a bulging fontanelle. Older children can present systemic manifestations like rash, conjunctivitis, pharyngitis, and diarrhoea which may provide clues to the underlying viral aetiology. Other signs of meningitis may be decreased level of consciousness, Brudzinski and Kerning's signs (40).

Viral meningitis is a self-limiting disease; the vast majority of patients recover in 1-2 weeks. About 5% of patients have residual deficits including fatigue, mild cognitive impairments, seizures and cranial nerve palsies. The most severe complications are those described with herpes virus infection including the progression to severe encephalitis, or the development of other organ involvement such as liver or myocardial necrosis (40).

Diagnosis and Management approach to meningitis

The clinical picture alone is not enough to diagnose viral meningitis. Patients need a lumbar puncture however the CSF profiles of bacterial and viral meningitis often overlap, particularly during the earliest stages of viral meningitis further making the differentiation difficult (39). **Table 3** summarizes clinical case definitions of meningitis according to the Centre for Disease Control and Prevention (CDC).

Table 3: Recommended case definitions of meningitis

	Viral meningitis	**Bacterial meningitis**
Definition of meningitis	Is a syndrome characterized by acute onset of meningeal symptoms, fever, and cerebrospinal fluid pleocytosis, with bacteriologically sterile cultures	Manifests most commonly with fever, headache, and a stiff neck; the disease may progress rapidly to shock and death. However, other manifestations may be observed.
Laboratory criteria for diagnosis	No evidence of bacterial or fungal meningitis	Isolation of a bacterial species from the cerebrospinal fluid
Confirmed case	A clinically compatible case diagnosed by a physician as aseptic meningitis, with no laboratory evidence of bacterial or fungal meningitis.	A clinically compatible case that is either laboratory confirmed or is accompanied by a positive blood culture

Adapted from the *Centre for Disease Control and Prevention (https://www.cdc.gov/mmwr/preview/mmwrhtml/00047449.htm).*

The introduction of new vaccines and antimicrobial drugs has been a huge accomplishment in the field of infectious diseases especially in bacterial meningitis, but there has been very little improvement in the diagnostic methodology (20, 41). The initial laboratory evaluation of a child with suspected meningitis includes blood tests and CSF examination. A confirmed diagnosis of viral meningitis requires a positive identification of a viral pathogen, most often in the context of a negative CSF culture for routine bacterial pathogens.

Laboratory diagnosis of bacterial meningitis

Examination of the CSF is necessary to establish a diagnosis of meningitis and to make a provisional diagnosis of bacterial, viral, or unclear aetiology. A lumbar puncture may also provide symptom relief by relieving the accompanying raised intracranial pressure in patients with viral meningitis (42). CSF should be sent for cell count, glucose, chloride, protein, gram stain and bacterial culture. The role of chloride in diagnosing meningitis is still unclear and has no bearing on the diagnosis of VM. Viral culture and Polymerase Chain Reaction (PCR) are usually done if the clinical picture suggests viral meningitis and if the resources are available.

CSF chemistry and cell count

Table 4 compares CSF findings in cases of bacterial and viral meningitis. This does not take the prior use of antibiotics into consideration.

TABLE 4 -- Cerebrospinal Fluid Analysis in Bacterial and Viral Meningitis.*

FINDING	Bacterial Meningitis	Viral Meningitis
Opening pressure	Usually elevated	Usually normal
WBC count/mm^3	Elevated, 500–10,000+	Elevated, 6–1000
Differential count	Polymorphonuclear predominance	Lymphocytic predominance
Glucose level	Decreased, 0–40 mg/dL	Usually normal
Protein level	Elevated, >50 mg/dL	Normal or slightly elevated

Adapted from Fong B, Van Bendegem J: Lumbar puncture. In Reichman E, Simon R (eds): Emergency Medical Procedures. New York: McGraw-Hill, 2004, p 875.
*This is only a guide and care must be taken when interpreting these parameters, especially **early in**

The level of glucose in viral meningitis is usually normal but a slight decrease is

not uncommon. In cases of TBM, fungal and bacterial meningitis, it is very low (43).

In patients who did not receive antibiotics before LP is done; CSF white blood cell count can be a useful and rapid diagnostic tool to distinguish between BM and VM in children only (44).Certain authors recommend that when in doubt, the patients must be admitted for intravenous antibiotics pending the CSF culture results or admit for observations and repeat LP in 24 hours (45).

In viral meningitis one may find mildly elevated CSF protein with normal glucose. The pleocytosis in VM shows a predominance of lymphocytes. Although the differential diagnosis includes TBM; the CSF in TBM usually has low glucose with significantly raised protein. When in doubt in the absence of viral culture and PCR, TB screening is recommended before calling it VM (46, 47).

There have been cases of VM that presented with polymorphonuclear cell predominance and that makes it difficult to distinguish it from BM. This has been reported to happen early during VM as confirmed by Thomson Jr et al. who reported cases of viral infections like herpes, enterovirus and arbovirus which were reported to have predominance in polymorphonuclear (PMN) leucocytes during the first 48 hours (48). This is when history taking becomes crucial so that one can correlate symptoms with the CSF findings and if in doubt then patients are treated pending the CSF culture results.

Confusion was reported by Potter et al. in his survey done in Cape Town, South Africa

of 213 children who presented with meningitis during winter months. At that time there was an enterovirus outbreak but still the most common cause of meningitis was *N. meningitidis*. Most concerning was that there was no difference in the initial CSF findings of both EV meningitis and those of *N. meningitidis*. Those who had purulent CSF with PMN predominance actually did not have BM and they recovered quickly (12). CSF should not have any erythrocytes unless there is a bloody tap or in cases of head trauma /haemorrhage.

In developing countries like South Africa, the most commonly used approaches for the detection and characterization of bacterial meningitis pathogens include bacterial culture and Gram stain (49). Viral cultures are however not available in most hospitals.

Gram stain

CSF Gram staining is a rapid, inexpensive, and requires relatively little training. It simply gives a clue as to the genus and species of the aetiological agent. However, accurate results are highly dependent on the operator's staining and interpretation skills. It has been long considered a mainstay of bacterial meningitis diagnosis. Previous studies report Gram stain sensitivities as high as 90% and specificities of 97% or more (50). In a different study Gram stain had a sensitivity of 98.2% and specificity of 98.7% (51).

Gram stain results are also subject to observer misinterpretation, therefore

broad- spectrum antimicrobial therapy should be continued until CSF culture results are available. It is reported as Gram-positive diplococci for *S. pneumoniae*, Gram-negative diplococci for N*. meningitidis* and Gram-negative coccobacilli for Hib (52).

Culture

Bacterial Culture

Isolation of a bacterial pathogen from the CSF culture confirms the diagnosis of bacterial meningitis. However, a negative culture of the CSF at a particular point in time does not exclude meningitis if CSF was taken too early of if taken after giving the patient antibiotics. The positive rate is relatively low due administration of antibiotics prior to lumbar puncture. CSF culture sensitivities have been reported to range from 70% to 90%. Bacterial culture yield may vary based on patient characteristics, laboratory practices, CSF volume and spectrum of bacterial pathogens likely contributing to the observed differences (51).

Kanegaye et al. demonstrated that CSF sterilization may occur more rapidly after initiation of parenteral antibiotics, with complete sterilization of meningococcus within two hours and the beginning of sterilization of pneumococcus by four hours into therapy (53). Nigrovic and collaborators looked at 159 patients with BM who had not been treated with antibiotics. Antibiotics dropped CSF culture sensitivity by 15% with the sensitivity of 84% in patients who had not received antibiotics before LP and 59% in those who had antibiotics before LP (54).

Lack of adequate culture material may result in inability to tailor therapy to

antimicrobial susceptibility or in unnecessarily prolonged treatment if the clinical presentation and laboratory data cannot exclude the possibility of bacterial meningitis. Kanagaye et al. recommend that if the child is stable; LP should be done before giving antibiotics if the facility has resources to do so (53).

