Clinical presentation, aetiology, and outcome of central nervous system infections in Yogyakarta, Indonesia

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by

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Declaration

Except for the assistance outlined in the acknowledgements, the work described is my own work and has not been submitted for a degree or other qualification to this or any other university.

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Abstract

Background

Central nervous system (CNS) infections, such as meningitis and encephalitis, remain life-threatening, especially in developing countries. The aetiology of CNS infections in much of Indonesia, including Yogyakarta, is frequently unknown. This is partly due to a lack of available diagnostic techniques other than Gram stain and culture. In addition, limited reports are available on the short- and long-term functional outcomes of patients with CNS infection in the country. Knowledge of the specific pathogen causing the disease and the impact of the disease on the patients is essential to guide appropriate hospital treatment and inform future vaccine strategies.

Methods

A prospective hospital-based study was conducted in patients aged ≥1 month, including children (aged ≤18 years) and adults (aged >18 years), at Dr Sardjito Hospital, a large tertiary referral hospital in Yogyakarta, from February 2015 – January 2018. I reviewed the clinical features and management of the patients. I then introduced a systematic testing of cerebrospinal fluid (CSF), including pathogen-specific polymerase chain reaction (PCR) and antibody/antigen tests, into the local laboratory to supplement the standard hospital diagnostic tests. I also assessed the patient's functional outcome at hospital discharge and at follow-up (ranging from 1-6 months).

Results

Three-hundred-and-fifty-five children and 195 adults with clinically suspected CNS infection were recruited to the study, of whom 247 (70%) children and 168 (86%) adults had syndromic CNS infection. The two most common types of syndromic CNS infection in both age groups were encephalitis and meningoencephalitis, accounting for 70.8% and 17.4% of paediatric and 49.4% and 22% of adult cases, respectively. The introduction of both pathogen-specific PCR and antibody/antigen tests, in addition to the standard hospital CSF testing, has increased the detection of pathogens causing CNS infection from 11% to 20% in children and from 6% to 27% in adults. I have identified new cases of *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, *Escherichia coli* and *Streptococcus agalactiae*, *Neisseria meningitidis* and *Haemophilus influenzae* by the PCR assays and new cases of dengue virus, scrub typhus and *Cryptococcus* by the antibody/antigen tests. *M. tuberculosis* was the most common pathogen

identified in CSF in both children and adults. The 6-month mortality was 21% in children and 52% in adults. Among those who were discharged alive, 88.0% of children and 61.8% of adults had functional impairment at hospital discharge, and this impairment continued in 75.8% of children and 42.4% of adults at follow-up (median 6 months). Behavioural disturbance was the most common impairment in children at both time points.

Conclusion

Patients with syndromic CNS infection had high mortality and morbidity, not only at the time of hospital discharge but also within 6 months post-discharge. The standard hospital diagnostic tests had a relatively poor yield in identifying the causative pathogens which might contribute to the poor patient outcome. Systematic CSF testing increased pathogen identification, and considerations should now be given for these tests to be incorporated into routine standard hospital testing. Follow-up assessment should be used to identify patients with long-term neurological sequelae.

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List of abbreviations

AIDS	Acquired immune deficiency syndrome
AFP	Acute flaccid paralysis
ATM	Acute transverse myelitis
ATS	American Thoracic Society
BLAST	Basic local alignment search tool
BTS	British Thoracic Society
CDC	Centers for Disease Control and Prevention
CFR	Case fatality rate
CFU	Colony forming unit
CMV	Cytomegalovirus
CNS	Central nervous system
Cq	Quantification cycle
CrAg LFA	Cryptococcal Antigen Lateral Flow Assay
CSF	Cerebrospinal fluid
CT	Computed tomography
DENV	Dengue virus
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
EV	Enterovirus
EVD	Extra ventricle drainage
FTD	Fast tract diagnostic
GOSE	Glasgow outcome score extended
HIV	Human immunodeficiency virus infection
HSV	Herpes simplex virus
ICU	Intensive care unit
IDSA	Infectious Disease Society of America
IFA	Immunofluorescence assay
IgM	Immunoglobulin M
IQR	Interquartil range
ISR	Immune status ratio
JERA	JE antigen
JEV	Japanese encephalitis virus
LJ	Lowenstein-Jensen
LoD	Limit of detection
LOS	Liverpool outcome score
LP	Lumbar puncture
mAb	Monoclonal antibody

MGIT	Mycobacteria growth indicator tube
MRCN	Medicines for Children Research Network
MRI	Magnetic resonance imaging
NCA	Normal cell antigen
ND	Not detected
NS1	Non-structural protein 1
NTC	No template control
OD	Optical density
PCR	Polymerase chain reaction
PRNT	Plaque reduction neutralisation test
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
TB	Tuberculous
TBM	Tuberculous meningitis
TORCH	Toxoplasmosis, rubella, cytomegalovirus, herpes simplex, HIV
TST	Tuberculous skin test
UK	United Kingdom
USA	United States of America
vs	versus
VZV	Varicella Zoster virus
WCC	White cell count
WHO	World health organisation
WNV	West Nile virus

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Chapter 1 – Introduction

1.1 Overview

Central nervous system (CNS) infections such as meningitis and encephalitis cause high morbidity and mortality globally,[1] particularly in developing countries where human immunodeficiency virus (HIV) infection is frequent. Despite the advanced diagnostic techniques, the majority of CNS infection cases still have an unknown aetiology.[2, 3] This is particularly true for developing countries, where advanced molecular techniques are often unavailable. In such settings, establishing the aetiological diagnosis of CNS infection relies on classic microbiological testing, such as Gram stain, Indian ink stain, Ziehl-Neelsen (ZN) stain and culture. The challenges are increasing as lumbar puncture (LP) is not always performed – particularly if the hospital laboratory does not support cerebrospinal fluid (CSF) analysis, antibiotics are used extensively, and access to neuroimaging is often limited.

The aforementioned challenges are relevant in Indonesia, a country with the fourth highest population in the world. Previous studies in Indonesia only examined specific CNS infections, such as Japanese encephalitis (JE), bacterial meningitis, tuberculous meningitis, cerebral toxoplasmosis, and cryptococcal meningitis; and focused on a particular group age (i.e. in children or adults only).[4-12] Moreover, most of these studies did not assess the long-term outcome of patients with CNS infection.

Therefore, with this background, I performed a study examining a broad spectrum of CNS infection, including the aetiology, clinical presentation, laboratory features, clinical management, and short- and long-term outcomes; among children and adults. This study was performed in a tertiary referral hospital in Yogyakarta, Indonesia, where such a study

has never been done previously. It is expected that the findings will allow for a better understanding of the burden of the disease in the local population and help clinicians to improve the diagnosis and patient management.

1.2 Introduction to the study site – Yogyakarta, Indonesia

1.2.1 Demography

Indonesia is an archipelagic country in South East Asia with 16,056 islands (figure 1.1).[13] There are five main islands and four archipelagos. The five main islands include Borneo, Sumatra, Papua, Sulawesi, and Java; whilst the four archipelagos are Riau, Bangka Belitung, Nusa Tenggara, and Maluku. In 2013, the country consisted of 34 provinces.[14] The country's capital city is Jakarta, located on Java Island.

Figure 1.1 Map of the Indonesian archipelago
(Adopted from: http://www.globalcitymap.com/indonesia/indonesia-political-map.html)

In 2017, Indonesia's population was approximately 262 million people.[13] Most of the population lives in Java (57%) and Sumatra (22%) Islands. Java is less than 7% of the total Indonesian area; therefore, it is the most populous island in Indonesia. The Indonesia population includes numerous ethnic groups, speaking 724 different languages and

dialects. The national language is Indonesian, which is used in most written communication, education, government, and business affairs.

Indonesia lies on the equator and has a tropical climate which provides an ideal environment for the circulation of a variety of pathogens.[15] Indonesia is home to numerous communicable diseases, including pneumonia, bacillary dysentery, cholera, diphtheria, dengue fever, influenza, HIV, leptospirosis, malaria, measles, pertussis, rabies, tetanus, soil-transmitted helminthiases, typhoid fever, and meningococcal disease.[16] Indonesia has two seasons, including dry and rainy seasons. The rainy season is from October to April.

Yogyakarta is one of the provinces in Indonesia. It is located on the main island of Java and has a population of 3.8 million.[13] The province is directly adjacent to four regencies/districts/municipalities of Central Java Province, including Purworejo Regency, Magelang Regency, Klaten Regency, and Wonogiri Regency (figure 1.2).[17] Yogyakarta consists of five regencies, including Yogyakarta Municipality, Sleman Regency, Kulon Progo Regency, Bantul Regency, and Gunungkidul Regency. Its terrain is mainly lowland, with a coastal area in the southern part and Merapi Volcano in the northern region.

Figure 1.2 Map of Yogyakarta and Central Java Provinces
(Adopted from: https://www.dreamstime.com/jawa-tengah-central-java-administrative-political-vector-map-indonesia-image139055248)

1.2.2 Health status

Indonesia has made an improvement in key population health indicators such as life expectancy and infant mortality. The life expectancy at birth improved from 70 years in 2010 to 72 years in 2018, whereas the infant mortality rate decreased from 28 per 1,000 live births in 2010 to 20.2 per 1,000 live births in 2019. In 2019, the mortality rates in neonates, under-5-years, adult females, and adult males were 12.4, 23.9, 125.7, and 177.5 per 1,000, respectively.[18]

Data from the Global Burden of Disease Study 2017 (GBD 2017)[19] showed stroke as the leading cause of death in Indonesia's population, causing 18.8% of all deaths in 2017. The next most common causes of mortality among Indonesians were ischaemic heart disease and diabetes, contributing to 14.3% and 5.9% of all deaths. Tuberculosis was the fourth leading cause of mortality, causing 5% of all deaths. Other infectious diseases which cause high mortality among Indonesians include diarrhoea and lower respiratory tract infection, accounting for 4.2% and 2.7% of all deaths, respectively. In terms of CNS infection, meningitis and encephalitis caused 0.33% and 0.14% of deaths among Indonesia's population.

The number of reported new HIV cases in Indonesia has increased over the last decade, from 3 per 100,000 population in 2007 to 12 per 100,000 population in 2010, and reached 17 per 100,000 population in 2018.[20-22] Mortality due to HIV was estimated to reach 40,468 people in 2017 with the case fatality rate of 1.08%.[20] The majority of HIV-infected individuals were male (63.6%). In 2018, the estimated HIV prevalence in individuals aged 15-49 years was 0.4%.[22] There is no published data on HIV prevalence among Indonesian children. The highest proportion (69.3%) of HIV-positive individuals was seen in the 25-49 years age group, followed by 20-24 years (17.1%). Three percent of HIV-positive individuals were children under 15 years of age.[20]

1.2.3 Health system

Health services in Indonesia are provided by the public and private sectors. The public sector consists of government-run hospitals, including district, provincial, tertiary, and specialist hospitals, as well as Community Health Centres (*Pusat Kesehatan Masyarakat* [*puskesmas*] in Indonesian) and their networks. The private sector comprises private hospitals as well as physician-run clinics/group practices, midwifery clinics, pharmacies, and clinical laboratories.[21]

The *puskesmas* acts as a primary health centre at the district level. There were 9,815 units of *puskesmas* across 34 provinces in Indonesia by 2017.[20] Although this number has been increasing year on year, the distribution of *puskesmas* remains unequal among the districts. For instance, the ratio of *puskesmas* per district in Jakarta Province, the most populous province in Indonesia, where the capital city of Jakarta is situated, was 7.7 in 2017. This number is much higher than in Yogyakarta Province, where the ratio was 1.6. Two provinces with the lowest proportion of *puskesmas* per district included Papua and West Papua, where the ratio was 0.7 for each province. This ratio can be used to describe public accessibility to *puskesmas*, which is affected by the geographical condition, the area's span, the region's development, and the availability of basic facilities and infrastructure. The *puskesmas* provide mainly outpatient facilities, with some also providing inpatient facilities.

Hospitals in Indonesia are stratified into four types: A, B, C, and D. Type A hospitals are top referral hospitals providing a comprehensive subspecialties service, including academic hospitals. They are owned by the Ministry of Health. Type B hospitals are established in each provincial capital, receive referrals from district hospitals, and provide limited subspecialties and a wide range of specialties. Additionally, type B hospitals include academic hospitals not classified as type A. Type C hospitals are referral points for

the puskesmas, providing limited specialties that include internal and paediatric medicine, obstetric, and surgery services. Type D hospitals are those developing into type C hospitals, providing general medicine and dental services only. Type D hospitals are also referral points for the *puskesmas*.[21] In 2017, there were 2.6% of type A, 14.3% of type B, 48.3% of type C, 26.6% of type D, and 8.3% of unclassified hospitals.

The majority of hospitals are located on Java Island (47.2%), where 57% of the Indonesian population lives. In contrast, 4% of hospitals are located in eastern Indonesia (i.e. North Maluku, Maluku, West Papua, and Papua Provinces), which covers 26% of the total Indonesian land area but is only populated by 3% of the total population.[14, 23] In general, hospitals outside Java and Bali islands have to serve a much larger area; therefore, access to hospital care in these outer regions is limited. Data from a national health survey in 2018 showed that over 50% of households living in Jakarta, Yogyakarta, Bali, and Riau Archipelago Provinces thought access to hospitals was easy. In contrast, over 50% of families residing in Papua, East Nusa Tenggara, and West Sulawesi Provinces thought it was very difficult.[24] The hospital bed occupancy rates between 2004 and 2012 were steady at just over 60%.[21]

There is a national health insurance programme, namely *Jaminan Kesehatan Nasional* (JKN), which was launched in 2014 and is administered by the Social Security Agency for Health (*Badan Penyelenggara Jaminan Sosial Kesehatan* / BPJS Health). The JKN membership has increased from 133.4 million members in 2014 to 187.9 million members (approximately 72% of the total population) in 2017.[20]

JKN patients can visit an appointed *puskesmas*/clinic without a prior appointment. If they seek further treatment in hospitals/specialist clinics, they need a referral letter, except in an emergency. In the latter case, the JKN members can go directly to any hospital,

including hospitals not collaborating with BPJS Health, without having to pay the cost of treatment. However, when their condition allows, they will be referred to a hospital that has a collaboration with BPJS Health.[21] The referral system is run in hierarchical order, from primary healthcare facilities to type C/D hospitals, then to type B hospitals, and finally to type A/specialist hospitals when required.

The referral system, however, has a downside where there is a long waiting time for obtaining some treatments, particularly for outpatient care. Moreover, limited numbers of hospitals, especially private hospitals, collaborate with BPJS Health. These factors have led to serious barriers to accessing hospitals. Most patients must come to the hospital early in the morning to take a registration number and wait a long time to obtain treatment.[21]

By contrast, non-JKN patients can get treatment from primary healthcare facilities or visit specialist doctors directly, although they have to pay their own fees. When they need hospital treatment, they may choose between public and private hospitals without requiring a referral letter, but they must pay the costs themselves. In many cases, these patients will receive some privileges in most hospitals, including bypassing queues for service and getting priority for hospital beds.[21]

Dr Sardjito General Hospital is a type A government-run hospital designated as a national referral hospital in Java Island. The hospital receives referral patients from other healthcare facilities throughout the Yogyakarta territory and some Central Java and East Java Provinces regencies. The hospital provides 806 beds, divided into several classes and specialised wards.[25] Hospitalised patients with neurological disorders are treated in specialised paediatric or adult neurological wards, depending on their age. The health expenses for inpatients are predominantly covered by the JKN (88%), whereas a small

proportion of other inpatients are covered by private insurance (4%) or self-payment (8%).[25]

In terms of healthcare supplies, there are discrepancies in the availability of medicines and vaccines among provinces. A previous report showed that among the regions with the highest percentage (100%) of medication and vaccines availability in 2016 were Bengkulu, West Sumatra, Bangka Belitung, Bali, West Nusa Tenggara, South Sulawesi, West Sulawesi, and Maluku; whereas the lowest percentage (52.4%) was reported from South Sumatra.[26] The availability of medicines and vaccines in Jakarta and Yogyakarta was 99.4% and 98.4%, respectively.

The availability of medicines and vaccines in 2016 ranged from 66.7-86.7% in Provincial Health Offices, 77-83.9% in District Health Offices, 84.1-89.5% in hospitals, 83.3-96.7% in *puskesmas* without beds, and 82.9-90.6% in *puskesmas* with beds.[27] Among the reported out-of-stock medicines included diazepam, amlodipine, antacid, allopurinol, and mefenamic acid. Vaccines/serums that were often unavailable included anti-snake-bite serum, anti-tetanus serum, inactivated polio vaccine (IPV), rabies vaccine, and tetanus toxoid (TT). Anti-tuberculosis medicines are only available in 90% of the *puskesmas*.[20]

In the JKN era, medicines and medical equipment are provided via an electronic catalogue. However, a recent report shows that approximately 8% of the medications listed in the national formulary are not included in the electronic catalogue.[27] Demands on medications by healthcare facilities are often not fulfilled. There are also problems with medicine purchasing by hospitals where there is an extended processing time between orders and deliveries – in several cases may take more than six months.[28]

Several studies in Indonesia reported patients presenting to healthcare facilities in the late stage of their disease. These include patients with brain infections, HIV, and avian

influenza. A previous study on avian influenza conducted in Persahabatan Hospital, a type A and national referral hospital in Jakarta, described patients sought first medical help at a local clinic/hospital on a median of 2 (range 0-7) days after illness onset. However, they presented to Persahabatan Hospital on a median of 7 (range 3-10) days after illness onset. A more recent study on brain infections conducted at Cipto Mangunkusumo Hospital, another type A and national referral hospital in Jakarta, reported patients presenting to the hospital after a median of 14 days (IQR 7-30 days) following their first neurological symptoms.

1.2.4 Aetiology of central nervous system infection in Indonesia

The aetiology of CNS infection in Indonesia is incompletely understood. Previous studies only investigated specific CNS infections in a particular age group. Gessner *et al.* reported that *Streptococcus pneumoniae* and *Haemophilus influenzae* were the two most common bacteria causing meningitis among children in Lombok, West Nusa Tenggara (proportion 7/373 (2%) each), followed by *Neisseria meningitidis* (proportion 3/373 (0.8%)).[4] A preliminary report from Jakarta showed that *H. influenzae* caused 2/16 cases of paediatric bacterial meningitis, whereas *N. meningitidis*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella ozaenae* caused 1/16 cases each.[5] However, a full report of this study is not available.

A hospital-based JE surveillance conducted by Kari *et al.* in Bali between July 2001 and December 2003 identified 86 confirmed and four probable JE cases among 599,120 children under 12 years of age.[29] The annualised JE incidence rate was 7.1 per 100,000.[29] Following this, a further JE surveillance was conducted across six provinces, including West Sumatra, West Kalimantan, East Java, West and East Nusa Tenggara, and Papua, from January 2005 to December 2006. Eighty-two (5.5%) JE cases were confirmed among

1,496 paediatric acute encephalitis syndrome cases included in the surveillance. JE cases were detected in all provinces. However, the proportion varied between 2% (West Sumatra and Papua) and 18% (West Kalimantan).[8] A more recent surveillance conducted in 2016 collecting 326 human sera from 11 provinces showed JE IgM positive in 43 (13%) samples.[30] Another viral CNS infection case reported from Indonesia was a confirmed poliomyelitis case in an 18-month-old child from West Java.[12]

There is no comprehensive data on the aetiology of paediatric CNS infection in Indonesia, unlike any other countries such as Vietnam and Thailand. Trung *et al.* reported that JE virus was the most common pathogen causing CNS infection in Vietnam, accounting for 23% of all cases, followed by *H. influenzae*, *S. pneumoniae*, and Enteroviruses (proportion 6% each).[3] In Thailand, Enterovirus and JE virus were the two most frequent aetiology of CNS infection, accounting for 30% and 21% of the cases. Interestingly, this study reported that the proportion of scrub typhus CNS infection cases (6%) was higher than *S. pneumoniae* and *H. influenzae* cases (proportion 3% each). The differences in CNS infection epidemiology in these two neighbouring countries of Indonesia suggest that the CNS infection aetiology in Indonesia, particularly in Yogyakarta and surrounding areas, may differ from other provinces and countries.

Similar to children, there are limited data on the causes of adult CNS infection in Indonesia, specifically from the Yogyakarta region. A previous study from West Java showed a significantly high proportion of tuberculous meningitis, accounting for 42% of all cases with suspected meningitis.[31] Another study from Jakarta reported a similar finding where *M. tuberculosis* was the most common pathogen causing CNS infection (16%), followed by *Cryptococcus* species (5%).[6] Among the most frequent viral causes included cytomegalovirus (CMV), herpes simplex virus (HSV), and Varicella-zoster virus (VZV), accounting for 1%, 0.7% and 0.7% of cases, respectively. These data are distinct

from the Vietnam study where *Streptococcus suis* was reported as the most frequent aetiology of adult CNS infection (24%), followed by *S. pneumoniae* (6%) and *M. tuberculosis* (5%).[3] A previous study from Cambodia showed *C. neoformans* and *M. tuberculosis* were the two most frequent causes of adult CNS infection, accounting for 15% and 8.5% of the cases.[32] Taking these data into account, the aetiology of adult CNS infection in Yogyakarta may differ from other countries and regions in Indonesia.

1.2.5 Current management of patients with central nervous system infection in the study setting

There are broad national guidelines for the management of children with suspected CNS infection in Indonesia published by the Indonesian Paediatrician Association.[33] More detailed guidelines are available at the hospital level (unpublished). Implementation of these guidelines varies among paediatricians. Those who work in type A hospitals, where the laboratory facilities for cerebrospinal fluid (CSF) investigation are usually available, generally perform a lumbar puncture (LP). However, paediatricians who work in type B hospitals do not always perform LPs, depending on whether the hospitals have the necessary laboratory facilities. To my knowledge, LPs in children are usually performed at any time in Dr Cipto Mangunkusumo and Dr Sardjito Hospitals. Dr Cipto Mangunkusumo Hospital is a type A and national referral hospital in Jakarta with CNS infection speciality care. Nevertheless, both Dr Cipto Mangunkusumo and Dr Sardjito Hospitals do not perform molecular diagnostic testing such as polymerase chain reaction (PCR) for paediatric CSF samples. Pathogen detection relies upon classic microbiological testing, including Gram stain, ZN stain, and bacterial cultures. Antibody and antigen testing are performed but usually in a single serum sample, not in a CSF sample.

In contrast, there are no such guidelines for the management of CNS infection in adults, except for tuberculous CNS infection.[34] There is a Neuroinfection Module book written by the Neuroinfection Study Group of the Indonesian Neurologists Association, which includes the management of adult patients with CNS infection.[35] However, the management guidelines are referenced from international guidelines. Similar to that in children, implementation of the referred international guidelines varied among neurologists. Adult LPs are usually performed regularly in type A hospitals but not in type B hospitals or below. To my knowledge, adult LPs are generally performed at any time in Dr Hasan Sadikin (located in Bandung, West Java) and Dr Cipto Mangunkusumo Hospitals. However, this practice is not implemented in the study setting, Dr Sardjito Hospital Yogyakarta, where LPs are usually performed during working hours (i.e. from Monday to Friday, 8 am – 4 pm). A more detailed explanation of this will be given in chapter 3.

Dr Hasan Sadikin and Dr Cipto Mangunkusumo Hospitals have been involved in other adult CNS infection studies. Therefore, molecular testing (e.g. PCRs and MTB/RIF GeneXpert), *M. tuberculosis* cultures, Indian ink staining, and *Cryptococcus* antigen testing are routinely performed, in addition to Gram staining, ZN staining, and bacterial cultures. In contrast, molecular testing, *M. tuberculosis* cultures, and *Cryptococcus* antigen testing are not performed in Dr Sardjito Hospital. Indian ink staining was introduced as a routine practice in the second year of my study. Furthermore, there is sometimes a delay between collecting and testing CSF samples in my study setting. In the meantime, the CSF samples are left at room temperature, which may result in CSF leucocytes and pathogen degradation. These facts highlight the importance of investigating the performance of the standard hospital testing in Dr Sardjito Hospital and investigating whether molecular and serological testing may improve the pathogen detection rate.

With the aforementioned limitation in diagnostics in Dr Sardjito Hospital, based on my initial observation prior to my study, clinicians typically use syndromic diagnosis as the patient's final diagnosis. In cases where there is CSF pleocytosis with no pathogen detected by the available diagnostic testing, the likely pathogen (i.e. bacterial, tuberculosis, or viral) is usually concluded based on the patient's history, clinical, laboratory, and neuroimaging findings where possible.

In terms of follow-up, patients are usually followed-up within the first week following hospital discharge, where patients should come to hospital outpatient clinics. The clinicians assess the patient's condition and schedule the subsequent follow-up on a case-to-case basis. Patients with neurological sequelae are referred to the (general) medical rehabilitation department, as there is no specific neurorehabilitation department in Dr Sardjito Hospital.

1.3 Central nervous system infection – epidemiology, clinical features and treatment

CNS infection is regarded as an infection involving the brain and spinal cord. Within the CNS, the infection can affect different tissues of the nervous system, including the membranes covering the brain (meninges), the brain parenchyma (encephalon), the spinal cord, and the covering of the nerve fibres within the cord (myelin).

Infection is typically associated with inflammation. Inflammation is a histopathological feature of infection, when leucocytes infiltrate the infected tissue to stop the spread of the invading pathogen. Inflammation can only be definitely confirmed by examination of tissue. However, there are multiple indirect markers of inflammation, such as increased leucocytes in the CSF – a fluid that surrounds the brain – or tissue swelling and changes in its density that can be detected by imaging (brain Computed Tomography [CT] or

Magnetic Resonance Imaging [MRI]). Inflamed brain tissues are often classified by putting the term 'itis' at the end of the affected tissue. Therefore, inflammation of the meninges is termed meningitis. Of course, infection is just one, albeit a common, cause of brain inflammation.

1.3.1 Classification of the central nervous system infection

1.3.1.1 Meningitis

Meningitis is an inflammation of the meninges, the layers which protect the brain and spinal cord.[36] It is a life-threatening inflammatory disorder with high morbidity and mortality despite aggressive treatment.[37] Meningitis-associated symptoms can also occur in meningism, a state of meningeal irritation regardless of its aetiological factors. Based on its aetiology, meningitis can be divided into infectious and non-infectious meningitis. Infectious meningitis can be caused by various pathogens, including bacteria, viruses, fungi and parasites. In contrast, the aetiology of non-infectious meningitis includes autoimmune conditions, drug reactions and cancers.[38]

1.3.1.2 Encephalitis

Encephalitis is described as an inflammation of the brain (encephalon) with clinical evidence of neurologic dysfunction.[39] It is often confused with encephalopathy, a state of altered mental status, including confusion, changing behaviour, disorientation, or other cognitive disturbances, with or without brain inflammation. Encephalitis can be caused by infection of the brain parenchyma, a post-infectious process such as acute disseminated encephalomyelitis (ADEM), or a non-infectious condition such as autoimmune encephalitis.

1.3.1.3 Myelitis

Myelitis is inflammation of the spinal cord which can be caused by direct infection, post-infectious processes, or other mechanisms. The concurrent presence of myelitis and encephalitis is often defined as encephalomyelitis.[40, 41] The disease severity scale may vary depending on the site of infection.[42] Infection of the cord, can be localised to motor tracts or involve sensory pathways as well, leading to sensory or autonomic dysfunction. For example, poliovirus typically infects the anterior horn cells in the spinal cord's grey matter, leading to signs and symptoms of motor dysfunction. It presents itself as a sudden loss of motor tone and function, known as acute flaccid paralysis (AFP). Another serious condition named acute transverse myelitis (ATM) may occur if an infection transverse across the cord involving both peripheral and central tissue. Patients with a syndrome of ATM typically experience sensory, motor, and possibly autonomic dysfunction below the level of the inflammation.[42]

1.3.1.4 Brain abscess

Brain abscess is a focal intra-cerebral infection that starts from a small area of the brain tissue and develops into a collection of pus enclosed by a well-vascularised capsule.[43] Frontal, temporal, frontal-parietal, parietal, cerebellar, and occipital lobes are the most commonly reported locations of brain abscess.[44]

1.3.2 Incidence and aetiology of central nervous system infection

1.3.2.1 Meningitis

Meningitis is the most common reported CNS infection worldwide, with an incident case of 5 million (95% CI 4.4 to 5.8 million) in 2017.[45] In the United Kingdom, McGill *et al*. reported the annual incidence rate of viral and bacterial meningitis was 2.73 cases/100,000 population and 1.24 cases/100,000 population, respectively.[2] A systematic

review and meta-analysis conducted by Ali *et al.* reported the mean annual incidence of bacterial meningitis cases in South Asian children was 105 cases/100,000 population (95% CI 53-173).[46] There is limited data on bacterial and viral meningitis incidence in Indonesia. The published studies only reported the number of brain infection cases among patients with suspected CNS infection in several cities. In Jakarta, 167 patients with laboratory-confirmed CNS infection were reported from 274 patients with suspected CNS infection.[6] In another study from Manado, 15% of patients with suspected CNS infection were found to have viral aetiology.[47]

Among meningitis causing-organisms, *Streptococcus pneumoniae* is the most commonly reported causative agent, with a global prevalence of 3.5 million cases in 2017 (including complications), followed by *Neisseria meningitidis* and *Haemophilus influenzae* type B with a global prevalence of 1.1 million and 0.9 million of cases, respectively.[45] Similar findings were also documented in a UK study suggesting *S. pneumoniae* and *N. meningitidis* as the most common bacterial cause, responsible for 8% and 5% of all meningitis cases, respectively.[2] In South East Asian studies, *Streptococcus suis* serotype 2 was the most frequently identified pathogen in adult patients with bacterial meningitis in Thailand (24%), whereas *Mycobacterium tuberculosis* was the leading cause of bacterial meningitis in Indonesia (42% in West Java and 16% in Jakarta).[3, 6, 31]

Specific aetiologies of meningitis are discussed in more detail below.

Viral meningitis

Several viruses have the propensity to infect humans in specific age groups. Enteroviruses are commonly related to viral meningitis in infants and elderly people; arboviruses in young children; poliovirus, measles and mumps in school-going children; mumps and

measles in young adults; and herpes virus in middle-aged people.[48, 49] The causative agents of viral meningitis are presented in table 1.1.

Table 1.1 Microorganisms causing viral meningitis[48, 49]

Proportion of cases	Causative agents
80-85%	**Enteroviruses** (Coxsackie viruses A and B, Echoviruses, polioviruses)
10-15%	**Arboviruses** families, including: 1. Alphaviruses (Eastern, Western, Venezuelan equine encephalitis) 2. Bunyaviruses (California encephalitis viruses, Jamestown Canyon viruses) 3. Flaviviruses (Japanese B encephalitis virus, Colorado tick fever virus, West Nile virus, St. Louis encephalitis virus, Murray valley viruses) **Herpes virus** family, including herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2), varicella-zoster virus (VZV), human herpes virus-6, Epstein-Barr virus (EBV) and cytomegalovirus (CMV). **Mumps**
≤5%	Measles, lymphocytic choriomeningitis viruses, human immunodeficiency virus (HIV) and adenoviruses

One of the most frequent causes of viral meningitis diagnosed in high-income countries, such as the UK, is enteroviruses.[2] Additionally, members of the Herpesviridae family, such as herpes simplex virus (HSV) and varicella-zoster virus (VZV), are frequent causes of viral meningitis.[2] Meningitis, including the recurrent type (Mollarett's meningitis), is more frequently caused by HSV-2, whilst HSV-1 is more often associated with encephalitis.[50]

Bacterial meningitis

Based on the origin of the infection, several bacteria causing meningitis can be classified into community-acquired and nosocomial (including neurosurgical). Community-acquired bacterial meningitis is caused by numerous bacteria. Different bacteria have different prevalence among different age groups. For example, in neonates (1 month of age), *Escherichia coli*, *Streptococcus agalactiae,* and *Listeria monocytogenes* are common causes in the UK. In contrast, *S. pneumoniae, N. meningitidis,* and *H. influenzae* are common causes of meningitis among UK adults. In nosocomial bacterial meningitis, Gram-positive cocci (*Staphylococcus aureus, Staphylococcus epidermidis,* and *coagulase-*

negative staphylococci), aerobic Gram-negative bacilli (*Pseudomonas aeruginosa*) and *Propionibacterium acnes* are identified as the major causative pathogens.[51, 52]

Tuberculous meningitis

A major cause of bacterial meningitis across the globe, particularly among immunocompromised patients, is *M. tuberculosis*. *M. tuberculosis* is transmitted through the air via respiratory droplets.[53, 54] Therefore, the first focus of tuberculosis (TB) infection is in the lungs. *M. tuberculosis* can escape from the lungs to the bloodstream via lymph nodes or a direct extension of local infection.[53] The escaped bacteria can cause diseases in many organs manifesting as extrapulmonary TB, including tuberculous meningitis.[54] Once the bacteria settle in the meninges or para-meningeal areas, they form a single, localised granuloma called the Rich focus.[53, 54] The rupture of this focus causes the extension of the infection and inflammation to the subarachnoid space, eventually spreading to the brain parenchyma and blood vessels.[54] The bacteria can form a tuberculoma or an abscess in the brain parenchyma. Infections in the brain vascular system can lead to vasculitis, which can cause a vascular obstruction and eventually stroke.[53, 54] The inflammation caused by *M. tuberculosis* can also lead to the accumulation of exudates in the vicinity of basal cistern, causing increased intracranial pressure and hydrocephalus.[54, 55] Together, these abnormalities (brain infarct, tuberculoma/abscess, cerebral vessel damage, signs of increased intracranial pressure/hydrocephalus) can be visible on the CT scan images and aid the diagnosis of tuberculous meningitis.[55] However, these signs usually only become apparent at the later stages, and by then, the infection may have already caused irreversible brain damage.[55, 56] Tuberculous meningitis is notoriously difficult to diagnose at its early stages, and the consequences are often fatal.[56, 57]

Fungal meningitis

Fungi causing meningitis can be categorised into three groups, as described in table 1.2.

Table 1.2 Classification of fungi which may cause meningitis[37, 58]

Group	Species
Pseudomycetes/ yeast	*Cryptococcus, Blastomyces, Candida, Coccidioides, Histoplasma, Paracoccidioides, Sporotrichum*
Septate mycetes	*Aspergillus, Cephalosporium, Cladosporium, Diplorhinotrichum, Homodendrum, Paecilomyces, Penicillium*
Nonseptate mycetes	*Absidia, Basidiobolus, Cunninghamella, Mucor, Rhizopus*

The most common CNS fungal infection worldwide is cryptococcal meningitis, affecting over 1 million individuals globally with 0.6 million annual fatalities.[59, 60] Ninety-five percent of cases in middle-and-low-income countries[61] and 80% of cases in high-income countries[62] are related to HIV. A previous study from Indonesia reported 93% cryptococcal meningitis cases were in HIV-positive patients.[6] *Cryptococcus neoformans* var. *grubii* (capsular serotype D) accounts for 82% of cryptococcosis globally. *C. neoformans* var. *neoformans* (capsular serotype A) is linked with HIV-associated cryptococcal meningitis, particularly in several northern European countries, including France, Italy, and Denmark.[63-65]

Parasitic meningitis

Several potential parasites causing meningitis include *Taenia solium*, *Angiostrongylus cantonensis, Trichinella spiralis, Trypanosomes, Plasmodium, Acanthamoeba, Naegleria fowleri*, and *Toxoplasma gondii*. The development of parasitic meningitis in developing and developed countries is influenced by various factors. Poor hygiene standards, limited healthcare facilities, poor quality of education and low socioeconomic status are predominantly responsible for spreading the disease in developing nations. On the other

hand, factors such as lymphoma, immunosuppression and diabetes mellitus are commonly found in developed countries.[66, 67]

1.3.2.2 Encephalitis

Encephalitis is the second most common CNS infection worldwide, causing 2.2 million cases in 2017.[45] Global data shows an increased incidence rate of acute encephalitis from 3.5-7/100,000 to 16/100,000 yearly.[68] In the UK, the Health Protection Agency reported the incidence rates of acute encephalitis in the general population and children were 1.5/100,000 and 2.8/100,000, respectively. The highest incidence rate was documented in infants less than one year of age, at 8.7/100,000.[69] In the USA, encephalitis leads to approximately 20,000 hospital admissions annually, which costs around 2 billion dollars per year.[70] Encephalitis has a case fatality rate (CFR) of about 3.8% - 7.4%, which is significantly higher in immunocompromised cases such as patients with HIV.[71, 72] In South East Asian studies, acute encephalitis is commonly caused by Japanese encephalitis virus (JEV), accounting for 31% to 45% of cases.[32, 73] In Indonesia, routine surveillance is performed by analysing sera from suspected Japanese encephalitis (JE) patients. The recent surveillance conducted in 2016 collecting 326 human sera from 11 provinces showed JE IgM positive in 43 samples.[30]

Encephalitis can be caused by various organisms, including bacteria, viruses, fungi and parasites (table 1.3). The proportion of pathogens which cause encephalitis has changed in recent years, mainly due to an increase in vaccine availability for several causative pathogens such as mumps, measles, rubella and varicella.[74, 75] Aetiological pathogens which are frequently found in one region might be uncommon in other areas.[76-78]

In industrialised nations, HSV-1 is the most common pathogen detected in viral encephalitis, whilst globally JEV is the most important cause of viral encephalitis.[79, 80] JEV

is reported to be endemic in 24 countries in Asia and the Western Pacific rim, predominantly in China and India.[80] The estimated annual incidence of JE was 67,900 cases, almost ten times higher than initially reported, with approximately 50% of these cases occurring in China.[81] Most JEV infections in humans are asymptomatic, with previous reports suggesting that less than 1% of JEV infections result in encephalitis. However, the consequence of JE is severe as approximately 20 – 30% of JE patients die, and between 30 – 50% of those who survive have severe neurological sequelae.[82]

Table 1.3 Causative agents of encephalitis in immunocompetent patients[83]

Most common causative agents	Less common causative agents
Viruses	**Viruses**
Enteroviruses	B virus
Epstein-Barr virus	Eastern equine encephalitis virus
HSV-1 and -2	Hendra virus
Japanese encephalitis virus (JEV)	Lymphocytic choriomeningitis virus
Dengue virus	Measles virus
La Crosse virus	Mumps virus
Rabies virus	Murray Valley encephalitis virus
St Louis encephalitis virus (SLE)	Nipah virus
Tick-borne encephalitis virus	Powasan virus
Varicella-zoster virus	Rubella virus
West Nile virus (WNV)	Vaccinia
	Venezuelan equine encephalitis virus
	Western equine encephalitis virus
Bacteria	**Bacteria**
Bartonella henselae	*Borrelia burgdorferi*
Mycobacterium tuberculosis	*Coxiella burnetii*
Ehrlichia/ Anaplasma	*Treponema pallidum*
Rickettsia rickettsii	*Trophyeryma whipplei*
	Protozoa
	Balamuthia mandrillaris
	Naegleria fowleri
	Helminths
	Baylisascaris procyonis
	Schistosomiasis

In addition to JEV, dengue infection represents a growing threat as its incidence has increased approximately 30-fold in the last 50 years.[84] Dengue viruses (DENVs) are endemic in multiple regions across the world located near the equatorial line including South and South East Asia, Central and South America, Equatorial Africa, and the Eastern Mediterranean region.[84] Clinical manifestations of dengue infection range from the asymptomatic state to severe dengue, which involves plasma leakage leading to shock, severe bleeding, and multiple organ impairment.[85] Not every case of dengue infection presents with neurological symptoms. Previous reports suggest that the proportion of dengue cases with neurological manifestation vary between 0.5% and 21%.[86, 87]

Despite a systematic paradigm for classifying the aetiological agents of encephalitis as described in table 1.3, many studies documented that no pathogen is detected in most cases. Studies conducted in different geographical regions on diverse populations demonstrated similar findings that 50-70% cases were aetiologically unidentified.[70, 88, 89] A previous study reported 10% of patients who were initially suspected to have encephalitis were discharged with a final diagnosis of a non-infectious condition. In this cohort, an autoimmune syndrome called anti-N-methyl D-aspartate receptor (anti-NMDAR) encephalitis was more than four times higher than the combination of other cases, including West Nile Virus (WNV), HSV-1 and VZV.[90]

1.3.2.3 Myelitis

One of the most frequent clinical presentations of myelitis is acute flaccid paralysis (AFP). AFP is typically caused by viral infection. Viral-related AFP accounts for an incidence rate of 4/100,000 persons per year globally.[91] In 1988, polioviruses were considered the major aetiology of AFP leading to more than 350,000 cases over 125 countries. However, the number of wild-type polio cases has been dramatically decreasing following a massive

vaccination campaign by World Health Organisation (WHO).[91] In 2006, AFP caused by polioviruses was only identified in approximately 5% of cases across 16 nations in South East Asia, the Middle East and Africa.[91] Emerging infections, including WNV, have been shown to be the major viral aetiology of acute spinal cord disease in the Western hemisphere. Moreover, following the first reported cases in 1999, North America has documented increasing WNV infections.[92] Therefore, although AFP has contributed to only 2-3% of all WNV infections reported in recent years, almost one-third of hospitalised patients are attributable to the syndrome.[93]

The annual incidence rate of acute transverse myelitis (ATM) is estimated at 1-4/1,000,000 population worldwide, with bimodal peaks between ages 10-19 and 30-39 years.[94, 95] Several factors such as sex, familial and ethnicity were not documented as the predisposition factors of ATM. Furthermore, there is no variation in the incidence rate of ATM geographically.[95]

Transverse myelitis may be linked to several causes but most often results from autoimmune disease (60% in children), direct infection, or acquired demyelinating conditions such as multiple sclerosis or defects associated with neuromyelitis optica.[96-98] Direct spinal cord invasion may occur with several pathogens, including *Borrelia burgdorferi* and VZV. Transverse myelitis can also be triggered by tuberculosis and syphilis.[99] Transverse myelitis may also be caused by a viral infection, contributing to approximately 20-40% of cases. Enteroviruses, including Coxsackie virus A7, A9 and A23, and Coxsackie virus B strains, are the most frequently reported to cause transverse myelitis.[100] Among the herpes viruses, HSV-1 is linked with myelitis in children, whereas HSV-2 is considered the major aetiology of myelitis in adult patients.[101, 102]

Enterovirus 71 (EV-71) infection may involve the spinal cord and result in either AFP or ATM. AFP is among the most common CNS manifestations of EV-71, in addition to aseptic meningitis and brainstem encephalitis.[41] The occurrence of AFP or ATM related to EV-71 has varied between 1% and 21% in different outbreaks.[103-105] Sporadic EV-71-related AFP has also been reported.[106, 107]

1.3.2.4 Brain abscess

Brain abscess occurrence is reported at 0.3-1.3 cases per 100,000 people a year,[108, 109] with a proportion of 2:1 to 4:1 between males and females.[110-112] The occurrence of brain abscess in the population is relatively low. However, the risk of developing brain abscess is considerably increased in some patient populations. Patients with a diffuse pulmonary arteriovenous malformation (AVMs) have the highest risk of developing a brain abscess (37%), followed by multiple pulmonary AVMs (16.4%), single pulmonary AVMs (7.7%), acute infective endocarditis (7.1%), penetrating craniocerebral trauma (4.2%), etc.[43]

In the pre-antibiotic era, the culture of pus from brain abscess documented several causative pathogens as follows: *Staphylococcus aureus* (25-30%), *Streptococci* (30%), coliforms (12%) and no growth (50%).[113, 114] With the improvement of technical diagnosis, the function of anaerobic agents in brain abscess is evident. A study reported that anaerobes grew in 14 out of 18 abscess samples, primarily *Streptococci* in 66%, with *Bacteroides* species in 60%.[115] The findings of seven studies from six countries (in children and adults) indicate several common causative organisms as follows: *Streptococci* (40.3%), *Enterobacteriaceae* (25.3%), *Bacteroides* species (21.8%), *Staphylococci* (12.6%), *Peptostreptococci* (5.9%), *Pasteurellaceae* (5.5%) and *Fusobacterium* species (4.9%).[43]

1.3.3 Clinical features

1.3.3.1 Meningitis

Viral meningitis

In the early stages of infection, patients may experience prodromal symptoms, including fever, general malaise, muscle pains and lethargy. Extraneural symptoms, including nausea, vomiting, sore throat, skin rashes, joint aches, abdominal pains, lymphadenopathy and upper respiratory infection, may also be present. The symptoms will then be accompanied by meningeal irritation, which contributes to headaches, neck stiffness, photophobia and vomiting. From the physical examination, clinicians may identify two typical signs in patients with meningitis (often referred to as meningeal signs), including Brudzinski's and Kernig's signs. Patients with positive Brudzinski's sign may reveal enhancement in the resistance to the passive flexion of the neck. When patients are unable to extend the knee after a passive flexion of the hip at 90 degrees, the condition is referred to as positive Kernig's sign. In the progression of the disease, patients may experience various symptoms such as disorientation, irritability, agitation, drowsiness, confusion, focal neurological defects, seizures or coma.[48, 49, 116]

Bacterial meningitis

Patients with acute bacterial meningitis may experience prodromal symptoms such as headache, fever, nausea, vomiting, neck stiffness and photophobia. This may be followed by more serious conditions including disorientation, confusion, drowsiness, irritability, seizure, focal neurological defects or coma. Forgetfulness, confusion, disorientation and altered personality may be identified in elderly patients. On the other hand, patients with chronic meningitis may experience symptoms of increased intracranial pressure, such as headaches, papilledema, vomiting, drowsiness and visual disturbance.[117]

In neonates, clinicians may analyse various parameters including altered vital signs, feeding habits, sound production, bodily status, limb movements, increased intracranial pressure (irritability, seizures and bulging fontanelle), symptoms of meningism (photophobia, phonophobia and nuchal rigidity), constitutional symptoms (nausea, vomiting, anorexia and lethargy) and signs of cerebral irritation (irritability, excessive crying, seizure and bulging fontanelle). Headache, photophobia, neck pains, exhaustion and bodily pain may be identified in young children. [118, 119]

Tuberculous meningitis

Several studies have extensively described the clinical features of patients with tuberculous meningitis, as listed in table 1.4.