Viral cultures

For children in whom viral meningitis is suspected, CSF viral culture is important. Stool and throat swabs may be sent for viral culture if available (55). Viral cultures are not routinely done at Red Cross War Memorial Children's Hospital. A diagnosis of viral meningitis is usually made in the absence of bacterial meningitis.

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) was developed in the mid- to late 1980s and is considered one of the most important methodological inventions in molecular biology. It is designed to permit selective amplification of a specific target DNA sequence(s) within a heterogeneous collection of DNA sequences (56).

PCR sensitivity has been shown to be superior to that of tissue-culture isolation for both bacteria and viruses. It is however very selective and detects only those pathogens for which primers have been designed. Therefore, a negative test does not exclude other pathogens as the cause of meningitis. PCR can also be compromised by false positive results due to contamination and negative results due to inhibitors of the amplification reaction present in the sample (57).

Khumalo et al. looked at the diagnostic accuracy of two multiplex real-time PCR assays for the diagnosis of meningitis in South African children. They analysed 292 CSF samples using two multiplex RT- PCRs which were developed to detect *S. pneumoniae, N. meningitidis, H. influenzae*, enteroviruses, mumps virus and herpes simplex virus. Bacterial DNA was detected in 12 (4.1%) and viral nucleic acids in 94 (32%).

All patients who tested culture positive for bacterial meningitis were also positive on RT-PCR; PCR also detected an additional four cases which were not picked up on microscopy/culture. Compared to CSF culture, the sensitivity and specificity of the bacterial RT-PCR was 100% and 97.2% with complete agreement in organism identification. None of the cases positive by viral RT-PCR had a bacterial cause confirmed on CSF culture (58).

Wu et al. compared real time-PCR to microscopy and culture results and it was found to be associated with high sensitivity, specificity, and predictive values to diagnose bacterial meningitis (51). **Table 5** summarises their findings (51, 59).

Table 5: Diagnostic methods for identification of micro-organism in meningitis

Methods	Sensitivity	Specificity	Turn-around time	Advantages	Disadvantages
Gram stain	High if not pre-treated with antibiotics 98.2%	High 98.7%	Hours	• Quick • Cheap	• Operator dependent • Prior antibiotic lowers the sensitivity • Cannot serotype the organism
CSF culture	Low 81.3%	High 99.7%	48 hours or more	• Specific • Identifies a wider range of organisms • Tests antibiotics sensitivity	• Prior antibiotic lowers the sensitivity
Bacterial Meningitis Score*	Low	Low	Hours	• Quick	• Not diagnostic • Not applicable if pre-treated with antibiotics • Not for neonates • Not for immunocompromised
PCR	95.7%	94.3%	Hours	• Quick detection • Reduced contamination risk	• Expensive • Identifies a limited number of organisms

Adapted from *Wu et al. (2013) and Nigrovic et.al (2012)*

The main risk factor for being culture-negative and PCR-positive is the presence of antibiotic in CSF (59). PCR can detect many of the pre-treated, culture-negative cases of meningitis (60, 61). If PCR is available then culture is only needed for testing bacterial sensitivity to antibiotics (62).

PCR has a quick turn-around time compared to culture (5 to 24 hours compared with 4 to 8 days) (63). PCR-based assays have become accessible to some South African hospitals especially in private practice to provide a rapid and accurate diagnosis of bacterial and viral meningitis.

Few international studies done on CSF PCR have shown a decrease in patient hospital length of stay therefore avoiding inappropriate exposure to antibiotics. It has also

been highly recommended particularly during the epidemic seasons (39, 64-66).

PCR may also be performed on blood, urine and stool, especially if CSF is not obtainable. Positive PCR in these specimens may suggest a positive CSF PCR provided that the patient is outside the neonatal period and there was no use of antibiotics before the specimen is collected (67).

Stool is also the preferred specimen in cases of suspected aseptic meningitis in a paediatric clinic because the sampling is not-invasive. Enteroviruses are transmitted through the intestine so one would expect high viral load in the stool sample. If only stool is available, PCR as well as cell culture are valuable; on the contrary, if CSF is available cell culture is significantly superior to PCR in attaining a positive result (39, 68, 69).

Challenges with the diagnosis of meningitis

Integrated Management of Childhood Illnesses (IMCI)

Development of a strategy of Integrated Management of Childhood Illness (IMCI) was started by World Health Organisation (WHO) and The United Nations Children's Fund (UNICEF) in 1992. Its main objective was to reduce mortality and morbidity associated with the major causes of childhood illness. At that time, it was estimated that about 10million children die before reaching their fifth birthday. The vast majority of deaths occurred in the developing world and are due to acute respiratory infections, diarrhoeal diseases, malaria measles and malnutrition (70).

The focus was mainly on the primary health services which are the first point of contact for sick patients. It did not make sense to only focus on the above five conditions, other common life-threatening conditions (including meningitis) were also included in their guidelines and the first version was completed in 1995. In a case of suspected meningitis IMCI suggests urgent treatment with injectable antibiotics and referral to the hospital. This has been done to mitigate against delaying antibiotics which has an impact in the outcome of BM.

Antibiotics use before doing a lumbar puncture

The probability of visualising bacteria on a Gram stained preparation of CSF is dependent on the number of organisms present. In most cases antibiotics either given orally or intravenously would kill the majority of those bacteria hence lowering rates of Gram stain and culture and this may occur within hours. This can drop the yield of positive CSF cultures from 70%-85% to below 50% in patients (especially in meningococcal infection), but the CSF inflammatory indices do not often change significantly (71).

Although the use of antimicrobial therapy before LP affects the CSF culture and perhaps the Gram stain, conventional teaching has been that a pathogen still can be identified in the CSF in the majority of patients up to several hours after the administration of antibiotics. Kanegaye et al. reported that in the case of meningococcus, sterility occurred as early as two hours after antibiotic therapy with a 3rd generation cephalosporin and for *S. pneumoniae* after four hours of antibiotic therapy (53).

A review of 128 children with bacterial meningitis specifically addressed this question and found that the time interval between antibiotic administration and negative CSF cultures may be shorter than appreciated for children who receive parenteral antibiotics (53). Oral antibiotics have been reported to have minimal effects on CSF cytology (70, 71).

In order to screen patients and avoid unnecessary admissions, Nigrovic came up with a Bacterial Meningitis Score (BMS). It is a clinical predictive model which can be used in an emergency room to screen patients who are at low risk of bacterial meningitis. It can be used in children with increased white cell count in the CSF. Children are classified as low risk for bacterial meningitis if they lack the following: positive CSF Gram stain, CSF absolute neutrophil count ≥1000 cells/microL, CSF protein ≥80 mg/dL, peripheral blood absolute neutrophil count ≥10,000 cells/microL and history of seizure before or at the time of presentation (72).

The sensitivity and specificity of the BMS for bacterial meningitis were 99.3 percent (95% CI 98.7-99.7 percent) and 62.1 percent (95% CI 60.5 to 63.7 percent), respectively when it was tested in a meta-analysis of data from eight validation studies (one of which was prospective) including 5312 patients. A negative BMS has a negative predictive value (NPV) for bacterial meningitis of 99.9%. This suggests that the BMS may be used in conjunction with clinical judgment to identify children with CSF pleocytosis who are at very low risk of bacterial meningitis and can be discharged from the emergency room (59).

The use of BMS is however limited especially in our context as it has not been

validated in children < 2 months old, those pre-treated with antibiotics, those with critical illness, purpura, ventriculo-peritoneal shunt, recent neurosurgery or immunosuppression (65, 72). Our other problem is the prevalence of HIV and malnutrition in our country (73).

Traumatic Lumbar Punctures

Another challenge we face is the high number of traumatic CSF taps that occur due to the fact that these patients may either present at night when doctors do LPs in a hurry because of the workload, lack of sedation and different levels of experience by doctors. Traumatic LP is common and it occurs when the spinal needle penetrates the vascular epidural space and blood contaminates CSF sample. Traumatic LPs also make interpretation of the WBCs (especially lymphocytes) of the CSF sample difficult since it is more likely that some WBCs in the CSF sample are from the blood rather than arising from meningeal inflammation. Children with a traumatic LP are often treated presumptively for meningitis pending results of CSF culture or a repeat LP after 48 hours. Even with a positive culture it is unclear whether one is culturing blood or CSF.