Table 1.4 The most common signs and symptoms in patients with tuberculous meningitis[120-125]

Symptom/Clinical sign	Proportion of patients affected
Symptom	
Headache	50-80%
Fever	60-95%
Vomiting	30-60%
Photophobia	5-10%
Anorexia	60-80%
Clinical sign	
Neck stiffness	40-80%
Confusion	10-30%
Coma	30-60%
Any cranial nerve palsy	5-15%
Cranial nerve III palsy	30-40%
Cranial nerve VI palsy	10-20%
Cranial nerve VII palsy	10-20%
Hemiparesis	10-20%
Paraparesis	5-10%
Seizures	Children: 50%; adults: 5%

Fungal meningitis

Several signs and symptoms generally detected in fungal meningitis include nausea, vomiting, headache, visual disturbance and papilledema. These may be followed by neck stiffness, fever and changes in personality. Cranial nerve palsies, seizures, altered sensorium, hydrocephalus, and coma may be present in more severe conditions.[37, 58]

1.3.3.2 Encephalitis

Encephalitis is less commonly established by pathology. Therefore, brain inflammation is analysed using signs and symptoms of neurological dysfunction. A condition mimics encephalitis, termed encephalopathy, is characterised as impairment of brain function with no evidence of brain inflammation.[126] Two important factors, the brain area involved and the severity of infection, determine what signs and symptoms will be experienced by patients. Fever and headache are commonly experienced by patients prior to altered mental status, which varies from mild confusion to obtundation.[127] Several neurological deficits which might be encountered in patients with encephalitis include focal paresis or paralysis, cranial nerve palsies, behavioural changes (such as psychosis) and movement disorders (such as chorea).[76]

Several causative agents exhibit tropism for specific anatomic sites; for instance, HSV-1 infection frequently affects one (or occasionally both) temporal lobes, occasionally to the extent of haemorrhagic necrosis. HSV-1 infection of these lobes is often associated with seizures.[128] Patients with JE often present with a distinct facial appearance, including a dull, flat mask-like face with wide unblinking eyes, tremors, generalised hypertonia, and cogwheel rigidity.[73, 80] Abnormal behaviour may be the only predominant symptom observed in JE patients, especially adults, leading to an initial diagnosis of mental illness.[73]

In children, JE may also cause convulsions and a poliomyelitis-like acute flaccid paralysis, usually manifest as asymmetrical lower limb weakness.[129, 130]

Microbiological diagnosis in patients with encephalitis can be established although evidence of inflammation or infection is identified at sites which are relatively distant from the CNS. Skin manifestations are closely related to WNV and VZV. Enterovirus infection may often be associated with ulcerative lesions and stomatitis in the mouth. Tuberculous and fungal meningoencephalitis are frequently associated with evidence of pulmonary disorders. Gastrointestinal symptoms, particularly diarrhoea, are typically present in viral infections, including adenovirus, enterovirus and rotavirus.[83]

1.3.3.3 Myelitis

Myelitis may be present in encephalitis patients (as encephalomyelitis) or those without it. Typical signs and symptoms of myelitis include motor weakness, ascending sensory deficit and early bowel and bladder involvement.[41, 131, 132] Myelitis may lead to upper and lower motor neuron weakness. Physical examination in patients with lesions of the upper motor neuron may reveal spasticity, hyperreflexia and extensor plantar reflexes. On the contrary, lesions of the lower motor neurons result in flaccid weakness and decreased or absent deep tendon reflexes. Acute flaccid paralysis frequently occurs in myelitis caused by several viruses, including enteroviruses (i.e. poliovirus and enterovirus 71) and flaviviruses (i.e. WNV, JEV and tick-borne encephalitis virus).[131, 133, 134]

When the inflammation transverses (across) the spinal cord, this condition is referred to as transverse myelitis.[132] Patients with transverse myelitis might develop neurological dysfunction involving motor, sensory and autonomic pathways. Disruption of both ascending and descending neuroanatomical pathways in the transverse plane of the spinal cord leads to various clinical signs, including paraesthesia, numbness, and radicular

dysesthesias with a corresponding sensory level. Interruption of the autonomic pathway may result in several symptoms, including urinary retention, bladder or bowel incontinence or constipation.[99]

1.3.3.4 Brain abscess

The classic triad suggestive of brain abscess incorporating headache, fever and focal neurological deficits is reported in less than 50% of cases. In most cases, signs and symptoms are attributable more to a growing intracerebral mass rather than infection. For instance, patients with multiloculated brain abscess tend to have more dominant neurological deficits (i.e. headache, hemiparesis) than those who develop uniloculated brain abscess.[135]

The prominent clinical manifestations may vary depending on the affected area by the brain abscess. Patients with a cerebellar abscess may experience fever, meningism and signs of elevated intracranial pressure (such as headache, vomiting and papilledema). In patients with frontal lobe abscess, headache, drowsiness, and mental deterioration are frequently present. In addition, ipsilateral headache typically occurs in patients with temporal lobe abscess. When the abscess is identified in the dominant hemisphere, the patients may experience aphasia or dysphasia.[43] Symptoms from the main site of infection (such as otitis, sinusitis or distant suppurative foci) can also prevail. Patients with abscess have an average duration from initial symptoms to hospital admission between 11-12 days.[136, 137]

1.3.4 Diagnosis

1.3.4.1 Cerebrospinal fluid parameters

Cerebrospinal fluid (CSF) analysis following the lumbar puncture remains the gold standard for diagnosing CNS infection. Specific findings in CSF analysis may vary depending on the causative pathogen, as described in table 1.6. An elevated number of white blood cells in the CSF (CSF pleocytosis) indicates inflammation of the CNS. CSF pleocytosis is often a prerequisite for a diagnosis of CNS infection.

The type of white cells migrating into the CSF can help suggest the type of pathogen causing infection. Typically, an acute bacterial infection is associated with neutrophil predominance in the CSF, whereas an acute viral infection is often associated with lymphocyte predominance. However, this is a rough guide, with many exceptions. For example, prior antibiotic treatment will lead to a shift towards lymphocyte predominance in acute bacterial CNS infection. In contrast, an early presentation of viral meningitis can be associated with a high neutrophil predominance.[79]

Bacterial CNS infection

In bacterial CNS infection, CSF leucocytes markedly increase with neutrophilic predominance.[138] Elevated opening pressure and CSF protein are commonly present. CSF glucose is generally detected at low concentrations. The glucose samples from both CSF and serum should be obtained simultaneously because CSF glucose is affected by serum glucose levels. CSF lactate can also be informative as a proxy marker of CNS infection. A study reported that the CSF lactate concentration from samples which is obtained before the initial administration of antibiotics could be used to distinguish bacterial form viral infection with a sensitivity and specificity of 0.93 (95% CI 0.89-0.96) and 0.96 (95% CI 0.93-0.98), respectively.[139]

Viral CNS infection

A lymphocyte pleocytosis can indicate viral CNS infection. However, neutrophils can be dominant in the early course of infection, such as in early enteroviral meningitis.[140, 141] A retrospective study evaluating CSF findings in 138 children with aseptic meningitis – a condition of meningitis with a sterile bacterial culture – demonstrated a neutrophil predominance in 57% of cases within the first 24 hours.[142] CSF leucocyte counts are commonly within the range of 5-500 cells/µL; however, they can reach 1000 cells/µL.[50, 138]

Tuberculous meningitis / CNS tuberculosis

CSF white blood cell analysis is often dominated by lymphocytes between 5-500 cells/mm^3, although polymorphonuclear cells may occasionally predominate during the first 10 days.[138, 143] In most cases, an increased CSF protein is present, and CSF glucose may be declined by 70%.[124, 138] Non-specific CSF findings are generally identified in patients with depressed cell-mediated immunity. A study suggested that acellular CSF may be present in elderly patients and those who are HIV positive.[144]

Fungal CNS infection

In most cases, mononuclear pleocytosis is commonly found in fungal CNS infections with a range of 5-500 cells/mm^3. However, a polymorphonuclear cell predominance may occasionally be identified in several cases, especially in CNS infection caused by *Aspergillus, Blastomyces, Pseudoallescheria*, and *Zygomycetes*.[145] *Coccidioides immitis* may be considered the causative agent if eosinophil predominance is identified. In immunosuppressed patients, the host CSF cellular counts may considerably decline at less than 20 cells/mm3. CSF protein concentrations are predominantly increased; however, glucose levels are commonly low.[146]

Table 1.5 Typical CSF findings of various causes of CNS infection[79, 138]

	Normal	Bacterial	Viral	Tuberculous	Fungal
Opening Pressure	12-20 cm CSF	Elevated	Normal/mildly elevated	Elevated	Elevated
Appearance	Clear	Turbid, cloudy, purulent	Clear	Clear or cloudy	Clear or cloudy
CSF WCC (cells/μL)	<5	Elevated (typically >100)	Elevated (typically 5-1000)*	Elevated (typically 5-500)*	Elevated (typically 5-500)*
Predominant cell type	n/a	Neutrophils**	Lymphocytes#	Lymphocytes‡	Lymphocytes
CSF protein (mg/dL)	<40	Elevated	Mildly elevated	Markedly elevated	Elevated
CSF glucose (mg/dL)	46.8-81	Very Low	Normal/slightly low	Very Low	Low
CSF:serum glucose ratio	>0.66	Very Low	Normal/slightly low	Very Low	Low

CSF – cerebrospinal fluid; CNS – central nervous system; WCC – white cell count; n/a – not applicable.

*Occasionally, the CSF WCC may be normal (especially in immunodeficiency or tuberculosis meningitis).

** may be lymphocytic if antibiotics are given prior to lumbar puncture (partially treated CNS infection), or with certain bacteria, i.e. *Listeria monocytogenes*.

May be neutrophilic in enteroviral meningitis (especially early in the course of the disease)

‡ May be neutrophils early in the course of the disease

Brain abscess

Lumbar puncture is also less helpful in establishing the diagnosis of brain abscess. CSF analysis following the lumbar puncture often produces nonspecific findings. Moreover, the technique has a major risk of cerebral herniation. Consequently, the method should not be done in those with a space-occupying brain abscess. Studies indicated that cerebral herniation might rapidly occur in 2.1% of patients following lumbar punctures.[44, 137, 147-149] CSF cultures were reported positive in only 6% of patients.[44, 110, 136, 137] This proportion, however, may increase up to 20% when the abscess ruptures into the ventricles or subarachnoid space.[136]

A definite diagnosis of brain abscess is established by histologic and microbiologic examination of the specimen from the lesion, where the sample is usually obtained by brain biopsy.[43] Although useful, the biopsy procedure is a rare practice in the study setting, where it is only performed alongside abscess excision.

1.3.4.2 Pathogen detection in cerebrospinal fluid

Viral CNS infection

Tissue culture for isolating viruses in CSF, blood or urine specimens remains the gold standard of viral meningitis diagnosis. However, the method has several limitations, including being time-consuming, high-cost and not always sensitive. In the last few decades, CSF polymerase chain reaction (PCR) has been considered the most valuable development in diagnosing viral CNS infection because it allows a rapid, sensitive and specific technique.[150] The PCR method is reported to have a 3- to 1000-fold higher sensitivity compared to viral culture, particularly in detecting enteroviruses, varicella zoster and herpes simplex virus.[151] Other approaches using serologic, blood and oral fluid

samples have also been applied for establishing the diagnosis of viral CNS infection, as described in table 1.7.

Table 1.6 Laboratory techniques used in diagnosis of viral meningitis/encephalitis[151-154]

Organism	First Line test Method (sample used)	Alternative tests Method (sample used)
Enteroviruses	PCR (CSF)	PCR, culture (Throat and rectal swabs)
Herpes simplex virus	PCR (CSF)	PCR, culture, immunofluorescence, electron microscopy, Tzanck smear (genital lesions)
Varicella zoster virus	PCR (CSF)	PCR, culture, immunofluorescence, electron microscopy, Tzanck smear (skin lesions)
Flaviviruses	PCR (CSF, serum) if specimens are collected ≤7 days after onset Serology (CSF, serum) if samples collected >7 days after onset	

CSF – cerebrospinal fluid; PCR – polymerase chain reaction.

Bacterial meningitis

Performing Gram staining and culture of the CSF samples may provide data about the causative pathogens and any antimicrobial resistance. [155, 156] The possibility to detect an organism, however, decreased by up to 44% if the LP is delayed until after antimicrobial therapy is administered.[157, 158] Despite its limited sensitivity, bacterial antigen tests may be beneficial to patients with clinical manifestations suggestive of bacterial meningitis but having negative results of CSF Gram staining and cultures.[155] Furthermore, PCR can be an effective tool in detecting causative bacteria, particularly in those who have negative CSF cultures.[159]

Tuberculous meningitis

The conclusion whether bacteriological or molecular methods are more beneficial for the diagnosis of tuberculous meningitis remains a controversy. An established study demonstrated that performing Ziehl-Neelsen staining for CSF specimens may reveal the

acid-fast bacilli of *M. tuberculosis.*[160] However, a recent study reported that the approach rarely produces positive results in current laboratory observations.[161] Kennedy and Fallon suggested that performing repetitive CSF sampling may increase the sensitivity of Ziehl-Neelsen staining by up to 80%.[162]

PCR technique is often used for establishing the diagnosis of tuberculous meningitis, although the method has a varied sensitivity. Pai *et al.* demonstrated that nucleic acid amplification approaches for diagnosing tuberculous meningitis have a sensitivity and specificity of 56% (95% CI 0.46-0.66) and 98% (0.97-0.99), respectively.[163] However, the molecular method seems to be sensitive for longer during anti-tuberculosis treatment compared to the bacteriological approach.[164]

GeneXpert MTB/RIF is the current gold standard for diagnosing pulmonary tuberculosis.[165] However, its sensitivity in detecting *M. tuberculosis* in CSF specimens varies between 51% and 90%, with specificity between 95% and 100%.[165] A more recent study by Chaidir *et al*. comparing five different techniques for the diagnosis of tuberculous meningitis, including PCR assay, microscopy (i.e. Ziehl-Neelsen stain), GeneXpert MTB/RIF, solid culture and liquid culture showed that the PCR assay had the highest sensitivity among all (64% versus 12.2%, 42%, 46% and 48.8%, respectively).[31]

Fungal meningitis

The laboratory techniques used for fungal meningitis diagnosis vary depending on the type of causative agent. In cryptococcal meningitis, the cryptococcal antigen (CrAg) lateral flow assay has the highest sensitivity and specificity combined compared to other assays, including culture, Indian ink microscopy, and latex agglutination assays.[166] A common form of cerebral mycosis, candidal meningitis, is established using CSF microscopic examination. Staining the CSF samples using PAS and Methanamine silver stains may

reveal Gram-positive yeast. Yeast-like fungi may be visualised in the prepared exudates (with 10% KOH) microscopically.[37, 167, 168]

1.3.4.3 Neuroimaging

The two most common types of brain imaging are Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). The picture resolution obtained from MRI is often regarded as superior to CT. However, CTs are much quicker to perform, so sedation (to ensure the patient is still) is not as likely to be needed to obtain good-quality images. MRI scans are also more complex to interpret and require expensive equipment and a stable power supply to support the magnets. Both types of imaging can be difficult to access in low-resource countries, but MRI is particularly rare and often only found in private health centres.

Imaging is used to localise areas of CNS inflammation or damage. Imaging can help support the diagnosis of CNS infection. Visual demonstration of tissue inflammation can help localise where the infection is. An injection of contrast solution into the bloodstream during or just before the scan can help highlight areas of inflammation of the brain tissue. Both CT and MRI images can be enhanced by the use of contrast.

A diagnosis of meningitis is supported by the visual demonstration of inflammation involving membranes covering the brain. Diagnosis of myelitis is supported by spinal imaging indicating inflammation of the cord. A diagnosis of encephalitis is supported by imaging showing areas of inflammation in the brain parenchyma.

Beyond localising where inflammation occurs, the patterns of inflammation or brain changes can help suggest the pathogen causing the infection. For example, tuberculous meningitis (TBM) often presents with a triad of imaging features: basal meningitis (inflammation of the meninges near the base of the brain), hydrocephalus (swelling of

ventricles [CSF chambers in the brain parenchyma]) and tuberculoma. Tuberculomas are local foci (or collections) of mycobacterium in the brain tissue surrounded by tissue oedema (swelling). The hydrocephalus in TBM is believed to occur, in part, due to the high protein content of the CSF during TBM infection, causing blockage of CSF flow in the CNS. Alternatively, HSV-1 encephalitis is typically associated with brain tissue inflammation localised to one (or occasionally both) temporal lobes. This inflammation is best seen on a contrast-enhanced MRI.

Several neuroimaging findings might guide clinicians to elaborate on the possible causative pathogens, as described in table 1.8.

Table 1.7 Possible causative pathogens based on neuroimaging results[83]

Neuroimaging results	Possible causative pathogens
Arteritis and infarctions	Varicella-zoster virus (VZV), Nipah virus, *Rickettsia rickettsii*, *Treponema pallidum*
Calcifications	Cytomegalovirus (cortical), *Toxoplasma gondii* (periventricular), *Taenia solium*
Cerebellar lesions	VZV, Epstein-Barr virus, *Mycoplasma pneumoniae*
Focal lesions in basal ganglia, thalamus and/or brain stem	Epstein-Barr virus, Eastern equine encephalitis virus, Murray Valley encephalitis virus, St Louis encephalitis virus, Japanese encephalitis virus, West Nile virus (WNV), enterovirus 71, influenza virus, human transmissible spongiform encephalopathies, *Tropheryma whipplei*, *Listeria monocytogenes*
Hydrocephalus	*Mycobacterium tuberculosis*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Balamuthia mandrillaris*
Space-occupying lesions	*Toxoplasma gondii*, *Balamuthia mandrillaris*, *Acanthamoeba spp.*, *Taenia solium*
Temporal and/or frontal lobe involvement	Herpes simplex virus, VZV, human herpesvirus 6, WNV, enteroviruses, *Treponema pallidum* (medial lobes)
White matter abnormalities	VZV, cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, human immunodeficiency virus (HIV), Nipah virus, human polyomavirus 2 (John Cunningham [JC] virus), measles virus, *Baylisascaris procyonis*, acute disseminated encephalomyelitis

1.3.4.4 Electroencephalography

In CNS infection, electroencephalography (EEG) is used to assess the impact of disease on brain function. In terms of diagnosis, some EEG findings suggest encephalitis. Lai and

Gragasin[169] reported the finding of periodic lateralising epileptiform discharge or repetitive sharp wave complexes on the temporal lobes in individuals with HSV-1 encephalitis. In contrast, Gutierrez *et al*.[170] reported bilateral synchronous periodic sharp and slow waves in those with subacute sclerosing panencephalitis. There is no definitive EEG feature of encephalitis. In patients with decreased consciousness, the EEG may be useful in identifying epileptiform discharge in subclinical seizure activity.[126]

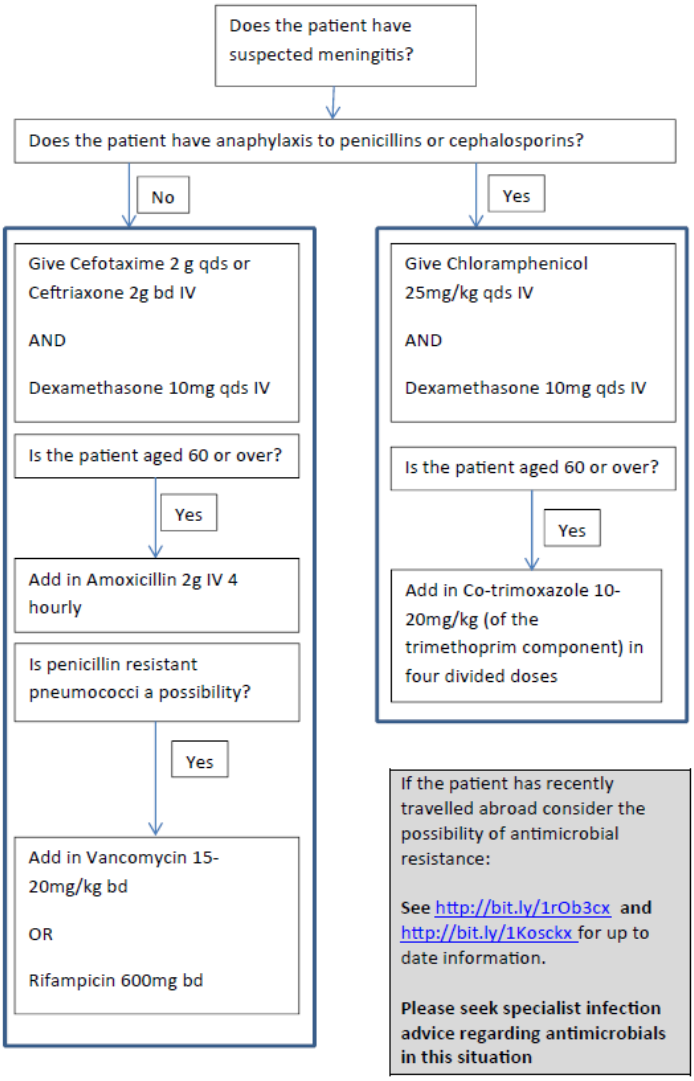
1.3.5 Treatment

1.3.5.1 Antibiotics

Three essential factors are considered when choosing an appropriate antibiotic treatment for patients with bacterial CNS infection, including its ability to penetrate the CSF, bactericidal effectiveness and clinical efficacy.[51] The selection of antibiotics is also influenced by predisposing factors in each age group of patients.[52] In patients with bacterial meningitis/encephalitis, antibiotics may be prescribed in three different situations.