There are suggested methods which can help with the interpretation of a CSF sample after a traumatic LP. True CSF WBC can be predicted comparing the ratio of WBC: RBC in the peripheral blood with that of the CSF. One formula for predicted CSF leucocytes is =CSF erythrocytes* (blood leucocytes/blood erythrocytes). True CSF leucocytosis is confirmed if the observed CSF WBC count exceeds the predicted count. Patients who have history of head trauma, haemorrhagic stroke, and

necrotizing encephalitis are excluded in this formula (74).

Alternatively in the absence of data on blood erythrocytes and leucocytes, when the CSF is not grossly blood stained using simple leucocyte: erythrocyte ratio, we subtract 1 WBC for every 1000 RBCs/microL. However, none of the formulae to correct the number of CSF WBC obtained in a traumatic tap can be used with total confidence to exclude meningitis (75, 76).

The CSF protein is also increased in children with traumatic LP because of the increased protein concentration in plasma and the release of proteins from lysed RBC. In children with traumatic LP the CSF protein concentration may be corrected by subtracting 1 mg/dL for every 1000 RBCs/microL (77, 78).

CSF co-infection with a virus and bacteria

There have been issues raised regarding co-infection in cases of meningitis but it is known that CSF is a sterile fluid. The only time this would be a possibility would be in cases of head trauma or patients with a foreign body such as ventriculo-peritoneal shunt. Nigrovic et al. also confirmed that children with CSF pleocytosis who have a positive EV-PCR result are at a very low risk of bacterial meningitis and might be safely treated as outpatients, if they appear well enough (79) .

Adjunctive tests for meningitis

CSF

CSF lactate level has been said to be a good predictor of bacterial meningitis at values above 3.5 mmol/L. Like microscopy and culture; it can also be affected by pre-treatment with antibiotics. Its routine use is not yet recommended because further prospective studies are needed (80).

Blood

Initial blood tests for a child with suspected meningitis should include blood cultures, full blood count (FBC) with differential and platelet count (15, 81). The measurement of inflammatory mediators C-reactive protein (CRP) and procalcitonin (PCT) have been evaluated to distinguish between viral and bacterial meningitis with several studies showing PCT to be superior to CRP in differentiating between the two (82-84). While an elevation in either CRP or PCT is more suggestive of bacterial infection, neither can establish, nor exclude the diagnosis of bacterial meningitis (82, 85). CRP lacks the specificity although at high levels above 40 has been shown a high specificity for invasive bacterial infection including meningitis (86).

Table 6: The effect of inflammatory markers in diagnosis of bacterial meningitis (38, 87-92)

	CRP	**PCT**	**WBC**
Rise in concentration	Slow	Hours	Hours
Sensitivity	High (depends on the time that the sample will be taken)	High (If between 0.5 and 2 ng/mL)	High
Specificity	High if above 40	High (If between 0.5 and 2 ng/mL)	Low
Prognostic value	No	Yes	No
Independent diagnostic value	No	No	No

Imaging

Computed tomography (CT) of the head is necessary before lumbar puncture in patients with signs or symptoms of increased intracranial pressure who are at risk of coning. It has however been used more in cases of bacterial meningitis especially TBM. Additional imaging studies like chest radiograph in children with a history of exposure to *Mycobacterium tuberculosis* may be useful in selected patients (80).

Meningitis and HIV

Cryptococcal and tuberculous meningitis remain a major cause of morbidity and mortality among patients living with HIV in sub-Saharan Africa, and in many cases it is potentially preventable (93).

Paediatric studies have reported CM cases in HIV-negative children too. It should however still be considered as a differential diagnosis in older children who are HIV-positive and have a stage 4 defining illness (94). TBM is another differential diagnosis for HIV positive patients presenting with meningitis. Making a diagnosis can be a challenge especially in severely immunocompromised patients (95). It has been well studied locally and the management guidelines are clear.

In 2005, WHO estimated that 0.7-1 million children under 5 years of age in developing world die of pneumococcal disease every year (39). Mortality was shown to be higher in the HIV positive population. A trial done in Soweto, South Africa showed a pneumococcal vaccine efficacy to be 83% in HIV uninfected and 65% in HIV positive group (93).

Meningitis treatment

Meningitis has three diagnostic categories which determine the management of an individual patient (refer Table 7):

Table 7: Meningitis diagnostic categories and the proposed treatment (11, 31, 80, 96)

	Suspected VM	Suspected BM	Unclear aetiology
CSF glucose	Normal	<40 mg/dL (2.2 mmol/L	Normal or low
CSF Protein	<80 to 100 mg/dL	>80mg/dL	Normal or high
CSF WBC	<500/microL with >50 percent mononuclear cells	>1000/microL with a predominance of neutrophils (PMN)	PMN predominance
CSF GS	Negative	**Positive**	Negative or delayed results
CSF culture	Negative	**Positive**	Negative or delayed results
Prior treatment with antibiotics	No	**No**	Yes
Proposed Management	Supportive therapy only: bed rest and analgesia	Treatment with wide-spectrum antibiotics should not be delayed while awaiting laboratory results	Admit, observe without treating but if deteriorates, treat. Repeat LP in 24-48 hours (Swingler, Red Cross protocol 2013).

As the clinical features of encephalitis can overlap with those of meningitis, all patients with an acute onset of fever with a change in their mental status or new onset of seizures (excluding simple febrile seizures) should be treated for suspected herpes simplex encephalitis with acyclovir (96).

Meningitis prevention

Primary prevention of meningitis predominantly through vaccination programs is of paramount importance, since mortality and long-term disabling sequelae remain substantial. Routine vaccination also offers herd immunity for the unvaccinated population (97).

Viral meningitis

MMR vaccine has been shown to be effective in decreasing VM cases caused by mumps virus. Improved public hygiene has also shown a decrease in VM incidence (18).

Bacterial Meningitis

Since the 1980s, many countries have included immunization against *Haemophilus influenzae type b* in their routine childhood vaccination schemes. This has practically eliminated this pathogen as a cause of meningitis in young children in those countries. In the countries where the disease burden is highest, however, the vaccine is still too expensive (98).

Routine vaccination against *Streptococcus pneumoniae* with the 7-valent pneumococcal conjugate vaccine (PCV) in South Africa was introduced in April 2009 and was replaced with the 13-valent PCV in April 2011. The pneumococcal polysaccharide vaccine, which covers 23 strains, is only administered in certain groups (e.g. those who have had a splenectomy). It does not elicit a significant immune response in all recipients, e.g. very young children (98).

There is strong evidence that daily oral penicillin prophylaxis greatly reduces the risk of pneumococcal infection in children with sickle cell anaemia under the age of three years and moderately strong evidence that its withdrawal at the age of five years did not result in any serious consequences in Nigeria (99).

Meningococcal vaccine is not readily available in the state sector in South Africa. As the cost of a meningococcal vaccine programme is expensive and the prevalence of meningococcal disease including meningitis is not increasing, some countries can justify a motivation for certain high-risk groups e.g. patients with asplenia and other immunodeficiency syndromes (100). There is an urgent need to have guidelines for meningococcal vaccination for high-risk groups e.g. HIV-infected. Early infant vaccination with BCG has been reported to significantly reduce the rate of tuberculous meningitis in children (101, 102).

Conclusion

Viral meningitis remains the most common cause of meningitis amongst children with meningitis in hospitals. Despite the revised South African Expanded Programme on Immunisation there are still cases of bacterial meningitis and this, together with the overlapping symptoms and signs of bacterial and viral meningitis, contributes to clinicians over diagnosing BM because they fear complications.

Other complicating factors such as bloody CSF tap and prior exposure to antibiotics affect our interpretation of CSF findings and CSF culture results. Septic markers like CRP, PCT and FBC are expensive and have not been shown to establish or exclude a diagnosis of bacterial meningitis.