First, patients with suspected CNS infection may be given empirical antibiotic treatment before CSF investigation results are obtained.[138, 171] It should be noted, however, that LP should be attempted as soon as reasonably possible prior to antibiotic treatment. All adult patients suspected with meningitis should receive cefotaxime 2 g IV/6h or ceftriaxone 2 g IV/12h and dexamethasone 10 mg IV/6h (figure 1.3).

Figure 1.3 Algorithm of the empirical treatment of suspected meningitis.
Taken from McGill *et al*.[135]



Those who have anaphylaxis to penicillins or cephalosporins can be given chloramphenicol 25 mg/kg IV every 6 hours and corticosteroid. Additional ampicillin/amoxicillin 2 g IV 6-hourly should be given to patients aged 60 or over and immunocompromised patients, including diabetics or patients with a history of alcohol misuse. If penicillin-resistant pneumococci is a possibility (e.g. travel history to countries where penicillin-resistant pneumococci are prevalent), vancomycin 15 – 20 mg/kg IV per 12h or rifampicin 600 mg IV or PO/12h should be added.[138, 171] The algorithm for the empirical treatment of meningitis is illustrated in Figure 1.3.

Second, patients may receive antibiotic treatment after obtaining the positive CSF Gram stain without a particular bacterium detected. In this scenario, a double antibiotic policy is routinely used, as described in table 1.9.[171]

Table 1.8 The antibiotic policy in patients with positive cerebrospinal fluid Gram staining but no evidence of specific pathogens

Causative organism	Drugs of choice
Gram-positive cocci	Continue with empiric treatment
Gram-negative cocci	Continue with empiric treatment
Gram-positive bacilli	Continue with empiric treatment Add ampicillin/amoxicillin 2 g IV 4-hourly
Gram-negative bacilli	Continue with empiric treatment Seek specialist advice on local microbial resistance If there is a high suspicion of extended-spectrum beta-lactamase (ESBL) resistance, switch to meropenem 2 g IV 8-hourly

Third, patients may be given a specific antibiotic after the definitive detection of causative pathogens.[138] The antibiotics may be administered intravenously to gain an effective treatment and an early bactericidal effect.[51, 52, 172-174]

1.3.5.2 Anti-tuberculosis therapy

The treatment regimen for tuberculous CNS infection is based on the treatment for pulmonary tuberculous infection but is typically given for 9-12 months.[175-177]. The treatment is given in two phases, an intensive phase (using four drugs for 2 months) and a continuation phase (continuing with two of these drugs for another 7-10 months). Recommendations for the drug regimens to be used for tuberculous meningitis, based on the British Infection Society and the joint committee between the Infectious Disease Society of America (IDSA), the American Thoracic Society (ATS), and Centres for Disease Control and Prevention (CDC), are described in table 1.12.[175, 177]

Table 1.10 International guidelines for treatment of tuberculous meningitis[175, 177]

Drug	Daily Dose		Route	Duration
	Children	**Adults**		
British Infection Society guidelines, 2009				
Isoniazid	10-20 mg/kg (max 500 mg)	300 mg	Oral	12 months
Rifampicin	10-20 mg/kg (max 600 mg)	450 mg (<50 kg) 600 mg (≥50kg)	Oral	12 months
Pyrazinamide	30-35 mg/kg (max 2 g)	1.5 g (<50kg) 2.0 g (≥50kg)	Oral	2 months
Ethambutol	15-20 mg/kg (max 1 g)	15 mg/kg	Oral	2 months
Guidelines of the joint committee of the ATS, IDSA, and CDC, 2003				
Isoniazid	10-15 mg/kg (max 300 mg)	5 mg/kg (max 300 mg)	Oral	9-12 months
Rifampicin	10-20 mg/kg (max 600 mg)	10 mg/kg (max 600 mg)	Oral	9-12 months
Pyrazinamide	15-30 mg/kg (max 2 g)	40-55 kg person: 1.0 g 56-75 kg person: 1.5 g 76-90 kg: 2.0 g	Oral	2 months
Ethambutol	15-20 mg/kg (max 1 g)	40-55 kg person: 1.0 g 56-75 kg person: 1.5 g 76-90 kg: 2.0 g	Oral	2 months

ATS – American Thoracic Society; IDSA – Infectious Diseases Society of America; CDC – Centres for Disease Control

According to the national guideline, treatment for CNS tuberculosis follows the principle for extrapulmonary tuberculosis treatment with a minimum duration of 12 months.[34] These include 2 months of isoniazid 5 mg/kg, rifampicin 10 mg/kg, pyrazinamide 25 mg/kg, and ethambutol, 15 mg/kg, followed by 10 months of isoniazid and rifampicin. This guideline is followed by the clinicians in Dr Sardjito Hospital (i.e. the current study setting).

Rifampicin plays a crucial role among the standard drugs for tuberculosis, as indicated by the high mortality in patients with rifampicin-resistant tuberculous meningitis.[178, 179] This leads to the question of whether an intensified regimen of rifampicin can improve patient outcomes. Addressing that question, several studies have been done in Indonesia and Vietnam.[180-184] The most recent double-blind, randomised, placebo-controlled phase II

trial from Indonesia compared standard 450 mg, 900 mg, or 1,350 mg (10, 20, and 30 mg/kg) of oral rifampicin combined with other anti-tuberculosis drugs for 30 days. The result showed the 6-month mortality was 35%, 45% and 15% in the aforementioned groups, respectively (p=0.12).[180] The authors recommend the use of 900-1,350 mg/day of oral rifampicin combined with other anti-tuberculosis drugs for the first month, followed by 450 mg/day of oral rifampicin plus other anti-tuberculosis drugs for the second month, then followed by 450 mg/day of oral rifampicin and standard dose of isoniazid for the following 7 months. With regard to this finding, the Indonesian national guideline for CNS tuberculosis treatment stated that the administration of the high-dose rifampicin regimen should be consulted with the expert.

In contrast, the Vietnam study comparing a standard 9-month tuberculosis regimen containing 10 mg/kg of rifampicin with an intensified regimen containing 15 mg/kg of rifampicin and 20 mg/kg of levofloxacin for the first 8 weeks of treatment showed that the intensified regimen was not associated with a higher survival rate among patients with tuberculous meningitis than the standard treatment.[183] A further investigation was performed by the Vietnam study comparing the effect of the aforementioned regimens to sub-groups of multidrug-resistant (MDR); isoniazid-resistant, rifampicin susceptible (INH-R); and susceptible to rifampicin and isoniazid (INH-S + RIF-S). The result showed that the intensified regimen significantly improved survival in patients with INH-R tuberculous meningitis (HR 0.34 [95% CI, 0.15–0.76], p=0.01).[184]

1.3.5.3 Antiviral treatment

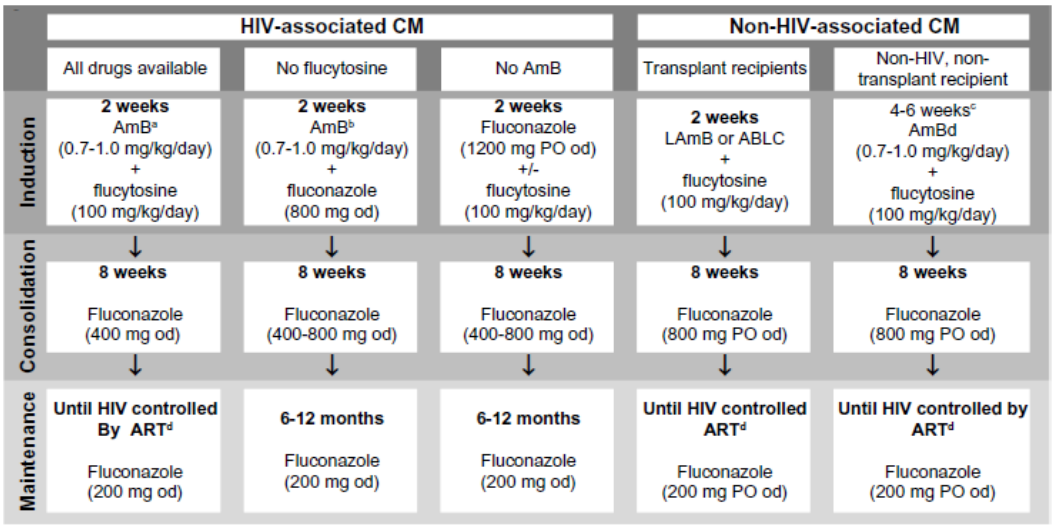
To date, no specific treatment is available for viral meningitis. Antiviral therapy such as acyclovir has only been proven effective in herpes encephalitis cases. When the patients exhibit signs of encephalitis, such as impaired consciousness, focal neurological signs, and

signs of brain parenchymal inflammation in the region of the temporal lobe on cranial imaging, acyclovir treatment could be started immediately.[171] Acyclovir is reported to have strong antiviral activity against HSV and related viruses such as VZV. Patients with suspected HSV encephalitis could be treated with intravenous acyclovir (10 mg/kg three times a day). The therapy is maintained for a minimum of 2 weeks, at which an LP should be performed to evaluate the presence of HSV DNA or until HSV infection has been excluded from the final diagnosis.

1.3.5.4 Antifungal treatment

Treatment for cryptococcal meningitis consists of three phases, including induction, consolidation, and maintenance. Current guidelines from the Infectious Guidelines of America (IDSA)[185] and World Health Organisation (WHO)[186] are summarised in figure 1.4.

Figure 1.4 Summary of current guidelines from the Infectious Disease Society of America and World Health Organisation for cryptococcal meningitis treatment. Taken from Sloan and Paris.[187]
HIV - human immunodeficiency virus; AmB - amphotericin B; LAmB - liposomal amphotericin B; ABLC - amphotericin B lipid complex; ART - antiretroviral therapy.



In HIV-associated cryptococcal meningitis, amphotericin B (AmB) is the fungicidal drug of choice and should be given intravenously for two weeks during the induction phase whenever possible. The recommended dose is 0.7-1.0 mg/kg for the most common

preparation, AmB deoxycholate (AmBd). The administration of AmB should be accompanied by intravenous or oral flucytosine at 100 mg/kg/day. If flucytosine is unavailable, WHO recommends oral fluconazole 800-1,200 mg once daily as an alternative drug. AmB-fluconazole induction therapy has shown to be cost-effective in resource-poor settings.[188] Following the induction phase, treatment is continued with a consolidation phase using fluconazole 400 mg/day for a further eight weeks, which may be increased to 800 mg when AmB-fluconazole is used in the induction phase. Monotherapy using oral fluconazole 1200 mg is recommended when fluconazole is the only drug available for induction and consolidation therapy. Fluconazole 200 mg once daily is used for maintenance therapy until HIV is controlled by antiretroviral therapy (ART) if an AmB-flucytosine regimen is used during the induction phase or for 6-12 months if AmB-fluconazole or single fluconazole regimens is used for the induction therapy.

Immune reconstitution inflammatory syndromes (IRIS) in patients with cryptococcal meningitis occurs when host immune restoration induces inflammatory reactions to persistent fungal antigen. In HIV-associated cryptococcal meningitis, IRIS is affected by the time of ART initiation. Recent studies investigating the optimal timing for ART introduction have shown variable results.[189-192] To date, starting ART 4-10 weeks after initiating antifungal treatment is considered the safest option.[187]

1.3.5.5 Corticosteroids

Tuberculous meningitis

The current protocol for TBM management recommends corticosteroids (either dexamethasone or prednisolone) should be given to all patients with TBM at any stage of the disease.[193] However, whether the regimens have beneficial effects for all groups of patients with tuberculous meningitis remains challenging to confirm.[194] Previous clinical

trials suggested that corticosteroid administration in TBM patients may cause a modest reduction in morbidity and mortality.[195] However, it remains unclear how corticosteroids produce these effects. Simmons *et al*.[196] showed that corticosteroid treatments significantly modulated acute CSF protein concentrations and mildly reduced IFN-γ concentrations. Nevertheless, the clinical parameters for inflammation remain unchanged. Similarly, Thwaites and colleagues found that corticosteroid treatment reduced complication rates during TBM (e.g. hydrocephalus or brain infarction).[197]

Bacterial meningitis

The advantages of corticosteroids in both children and adults with bacterial meningitis have been reported in clinical trials.[198] Dexamethasone is beneficial in adults with bacterial meningitis, especially when given early in the disease course and ideally before / at the same time as antibiotics.[198] A more recent meta-analysis to assess the benefit of corticosteroid treatment in children and adults with bacterial meningitis showed that although corticosteroid treatments did not reduce the overall mortality, they significantly reduced hearing loss and neurological sequelae.[199]

Dexamethasone therapy is suggested for treating infants and children 2 months and older with suspected or proven bacterial meningitis. This drug should be administered with a dose of 0.4 mg/kg every 12 hours for three to four days.[117, 200] Other studies indicated that administration of dexamethasone for only two days was also effective therapy.[201, 202] However, the adverse effects of corticosteroid use, including gastrointestinal bleeding and hyperglycaemia should be monitored closely in children. The intravenous H2 receptor antagonist is considered an effective drug to prevent gastrointestinal haemorrhage. In adults, dexamethasone should be given at 10 mg IV 6-hourly 15 – 20 mins before or together with the first dose of the antibiotic.[198] Up until 12 hours after the initial dose of

antibiotics, dexamethasone can still be started, but the impact of this on the outcomes has not been investigated. It has been suggested that dexamethasone treatment should be considered to be stopped if the causal pathogen is neither *S. pneumoniae* nor *M. tuberculosis*.[171]

Viral meningitis/encephalitis

Corticosteroid use in viral meningitis/encephalitis remains controversial as there is no sufficient evidence to support its use.[79] Corticosteroids are often given along with acyclovir to patients with signs of cerebral oedema, brain shift, or increased intracranial pressure as they can reduce swelling. However, they also have strong immunomodulatory properties, which in theory, can increase viral replication and worsen the infection.[79] A European-wide randomised controlled trial is currently being carried out to provide unbiased evidence of the benefit of corticosteroid use in viral meningitis/encephalitis.[203]

Brain abscess

The administration of corticosteroids in patients with brain abscesses remains problematic due to their ability to interrupt the penetration of antimicrobial agents into brain tissue or brain abscess.[204] There is no data to support the beneficial effect of steroids in brain abscess therapy. Adjunctive corticosteroids should only be given to several conditions, including patients with progressive neurologic deterioration and impending cerebral herniation. In addition, corticosteroids should be considered in patients with abscesses in sites where oedema may rapidly become severe. It is advised to do a tapering plan of the corticosteroid dose when a clinical response has been achieved.[43]

1.3.6 Outcome

Meningitis

Patients with viral meningitis commonly have a better prognosis than other infective causes, with total recovery within 2 to 4 weeks. However, this does not occur in infants and young children. Complicated viral meningitis/meningoencephalitis in children is the fifth most common cause of infantile morbidity and mortality. Effective detection and management may result in fewer symptoms, lower morbidity and decreased death.[48, 49] Several factors, including age, immune status, pathogen type and virulence, inoculum dose and clinical severity, affect the prognosis of patients with bacterial meningitis. The application of effective early management may help many patients to recover with no residual disability.[172, 205]

Bacterial meningitis generally has relatively high morbidity and mortality rates of 15-25% and 10-20%, respectively. However, the rates may vary depending on the age group of patients. The vulnerable groups (neonates, infants and elderly people) have the highest morbidity and mortality rates of 20-30% and 10-20%, respectively. However, the morbidity and mortality rates in adult patients are about 12-15% and 7-15%, respectively.[119]

In terms of causative organisms, *S. pneumoniae* contributes to the highest combined morbidity and mortality rates by 30-40%, followed by *L. monocytogenes* (25-30%), *N. meningitidis* (15-20%) and *H. influenzae* (5-10%). Furthermore, patients with tuberculous meningitis still have considerable morbidity and mortality rate, although they attain the proper therapy.[51, 174]

Encephalitis

The prognosis of patients with encephalitis varies depending on the cause. A study attempted to compare the prognosis of HSV-1 and enterovirus encephalitis concluded that a worse prognosis occurs in HSV-1, with more than 35% of herpes simplex encephalitis having severe sequelae or death.[206] Enterovirus is linked to neurological sequelae and death, although patients with enteroviral encephalitis are frequently reported to have a good outcome.[207] Among the bacteria causing encephalitis, *M. tuberculosis* and *L. monocytogenes* are documented to have relatively high morbidity and mortality rates.[208, 209] A study conducted in France demonstrated that *M. tuberculosis* and *L. monocytogenes* contribute to the major part of case fatalities caused by encephalitis (in combination, 12 out of 26 death).[77]

Brain abscess

Recent studies indicated that the mortality rate of brain abscesses ranges from 10% to 19%.[112, 210-212] Brain abscess may lead to serious long-term complications, including seizures and focal neurologic deficits. Numerous studies documented that the incidence of seizures after hospitalisation may vary depending on the follow-up interval. Within one year after discharge, seizures occurred in 86% of patients.[213] Within two and four years after discharge, the patients who experienced seizures were documented at 78% and 76%, respectively.[214, 215] Several major neurological deficits, including pareses and aphasia, were reported in 18% of patients, whereas minor deficits, including unilateral deafness, eye muscle palsies and visual field defects, were experienced by 9% of patients.[216]

1.4 Scope of Thesis

1.4.1 Study setting

My study was undertaken at Dr Sardjito Hospital. As described earlier in this chapter, it is a Type A specialised referral hospital in Yogyakarta. Lower-tier hospitals refer suspected meningitis and encephalitis cases to Dr Sardjito Hospital. The hospital has clinical laboratories. Lumbar punctures are performed by clinical staff in the paediatric and adult neurology departments. Infection diagnosis is routinely performed using classical microbiological techniques (Gram, ZN, Indian ink stain, and microbiological culture). Pathogen-specific PCR is not undertaken. A few antibody tests are performed [e.g. adult HIV, adult TORCH (i.e. *Toxoplasma*, Rubella, CMV, HSV-1 and -2), child CMV, dengue rapid IgM and IgG tests, Tubex TF, and Leptospira]. Appropriate antibiotics are available and prescribed by clinical staff.

1.4.2 Study Aim and Objectives

1.4.2.1 Aim

To describe the aetiology and outcome of CNS infection among children and adults attending Dr Sardjito Hospital, and explore whether the introduction of additional diagnostic testing at the study site can increase diagnosis among suspected CNS infection patients.

1.4.2.2 Objectives

1) Describe the clinical features and management of paediatric and adult patients with suspected CNS infection attending Dr Sardjito Hospital.
2) To determine whether introducing pathogen-specific PCR to the study setting, as a research tool, can increase the diagnosis of CNS infection (above that detected by standard hospital testing) among children and adults attending the hospital.

3) To determine whether the introduction of antibody/antigen testing to the study setting, as a research tool, can increase the diagnosis of CNS infection (above that detected by standard hospital testing) among children and adults attending the hospital.
4) To describe the aetiology of CNS infection in the study setting using these additional diagnostic tools alongside standard hospital testing.
5) To assess the outcome of children and adults with suspected CNS infection at and after hospital discharge.

Chapter 2 – Materials and Methods

I established a prospective observational cohort study entitled 'The UK-Indonesia Study on the Epidemiology of Neurological Infectious Diseases in Yogyakarta (abbreviated as The UNITY-NeuroID Project)', which became the foundation of all the work in my PhD thesis. The study was the first study in Indonesia that employed a systematic and comprehensive approach in identifying the clinical presentation, aetiology, and functional outcome of central nervous system (CNS) infection among both children (aged ≤18 years) and adults (aged >18 years). This study provided the clinical data for chapters 3 and 6 and the clinical samples for the work in chapters 4 and 5.

I developed the study protocol, ethics submissions, polymerase chain reaction (PCR) assay and optimisation. I performed subject screening, subject recruitment, completion of the paper-based case report form (CRF), sample and data collection, as well as outcome assessments (Liverpool Outcome Scores [LOS]). I also performed PCR testing, antibody and antigen testing, as well as clinical and laboratory data analysis. In the third month of active patient recruitment, I recruited seven (part-time) research assistants to help carry out subject screening, recruitment and outcome assessments (LOS). They also entered CRF data into an electronic database (RedCap). A few aspects of the laboratory work were performed by my colleagues, Dr Janet Flatley and Dr Tessa Prince, at the Liverpool Brain Infections Group. This included PCR limit of detection tests, development of *Klebsiella pneumoniae* and *Salmonella* PCR assays, as well as several batches of DNA/RNA extraction and enzyme-linked immunosorbent assays (ELISAs). Plaque reduction neutralisation tests (PRNT) were performed by colleagues from The Eijkman Institute for Molecular Biology.