Real-time multiplex PCR remains the investigation of choice in diagnosing meningitis and quickly deciding which patients will need admission for treatment.

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Chapter 2: Publication-Ready Manuscript

Use of real-time polymerase chain reaction to diagnose meningitis in children admitted to Red Cross War Memorial Children's Hospital

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Abstract

Background

Viral meningitis is a major cause of childhood meningitis. Bacterial meningitis, though a more severe infection with high mortality rate, is relatively uncommon. Routine cerebrospinal fluid microscopy and culture tests have low sensitivity for diagnosing bacterial meningitis particularly in the face of antibiotic administration. Many children with viral meningitis are misdiagnosed and treated for bacterial meningitis. Our research explored the efficacy of polymerase chain reaction (PCR) for diagnosing meningitis in children older than two months of age.

Methods

The study included children with an abnormal cerebrospinal fluid (CSF) result, defined as raised CSF white cell counts, who presented to the emergency unit of Red Cross War Memorial Children's Hospital (RCWMCH) between November 2012 and October 2013.

Data obtained from patient records included clinical data (presentation, prior use of antibiotics and HIV status), blood culture, C-reactive protein levels and routine cerebrospinal fluid analysis results were reviewed. PCR for bacterial pathogens namely: *Neisseria meningitidis*, *Haemophilus influenzae type b* and *Streptococcus pneumoniae* and viral pathogens enterovirus, herpes simplex and mumps virus was conducted on the CSF.

Results

The study included 286 children with a median age of 19 months (IQR=2-65 months). Viral PCR detected 86 (30.1%) cases (enterovirus = 84, mumps=2) while 14 (4.9%) had confirmed bacterial meningitis by either PCR or routine culture. Median C-reactive protein was 46.2 (19.6 – 173.5) mg/L in confirmed bacterial meningitis and 12.1 (6.0 - 28.4) mg/L in confirmed viral meningitis; **p=0.009.** CSF chemistry, cell counts were similar in viral meningitis and bacterial meningitis. Forty (46.5%) of the 86 children with confirmed viral meningitis were initially treated as bacterial meningitis.

Conclusions

Viral meningitis is the commonest cause of meningitis seen at RCWMCH but most of the cases are wrongly managed as bacterial meningitis due to poor diagnostic confirmation. PCR takes shorter time and improves diagnostic yield of viral meningitis and healthcare services should consider making it standard of care.

Introduction

Viral meningitis (VM) is the leading cause of meningitis outside the neonatal period with enteroviruses being the most common pathogens (1). VM is usually a self-limiting illness but remains an important cause of financial burden and it requires improvement in its diagnostic, treatment, and prevention options (2).

On the other hand bacterial meningitis (BM) is a severe infection that can rapidly progress to multiple organ failure and death if untreated.

Prior to the introduction of vaccines, BM was rated one of the top ten causes of death among all infectious diseases and the mortality varies from 10 to 20% if untreated (3). Among survivors, up to 50% can have severe lifelong disability. Permanent sequelae include hearing loss, neurological disability and limb loss (4). The fear of missing BM has led to a large proportion of children with VM being misdiagnosed as BM and subsequently receiving inappropriate treatment.

Development of the Integrated Management of Childhood Illness (IMCI) strategy was started by World Health Organisation (WHO) and The United Nations Children's Fund (UNICEF) in 1992. Its main objective was to reduce mortality and morbidity associated with the major causes of childhood illness. In a case of suspected meningitis IMCI suggests urgent treatment with injectable antibiotics before referring to the hospital. As a result, these children receive antibiotics prior to CSF sampling.

Reduction in cases of *Haemophilus influenzae* has been attributed to the introduction of the Hib conjugate vaccine into the Expanded Programme on Immunisation (EPI) for South Africa in 1999 (5). Similarly, the introduction of 7-valent pneumococcal conjugate vaccine (PCV) in April 2009 and 13-valent PCV in April 2011 has contributed to a substantial reduction in the incidence of pneumococcal meningitis in children <5 years of age.

Neisseria meningitidis is the only major cause of bacterial meningitis outside the

neonatal period for which routine immunisation is currently not available in SA. According to the GERMS 2016 report, a total of 131 cases of laboratory-confirmed meningococcal disease were identified by the surveillance system. Compared to the 2015 report, the incidence of meningococcal meningitis was slightly lower (0.23 vs 0.28 cases per 100 000 population) (6).

Even with the noted change in epidemiology of meningitis following introduction of vaccines, most children are admitted for intravenous antibiotics while awaiting laboratory confirmation for bacterial meningitis. Traditional laboratory methods include Gram stain and culture of cerebrospinal fluid (CSF). These have long turnaround times and usually show negative results especially in children who receive antibiotics prior to CSF sampling (4, 7). In a previous study, antibiotic treatment was retrospectively judged to be unjustified in over 80% of children treated for longer than two days (8). Inappropriate use of antibiotics may lead to future antibiotics resistance; it may also cause adverse antibiotic effects (9, 10).

Rapid diagnosis is essential to confirm the cause of meningitis and institute appropriate timeous treatment. Modern molecular methods that are based on detection of nucleic acid sequences can provide rapid identification of both viral and bacterial pathogens. Real time Polymerase Chain Reaction (PCR) can detect responsible pathogens independent of culture even after antibiotics have been commenced. Currently PCR is not standard of care at RCWMH as well as at most public hospitals in South Africa.

Our study aimed to describe the clinical features and outcomes of patients who had

confirmed viral meningitis on PCR testing. Secondarily the study compared the clinical features and laboratory findings in children with confirmed viral meningitis with children with confirmed bacterial meningitis and children in whom the aetiology of meningitis could not be determined.

Materials and methods

Our methods have been previously published (11). Briefly, residual CSF samples received by the microbiology laboratory from the emergency department of the Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town, South Africa, were prospectively collected between 1 November 2012 and 30 October 2013. The National Health Laboratory Services (NHLS) database was used to identify all specimens with abnormal white cell counts (defined as presence of any polymorphs or more than five lymphocytes in CSF). All children older than 60 days of age with abnormal CSF findings were eligible for inclusion if they had sufficient residual CSF for analysis (if they had at least 1 ml of residual CSF). Children with head injury or existing skull defects (e.g. base of skull fracture or ventricular-peritoneal shunt in situ) were excluded.

A retrospective review of folders was conducted on all subjects who met the inclusion criteria. Data including history of clinical presentation, prior exposure to antibiotics and indication for lumbar puncture (LP) were sourced from the patient's record. Examination findings at presentation prior to sampling of CSF were obtained from admission notes. Results of laboratory investigations including CSF Gram stain and culture, blood culture, a full blood count and C-reactive protein were recorded. Where available, the patient's HIV status was also recorded. The HIV status was classified as

HIV-infected if the children had a positive HIV PCR if younger than 18 months of age or had two positive ELISA tests if older. The children's management plans, duration of therapy as well as discharge diagnosis were recorded.

Laboratory diagnosis

A diagnosis of confirmed bacterial meningitis was made in the case of either a positive CSF Gram stain or culture or a positive blood culture result. A validated in- house real-time multiplex PCR assay was used to test for the presence of the three common bacterial pathogens namely, *Neisseria meningitidis* (ctrA gene), *Haemophilus influenzae* (hpd gene) and *Streptococcus pneumoniae* (lytA gene) as well as three selected viral pathogens: *enterovirus* (5' UTR), *herpes simplex* virus (UL30 gene) and *mumps virus* (Fusion protein gene). PCR was carried out on the Biorad CFX 96 realtime instrument (11).

In addition, routine molecular diagnostic testing was carried out for *Mycobacterium tuberculosis* complex (GeneXpert) on CSF and/or sputum if tuberculous meningitis (TBM) was suspected. Viral and bacterial PCRs were done on batched specimen samples long after the children had been discharged.

Data analysis

Descriptive statistical data were generated for all relevant variables. Proportions were used to depict frequencies of categorical data while means with standard deviation (SD) or medians with interquartile ranges (IQR) were used for all continuous data as appropriate. Comparisons were made between laboratory data of subjects with

confirmed viral and bacterial meningitis as well as between viral and unconfirmed meningitis using a Mann-Whitney test. A two-tailed $p<0.05$ was used as the cut-off point for significance at each instance.

All statistical computations were done on STATA software version 13 (STATA Corporation, College Station, TX).

Ethics

Approval for the study was granted by the Human Research Ethics Committee of the University of Cape Town (HREC REF 223/2015).

Results

Baseline characteristics of the study population

Of 3247 CSF samples that were collected over the period, 517 (20%) were abnormal of which 292/517 (56.5%) had enough residual CSF sample to be included in the analysis and 286/292 (97.9%) children had folders available for review. (**Table 1**). Blood cultures were available for 57 (19.9). Viral PCR detected 86 (30.1%) cases (enterovirus = 84, mumps=2) while 14 (4.9%) had confirmed bacterial meningitis by either PCR or routine culture.

Of the 286 children with sufficient data to be included in the analysis 152 (53.1%) were male. The median age of the participants was 19 (IQR 6 – 65) months. HIV status was known in 178 (62.2%) children of whom 10 (3.5%) were HIV infected.

A total of 86 (30.1%) study subjects had received antibiotics before lumbar puncture was performed. In 41 (47.7%) children only oral antibiotic had been received prior to CSF sampling. A similar number had received parenteral antibiotics only while four (4.6%) had received both oral and parenteral antibiotics. The commonest oral antibiotic received was amoxycillin while almost all the patients who received parenteral antibiotics were given ceftriaxone **(Table 1).**

Table 1: Baseline characteristics of the study population

Baseline characteristics	*N=286*
Median (IQR) months	19 (2-65)
	N (%)
Gender	
Female	134 (46.9)
Male	152 (53.1)
HIV status	
Unknown	108 (37.8)
Negative	168 (58.7)
Positive	10 (3.5)
Pre-hospital antibiotics	
None	200 (69.9)
Oral amoxycillin	24 (8.4)
Ceftriaxone	41(14.3)
Unknown antibiotic	21 (7.3)

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1382 # Clinical presentation

Table 2: Clinical presentation of participants by PCR diagnostic confirmation

Clinical	All(n=286)	BM(n=14)	VM(n=86)	Unconfirmed (n=186)	P1	P2	P3
Fever	227(79.4)	13 (92.9)	77 (89.5)	137 (73.7)	1.000	**0.003**	0.196
Headache	111(38.8)	2 (14.3)	57 (66.3)	52 (28)	**<0.001**	**<0.001**	0.360
Seizures	57 (19.9)	2 (14.3)	4 (4.7)	51 (27.4)	0.197	**<0.001**	0.362
Vomiting	153(53.5)	7 (50.0)	62 (72.1)	84 (45.2)	0.122	**<0.001**	0.726
Diarrhoea	63 (22.0)	2 (14.3)	7 (8.1)	54 (29)	0.609	**<0.001**	0.357
Meningeal irritation	23 (8.0)	5 (35.7)	14(16.3)	4 (2.2)	0.134	**<0.001**	**<0.001**
Photophobia	21 (7.3)	0 (0.0)	15 (17.4)	6 (3.2)	0.120	**<0.001**	1.000
Altered mental	13 (4.5)	9 (64.3)	1 (4.7)	3 (1.6)	**<0.001**	1.000	**<0.001**
Irritability	56 (19.6)	3 (21.4)	19 (22.1)	34 (18.3)	1.000	0.460	0.726
Poor feeding	60 (21.0)	6 (42.9)	20 (23.3)	34 (18.3)	0.185	0.339	**0.038**
Apnoea	2 (0.7)	0 (0.0)	0 (0).0	2 (1.1)	**N/C**	1.000	1.000
Bulging fontanelle	19 (6.6)	2 (14.3)	3 (3.5)	14 (7.5)	0.143	0.201	0.310

VM=viral meningitis; BM=bacterial meningitis;

P values: **P1**= VM vs BM, **P2**= VM vs unconfirmed, **P3**= BM vs unconfirmed, N/C=not calculated

Table 2 summarises the most common clinical manifestations of the study population. It also compares signs and symptoms between VM and BM. Most children presented with fever in 227 (79.4%) cases followed by vomiting 153 (53.5%) and reported headache in 111 (38.8%). Seizures were reported in 57 (19.9%) of the children of which 51 (89.5%) were generalised tonic-clonic seizures; 34 (59.6%) of these were diagnosed with febrile seizures. Only 32 (11.2%) children had signs of meningeal irritation. Apnoea was documented in two (0.7%) children, both younger than three months of age.

Diagnosis

The diagnosis was made by the attending clinicians based on CSF cell counts, biochemical analysis and routine culture in addition to clinical presentation. Discharge diagnosis of VM was made in 96 (33.6%) of the children while 94 (32.8%) children had a diagnosis of BM. In the remaining 96 (33.6%) children the diagnosis of meningitis was excluded by the attending clinician.

The cause of meningitis was confirmed by PCR in 100 (35.0%) of the patients; 14 (14.0%) were confirmed to have BM while 86 (86.0%) had confirmed VM. Routine CSF or blood culture were positive in 7 (50%) of the 14 confirmed cases of BM. Three of the culture confirmed cases (one on blood culture alone and two on CSF culture) were due to *S. pneumoniae*, with one each due to *N. meningitidis* and *H. influenzae*. The other two cases had TBM **(Figure 1).** Of 86 patients who had had antibiotics before LP was done, only one had a positive culture on CSF.

It took an average time of 9.2 (SD ±4.7) hours for CSF to show bacterial growth while

blood cultures flagged positive in 17.4 (SD ±6.0) hours. The two CSF specimens positive for M. tuberculosis took 34 and 18 days respectively to show growth. One organism was cultured in the CSF in 1 of the 86 children who received prior antibiotics. PCR detected an additional 7 (50%) children with BM as shown in **figure 1**.

A total of 100 (35.0%) children had a confirmed diagnosis of meningitis on PCR. Fifty-one (51.0%) had been managed as BM based on clinical features, routine microscopy and culture, 42 (42.0%) were managed as VM and seven (7.0%) were treated for a non-meningitis diagnosis (Table 3). Forty (46.5%) of the children treated for BM were later confirmed to have VM on PCR. The use of clinical evidence and routine laboratory detected VM with a sensitivity of 48% (95% CI 37- 59%) and a specificity of 93% (95% CI 66-100%) compared to PCR.

Table 3. Clinical diagnosis of confirmed meningitis (N=100)

Clinical diagnosis#	Bacterial	Viral (n=86)	Total
	n (%)	n (%)	
Viral Meningitis	1 (7.1)	41 (46.7)	**42**
Bacterial Meningitis	11 (78.6)	40 (46.5)	**51**
Non-meningitis	2 (14.3)	5 (5.8)	**7**

#Included use of routine CSF cell count, chemistry, microscopy and culture

Real-time multiplex PCR assay detected an additional seven cases of BM comprising five cases of *S. pneumoniae* and two cases of *N. meningitidis*. All positive CSF cultures were also positive on PCR. Negative CSF bacterial culture results and positive viral PCR on CSF confirmed the diagnosis of VM. Viral PCR was positive in 86 (30.1%) CSF samples of which 84 (97.7%) had enteroviruses, two (2.3%) had mumps virus and no cases of herpes simplex virus (HSV) **Figure 1**.

The cause of meningitis could not be determined in 186 (65.0%) samples as they tested negative on microscopy, culture and on PCR for the organisms of interest. Apart from *M. tuberculosis* cultured in two CSF specimens, no organism was cultured in the CSF of the 86 children who received prior antibiotics. Only four (4.7%) of them had positive PCR results for BM.