2.1 Study population

Patients were eligible for the study if they met the following criteria:

a. Aged 1 month or over (including children and adults)

b. Presented to Dr Sardjito General Central Hospital between February 2015 and January 2018 with the following conditions :

   (1) had an acute onset of fever or a recent history of fever, AND

   (2) had any neurological signs (including change in mental status, new onset of seizures [except simple febrile seizures], cranial nerve palsy, or abnormal movement), AND/OR

   (3) had impaired consciousness, AND/OR

   (4) had neck stiffness, AND/OR

   (5) had increased cerebrospinal fluid (CSF) leucocyte count following lumbar puncture (LP) / extra-ventricular drainage (EVD)

Patients with simple febrile seizures (i.e. those who exhibited full recovery without neurological sequelae within one hour of seizures) and those who did not provide consent were excluded from the study.

2.2 Methods

Potential patients were identified by screening the patient register of initial diagnoses and engaging with paediatric and adult neurology trainees when seeing patients with suspected CNS infection (as defined by the inclusion criteria above). Patient information sheets were used to inform the patients (or their responsible consultee – patient parent/carer/other family members) regarding the study [see appendices 8.1 and 8.2, pgs. 350-363]. Written informed consent was provided. If an adult patient lacked capacity to undertake informed consent, a responsible consultee (patient carer/family member)

declaration was sought. If the patient regained capacity, they had the choice to review the consultee's decision and participate or leave the study. For all children (with or without capacity), a responsible consultee (guardian/parent) declaration was sought. Declaration was also sought from adolescent patients whenever possible.

2.2.1 Systematic testing to identify pathogens causing CNS infection in Yogyakarta

Clinical data were initially recorded on paper-based case report forms and entered into a secure online database (REDCap).[217] Specimens, including CSF, whole blood, early (acute) and late (follow-up/convalescent) sera, were collected. CSF was obtained as part of patient standard care, whilst blood samples (including sera) were taken at the same time as routine venepuncture where possible. For children, an appropriate and safe volume of blood was collected according to their age and/or weight in accordance with the guidelines from the Medicines for Children Research Network (MCRN).[218] Similarly, an appropriate and safe volume of CSF was collected according to their age groups based on British Infection Society guidelines.[175] In cases where fresh serum and/or CSF could not be collected, leftover samples (i.e. the remainder of clinical samples after being utilised for routine investigations) were sought from the hospital's lab. Once collected, specimens were immediately stored at -20 °C or -80 °C until tested. A series of assays, including pathogen-specific real-time polymerase chain reaction (PCR) and antibody/antigen tests, were performed to identify pathogens causing CNS infection. All assays involving patient specimens were conducted in the laboratories in the Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada unless otherwise stated. None of the specimens was transferred outside the country.

2.2.1.1 Pathogen-specific real-time PCR

The target pathogens for PCR included enterovirus, herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2), cytomegalovirus (CMV), varicella zoster virus (VZV), *Streptococcus pneumoniae, Haemophilus influenzae*, *Neisseria meningitidis, Mycobacterium tuberculosis, Streptococcus suis, Escherichia coli, Streptococcus agalactiae, Klebsiella pneumoniae,* and *Salmonella* species. The pathogen-specific PCR assays were performed on all CSF samples with pleocytosis and a few samples without pleocytosis, with the exception of *E. coli* and *S. agalactiae* PCR assays which were only performed on paediatric samples. Details on why those pathogens were selected as the target pathogens and which clinical samples were tested by the PCR assays will be further described in chapter 4. Additionally, details on PCR testing procedures will be described in chapter 4.

2.2.1.2 Antibody and antigen tests

Enzyme-linked immunosorbent assays (ELISAs) were used to detect IgM antibodies against dengue virus (DENV) 1-4, Japanese encephalitis (JE) virus and *Orientia tsutsugamushi. Cryptococcus* antigen detection was performed using lateral flow assays. The reasons why these pathogens were targeted and which clinical samples were tested will be explained in chapter 5. Details on the procedures for antibody/antigen tests are described in chapter 5.

2.2.2 Functional outcome of CNS infection in Yogyakarta

Patient's functional outcomes were assessed using the Liverpool Outcome Score (LOS)[219] for children and Glasgow Outcome Score Extended (GOSE)[220] for adults. The functional outcomes were evaluated at two time points, including at hospital discharge and at follow-up. As mentioned previously, the subject recruitment period for this study was between February 2015 and January 2018. Due to time constraints, follow-up

assessments were completed by the end of March 2018. Patients who were admitted to the hospital before October 2017 were followed-up for six months, whilst those who were admitted to the hospital between October 2017 and January 2018 were followed up for 1-5 months. Follow-up assessments were undertaken by telephone interview. Detailed methods used for the outcome assessment will be explained in chapter 6.

2.3 Statistical analysis

Data were analysed using SPSS v24 and GraphPad Prism v6. Data were checked for normality, and normally distributed data were analysed using a *t*-test. Mann Whitney U or Kruskal-Wallis tests were used to analyse non-parametric continuous data. Chi Square tests (or Fisher's exact where expected counts were less than five) were used for categorical data. For outcome analysis, differences between outcomes at hospital discharge and follow-up were analysed using McNemar tests. For all data, two-sided tests at 95% confidence level were used to define statistical significance. A *p*-value less than 0.05 ($p < 0.05$) was defined as statistically significant.

2.4 Ethics and governance

Ethical approval for the UNITY-NeuroID Project was given by the Medical and Health Research Ethics Committee of the Faculty of Medicine UGM (KE/FK/1318/EC) and reviewed annually. Approval was also given by Dr Sardjito General Central Hospital through its education and research department.

2.5 Funding

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Chapter 3 – Syndromic classification, clinical features, and management of patients with central nervous system infections in Yogyakarta

3.1 Introduction

The common causes of central nervous system (CNS) infection in Indonesia are incompletely understood. Previous studies have highlighted the burden of meningococcal and tuberculous meningitis in some regions in Indonesia,[4-7] whilst other studies have demonstrated a higher than anticipated prevalence of Japanese encephalitis in parts of the country.[8, 9] Other causes of CNS infection, including *Cryptococcus neoformans*, *Toxoplasma gondii*, chikungunya virus and poliovirus have also been reported from previous studies in Indonesia.[10-12]

The widespread use of antibiotics, delay in lumbar puncture, and lack of diagnostic techniques beyond classical microbiological testing, such as Gram stain and culture, have been shown to reduce successful detection of the pathogens among cases with suspected CNS infection.[158, 221] The percentage of CSF or blood samples in which no pathogen could be identified among patients with suspected CNS infection is generally high.[3, 6] The use of molecular techniques such as pathogen-specific PCR can improve pathogen detection.[222-226] However, molecular methods are largely unavailable on a routine basis in much of Indonesia, including in our local government hospitals in Yogyakarta.

To better estimate the prevalence of known pathogens causing CNS infection among adult and child patients attending the main University associated hospital in Yogyakarta with suspected CNS infection, I conducted the UK-Indonesia Study on the Epidemiology of Neurological Infectious Diseases in Yogyakarta (The UNITY-NeuroID Project). This project

was a prospective observational study based at Dr Sardjito Hospital (the main hospital associated with Universitas Gadjah Mada) in Yogyakarta. In this chapter, I aim to describe the syndromic categories, clinical features and management of patients with suspected CNS infection.

My objectives for this chapter are as follows:

1) To describe the demographic and clinical presentation of paediatric and adult patients with suspected CNS infection attending Dr Sardjito Hospital during the study period (2015 – 2018);
2) To describe investigations performed at the hospital as well as the results of the investigation;
3) To describe the treatment of these patients;
4) To describe the early outcome in terms of the admission length and the critical care requirement;
5) To describe the syndromic clinical classifications for these patients.

3.2 Methods

The data for this chapter were provided from the UNITY-NeuroID project, as described in the previous chapter.

3.2.1 How syndromic classifications were applied to the study group

Case definitions used in the study are described in table 3.1. Clinical definitions of CNS infection and their sub-types (e.g. meningitis or encephalitis) were identified from peer-reviewed publications. Typically, guideline publications were chosen. These definitions were **retrospectively** applied to each patient with reference to their clinical and laboratory data collected through the study case record form. The working diagnoses for

each patient were also examined using the clinical diagnoses provided in the hospital records.

3.2.2 Determination of aetiological diagnoses

Initial pathogen diagnoses were determined based on the data obtained from routine investigations carried out at the hospital. The standard hospital diagnostic tests included the following: (1) Gram stain and microbiological culture in blood or CSF. The microbiological culture that was performed was typically for bacteria. Fungal culture was only performed based on a clinician's request or if fungi were seen from the Gram stain. Detection of *M. tuberculosis* in CSF was performed by Ziehl-Neelsen stain. *M. tuberculosis* culture was done using Lowenstein-Jensen (LJ) medium before the *Mycobacteria* growth indicator tube (MGIT) method was introduced at Dr Sardjito Hospital as an alternative in 2017. Nonetheless, the LJ culture was still infrequently performed for CSF samples. This was anecdotally reported due to the large CSF volume required to perform culture successfully. (2) Antibody-mediated testing was performed in serum but not CSF samples. These included tests for IgM and/or IgG against *T. gondii*, rubella virus, CMV, HSV-1 and HSV-2 [often shortly named as TORCH]; dengue virus, *Salmonella* and *Leptospira*. Additionally, dengue non-structural protein 1 (NS1) antigen testing was also performed for the detection of dengue infection. The dengue, *Leptospira* and *Salmonella* testing were performed using rapid tests, whilst the TORCH testing was performed by enzyme-linked immunosorbent assay (ELISA). These assays were performed on selected patients based on the treating clinician's decision and were typically tested in single rather than paired (acute and convalescent) serum samples. Indian ink stain was only introduced in the second year of my study period. GeneXpert MTB/RIF, PCR testing for *M. tuberculosis,* became available at the hospital in July 2017. The test was primarily used to test sputum

samples. Occasionally, GeneXpert MTB/RIF was performed on CSF at the special request of the clinician.

3.2.3 Statistical analysis

Data were analysed using SPSS version 24, as described in chapter 2. Results were recorded as n/number available (n/N), where the number available did not always represent the total number of patients due to missing data for some patients. Graphs in this chapter were generated using draw.io (www.draw.io) and Microsoft Excel.

Table 3.9 Clinical case definitions

Diagnosis	Definition	References
Suspected CNS infection	Patients with acute onset of fever or recent history of fever and one or more of the following: neurological signs (including change in mental status, new onset of seizures [except simple febrile seizures], cranial nerve palsy, or abnormal movement); impaired consciousness; neck stiffness; and increased CSF leucocyte count following lumbar puncture (LP) / extra-ventricular drainage (EVD)	
Syndromic CNS infection	Patients with suspected CNS infection who fulfilled the criteria for having meningitis or encephalitis or myelitis or shunt infection, or brain abscess	
Not CNS infection	Patients with suspected CNS infection who had confirmed alternative diagnoses other than CNS infection	
Sub-types of CNS infection		
Meningitis	Patients with symptoms compatible with meningitis as deemed by the treating clinical team and a CSF leucocyte count >4 cells/µL (for patients ≥6 weeks of age) or >14 cells/µL (for patients <6 weeks of age) OR Patients with symptoms compatible with meningitis as deemed by the treating clinical team, CSF leucocyte count ≤4 cells/µL (for patients ≥6 weeks of age) or ≤14 cells/µL (for patients <6 weeks of age), AND detection of an appropriate pathogen in CSF or blood by PCR or culture or Gram stain or serology	McGill *et al.*,[2] Olsen *et al*.[227]
Encephalitis	Altered consciousness for >24 hours (including lethargy, irritability, and a change in personality or behaviour) and two or more of the following: fever (≥38°C) or history of fever during the current illness; seizures or focal neurological findings (with evidence of brain parenchyma involvement); CSF leucocyte count >4 cells/µL (for any age); EEG findings suggesting encephalitis; and abnormal neuroimaging findings (CT or MRI) suggestive of encephalitis	Granerod *et al.*[78]
Myelitis	Impairment of sensory, motor, or autonomic functions attributable to the spinal cord and one or more of the following: fever (≥38°C) or history of fever during the current illness; CSF leucocyte count >4 cells/µL (for patients ≥6 weeks of age) or >14 cells/µL (for patients <6 weeks of age); neuroimaging findings showing acute inflammation (with or without meninges involvement) or demyelination of the spinal cord	Adapted from Sejvar *et al*.[228]
Meningoencephalitis	Patients with CNS infection who fulfilled the criteria for both meningitis and encephalitis	
Encephalomyelitis	Patients with CNS infection who fulfilled the criteria for both encephalitis and myelitis	
Cerebral toxoplasmosis (aka *Toxoplasma* encephalitis)	Definite: HIV positive and presence of 1 or more cerebral mass lesions on CT or MRI and detection of *Toxoplasma gondii* in CSF by PCR and a documented clinical response to anti-toxoplasmosis treatment	Imran *et al*.[6]
	Presumptive: HIV positive and presence of 1 or more cerebral lesions on CT or MRI and a documented clinical response to anti-toxoplasmosis treatment	
Shunt infection	Definite: Compatible clinical syndrome (i.e. fever or history of fever, vomiting, neurological deficits, and/or altered consciousness) and detection of a bacterial pathogen from CSF or other appropriate sites (e.g. device puncture, overlying shunt wound, shunt tube, or cellulitis)	Overturf *et al.*[229]

Shunt infection	Probable: Compatible clinical syndrome (i.e. fever or history of fever, vomiting, neurological deficits, and/or altered consciousness) and CSF findings consistent with bacterial infection and negative blood, CSF, and device culture for bacteria	Overturf *et al.*[229]
Brain abscess	Patients with headache, fever or history of fever, focal neurological signs, and/or altered consciousness; and neuroimaging suggestive of abscess or surgical/anatomic evidence or positive cultures/histology from site or autopsy confirmation	Overturf *et al.*[229]
CNS infection - unclassified	Patients with symptoms, signs and/or neuroimaging compatible with CNS infection as deemed by the treating clinical team but did not fulfil the criteria for any of the CNS infection sub-types mentioned above	
Bacterial meningitis/encephalitis	Meningitis/encephalitis/meningoencephalitis and detection of an appropriate bacterial pathogen in CSF or blood by PCR, culture, or Gram stain	McGill *et al.*,[2] Granerod *et al.*[78]
Viral CNS infection	Meningitis/encephalitis/myelitis and detection of an appropriate viral pathogen by PCR or detection of viral-specific IgM by serologic test in CSF	Tan *et al.*[230]
Tuberculous meningitis (TBM), also referred to as tuberculous CNS infection in this study	Definite: Patients with one or more of the following symptoms and signs: fever, headache, neck stiffness, vomiting, altered consciousness (including lethargy and irritability), seizures, or focal neurological deficits; and detection *Mycobacterium tuberculosis* in CSF by microscopy, culture, or PCR	Marais *et al.*[231]
	Probable: Patients with symptoms and signs as described above and a TBM diagnostic score¶ of ≥10 (without available neuroimaging) or ≥12 (with neuroimaging) –where 2 points should either be obtained from CSF or neuroimaging and exclusion of alternative diagnoses	
	Possible: Patients with symptoms and signs as described above and a TBM diagnostic score¶ of 6-9 (without available neuroimaging) or 6-11 (with neuroimaging) and exclusion of alternative diagnoses	
Presumptive scrub typhus CNS infection	Meningitis/encephalitis and detection of *Orientia tsutsugamushi* specific IgM in serum with no other aetiological agents found in CSF	Adapted from Varghese *et al.*[232]
Cryptococcal CNS infection	Meningitis/encephalitis and identification of *Cryptococcus neoformans* in CSF by culture or antigen testing	Boulware *et al.*[233]
CNS infection of unknown aetiology	Patients with meningitis or encephalitis or myelitis with no aetiology found	

¶TBM diagnostic score was assessed based on 4 criteria categories: clinical symptoms and signs, CSF characteristics, neuroimaging findings, and evidence of tuberculosis elsewhere. Each category consisted of several criteria where each assigned a certain score. The clinical criteria category consisted of the presence of symptoms as described above for >5 days (score 4); one or more symptoms suggestive of systemic tuberculosis, including weight loss, night sweats, or persistent cough for >2 weeks (score 2); history of close contact with an individual with pulmonary tuberculosis or a positive tuberculous skin test (TST) or interferon-gamma release assay (IGRA) within the past year (for children aged <10 years) (score 2); focal neurological deficits –excluding cranial nerve palsies (score 1); cranial nerve palsy (score 1), and altered consciousness (score 1) with a maximum score of 6 for this clinical category. The CSF criteria category included clear appearance (score 1), CSF leucocyte count of 10-500/µL (score 1), CSF lymphocyte >50% (score 1), CSF protein >100 mg/dL (score 1), and CSF:blood glucose ratio <0.5 or an absolute CSF glucose <40 mg/dL (score 1) with a maximum score of 4 for this category. The neuroimaging criteria consisted of a finding of hydrocephalus (score 1), basal meningeal enhancement (score 2), tuberculoma (score 2), infarct (score 1), or pre-contrast basal hyperdensity (score 2) with a maximum score of 6. The evidence of tuberculosis elsewhere included chest radiographs showing signs of tuberculosis (score 2) or miliary tuberculosis (score 4); CT/MRI/ultrasound findings suggestive of tuberculosis outside the CNS (score 2); identification of *Mycobacterium tuberculosis* from extra-neural specimens by culture or microscopy (score 4) or by nucleic acid amplification test (score 4); with a maximum score of 4 for this category.

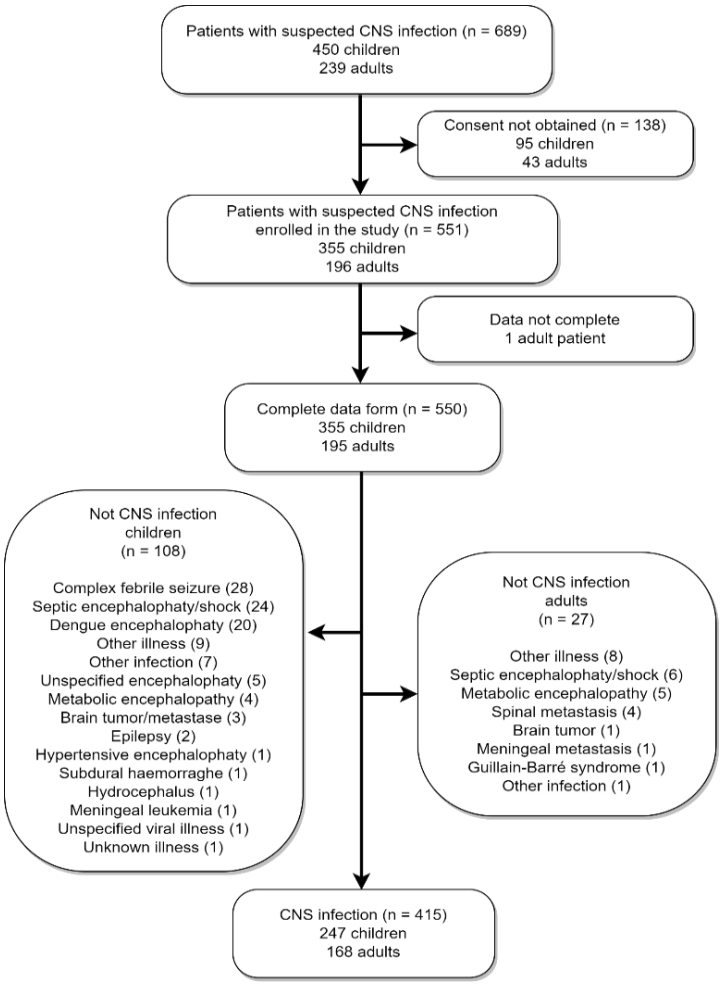
CNS – central nervous system; CSF – cerebrospinal fluid; PCR – polymerase chain reaction; EEG – electroencephalography; CT – computerised tomography; MRI – magnetic resonance imaging; HIV – human immunodeficiency virus.

3.3 Results

3.3.1 Demographics, clinical features, and management of overall paediatric and adult cohort

Over the 3-year study recruitment period, 689 patients, including 450 children and 239 adults, fulfilled the study inclusion criteria. Of these, 355 children and 196 adults were enrolled in the study. Sufficient data were available from 355 children and 195 adults, and therefore, these patients were included in the analysis (figure 3.1). Two-hundred-and-forty-seven (70%) of children and 168 (86%) of adults had syndromic CNS infection (as defined in table 3.1). Sub-types of the paediatric and adult CNS infection cases will be described separately in the following sub-section.

Figure 3.5 Study flow chart



The remaining children (n=108, 30%) and adults (n=27, 14%) did not have CNS infection; instead, a variety of final diagnoses for these patients were identified based on the final diagnoses made by the treating clinicians as documented in the patient's medical records. Of these, the three most common diagnoses were complex febrile seizure, septic encephalopathy and dengue encephalopathy in children; and other illnesses, septic encephalopathy, as well as metabolic encephalopathy in adults (figure 3.1). The complex febrile seizure was defined as a seizure related to febrile illness in children aged six months to five years without the presence of a CNS infection or acute electrolyte imbalance, which lasted >10 minutes, occurred twice or more during a febrile illness, or occurred with focal onset. Encephalopathy was described as altered consciousness (including lethargy, irritability, and a change in personality or behaviour) lasting >24 hours. Septic encephalopathy was clinically defined as encephalopathy, which occurred in patients with sepsis syndrome, whilst dengue encephalopathy was described as encephalopathy in patients with systemic dengue infection. Moreover, encephalopathy caused by chemical imbalance in the blood was defined as metabolic encephalopathy.

3.3.1.1 Demographics

Characteristics of the paediatric patients are presented in table 3.1. The median age of all children was 1.7 years (IQR 0.7-6.4 years). There was no significant difference in the median age of children who had syndromic CNS infection and those who did not (median age 1.7 years for both of these groups, p=0.576). The highest proportion of paediatric CNS infection cases occurred in children under one year of age, which accounted for 91/247 (37%) of cases. The majority (199/355 (56%)) of paediatric patients were male. There was no significant difference in the proportion of males between children with CNS infection and those without CNS infection (53.4% versus 62%, p=0.133).

In the adult group, the median age of all adults was 37 years (IQR 27-48 years) [table 3.2]. Adults with syndromic CNS infection were younger than those without CNS infection (median age 35 years versus 48 years, p=0.007). CNS infection occurred most frequently in younger adults; 115/168 (68%) cases were seen in those under 45 years of age. Sixty-five percent (65% (127/195)) of adults enrolled in the study were male. There was no significant difference in the proportion of males between adults who had CNS infection and those who did not (66% versus 63%, p=0.799).