Four (15.4%) of the 26 children that underwent brain computed tomography (CTB) scanning showed abnormal findings. One CTB showed subarachnoid haemorrhage with acute hydrocephalus, the second one showed bilateral basal ganglia infarcts, diffuse brain swelling and a right subdural hygroma. The CSF PCR for the latter patient was positive for *S. pneumoniae.* The other two abnormal scans showed basal meningeal enhancement and communicating hydrocephalus, features in keeping with *TBM*. Only three (3.5%) of the 86 children with confirmed VM were scanned, all of whom showed normal CT findings.

Of the 10 HIV-infected children, only two (20%) had confirmed BM while the other eight (80%) had negative PCR results. No further analysis was possible to compare HIV infected and uninfected children due to the small sample size.

Other laboratory findings in PCR positive and PCR negative groups.

The median blood C-reactive protein was 46.2 mg/L (IQR: 19.6 – 173.5 mg/L) in confirmed BM and 12.1 mg/L (IQR: 6.0 - 28.4 mg/L) in confirmed VM; **p=0.009**. CSF white cell count was similar in both groups, but a low CSF glucose **(p=0.044)** and a

high CSF protein **(p=0.001)** seem to be an important finding in children with BM compared to VM or unclassified **(Table 4).**

Table 4. Laboratory results by PCR confirmed cause of meningitis

Laboratory test	BM (n=14)	VM (n=86)	Unconfirmed (n=186)	P1	P2	P3
CRP (mg/L)	46.2 (19.6-173.5)	12.1 (6 - 28.4)	11.6 (2.2-46)	**0.009**	0.869	**0.0167**
WBC (*10^9)	17.7 (8.7 - 26.4)	13.6 (10.4 - 17.1)	12.9 (10.2 - 19.6)	0.334	0.631	0.6441
Polymorphs (/uL)	38 (1 - 1680)	43 (10 - 141)	2 (0 - 11)	0.960	**<0.001**	**0.0044**
Lymphocytes (/uL)	110 (9 - 280)	50.5 (14 - 139)	8 (5 - 30)	0.456	**<0.001**	**0.0090**
Glucose (mmol/L)	3.1 (1.7 - 3.9)	3.6 (3.3 - 3.9)	3.6 (3.1 - 4.2)	**0.044**	0.542	**0.0240**
Protein (g/L)	0.9 (0.4 - 1.1)	0.3 (0.2 - 0.4)	0.3 (0.2 - 0.4)	**0.001**	0.061	**0.0003**

CRP= C-reactive protein; WBC= White blood cell count; p<0.05 shown in Bold typeset; BM=bacterial meningitis; VM=viral meningitis; P values: P1= VM vs BM, P2= VM vs unconfirmed, P3= BM vs unconfirmed

Management and outcome of PCR confirmed meningitis

Children presumed to have VM, based on the CSF picture and rapid clinical improvement, were treated for a median period of 1 day (IQR 1-2 days) while those with BM were treated for a median of 5days (IQR 3-6 days); **p<0.001**. The usual treatment for presumed viral meningitis was supportive care and analgesia. Two children with TBM required ventricular-peritoneal shunt insertion for hydrocephalus as well as a prolonged in-patient course of anti-tuberculous treatment (85 and 197 days respectively). One (2%) child who was managed initially as VM was later confirmed to have *N. meningitidis* on PCR. This patient received two doses of 100mg/kg of ceftriaxone over two days and did not return to the hospital after discharge.

Of 86 children with PCR confirmed VM, 19 (22.1%) were admitted for 24 hours; 31

(36.1%) were admitted for 24-48 hours and 16 (18.6%) were admitted for more than 48 hours. Only 20 (23.3%) children were discharged home without antibiotic treatment. Of those children who had BM; only one was admitted for 24 hours, the other 12 (85.7%) were hospitalised for more than 72 hours and one died within 24 hours of admission.

A total of two (0.7%) children in the study died. One had pneumococcal meningitis and died within 24 hours of intensive care admission. The second child had been diagnosed with presumed septic shock requiring ICU admission, cefotaxime, ventilation and inotropes. His CTB showed sub-arachnoid haemorrhage with acute hydrocephalus requiring drainage in theatre. His blood culture, CSF Gram stain, culture and PCR were all negative. He died after four days of admission.

Discussion and conclusion

Our study shows that over 30% of patients with abnormal CSF had confirmed viral meningitis on real-time multiplex PCR testing while bacterial meningitis was confirmed in only 5%. Viral meningitis accounted for 85% of all confirmed meningitis cases where aetiology was found. Most of the cases (98%) were due to enteroviruses with a small proportion due to mumps virus. This finding is in keeping with the reported prevalence of meningitis which indicates that viral infections are the commonest causes of meningitis. The fewer cases of BM are attributed to the widespread use of vaccines targeting common bacterial causes of meningitis in children. (12-14).

In this study clinical signs of bacterial and viral meningitis overlapped significantly, and

clinicians had difficulty in distinguishing between the two. Comparing children with VM with those who had BM, headache and altered mental state gave statistical different results **($p<0.05$).** Fever, headache, seizures, vomiting, diarrhoea, meningeal irritation and photophobia also gave significant results between VM and the unclassified group. Between and the unclassified group, only meningeal irritation, decreased mental state and poor feeding were suggestive of BM as shown in **table 2**.

Additional laboratory support is thus required to determine aetiology. A large proportion of children later confirmed to have VM (46%) were admitted and treated with antibiotics as for BM.

According to de Crom et al. the acute onset of fever, headache, nausea, vomiting, stiff neck, and photophobia are common presenting symptoms of VM (15). Because of the retrospective nature of our study, we could not compare patients' clinical presentations in detail. In another study the frequency of seizures was found to be higher in BM and encephalitis than VM (16), in our study there was no difference.

CSF Gram stain and culture with clinical input has poor sensitivity in detecting meningitis. Our study showed no detectable differences in the CSF cell counts of VM and BM. Although predominance of polymorphs is regarded as a feature of BM, there is good evidence that early VM can have polymorph predominance (4, 6). In a study conducted by Potter et al, children with purulent CSF and polymorph predominance turned out to have VM and recovered quickly (17).

We found statistically significant differences in CSF chemistry between VM and BM

cases (low glucose and high protein in BM) as previously described(18).While chemistry can be used as a guide for differentiating VM from BM, it is not diagnostic for either condition on its own.

In the absence of laboratory viral confirmation, VM diagnosis is made by excluding BM in the absence of bacteria on CSF Gram stain and culture. After LP, children with CSF pleocytosis are routinely admitted and put on intravenous antibiotics for 48 hours pending the outcomes of bacterial culture. The ones who received antibiotics prior to LP often end up with a full course of BM treatment as negative culture becomes unreliable in excluding BM.

Our study found that a large proportion of children (47%) with confirmed VM were diagnosed and managed as BM. This misdiagnosis resulted in prolonged hospital stay and inappropriate usage of antibiotics. This imposes an undue financial burden on healthcare services as well as an increase the risk of resistance to antibiotics. Gram stain and bacterial culture also missed cases of PCR confirmed BM even in children without exposure to antibiotics prior to CSF collection.

CRP has been used as an additional test and has been shown to have a high sensitivity for bacterial infection depending on when the sample is taken. Its specificity is high with values above 40mg/L (19). In our study, there were significantly higher average levels of CRP in BM compared to VM. The small sample of BM cases in our study did not allow us to establish a diagnostic cut-off point for BM in our population. All other laboratory findings, including WCC did not differ significantly between confirmed BM and VM.

Blood culture results have been used to aid with the diagnosis of BM. Only 57 (20%) of our children had blood cultures taken. All children in our study with positive blood cultures showed the same bacteria in the CSF. PCR detected VM meningitis cases that should otherwise not need admission and antibiotics. It also picked up an additional four cases of BM which were missed on CSF Gram stain and culture. This indicates that PCR sensitivity is superior to that of CSF- culture isolation for confirming meningitis due to both bacteria and viruses (19, 20).

The main risk factor for false negative culture is prior exposure to antibiotic (21, 22). PCR can detect organisms in antibiotic-exposed patients who would have negative culture hence decreasing patient hospital length of stay and reduce inappropriate use of antibiotics (23- 26).