3.3.1.2 Clinical presentation

The median symptom duration prior to Dr Sardjito Hospital admission for all children with suspected CNS infection was 5 days (IQR 2-10 days). Children with syndromic CNS infection had a longer symptom duration than those with no CNS infection (median 5 versus 4 days, p=0.004). Ninety-three percent (330/355) of children with suspected CNS infection sought medical treatment before admission to Dr Sardjito Hospital. Of these, (299/355)84% went to other hospital(s) either as inpatients or outpatients. These included 221 (62%) children who went to hospital(s) only; 31 (9%) children who went to both private practice and hospital(s); 30 (8%) children who went to both primary health care service and hospital(s); 11 (3%) children who went to both general practitioner and hospital(s); and eight (2%) children who went to hospital(s) and two other health providers, either general practitioner, private practice, or primary health care service. The remaining children who sought medical treatment prior to Dr Sardjito Hospital admission went to private practice only (n=16, 5%), primary health care only (n=8, 2%), general practitioner only (n=2, 0.6%), general practitioner plus primary health care (n=2, 0.6%), and private practice plus primary health care (n=1, 0.3%).

In the adult group, the median symptom duration prior to Dr Sardjito Hospital admission for all adults with suspected CNS infection was 14 days (IQR 7-28 days). Symptom duration

was significantly longer than for children (p<0.001). There was no significant difference in the median symptom duration between those with syndromic CNS infection and those who were later found to have no CNS infection (median 14 versus 9 days, p=0.379). Eighty percent (156/195) of adults with suspected CNS infection had sought medical treatment prior to hospital admission. Of these, 134 (69%) adults went to other hospital(s), either as inpatients or outpatients, including 117 (60%) adults who went to hospital(s) only, 13 (7%) adults who went to both general practitioner and hospital(s), six (3%) adults who went to both primary health care and hospital(s), and an adult who went to both a private specialist practice and a hospital. The remaining adults who sought medical help prior to Dr Sardjito Hospital admission went to primary health care only (n=10, 5%), general practitioner only (n=4, 2%), private specialist practice only (n=4, 2%), and general practitioner plus primary health care (n=1, 0.5%).

Symptom history

The majority of children with suspected CNS infection presented to the hospital with a history of confusion, accounting for 321/354 (90%) of cases. The second most common symptom was a history of seizures, which was experienced by 258/353 (73%) children. There were some significant differences in the symptom history between children who had CNS infection and those who did not (table 3.1). History of neck stiffness and confusion were significantly more common in children with syndromic CNS infection (70/240 (29%) and 236/247 (96%), respectively) than in those with no CNS infection (10/106 (10%) and 85/108 (79%), p<0.001 for both of these symptoms). Moreover, children with syndromic CNS infection were much more likely to have a history of vomiting (90/245 (37%) versus 52/108 (48%), p=0.044)) and seizures (191/246 (78%) versus 67/107 (63%), p=0.003)) compared to those with no CNS infection.

Table 3.1 Demographic and clinical features of children with suspected CNS infection enrolled in the study

	All paediatric patients (n=355)	Syndromic CNS infection (n=247)	Not CNS infection (n=108)	P value
Demographics				
Age (years)	1.7 (0.7,6.4)	1.7 (0.6,6.8)	1.7 (0.8,6.1)	0.576
Percentage male	199/355 (56%)	132/247 (53.4%)	67/108 (62%)	0.133
Symptoms				
Headache	58/304 (19%)	45/210 (21.4%)	13/94 (14%)	0.119
Neck stiffness	80/346 (23%)	70/240 (29%)	10/106 (10%)	**<0.001**
Hypersensitivity to light	6/293 (2%)	5/206 (2%)	1/87 (1%)	0.673
History of rash	28/342 (8%)	20/239 (8%)	8/103 (8%)	0.852
Confusion[¥]	321/354 (90%)	236/247 (96%)	85/108 (79%)	**<0.001**
Respiratory symptoms[§]	156/351 (44%)	108/244 (44%)	48/107 (44%)	0.917
Vomiting	142/353 (40%)	90/245 (37%)	52/108 (48%)	**0.044**
Diarrhoea	132/350 (38%)	92/243 (38%)	40/107 (37%)	0.932
Genital ulcer	0/339 (0%)	0/238 (0%)	0/101 (0%)	N/A
Seizures	258/353 (73%)	191/246 (78%)	67/107 (63%)	**0.003**
Previous history of CNS infection	34/338 (10%)	26/236 (11%)	8/102 (8%)	0.373
Signs				
Fever[¶] (≥38 °C)	190/355 (54%)	130/247 (53%)	60/108 (56%)	0.611
Meningeal sign(s) positive[‡]	70/342 (20%)	56/237 (24%)	14/105 (13%)	**0.030**
Cranial nerve palsy	25/294 (9%)	22/203 (11%)	3/91 (3%)	**0.032**
Power abnormality	93/273 (34%)	73/185 (40%)	20/88 (23%)	**0.006**
Coma (GCS ≤8)	111/355 (31%)	89/247 (36%)	22/108 (20%)	**0.003**
Laboratory results				
HIV-positive	4/9 (44%)	4/8 (50%)	0/1 (0%)	1.000
Blood leucocyte count (x 10^9/L)	10.6 (7.2,15.2) [n=352]	10.7 (8,16) [n=245]	10.3 (6.2,14) [n=107]	0.117
Blood neutrophil (%)	67 (49,78) [n=345]	69 (52,81) [n=244]	59 (41,74) [n=101]	**0.001**
CSF pleocytosis[†]	110/251 (44%)	108/208 (52%)	2/43 (5%)	**<0.001**
CSF leucocyte count (cells/µL)	3 (0,36) [n=251]	5 (1,54) [n=208]	0 (0,1) [n=43]	**<0.001**
CSF neutrophil (%)	10 (0,45) [n=250]	18 (0,58) [n=208]	0 (0,4) [n=42]	**<0.001**
CSF protein (mg/dL)	50 (20,90) [n=251]	50 (20,100) [n=208]	40 (20,60) [n=43]	0.103
CSF glucose (mg/dL)	68 (54,83) [n=251]	68 (50,83) [n=208]	70 (62,80) [n=43]	0.116
CSF:blood glucose ratio	0.6 (0.4,0.7) [n=212]	0.6 (0.4,0.7) [n=179]	0.6 (0.4,0.8) [n=33]	0.171
Neuroimaging				
Abnormal CT scan result	204/216 (94%)	165/176 (94%)	39/40 (98%)	0.701
Abnormal MRI result	6/8 (75%)	6/7 (86%)	0/1 (0%)	0.250

Values are median (IQR) for continuous data and n/number evaluable (%) for categorical data. [¥]Includes confusion, irritability, lethargy and decreased consciousness. [§]Includes coryzal symptoms, sore throat, and chronic cough. [¶]Fever at the time of admission. [‡]Includes nuchal rigidity, Kernig's sign and Brudzinski's signs. [†]CSF leucocyte count >4 cells/µL (for patients ≥6 weeks of age) or >14 cells/µL (for patients <6 weeks of age).

CNS – central nervous system; vs – versus; GCS – Glasgow Coma Scale; CSF – cerebrospinal fluid; CT – computerised tomography; MRI – magnetic resonance imaging.

Table 3.10 Demographic and clinical features of adults with suspected CNS infection enrolled in the study

	All adult patients (n=195)	Syndromic CNS infection (n=168)	Not CNS infection (n=27)	P value
Demographics				
Age (years)	37 (27,48)	35 (22,57)	48 (24,56)	**0.007**
Percentage Male	127/195 (65%)	110/168 (66%)	17/27 (63%)	0.799
Symptoms				
Headache	130/192 (68%)	120/165 (73%)	10/27 (37%)	**<0.001**
Neck stiffness	57/189 (30%)	51/163 (31%)	6/26 (23%)	0.397
Hypersensitivity to light	23/186 (12%)	22/159 (14%)	1/27 (8%)	0.208
History of rash	18/183 (10%)	14/142 (9%)	4/27 (15%)	0.311
Confusion[¥]	152/195 (79%)	139/168 (83%)	15/27 (56%)	**0.001**
Respiratory symptoms[§]	80/195 (41%)	68/168 (41%)	12/27 (44%)	0.697
Vomiting	73/192 (38%)	69/167 (41%)	4/25 (16%)	**0.015**
Diarrhoea	29/192 (15%)	26/166 (16%)	3/26 (12%)	0.772
Genital ulcer	7/167 (4%)	7/142 (5%)	0/25 (0%)	0.596
Seizures	59/194 (30%)	56/167 (34%)	3/27 (11%)	**0.019**
Previous history of CNS infection	17/186 (9%)	13/161 (8%)	4/25 (16%)	0.254
Signs				
Fever[¶] (≥38 °C)	64/195 (33%)	55/168 (33%)	9/27 (33%)	0.951
Meningeal sign(s) positive[‡]	75/191 (39%)	67/164 (41%)	8/27 (30%)	0.268
Cranial nerve palsy	60/145 (41%)	55/123 (45%)	5/22 (23%)	0.054
Power abnormality	87/140 (62%)	75/120 (63%)	12/20 (60%)	0.831
Coma (GCS ≤8)	42/195 (22%)	38/168 (23%)	4/27 (15%)	0.360
Laboratory results				
HIV-positive	71/169 (42%)	66/148 (45%)	5/21 (24%)	0.071
Blood leucocyte count (x 10^9/L)	9.7 (6.7,14) [n=194]	9.7(6.7,18.1) [n=167]	10 (7.9,14.3) [n=27]	0.410
Blood neutrophil (%)	81 (71,87) [n=186]	81 (71, 92) [n=161]	78 (72,92) [n=25]	0.968
CSF opening pressure (cm CSF)	13 (9,22) [n=117]	14 (9.5,23) [n=102]	10.5 (8.5,16.5) [n= 15]	0.164
CSF pleocytosis[†]	90/143 (63%)	86/127 (68%)	4/16 (25%)	**0.001**
CSF leucocyte count (cells/µL)	8 (1,70) [n=143]	9 (2,84) [n=127]	1 (0,5) [n=16]	**<0.001**
CSF neutrophil (%)	15 (2.8,40) [n=142]	15 (6,40) [n=126]	6.5 (0,54) [n=16]	0.357
CSF protein (mg/dL)	80 (50,180) [n=143]	80 (50,170) [n=127]	85 (50,257.5) [n=16]	0.455
CSF glucose (mg/dL)	67 (50,85) [n=143]	67 (50,84) [n=127]	68.5 (55,95) [n=16]	0.363
CSF:blood glucose ratio	0.5 (0.4,0.6) [n=139]	0.5 (0.4,0.6) [n=123]	0.6 (0.5,0.7) [n=16]	**0.035**
Neuroimaging				
Abnormal CT scan result	70/83 (84%)	63/73 (86%)	7/10 (70%)	0.350
Abnormal MRI result	49/55 (89%)	42/46 (91%)	7/9 (78%)	0.251

Values are median (IQR) for continuous data and n/number evaluable (%) for categorical data. [¥]Includes confusion and decreased consciousness. [§]Includes coryzal symptoms, sore throat, and chronic cough. [¶]Fever at the time of admission. [‡]Includes nuchal rigidity, Kernig's sign and Brudzinski's signs. [†]CSF leucocyte count >4 cells/µL (for patients ≥6 weeks of age) or >14 cells/µL (for patients <6 weeks of age).

CNS – central nervous system; vs – versus; GCS – Glasgow Coma Scale; CSF – cerebrospinal fluid; CT – computerised tomography; MRI – magnetic resonance imaging.

Similar to the paediatric group, most adults with suspected CNS infection (152/195 (79%)) had a history of confusion. Furthermore, adult patients commonly presented to the hospital with a history of headache, accounting for 130/192 (68%) cases. Comparing the adults who had syndromic CNS infection and those who did not, the former group was significantly more likely to present with a history of headache (120/165 (73%) versus 10/27 (37%), p<0.001), confusion (139/168 (83%) versus 15/27 (56%), p=0.001), vomiting (69/167 (41%) versus 4/25 (16%), p=0.015), and seizures (56/167 (34%) versus 3/27 (11%), p=0.019) (table 3.2).

Clinical signs

Fever (≥38 °C) at the time of admission was the most common clinical sign found in children with suspected CNS infection, where over half (199/355 (54%)) of them had it. The next most common clinical signs were power abnormality and coma (Glasgow Comma Scale / GCS <8), which accounted for 93/273 (34%) and 111/355 (31%) of cases, respectively. Comparing children with syndromic CNS infection to those with no CNS infection, the former group was significantly more likely to have a positive meningeal sign(s) (56/237 (24%) versus 14/105 (13%), p=0.030), cranial nerve palsy (22/203 (11%) versus 3/91 (3%), p=0.032), power abnormality (73/185 (40%) versus 20/88 (23%), p=0.006), and Glasgow Coma Scale (GCS) ≤8 (89/247 (36%) versus 22/108 (20%), p=0.003) (table 3.1).

In the adult group, the most common clinical sign found in all adults with suspected CNS infection was power abnormality (87/140 (62%)), followed by cranial nerve palsy (60/145 (41%)) and meningeal sign(s) positive (75/191 (39%)) (table 3.2). Fever at the time of admission was less common in adults than in children with suspected CNS infection, where only 64/195 (33%) of adults had it. There were no significant differences in the

clinical signs between adults who had syndromic CNS infection and those who did not (table 3.2).

3.3.1.3 Investigations performed

Blood and CSF investigations

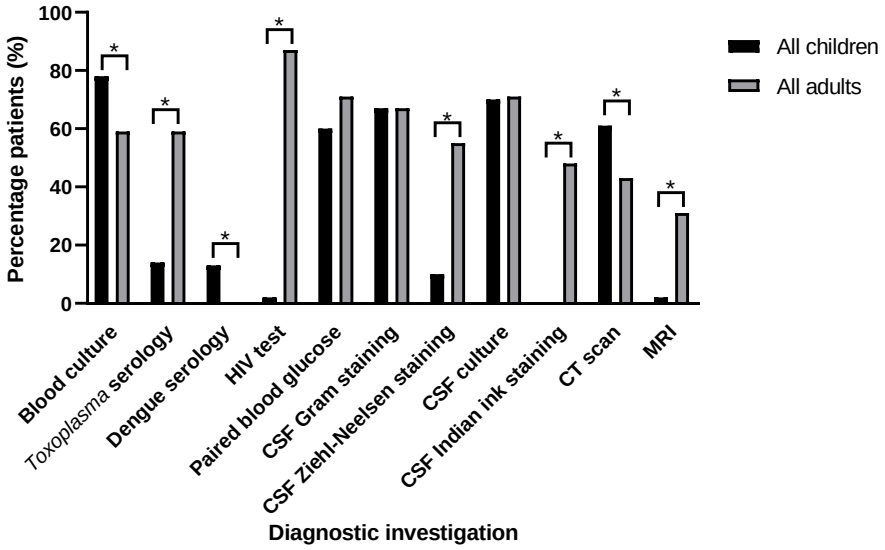
In the paediatric group, blood culture was the most frequent investigation done in those with suspected CNS infection, where it was performed in 278/355 (78%) children (figure 3.2). *Toxoplasma* and dengue serology tests were performed in 51/355 (14%) and 46/355 (13%) children. Both tests were performed on serum samples and often in single rather than paired samples. Human immunodeficiency virus (HIV) testing was not performed on a routine basis in children with suspected CNS infection, where only 9/355 (3%) children had it performed (figure 3.2). Paired blood glucose (i.e. to measure CSF:blood glucose ratio) was performed in 212/355 (60%) children.

Lumbar puncture (LP) / extra-ventricular drainage (EVD) was performed in 251/355 (71%) children enrolled in the study with a median of one day (IQR 0.5-3 days) from the day of admission. The median time from first antibiotic administration to LP/EVD was one day (IQR 0-4 days), whilst the median time from neuroimaging to LP/EVD was also one day (IQR -1 to 5 days). Among the children who did not have an LP performed, it was contraindicated by neuroimaging in 6/104 (6%) children and uncontrolled seizures in two (2%) children. Moreover, LP was not performed in 93/104 (89%) children due to a decision taken by the treating clinician for other conditions not mentioned above. Two of 104 (2%) children's families refused to undergo LP, while the reason for not performing LP was not recorded in two (2%) children.

CSF culture was the most frequent CSF investigation done in children, where it was performed in 249/355 (70%) children and in 249/251 (99%) of all children who underwent

LP/EVD (figure 3.2). The second most frequent CSF investigation was the CSF Gram stain, performed in 237/355 (67%) children. CSF Ziehl-Neelsen stain was not performed regularly in children where only 36/355 (10%) children had it performed, and none of these children had acid-fast bacilli detected in their CSF samples. Moreover, none of the children had CSF Indian ink stain performed in their samples.

Figure 3.6 Comparison of children and adults having certain diagnostic investigations. The graph shows the proportion (%) of children (n=355) and adults (n=168) with suspected CNS infection having specific diagnostic investigations at the hospital. *Significant difference ($p<0.05$) between the proportion of children and adults who underwent a specific diagnostic investigation.



In the adult group, blood culture and Toxoplasma serology tests were performed only in 116/195 (59%) patients. Interestingly, none of the adult patients had a dengue serology test performed on their samples. HIV testing was done more regularly in the adult group than in the paediatric group. It was performed in 169/195 (87%) adults overall. One-hundred-and-thirty-nine of 195 (71%) adults with suspected CNS infection had a paired blood glucose taken.

LP/EVD was performed in 143 of 195 (73%) adults with a median of three days (IQR 1-5 days) from the admission day. The median time to LP/EVD from the first antibiotic

administration was one day (IQR 0-3 days), whereas the median time to LP/EVD from neuroimaging was zero day (IQR -2 to 5 days). Of 52 adults who did not have an LP performed, it was contraindicated by neuroimaging in three (6%) patients and by uncontrolled seizures in three (6%) patients. Furthermore, LP was not performed in 25/52 (48%) adults due to a decision taken by the treating clinician for reasons other than the aforementioned conditions. Seven of 52 (13%) adults (or their families) refused to undergo LP, whereas the reason for not performing LP was not documented in 16 (29%) adults.

Similar to the paediatric group, CSF culture was the most frequent CSF investigation done in adults, where it was performed in 138/168 (71%) patients overall and in 138/143 (97%) patients who underwent LP/EVD. CSF Gram stain was performed in 130/195 (67%) adults with suspected CNS infection. Furthermore, the CSF Ziehl-Neelsen stain was performed in 107/195 (55%) adults, but it failed to detect acid-fast bacilli in any CSF samples.

CSF Indian ink stain was introduced as a routine test at Dr Sardjito Hospital for adults with suspected CNS infection in February 2016 and therefore was only done in 95/195 (49%) patients. A positive result was found in 11 patients, including ten with CNS infection and one without CNS infection. In the latter case, the CSF culture was positive for *Candida albicans*; however, the patient was not treated with any antifungal drugs, and a pathological anatomy investigation of the brain tissue showed a malignancy. Moreover, the patient was discharged alive with a final hospital diagnosis of brain tumour. Of the ten patients with CNS infection with a positive Indian ink stain result, only one had a positive CSF culture for *C. neoformans*.

Comparing the laboratory investigations in all children and all adults, there were many significant differences in the frequency of investigations performed in both age groups

(figure 3.2). The frequencies of blood culture and dengue serology tests were much higher in children than in adults ($p<0.001$ for both of these investigations). In contrast, the frequencies of paired blood glucose, CSF culture, CSF Ziehl-Neelsen, and Indian ink stain, *Toxoplasma* serology, and HIV tests were much higher in adults than in children ($p=0.007$ for paired blood glucose and $p<0.001$ for the remaining investigations).

Neuroimaging

Neuroimaging was performed in 219/355 (62%) children overall, of which 211 (59%) had a CT scan only, two (1%) had an MRI only, and six (2%) had both. CT scan was performed in 61% (217/355) of children with suspected CNS infection (figure 3.2). In contrast, MRI was performed only in 8/355 (2%) children. Of 251 children who had LP/EVD, 80 (32%) had neuroimaging performed before LP/EVD. Sixty of 80 (75%) children had a recognised indication for this, including Glasgow Coma Scale (GCS) ≤12 in 32 (41%) cases, focal neurological signs in 11 (14%) cases, and both low GCS and focal neurological deficit in 17 (21%) cases. The remaining children (20/80 (25%)) did not have a recognised contraindication to have neuroimaging performed before LP/EVD.

In the adult group, neuroimaging was performed in 126/195 (65%) patients, of which 66 (34%) had a CT scan only, 43 (22%) had an MRI only, and 17 (9%) had both CT scan and MRI performed. There was a significant difference in the proportion of adult and paediatric patients with suspected CNS infection who underwent CT scans (83/195 (43%) versus 61% (217/355), $p<0.001$) (figure 3.2). In contrast, the proportion of adults with suspected CNS infection who underwent MRI was much higher than that of children with suspected CNS infection (60/195 (31%) versus 8/355 (2%), $p<0.001$).

Of the 143 adults who underwent LP/EVD, 38 (27%) had neuroimaging performed before LP. Twenty-eight of 38 (74%) patients had a justified indication for this, of which the most

common was focal neurological signs in 16 (42%) patients, followed by a combination of both focal neurological signs and GCS ≤12 in seven (18%) and a low GCS only in 5 (13%) patients. The remaining adult patients (10/38 (26%)) did not have a justified contraindication for performing neuroimaging before LP, two of whom were immunocompromised.