There is an urgent need to consider routine use of PCR in the diagnosis of meningitis in children. Real-time multiplex PCR offers value in accurately detecting common viral and bacterial pathogens thus allowing for appropriate patient management. Even where limited resources do not allow for two assays our study suggests that viral PCR alone would identify a large number of children with VM who do not require antibiotics. PCR testing, however, should be done in addition to culture, not as a replacement. Otherwise one risks losing the organisms not included in the PCR assay, as well as the ability to do susceptibility testing and strain typing. An economic evaluation would be useful in making this decision. The current cost of the PCR is R586 (±USD 40) - relative to a CSF cell count, microscopy and culture which cost R114.83.

Our study highlights the need for using currently available methods for the diagnosis of

viral meningitis children with emphasis on excluding or confirming viral meningitis to reduce the need for unnecessary admission and treatment with antibiotics.

Our study was limited by the use of retrospective data. As a result, key clinical data was missing in some instances and detailed analysis could not be carried out. As the PCR assays used only tested for preselected pathogens, a negative test does not exclude meningitis due to other pathogens. This may account for the large proportion of unconfirmed meningitis.

A prospective study is needed to evaluate the narrow economic as well as the broader societal cost effectiveness of PCR testing in managing children with suspected viral meningitis. Such a study should be sufficiently powered to assess the cost of using PCR to diagnose meningitis compared to traditional methods and the resultant impact on paediatric admissions for meningitis, hospital length of stay and antibiotic usage.

In conclusion, this study highlights the potential of PCR in improving diagnostic accuracy and reducing the cost of managing children with suspected viral meningitis.

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1677 **Figures**

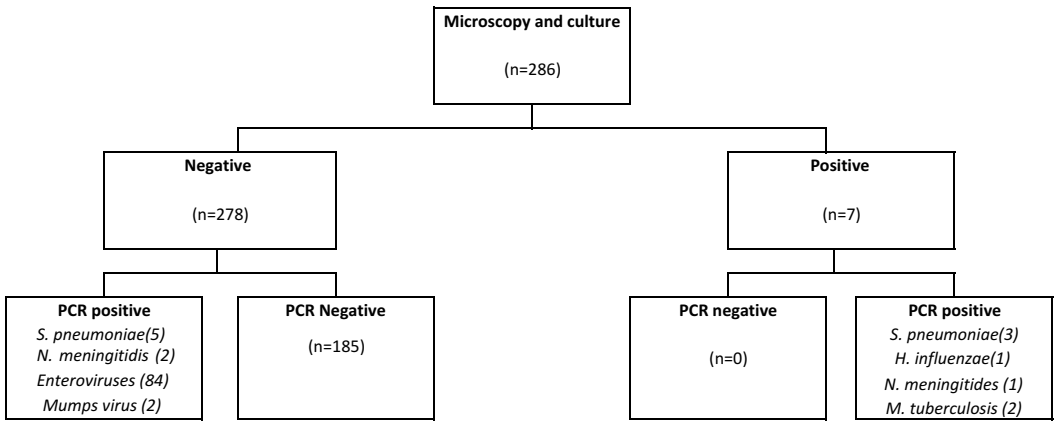

Figure 1: Comparing cerebrospinal fluid(CSF) micropscopy and culture to CSF polymerase chain reaction
NB. An extra case of *S. pneumoniae* was confirmed on blood culture only

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Meningitis Review CRF – Part I

Meningitis study number: __ __ __ __ __ __ *Date of folder review* __ __ / __ __ / 2 0 __ __

Patient information and background data

1.	Patient name	
2.	Folder Number	
3.	Sex	☐ *Male* ☐ *Female*
4.	Date of birth	__ __ / __ __ / 2 0 __ __ *(dd/mm/yyyy)*
5.	Suburb/Township of residence	
	Previous History	
6.	Immunization status If *Missing* which immunizations?	☐ *Up to date* ☐ *Missing* ☐ *Not recorded* _____
7.	Is the child failing to thrive?	☐ *Yes* ☐ *No* ☐ *Unsure*
8.	Birth weight	__ . __ __ kg ☐ *Unknown*
9.	Gestational age	☐ Term ☐ Premature (<37 weeks) ☐ *Unknown*
10.	What sort of house does child live in?	☐ Bricks ☐ Informal ☐ Unknown
11.	Does family use any of the these for heating and/or cooking in the house? (*check all that apply*)	☐ Electricity ☐ Gas ☐ Paraffin ☐ Coal ☐ Wood ☐ Unknown ☐ Other: _____
12.	What is the main source of water in the household?	☐ In-door tap water ☐ Outdoor/communal tap ☐ Unknown ☐ Other: _____
13.	What type of toilet does family use?	☐ *Flush toilet* ☐ *Bucket system* ☐ *Unknown* ☐ *Other* _____
14.	During the first 4 months of life, how was the child fed?	☐ *Breast milk* ☐ *Breast milk & formula* ☐ *Formula* ☐ *Unknown*
15.	If older than 4 months: how is child being fed at time of diagnosis?	☐ *Breast milk* ☐ *Breast milk & formula* ☐ *Formula* ☐ *Other* _____ ☐ *Unknown*
16.	Presence of pre-admission diagnosis (This refers to a preexisting diagnosis – check all that apply)	☐ *Previous IPPV* ☐ *Head injury* ☐ *High dose steroid* ☐ *Prematurity* ☐ *VP shunt* ☐ *Malnutrition* ☐ *Immunosuppressive therapy* ☐ *Not recorded* ☐ *Other, specify*
17.	Previous admissions in past 1 year If *Yes* Date of last admission Discharge date at last admission	☐ *Yes* ☐ *No* ☐ *Not recorded* *No. of previous admissions* _____ __ __ / __ __ / 2 0 __ __ *(dd/mm/yyyy)* __ __ / __ __ / 2 0 __ __ *(dd/mm/yyyy)*
18.	Diagnosis at last admission.	☐ *Not Applicable*

Meningitis study number: __ __ __ __ __ __

History at the time of testing the child for meningitis

1.	*Did the child have any of the following symptoms during the current illness?*		
1.1.1	Fever	☐ Yes ☐ No ☐ Unknown	*(skip to 1.2 if No or Unknown)*
1.1.2	*If yes:* Duration	_____ days ☐ Unknown	
1.2	Headache	☐ Yes ☐ No ☐ Unknown	*(skip to 1.3 if No or Unknown)*
1.2.2	*If yes:* Duration	_____ days ☐ Unknown	
1.2.3	Neck pain	☐ Yes ☐ No ☐ Unknown	
1.2.4	Neck stiffness	☐ Yes ☐ No ☐ Unknown	
1.2.5	Photophobia	☐ Yes ☐ No ☐ Unknown	
1.2.6	Vomiting	☐ Yes ☐ No ☐ Unknown	
1.2.7	Diarrhoea	☐ Yes ☐ No ☐ Unknown	
1.2.8	Myalgia	☐ Yes ☐ No ☐ Unknown	
1.2.9	Rash	☐ Yes ☐ No ☐ Unknown	
1.2.10	Seizures (describe)	☐ ☐ ☐	
1.2.11	Confusion	☐ ☐ ☐	
1.2.12	Poor feeding	☐ ☐ ☐	
1.2.13	Irritability	☐ ☐ ☐	
1.2.14	Other	☐ ☐ ☐	
1.3	Apnoea *(stopping breathing)*	☐ Yes ☐ No ☐ Unknown	
2.	Did child seek prior health care?	☐ Yes ☐ No ☐ Unknown	*(skip to 3 if not Yes)*
2.1	*If Yes,* did child go to:	☐ Clinic/Day Hospital ☐ Traditional healer ☐ General practitioner ☐ Other: _____	
3.	Did the child receive antibiotics before coming to the hospital?	☐ Yes, oral antibiotic ☐ Unknown ☐ Yes, injection ☐ No	*(skip to 4 if No or Unknown)*
3.1	*If Yes,* name of antibiotic	_____ ☐ Unknown	
3.2	*If Yes,* number of antibiotics days?	_____ days ☐ Unknown	
8.1	HIV status of child	☐ *Negative* ☐ *Infected* ☐ *Unknown* ☐ *Exposed Uninfected*	
8.2	If HIV exposed, did child receive PMTCT	☐ *Yes* ☐ *No* ☐ *Not Applicable* ☐ *Unknown* ☐ *Mother on HAART*	
8.3	HIV treatment	☐ *1st line* ☐ *2nd line* ☐ *Not Applicable*	
	How long is child on treatment?	_____ *months* ☐ *Not Applicable*	
8.4	Nevirapine prophylaxis	☐ *Yes* ☐ *No* ☐ *Not Applicable*	
8.5	Most recent CD4 count - child	__ __ __ __ Absolute CD4 ☐ *Not Applicable*	
8.6		__ __.__ % of lymphocytes	
8.7	If applicable, date of last CD4	__ __ / __ __ / 2 0 __ __ *(dd/mm/yyyy)*	
8.8	Most recent viral load - child:		
8.9	If applicable, date of last viral load	__ __ __ __ __ __ copies/ml ☐ *Not Applicable* / / 2 0 *(dd/mm/yyyy)*	

Meningitis Review CRF – Part III

Meningitis study number: __ __ __ __ __ __

Medical Records *(The following questions relate to the period closest to taking the diagnostic sample. Temperature, heart rate and respiratory rate should be the maximum recorded within 24 hours)*

1.	Date of admission	*___ ___ / ___ ___ / 2 0 ___ ___ (dd/mm/yyyy)*
2.	Time of admission	*___ ___ : ___ ___ ☐ Am ☐ Pm ☐ Unknown*
3.	Admission height/length	*__ __ _ , __ cm ☐ Not recorded*
4.	Admission weight	*__ __ . __ kg ☐ Not recorded*
5.	Head Circumference	*__ __ , __ cm ☐ Not recorded*
6.	Temperature	*__ __ . __ °C ☐ Not recorded*
7.	Heart rate	*___ ___ ___ beats / min ☐ Not recorded*
8.	Respiratory rate	*___ ___ ___ breaths / min ☐ Not recorded*
9.	Oxygen saturation	*___ ___ ___ % ☐ Room air ___ ___ ___ % ☐ On oxygen ☐ Not recorded*
10.	Signs o Neck stiffness o Photophobia o Rash o Oral lesions o Bulging fontanelle o Irritable o Brudzinski o Kernings o Decreased LOC o Other	***Yes*** ***No*** ***Not recorded*** ☐
11.	Did the child receive any of: NSAIDs Steroids Antivirals Antibiotics *If Yes*, name(s) of antibiotic(s) and duration Other	*Yes* *No* *Duration* ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

12.	Outcome of child	☐ *Discharged* ☐ *Refused Hospital Treatment (RHT)* ☐ *Died* ☐ *Transferred to another hospital*
13.	Date of discharge/ death/ transfer/RHT	*___ ___ / ___ ___ / 2 0 ___ ___ (dd/mm/yyyy)*
14.	If discharged, on what treatment was child discharged? *(mark all that apply)*	☐ *Antibiotics, specify:_____* ☐ *Antivirals* ☐ *Steroids* ☐ *Anti TB treatment* ☐ *Other, specify* _____
15.	Discharge diagnosis *(mark all that apply)*	☐ *Viral meningitis* ☐ *Bacterial Meningitis* ☐ *TB meningitis* ☐ *Partially treated meningitis* ☐ *Normal* ☐ *Not recorded* ☐ *Other, specify* _____
16.	Any readmissions after discharge If *Yes*, was it for a meningitis Dx? Date of admission Date of discharge	☐ *Yes* ☐ *No* ☐ *Yes* ☐ *No specify* _____ *___ ___ to ___ ___ / 2 0 ___ ___ (dd/mm/yyyy)* *___ ___ to ___ ___ / 2 0 ___ ___ (dd/mm/yyyy)*

Staff initials: __ __ __ Date completed: __ __ / __ __ / 20 __ __

Meningitis Review CRF – Part IV

Meningitis study number: __ __ __ __ __ __

Investigations & Results Form

1.	**Cerebrospinal fluid**			
	Test	Date of test		
1.1	Appearance ○ Polymorphs _____ ○ Lymphocytes_____ ○ Erythrocytes_____ ○ Glucose_____ ○ Protein_____ ○ Chloride_____ **Gram stain** ○ Gram positive cocci in chains ○ Gram positive cocci in chains ○ Gram negative cocci ○ Gram negative bacilli ○ Gram positive bacilli ○ Yeasts ○ Other **Culture** **days to grow** ○ H Infuenza ○ Staph pneumonia ○ Neisseria meningitis ○ Other			
2.	**CRP (C-reactive protein)** *(<72 hrs of diagnostic sampling)*			☐ **Not done** *(skip to 3)*
	Date __ __ / __ __ / 2 0 __ __	Time__ __ : __ __ ☐ Am ☐ Pm ☐ Not recorded		Result___ ___ ___ mg/l
3.1	**Blood glucose**_____			
3.2	**Haematology**	**Result**		☐ **Not done** *(skip to 5)*
3.1	Haemoglobin	_____		
3.2	Platelets	_____		
3.3	White cell count	_____ (Total)		
4.	Differential count	Lymphocytes _____	Absolute	Percentage _____
4.1		Neutrophils _____	Absolute	Percentage _____
4.2		Monocytes _____	Absolute	Percentage _____
	☐ **Not done** *(skip to 5)*			
4.3		Bands _____	Absolute	Percentage _____
4.4	Blood culture	Y		N
	Date taken			
	Results			

Staff initials: __ __ __ Date completed: __ __ / __ __ / 2 0 __ __

Gram stain

- Gram positive cocci in chains
- Gram positive cocci in chains
- Gram negative cocci
- Gram negative bacilli
- Gram positive bacilli
- Yeasts
- Other

Culture **days to grow**

- H Infuenza
- Staph pneumonia
- Neisseria meningitis
- Other

6. Other *(skip to 8 if not done)*

	Test	Date of test	Result
6.1	☐ Albumin	__ __ / __ __ / 2 0 __ __	___ ___ . ___ g/dL
6.2	☐ Total protein	__ __ / __ __ / 2 0 __ __	___ ___ . ___ g/dL
6.3	☐ Chemistry	__ __ / __ __ / 2 0 __ __	Na ____ K ____ Urea ____ Creatine ____

7. Radiology

Date of chest x-ray __ __ / __ __ / 2 0 __ __ ☐ **Not done**

Date of CT scan (If done, please download electronic copy for later reporting). **Not done**

Extra Notes

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UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

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13 April 2015

HREC/REF: 223/2015

Dr R Muloiwa
Paediatrics
5 th Floor ICH Building
Red Cross War Memorial Children's Hospital
Rondebosch

Dear Dr Muloiwa

Project Title: DETECTION OF BACTERIAL AND VIRAL CAUSES OF MENINGITIS USING MOLECULAR BASED TECHNIQUE - Sub-study linked to 370/2011 (MMed candidate - Dr P Mteshana)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year until the 28 April 2016.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

We acknowledge that the following student:- Dr Phindile Mteshana is also involved in this project.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Hrec/ref:223/2015

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Dr TA Blake
Manager: Medical Services
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Tel: +27 21 658 5788 fax: +27 21 658 5166
30 April 2013

DR R MULOIWA

Dear DR MULOIWA,

RE: MENINGITIS RESEARCH

Your application to do research at the Red Cross War Memorial Children's Hospital is hereby approved.
Note that should you want to remove folders from the Medical Records Department, not more than 5 may be removed at any one time, and if you do remove any, they must be returned by close of business the same day.

Yours faithfully,

DR T A BLAKE
DR T A BLAKE

CHAIRPERSON

HOSPITAL RESEARCH REVIEW COMMITTEE

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