3.3.1.4 Results of investigations

Results of blood and CSF parameters

The median blood leucocyte count in all children with suspected CNS infection was 10.6 x 10^9 cells/L (IQR 7.2-15.2 10^9 cells/L), whilst the median blood neutrophil percentage was 67% (IQR 49-78%). Children with syndromic CNS infection had a significantly higher blood neutrophil percentage than those with no CNS infection (median 69% versus 59%, p=0.001) (table 3.1). In the CSF, CSF pleocytosis was present in 110/251 (44%) children with suspected CNS infection. The median (IQR) CSF leucocyte count and CSF neutrophil percentages were 3 (0-36) cells/µL and 10% (0-45%). Sixty-three of 251 (25%) children who underwent LP/EVD, including 37/208 (18%) with syndromic CNS infection and 26/43 (60%) with no CNS infection, had no cells on CSF leucocyte cell count. Furthermore, the median (IQR) CSF protein, CSF glucose, and CSF:blood glucose ratio were 50 (20-90) mg/dL, 68 (54-84 mg/dL), and 0.6 (0.4-0.7) mg/dL, respectively. Comparing children who had syndromic CNS infection and those who did not, those with syndromic CNS infection were much more likely to have CSF pleocytosis (108/208 (52%) versus 2/43 (5%)) and had a notably higher CSF leucocyte count (median 5 cells/µL versus 0 cell/µL) and higher neutrophil percentage (median 18% versus 0%) (p<0.001 for all features).

In the adult group, the median blood leucocyte count and blood neutrophil percentage of all adults with suspected CNS infection was 9.7 x 10^9 (IQR 6.7-14 x 10^9) cells/L and 81%

(IQR 71-87%). Unlike in children, there were no significant differences in blood parameters between adults who had syndromic CNS infection and those who did not (table 3.2). Sixty-three percent (90/143) of adults with suspected CNS infection had CSF pleocytosis. The median (IQR) CSF leucocyte count and CSF neutrophil percentages were 8 (1-70) cells/µL and 15% (2.8-40%). Ten of 143 (7%) adults who underwent LP/EVD, including 6/127 (5%) with syndromic CNS infection and 4/16 (2.5%) with no CNS infection, did not have any leucocyte in CSF. Additionally, the median (IQR) CSF protein, CSF glucose, and CSF:blood glucose ratio of all adults was 80 (50-180) mg/dL, 67 (50-85)mg/dL, and 0.5 (0.4-0.6), respectively. There were a few notable differences in CSF parameters between adults with syndromic CNS infection and those with no CNS infection, where the former group had a significantly higher proportion of CSF pleocytosis (86/127 (68%)) versus 4/16 (25%), p=0.001), higher CSF leucocyte count (median 9 cells/µL versus 1 cells/µL, p<0.001) and lower CSF:blood glucose ratio (median 0.5 versus 0.6, p=0.035).

Results of pathogen detection testing at the hospital

Four of 9 (44%) children with suspected CNS infection who tested for HIV were HIV-positive, all of whom had syndromic CNS infection. The median CD4 count was 33 (IQR 6-493).

To evaluate the yield of the standard hospital diagnostic testing in identifying the pathogen causing CNS infection in children, I summarised all detected pathogens in table 3.3. Fifty-two of 247 children with syndromic CNS infection had a pathogen detected by the CSF culture, CSF Gram stain, pus culture from the underlying wound (in a neurosurgical case), or blood culture (in those who did not undergo LP).

Eighteen cases had a CSF pleocytosis and grew a pathogen in their CSF commonly reported to cause CNS infection in children or infants (under 1 year of age): *Staphylococcus*

haemolyticus[234, 235] (n=6), *Escherichia coli*[236-238] (n=3), *Staphylococcus aureus*[239, 240] (n=2), Gram-negative bacilli[241] [organism not specified] (n=2), *Salmonella paratyphi type B*[242, 243] (n=1), *Salmonella* species[242, 243] (unspecified) [n=1], *M. tuberculosis*[244] (n=1), *Enterococcus faecium* (n=1)[245-247], and coagulase-positive *Staphylococci* species[248, 249] (organism not specified) [n=1].

Staphylococcus haemolyticus is a coagulase-negative *Staphylococci* (CONS) that has been associated with sepsis and meningitis in neonates and infants. This pathogen can cause infection in older children, in particular hospital-associated infection, mainly in those with immunocompromised conditions. All of the children whose CSF culture was positive for *Staphylococcus haemolyticus* were older than 1 year of age and immunocompetent. Therefore, the positive *Staphylococcus haemolyticus* culture result was more likely to be a contaminant than the actual causative pathogen for their CNS infection. *E. coli,* Gram-negative bacilli, and *Salmonella* species are common aetiology of CNS infection in infants. All children who tested positive for the aforementioned pathogens were under 1 year of age; thus, the pathogens were likely to be the definite cause of their CNS infection. *M. tuberculosis, Staphylococcus aureus*, coagulase-positive *Staphylococci,* and *Enterococcus faecium* have been linked with paediatric CNS infection at any age; therefore, these pathogens are considered the cause of their CNS infection. In summary, all of the pathogens mentioned above, except for *Staphylococcus haemolyticus* (n=6), are considered the aetiology of CNS infection (n=12).

Eleven children were diagnosed with a neurosurgical infection, ten with shunt infection and one with post-neurosurgical encephalitis. Nine children had bacteria grown in their CSF; the list is presented in table 3.3. A case of *Staphylococcus haemolyticus* neurosurgical infection was identified by pus culture from the underlying neurosurgical wound, whilst the Gram-negative diplococci case was detected by CSF Gram stain.

Table 3.11 Pathogens identified by the standard hospital diagnostic testing in paediatric cases

Pathogens detected by the standard hospital testing in paediatric cases		Number of cases
1.	**Cases with CSF pleocytosis and pathogens commonly associated with non-neurosurgical CNS infection**	
	Staphylococcus haemolyticus	6
	Escherichia coli	**3**
	***Salmonella* species ¥**	**2**
	Staphylococcus aureus	**2**
	Gram-negative bacilli (organism not specified) †	**2**
	Mycobacterium tuberculosis*	**1**
	Coagulase-positive *Staphylococcus* (organism not specified)	**1**
	Enterococcus faecium	**1**
2.	**Cases associated with neurosurgical infection**	
	Staphylococcus haemolyticus	**2 ¶**
	Klebsiella pneumoniae	**1**
	Enterobacter cloacae	**1**
	Acinetobacter baumanii	**1**
	Staphylococcus warneri	**1**
	Staphylococcus epidermidis	**1**
	Pseudomonas aeruginosa	**1**
	Kocuria kristinae	**1**
	Gram-negative *diplococci* (organism not specified) †	**1**
	Pseudomonas stutzeri	**1**
3.	**Cases with CSF pleocytosis but pathogen infrequently associated with non-neurosurgical CNS infection in children**	
	Staphylococcus hominis	4
	Bacillus anthracis	**1**
4.	**Cases with CSF pleocytosis but pathogen unlikely to be associated with non-neurosurgical CNS infection in children**	
	Acinetobacter lwoffii	1
	Leuconostoc mesenteroides ssp. cremoris	1
	Pasteurella canis	1
5.	**Cases with absent CSF pleocytosis**	
	Staphylococcus haemolyticus	3
	Coagulase-negative *Staphylococcus* (organism not specified)	1
	Brevundimonas diminuta/vesicularis	1
	Staphylococcus cohni ssp. urealyticum	1
	Pseudomonas stutzeri	1
	Rhizobium radiobacter	1
	Pantoea species (unspecified)	1
	Acinetobacter baumanii	1
	Tetrads Gram-positive cocci (organism not specified) †	1
6.	**Cases with syndromic CNS infection but did not undergo lumbar puncture – bacteria detected by blood culture**	
	Escherichia coli	**1**
	Sphingomonas paucimobilis	**1**
	Staphylococcus haemolyticus	1

Cronobacter sakazakii	1

Organisms written in bold were considered as the pathogens causing CNS infection in the present study, whilst the remaining organisms not written in bold were considered contaminants.

†Organisms were detected by CSF Gram stain. *Mycobacterium tuberculosis was detected by CSF MTB/RIF GeneXpert. ¥Include *Salmonella paratyphi type B* (n=1, detected by CSF culture) and *Salmonella* species (species not specified, n=1, detected by blood culture in a patient with CSF pleocytosis). ¶*Staphylococcus haemolyticus* was identified by CSF culture in 1 case and by pus culture from the underlying wound in 1 case.

CSF – cerebrospinal fluid; CNS – central nervous system.

Four children had a CSF pleocytosis and grew a pathogen in their CSF infrequently reported (e.g. reported in case series) to cause CNS infection; *Staphylococcus hominis*[250, 251] (n=4) and *Bacillus anthracis*[252, 253] (n=1). Like *Staphylococcus haemolyticus*, *Staphylococcus hominis* is a CONS that is unlikely to cause CNS infection in immunocompetent children aged >1 year. All children who tested positive for *Staphylococcus hominis* were over 1 year of age and immunocompetent. Thus, in these cases, *Staphylococcus hominis* was likely to be a contaminant. *Bacillus anthracis* is included as a possible cause of CNS infection.

Three children had a CSF pleocytosis and grew a pathogen in their CSF very rarely (a single case report) or not reported to cause CNS infection. The pathogens included *Acinetobacter lwoffii*[254] (n=1), *Leuconostoc mesenteroides ssp. cremoris*[255] (n=1) and *Pasteurella canis* (n=1). These pathogens are presumed to be contaminants and excluded. Eleven cases did not exhibit CSF pleocytosis. Therefore, the pathogens grown in CSF are presumed to be contaminants and excluded. The bacteria grown are listed in table 3.3.

In summary, 26/247 (11%) children had a pathogen associated with CNS infection detected by standard hospital testing. Therefore, these cases are categorised into paediatric CNS infection with a known cause. The pathogens were detected by CSF culture (n=18, including six children who had a CSF Gram stain result consistent with the CSF culture result and a child who had CSF Gram stain and blood culture results consistent

with the CSF culture result), CSF Gram stain only (n=3), blood culture only (n=3), CSF GeneXpert (n=1), and pus culture from the underlying VP shunt wound (n=1). Among children with syndromic CNS infection, CSF culture and blood culture were performed in 206 children each; whilst CSF Gram stain was performed in 194 children. Therefore, the positivity rates of CSF culture, CSF Gram stain, and blood culture were 8.7% (18/206), 5.2% (10/194), and 1.9% (4/206), respectively. *E. coli* was the most common pathogen detected in children, accounting for four cases overall. The other pathogens detected in the remaining 26 paediatric cases were presumed to be contaminants. Thus, these cases and all other cases with no pathogen detected by the standard hospital testing (n=195) are categorised as paediatric CNS infection with an unknown cause (n=221, 89%).

In the adult group, 71/169 (42%) adults with suspected CNS infection who tested for HIV were HIV-positive with a median CD4 count of 18 (IQR 11-67). These included 66/148 (45%) adults with syndromic CNS infection and 5/21 (24%) with no CNS infection (table 3.3). Among adults with syndromic CNS infection, 37 patients had a pathogen detected at the hospital by CSF culture and blood culture/smear (in those who did not have an LP performed) [table 3.4]. Five cases had a CSF pleocytosis and grew a pathogen in their CSF commonly reported to cause CNS infection in adults, including *Acinetobacter baumanii*[245, 261] (n=2), *S. pneumoniae* (n=1), *C. neoformans* (n=1) and *Pseudomonas* species[262, 263] (unspecified) [n=1]. One of the *Acinetobacter baumannii* cases had a positive Gram stain result consistent with the pathogen. Sixteen cases had a CSF pleocytosis but grew a pathogen rarely reported as a cause of adult CNS infection. The pathogens are listed in table 3.4 and are presumed to be contaminants. Ten adult cases in whom a pathogen was grown from their CSF did not have a CSF pleocytosis. Thus, the pathogens (listed in table 3.4) are presumed contaminants.

Table 3.12 Pathogens identified by the standard hospital diagnostic testing in adult cases

Pathogens detected by the standard hospital testing in adult cases	Number of cases
1. Cases with CSF pleocytosis and pathogens commonly associated with non-neurosurgical CNS infection in adults	
Acinetobacter baumannii	**2**
Streptococcus pneumoniae	**1**
Cryptococcus neoformans	**1**
***Pseudomonas* species (unspecified)**	**1**
2. Cases with CSF pleocytosis but pathogen unlikely to be associated with non-neurosurgical CNS infection in adults	
Staphylococcus haemolyticus	5
Brevundimonas diminuta/vesicularis	2
Enterococcus faecium	1
Staphylococcus epidermidis	1
Staphylococcus capitis	1
Kocuria varians	1
Enterobacter aerogenes	1
Acinetobacter lwoffii	1
Staphylococcus arlettae	1
Micrococcus tetragenus	1
Staphylococcus sciuri	1
3. Cases with absent CSF pleocytosis	
Staphylococcus hominis	2
Staphylococcus epidermidis	1
Acinetobacter baumannii	1
Staphylococcus warneri	1
Pseudomonas fluorescen	1
Pseudomonas stutzeri	1
Pantoea species (unspecified)	1
Staphylococcus arlettae	1
Kocuria rosea	1
4. Cases with syndromic CNS infection but did not undergo lumbar puncture – bacteria detected by blood culture (n=5) or blood smear (n=1)	
Klebsiella pneumoniae	**2**
***Shigella* group (species not specified)**	**1**
Staphylococcus hominis	**1**
Staphylococcus saprophyticus	1
***Plasmodium falciparum*¶**	**1**

Organisms written in bold were considered as the pathogens causing CNS infection in the present study, whilst the remaining organisms not written in bold were considered contaminants.

¶Organism was detected by blood smear.

CSF – cerebrospinal fluid; CNS – central nervous system.

Five adults with syndromic CNS infection did not have an LP performed, but each had a positive blood culture for the following pathogens: *K. pneumoniae* (n=2), *Shigella* group (species not specified) [n=1], *Staphylococcus hominis* (n=1), *Staphylococcus saprophyticus* (n=1). *K. pneumoniae* has commonly been reported in bacterial meningitis among adults,[264] whilst Shigella groups[265, 266 265-269] have been infrequently reported in such cases. Additionally, one adult had a positive blood smear for *Plasmodium falciparum*, a pathogen known to cause cerebral malaria. Both *Staphylococcus hominis* and *Staphylococcus saprophyticus* are CONS and very rarely cause non-neurosurgical CNS infection in immunocompetent adults.[268-270] A patient whose blood culture was positive for *Staphylococcus hominis* was HIV-positive; therefore, the pathogen is presumed to be the aetiology of his CNS infection. Another patient who tested positive for *Staphylococcus saprophyticus* was immunocompetent; thus, the pathogen is presumed to be a contaminant.

In summary, 10/168 (6%) adults had a pathogen consistent with the aetiology of CNS infection detected in their CSF/blood and thus classified as adult CNS infection with a known cause. The pathogens were detected by CSF culture (n=4), both CSF culture and CSF Gram stain (n=1), blood culture (n=4), and blood smear (n=1). The positivity rates of CSF and blood cultures were 4.1% (5/122) and 3.9% (4/103), respectively. *Acinetobacter baumannii* and *K. pneumoniae* were the two most common pathogens identified by the standard hospital testing, accounting for two cases each. The remaining 27 adults were presumed to have contaminants rather than causative pathogens detected in their CSF. These patients, in addition to the remaining (n=131) adults who did not have a pathogen detected by the standard hospital testing, are classified as adult CNS infection with an unknown cause (n=158, 94%).

3.3.1.5 Treatments

Antibiotics

Three-hundred-and-forty-three children with suspected CNS infection (97%) received at least one dose of antibiotics, 273 (69%) of whom received more than one type of antibiotic. Of 248 children who underwent LP/EVD, 228 (92%) received antibiotics prior to LP/EVD with a median (IQR) of three (1-10) doses. Cefotaxime was the most common antibiotic given to children (267/355 (78%)), followed by ampicillin (113/355 (33%)).

In the adult group, 186 patients with suspected CNS infection (95%) received at least one dose of antibiotics, 103 (53%) of whom received multiple antibiotics. One-hundred-and-eleven of 143 (78%) adults who underwent LP/EVD received antibiotics before LP with a median of four (1-8) doses. In contrast to the children group, the two most frequent antibiotics administered in adults were ceftazidime (88/186 (47%)) and ceftriaxone (86/186 (44%)).

Anti-tuberculosis

Among 355 children with suspected CNS infection, 12 (3%) received anti-tuberculosis treatment. These included nine children with syndromic CNS infection and three children who were later categorised as having no CNS infection. The latter group had final neurological diagnoses of brain tumour (n=1), febrile seizure (n=1), and metabolic encephalopathy (n=1).

Forty of 195 (21%) adults with suspected CNS infection received anti-tuberculosis treatment. These consisted of 33 adults with syndromic CNS infection and seven with no CNS infection. Twenty-one of the 33 adults with syndromic CNS infection who received anti-tuberculosis treatment had an abnormal chest X-ray result, of which ten were described as pulmonary TB imaging by radiologists. Among those with no CNS infection,

the final neurological diagnoses included spinal metastasis (n=2), metabolic encephalopathy (n=2), septic encephalopathy (n=1), brain tumour (n=1), and other illness (n=1). Six of these patients had an abnormal chest X-ray result on hospital admission, three of which were interpreted as pulmonary TB pictures by the radiologist.

Anti-toxoplasmosis

Thirty-three adults with suspected CNS infection received anti-toxoplasmosis treatment, including 32 adults (30 HIV-positive and two HIV-negative) with syndromic CNS infection and one HIV-positive adult later diagnosed with no CNS infection. Of the 30 HIV-positive adults with syndromic CNS infection who received anti-toxoplasmosis treatment, two had increased anti-toxoplasma IgM, and all had increased anti-toxoplasma IgG. Twelve of those 30 HIV-positive adults had a neuroimaging result consistent with cerebral toxoplasmosis, eight had a neuroimaging result not consistent with cerebral toxoplasmosis, and ten did not have CT or MRI performed.

Antivirals

Forty-seven children with suspected CNS infection (13%) received at least one dose of antivirals (acyclovir, ganciclovir, or valganciclovir), 36 (77%) of whom received them for five or more days. The median (IQR) duration for antiviral treatment in children was 11 (6-14) days. Among four HIV-positive children, one (25%) received anti-retroviral (ARV) treatment, whilst the HIV-treatment status of the remaining children was not known (i.e. not recorded in case record forms).

Of 195 adults with suspected CNS infection, 25 (13%) received at least one dose of antivirals. Of these, 20 (80%) received the antivirals for five or more days. The antiviral treatment in adults was given for a median (IQR) duration of eight (5-11) days. Seventeen of 71 (24%) adults with suspected CNS infection who were HIV-positive were on ARV

treatment, all of whom had syndromic CNS infection. The HIV treatment status of the remaining HIV-positive adults was unknown (i.e. not recorded in case record forms).

Antifungal

None of the children with suspected CNS infection received antifungal treatment. In the adult group, four patients with suspected CNS infection received antifungal treatment for their CNS condition, three of whom had an Indian ink stain result consistent with *Cryptococcus* species. The remaining adult patient who received antifungal treatment had a yeast (unspecified) detected from CSF stain but no organism grew from CSF culture, and was clinically diagnosed as having brain abscess by the treating clinician. Of the eight remaining adults who had a positive Indian ink stain result, seven had syndromic CNS infection but did not receive antifungal treatment, and one was later diagnosed as having a brain tumour.

Steroids

Steroids were given to 235/355 (66%) children enrolled in the study, including 188/247 (76%) children with syndromic CNS infection and 47/108 (44%) children with no CNS infection. Of these, 137 (58%) children received steroids before LP/EVD, and 108 (46%) had steroids administered before undergoing neuroimaging. Additionally, 14/233 (6%) children who received both steroids and antibiotics had the steroids given before antibiotic administration.

The proportion of patients who received steroids was higher in the adult group, where 159/195 (82%) patients with suspected CNS infection received them. These included 143/168 (85%) patients with syndromic CNS infection and 16/27 (59%) with no CNS infection. Of 159 patients who received steroids, 106 (67%) received them before undergoing LP/EVD, and 61 (38%) received them before having neuroimaging performed.

Of 153 adults who received both steroids and antibiotics, 70 (46%) had the steroids administered before antibiotics.

3.3.1.6 Outcome

Hospital stay

The median length of hospital stay in all children was 14 days (IQR 7-25 days). Children with syndromic CNS infection stayed in the hospital longer than those with no CNS infection (15 days versus 10 days, $p<0.001$).

In the adult group, the median length of stay was 13 days (IQR 7-19 days). There was no significant difference in the length of hospital stay between adults with syndromic CNS infection and no CNS infection (12 days versus 16 days, $p=0.265$).

Critical care requirement

One-hundred-and-sixty-nine of 355 (48%) children, including 121 with syndromic CNS infection and 48 with no CNS infection, required critical care during their hospital stay with a median duration of seven days (IQR 4-12 days).

Twenty-three of 195 (12%) adults, including 18 with syndromic CNS infection and five with no CNS infection, required critical care with a median duration of three days (IQR 2-7 days). Additionally, two more patients with CNS infection required critical care; however, no space was available at the intensive care unit. One of these patients' treatment was continued on the ward, whereas the other patient was referred to the intensive care unit in another hospital.

Mortality during hospitalisation

Fifty-one of 355 (14%) children with suspected CNS infection died during hospitalisation. There was no significant difference in mortality between children who had syndromic CNS infection (39/247 (16%)) and those who did not (12/108 (11%), $p=0.248$).

Mortality was significantly higher in adults than in children, where 85/195 (44%) adults died during hospitalisation (p<0.001). There was no significant difference in mortality at discharge between adults who had syndromic CNS infection (76/168 (45%)) and those who did not (9/27 (33%), p=0.247). Moreover, seven more adults were confirmed to have died within two weeks after discharge, including six with syndromic CNS infection and one with no CNS infection.

3.3.2 Syndromic clinical classification

3.3.2.1 Syndromic clinical classification in children

The syndromic clinical categories of paediatric CNS infection (n=247) were classified into those which occurred in assumed immunocompetent (n=237, 96%) and known immunocompromised children (n=10, 4%) (table 3.5). The latter group consisted of 5 (2%) children with malignancy, four (1.6%) children who tested HIV-positive, and one (0.4%) child with pre-existing autoimmune disease.

Table 3.13 Syndromic clinical classification for children with CNS infection

Clinical syndromic diagnosis	Assumed immunocompetent children (n=237)	Known immunocompromised children¥ (n=10)	Total (n=247)
Encephalitis	171 (72.2%)	4 (40%)	175 (70.8%)
Meningoencephalitis	40 (16.9%)	3 (30%)	43 (17.4%)
Definite shunt infection	9 (3.8%)	1 (10%)	10 (4%)
Meningitis	6 (2.5%)	1 (10%)	7 (2.8%)
Brain abscess	5 (2.1%)	1 (10%)	6 (2.4%)
CNS infection - unclassified	4 (1.7%)	-	4 (1.6%)
Encephalomyelitis	2 (0.8%)	-	2 (0.8%)
Myelitis	-	-	-
Presumptive cerebral toxoplasmosis	-	-	-

Values are n/number available (%). ¥ Includes patients with HIV positive (n=4), autoimmune disease (1), and malignancy (n=5).

The vast majority of children had encephalitis (n=175, 70.8%), including 171/237 (72.2%) children with an assumed immunocompetent condition and 4/10 (40%) children with

known immunocompromised conditions. Meningoencephalitis was the second most common sub-type of CNS infection in children, accounting for 40/237 (16.9%) cases in assumed immunocompetent children and 3/10 (30%) cases in known immunocompromised children. Other notable sub-types of paediatric CNS infection included definite shunt infection, meningitis, and brain abscess, which were responsible for 10 (4%), seven (2.8%), and six (2.4%) of the total paediatric CNS infection cases. No cases of myelitis and cerebral toxoplasmosis were found in children.

The proportions of children who fulfilled the criteria for the classification of encephalitis, meningitis and meningoencephalitis are shown in table 3.6. Patients with a CSF leucocyte count ≤4 cells/μL could be classified as having encephalitis if fulfilling the other criteria for encephalitis (table 3.1).

Table 3.14 Proportions of children fulfilling the criterion for the classification of encephalitis, meningitis, and meningoencephalitis

Criteria	Encephalitis (n=175)	Meningitis (n=7)	Meningoencephalitis (n=43)
Criteria for encephalitis/meningitis			
Fever at admission or history of fever prior to admission	175/175 (100%)	7/7 (100%)	43/43 (100%)
Criteria for encephalitis			
Altered consciousness[¥]	175/175 (100%)	0/7 (0%)	43/43 (100%)
Abnormal CT and/or MRI findings	110/116 (95%)	5/7 (71%)	32/36 (89%)
Abnormal EEG findings	24/26 (92%)	N/A	6/6 (100%)
Seizures	147/174 (84%)	4/7 (57%)	30/43 (70%)
Focal neurological signs[§]	55/125 (44%)	1/5 (20%)	14/29 (48%)
CSF leucocyte count >4 cells/μL	48/141 (34%)	7/7 (100%)	42/42 (100%)
Criteria for meningitis			
CSF pleocytosis[†]	48/141 (34%)	7/7 (100%)	42/42 (100%)
Neck stiffness	32/169 (19%)	4/7 (57%)	28/42 (67%)
Meningeal sign(s) positive [¶]	25/167 (15%)	3/6 (50%)	25/43 (58%)
Headache	19/145 (13%)	2/7 (29%)	17/37 (46%)
Detection of an appropriate pathogen in CSF or blood	6/175 (3%)	1/7 (14%)	8/43 (19%)

Values are number (n) of patients per group (%). [¥]Includes confusion, irritability, lethargy, and decreased consciousness. [§]Includes cranial nerve palsies and power abnormality. [¶]Includes nuchal rigidity, Kernig's sign, and Brudzinski's signs. [†]CSF leucocyte count >4 cells/μL (for patients ≥6 weeks of age) or >14 cells/μL (for patients <6 weeks of age).
CSF – cerebrospinal fluid; EEG – electroencephalography; CT – computerised tomography; MRI – magnetic resonance imaging; N/A – not applicable. None of the children with meningitis had an EEG performed.

In this study, all of the children with encephalitis (175/175 (100%)) had altered consciousness and fever at admission or a history of fever prior to admission (table 3.6). Other criteria by which the majority of the children with encephalitis were classified included abnormal neuroimaging (CT and/or MRI) findings and seizures, which were present in 110/116 (95%) and 147/174 (84%) cases, respectively. The proportion of children with encephalitis who had abnormal EEG findings was high (92%). However, it should be noted that EEG was only performed on 26 children with encephalitis overall. Interestingly, only 48/141 (34%) of all children with encephalitis who underwent LP/EVD had a CSF leucocyte count >4 cells/µL. Moreover, 32/169 (19%) children with encephalitis had neck stiffness, and 25/167 (15%) children had positive meningeal signs, which are classic symptoms and signs of meningitis. These children were not classified as having meningoencephalitis because they did not have CSF pleocytosis nor an appropriate pathogen identified in their CSF or blood.

Patients were classified as having meningitis if they had symptoms compatible with meningitis as deemed by the treating clinical team and had CSF pleocytosis (i.e. a CSF leucocyte count >4 cells/µL (for patients ≥6 weeks of age) or >14 cells/µL (for patients <6 weeks of age)). Those who did not have CSF pleocytosis but had an appropriate pathogen detected in CSF or blood were also classified into meningitis cases (table 3.1). The classic symptoms of meningitis include fever, headache, and neck stiffness. In the present study, all of the children with meningitis (7/7 (100%)) had fever or history of fever and CSF pleocytosis; whilst neck stiffness, positive meningeal signs, and headache were only present in 4/7 (57%), 3/6 (50%) and 2/7 (29%) cases, respectively (table 3.6). Nevertheless, the treating clinical team diagnosed all of these children with meningitis.

Patients who fulfilled both of the criteria for encephalitis and meningitis were classified as having meningoencephalitis (table 3.1). As expected, in the present study, all children

with meningoencephalitis (43/43 (100%)) had altered consciousness and fever. In keeping with the case definition for meningitis, 42/42 (100%) of the children had CSF pleocytosis. The remaining child did not undergo LP, but a causative pathogen was identified from blood culture.

3.3.2.2 Syndromic clinical classification in adults

Similar to the paediatric cases, the syndromic clinical classification of adult CNS infections (n=168) was categorised into those which occurred in assumed immunocompetent (n=101, 60%) and known immunocompromised (n=67, 40%) adults (table 3.7). The latter group included 66 (39%) adults diagnosed as HIV-positive and one adult with end-stage renal disease. Similar to the paediatric group, the two most frequent sub-types of CNS infection in adults were encephalitis and meningoencephalitis. They were responsible for 83/168 (49.4%) and 37/168 (22%) cases, respectively. On the contrary, presumptive cerebral toxoplasmosis was the third most prevalent CNS infection sub-type found in adults, accounting for 12/168 (7.1%) cases. Other notable sub-types of CNS infection in adults included brain abscess and myelitis, which accounted for 9/168 (5.3%) and 8/168 (4.8%) cases, respectively. No cases of definite shunt infection were found in adults.

Within the assumed immunocompetent group, the three most common sub-types of adult CNS infection included encephalitis (50/101 (49.5%)), meningoencephalitis (25/101 (24.7%)), and myelitis (8/101 (8%)). In contrast, the three most frequent CNS infection sub-types in known immunocompromised adults were encephalitis (33/67 (49.2%), meningoencephalitis (12 (17.9%)), and presumptive cerebral toxoplasmosis (12 (17.9%)).

Table 3.15 Syndromic clinical classification for adults with CNS infection

Clinical syndromic diagnosis	Assumed immunocompetent adults (n=101)	Known immunocompromised adults[¥] (n=67)	Total (n=168)
Encephalitis	50 (49.5%)	33 (49.2%)	83 (49.4%)
Meningoencephalitis	25 (24.7%)	12 (17.9%)	37 (22%)
Presumptive cerebral toxoplasmosis	-	12 (17.9%)	12 (7.1%)
Brain abscess	4 (4%)	5 (7.5%)	9 (5.3%)
Myelitis	8 (8%)	-	8 (4.8%)
Encephalomyelitis	6 (5.9%)	1 (1.5%)	7 (4.2%)
CNS infection - unclassified	4 (4%)	3 (4.5%)	7 (4.2%)
Meningitis	4 (4%)	1 (1.5%)	5 (3%)
Definite shunt infection	-	-	-

Values are n/number available (%). [¥]Includes patients with HIV positive (n=66) and end-stage renal disease (n=1).

Table 3.16 Proportions of adults fulfilling the criterion used for classification of encephalitis, meningitis and meningoencephalitis

Criteria	Encephalitis (n=83)	Meningitis (n=5)	Meningoencephalitis (n=37)
Criterion for encephalitis/meningitis			
Fever at admission or history of fever prior to admission	83/83 (100%)	5/5 (100%)	37/37 (100%)
Criteria for encephalitis			
Altered consciousness[¥]	83/83 (100%)	0/5 (0%)	37/37 (100%)
Abnormal EEG findings	9/10 (90%)	0/1 (0%)	6/6 (100%)
Abnormal CT and/or MRI findings	38/47 (81%)	3/3 (100%)	19/22 (86%)
Focal neurological signs[§]	41/83 (49%)	2/5 (40%)	17/37 (46%)
Seizures	35/82 (43%)	1/5 (20%)	12/37 (32%)
CSF leucocyte count >4 cells/µL (at any age)	25/51 (49%)	5/5 (100%)	37/37 (100%)
Criteria for meningitis			
CSF pleocytosis[†]	25/51 (49%)	5/5 (100%)	37/37 (100%)
Headache	53/82 (65%)	5/5 (100%)	35/37 (95%)
Meningeal sign(s) positive[¶]	32/80 (40%)	2/5 (40%)	29/36 (81%)
Neck stiffness	24/80 (30%)	1/5 (20%)	20/35 (57%)
Detection of an appropriate pathogen in CSF or blood	5/83 (6%)	0/5 (0%)	3/37 (8%)

Values are number (n) of patients per group (%). [¥]Includes confusion and decreased consciousness. [§]Includes cranial nerve palsies and power abnormality. [¶]Includes nuchal rigidity, Kernig's sign, and Brudzinski's signs. [†]CSF leucocyte count >4 cells/µL (for patients ≥6 weeks of age) or >14 cells/µL (for patients <6 weeks of age).
CSF – cerebrospinal fluid; EEG – electroencephalography; CT – computerised tomography; MRI – magnetic resonance imaging

The proportions of adults fulfilling the criteria for the classification of encephalitis, meningitis, and meningoencephalitis are shown in table 3.8. As expected, all adults with encephalitis (83/83 (100%)) had altered consciousness and a fever or a history of fever.

Among ten adults with encephalitis who underwent EEG, nine (90%) had abnormal EEG findings. Other notable criteria of encephalitis by which the adult patients were classified included abnormal neuroimaging (CT and/or MRI) findings and focal neurological signs, which were present in 38/47 (81%) and 41/83 (49%) of the cases. Among 51 adults with encephalitis who underwent LP/EVD, 25 (49%) had CSF pleocytosis. Furthermore, classic symptoms and signs of meningitis, including headache, positive meningeal signs, and neck stiffness, were commonly found in adults with encephalitis, presenting in 53/82 (65%), 32/80 (40%), and 24/80 (30%) of the cases, respectively. These patients were not categorised as having meningoencephalitis because they did not have either CSF pleocytosis nor an appropriate pathogen identified in their CSF or blood.

All of the adult patients with meningitis (5/5 (100%)) had a fever or a history of fever, headache, and CSF pleocytosis; whilst all of the adults with meningoencephalitis (37/37 (100%)) had fever or history of fever, altered consciousness, and CSF pleocytosis. Additionally, headache, positive meningeal signs, and neck stiffness were also commonly present in the latter group, accounting for 35/37 (95%), 29/36 (81%), and 20/35 (57%) of the cases.

3.3.3 Comparison of demographics, clinical presentation, laboratory investigations, and outcomes among different syndromic clinical diagnoses

Demographics, clinical presentation, laboratory investigations, and outcomes of patients with major sub-types of CNS infection are described separately for each group age. The major sub-types in children include encephalitis (n=175), meningoencephalitis (n=43), definite shunt infection (n=10), and meningitis (n=7). In adults, the major sub-types include encephalitis (n=83), meningoencephalitis (n=37), presumptive cerebral toxoplasmosis (n=12), brain abscess (n=9), and myelitis (n=8).

3.3.3.1 Comparison of demographics

Children with meningoencephalitis were much older (median age 9 years) than those with encephalitis, definite shunt infection, and meningitis (median age 1.8 years, 0.5 year, and 2.4 years, respectively) ($p<0.001$, table 3.9). In the adult CNS infection group, there was a significant difference in the median age of adults with myelitis (41 years), meningoencephalitis (38 years), brain abscess (36 years), encephalitis (35 yeas), and presumptive cerebral toxoplasmosis (31 years, $p=0.043$).

3.3.3.2 Comparison of clinical presentation

Symptom history

There was no significant difference in the median symptom duration among children with encephalitis, meningoencephalitis, definite shunt infection, and meningitis (median 4 versus 8 versus 4 versus 10 days, $p=0.085$). Children with meningoencephalitis and meningitis were more likely to have a history of headache and neck stiffness than those with encephalitis and definite shunt infection (table 3.9). As expected, children with encephalitis, meningoencephalitis, and definite shunt infection (proportion 100% for all of these groups) were much more likely to have a history of confusion than those with meningitis (proportion 0%, $p<0.001$). Furthermore, a history of seizures was more common in children with encephalitis (85%), meningoencephalitis (70%), and meningitis (57%) than those with definite shunt infection (30%, $p<0.001$). A previous history of CNS infection was the only clinical feature associated with shunt infection.

Within the adult CNS infection group, there was a significant difference in the median symptom duration among patients with encephalitis (10 days), meningoencephalitis (14 days), presumptive cerebral toxoplasmosis (26 days), brain abscess (42 days), and myelitis (18 days, $p=0.009$). Moreover, there were some notable differences in the symptom

history among patients with those syndromic diagnoses. Headache was more common in adults with meningoencephalitis (95%), presumptive cerebral toxoplasmosis (92%), brain abscess (89%), and encephalitis (65%) compared to those with myelitis (25%, p<0.001). In contrast, history of neck stiffness was more frequent in adults with meningoencephalitis (57%) than in those with encephalitis (30%), presumptive cerebral toxoplasmosis (25%), myelitis (13%), and brain abscess (0%, p=0.003) (table 3.10).

As expected, adults with encephalitis and meningoencephalitis (100% for both groups) were more likely to have a history of confusion compared to those with presumptive cerebral toxoplasmosis (67%), brain abscess (44%), and myelitis (0%, p<0.001) (table 3.10). Additionally, a history of vomiting was more frequently found in adults with meningoencephalitis (61%), brain abscess (44%), and encephalitis (41%) than in those with presumptive cerebral toxoplasmosis (25%) and myelitis (0%, p=0.009).

Clinical signs

Within the paediatric CNS infection group, as expected, positive meningeal sign(s) were more likely to present in children with meningoencephalitis (58%) and meningitis (50%) than in children with encephalitis (15%) and definite shunt infection (0%, p<0.001) (table 3.9).

Table 3.17 Demographic and clinical features of children with suspected CNS infection enrolled in the study

	Sub-type of CNS infection				P value
	Encephalitis (n=175)	**Meningoence-phalitis (n=43)**	**Definite shunt infection (n=10)**	**Meningitis (n=7)**	
Demographics					
Age (years)	1.8 (0.8,4.8)	9.0 (1.8,13.5)	0.5 (0.4,2.5)	2.4 (0.5,13)	**<0.001**
Percentage male	92/175 (53%)	24/43 (56%)	4/10 (40%)	4/7 (57%)	0.847
Symptoms					
Headache	19/146 (13%)	17/36 (47%)	1/9 (11%)	2/7 (29%)	**<0.001**
Neck stiffness	32/169 (19%)	28/42 (67%)	1/10 (10%)	4/7 (57%)	**<0.001**
Hypersensitivity to light	2/144 (1%)	2/36 (7%)	0/9 (0%)	0/6 (0%)	0.406
History of rash	13/167 (8%)	6/43 (14%)	0/10 (0%)	0/7 (0%)	0.444
Confusion[¥]	175/175 (100%)	43/43 (100%)	10/10 (100%)	0/7 (0%)	**<0.001**
Respiratory symptoms[§]	80/173 (46%)	16/43 (39%)	2/9 (22%)	4/7 (57%)	0.336
Vomiting	56/174 (32%)	20/42 (48%)	6/10 (60%)	4/7 (57%)	0.056
Diarrhoea	74/172 (43%)	12/42 (29%)	3/10 (30%)	1/7 (14%)	0.176
Genital ulcer	0/167 (0%)	0/44 (0%)	0/10 (0%)	0/7 (0%)	N/A
Seizures	147/174 (85%)	30/43 (70%)	3/10 (30%)	4/7 (57%)	**<0.001**
Previous history of CNS infection	16/166 (10%)	4/42 (10%)	4/9 (44%)	1/7 (14%)	**0.029**
Signs					
Fever[¶] (≥38 °C)	94/175 (54%)	22/43 (51%)	6/10 (60%)	3/7 (43%)	0.905
Meningeal sign(s) positive[‡]	25/167 (15%)	25/43 (58%)	0/9 (0%)	3/6 (50%)	**<0.001**
Cranial nerve palsy	14/147 (10%)	6/34 (18%)	1/7 (14%)	0/5 (0%)	0.391
Power abnormality	49/129 (38%)	12/31 (39%)	5/8 (63%)	1/6 (17%)	0.398
Coma (GCS ≤8)	69/175 (39%)	15/43 (35%)	2/10 (20%)	0/7 (0%)	0.115
Laboratory results					
HIV-positive	3/4 (75%)	0/2 (0%)	N/A	N/A	0.400
Blood leucocyte count (x 10^9/L)	10.4 (7.0,14.3) [n=173]	12.6 (4.8,18.1) [n=43]	13.7 (9.2,17.1) [n=10]	16 (4.8,19.7) [n=7]	0.118
Blood neutrophil (%)	72 (62,81) [n=172]	75 (70,87) [n=43]	61 (44,82) [n=10]	66 (33,85) [n=7]	**0.006**
CSF pleocytosis[†]	48/141 (34%)	42/42 (100%)	9/10 (90%)	7/7 (100%)	**<0.001**
CSF leucocyte count (cells/µL)	2 (0,8) [n=141]	92 (11,299) [n=42]	113 (33,989) [n=10]	166 (9,250) [n=7]	**<0.001**
CSF neutrophil (%)	2 (0,45) [n=141]	36 (14,71) [n=42]	35 (13,78) [n=10]	34 (23,56) [n=7]	**<0.001**
CSF protein (mg/dL)	30 (20,53) [n=141]	110 (45,205) [n=42]	190 (59,472.5) [n=10]	70 (50,100) [n=7]	**<0.001**
CSF glucose (mg/dL)	76 (62,94) [n=141]	64 (28,77) [n=42]	34 (16,48.5) [n=10]	38 (19,52) [n=7]	**<0.001**
CSF:blood glucose ratio	0.7 (0.6,0.8) [n=127]	0.6 (0.3,0.8) [n=36]	0.4 (0.3,-) [n=2]	0.3 (0.2,0.4) [n=7]	**<0.001**
Neuroimaging					
Abnormal CT scan result	110/115 (96%)	32/36 (89%)	8/8 (100%)	5/7 (71%)	0.054
Abnormal MRI result	3/3 (100%)	1/2 (50%)	N/A	N/A	0.400

Values are median (IQR) for continuous data and n/number evaluable (%) for categorical data. [¥]Includes confusion, irritability, lethargy and decreased consciousness. [§]Includes coryzal symptoms, sore throat, and chronic cough. [¶]Fever at the time of admission. [‡]Includes nuchal rigidity, Kernig's sign and Brudzinski's signs. [†]CSF leucocyte count >4 cells/µL (for patients ≥6 weeks of age) or >14 cells/µL (for patients <6 weeks of age).

CNS – central nervous system; vs – versus; GCS – Glasgow Coma Scale; CSF – cerebrospinal fluid; CT – computerised tomography; MRI – magnetic resonance imaging.

Table 3.18 Demographic and clinical features of adults with suspected CNS infection enrolled in the study

	Sub-type of CNS infection					P value
	Encephalitis (n=83)	**Meningoence-phalitis (n=37)**	**Presumptive cerebral toxoplasmosis (n=12)**	**Brain abscess (n=9)**	**Myelitis (n=8)**	
Demographics						
Age (years)	35 (27,47)	38 (25,47)	31 (27,35)	36 (34,48)	41 (28,54)	**0.043**
Percentage Male	53/83 (64%)	23/37 (62%)	10/12 (83%)	8/9 (89%)	4/8 (50%)	0.305
Symptoms						
Headache	53/82 (65%)	35/37 (95%)	11/12 (92%)	8/9 (89%)	2/8 (25%)	**<0.001**
Neck stiffness	24/80 (30%)	20/35 (57%)	3/12 (25%)	0/9 (0%)	1/8 (13%)	**0.003**
Hypersensitivity to light	7/79 (9%)	6/34 (18%)	1/12 (8%)	2/8 (25%)	2/8 25%)	0.240
History of rash	7/81 (9%)	2/32 (6%)	2/9 (22%)	2/8 (25%)	0/8 (0%)	0.216
Confusion[¥]	83/83 (100%)	37/37 (100%)	8/12 (67%)	4/9 (44%)	0/8 (0%)	**<0.001**
Respiratory symptoms[§]	36/83 (43%)	9/37 (24%)	4/12 (33%)	4/9 (44%)	4/8 (50%)	0.293
Vomiting	34/83 (41%)	22/36 (61%)	3/12 (25%)	4/9 (44%)	0/8 (0%)	**0.009**
Diarrhoea	16/81 (20%)	4/37 (11%)	3/12 (25%)	1/9 (11%)	1/8 (13%)	0.716
Genital ulcer	6/72 (8%)	1/33 (3%)	0/6 (0%)	0/6 (0%)	0/8 (0%)	0.837
Seizures	35/82 (43%)	12/37 (32%)	4/12 (33%)	2/9 (22%)	0/8 (0%)	0.122
Previous history of CNS infection	8/80 (10%)	2/36 (6%)	2/11 (18%)	1/8 (12.5%)	0/7 (0%)	0.553
Signs						
Fever[¶] (≥38 °C)	32/83 (39%)	16/37 (43%)	1/12 (8%)	1/9 (11%)	0/8 (0%)	**0.014**
Meningeal sign(s) positive[‡]	32/80 (40%)	29/36 (81%)	3/12 (25%)	0/9 (0%)	0/8 (0%)	**<0.001**
Cranial nerve palsy	27/57 (47%)	12/21 (57%)	5/12 (42%)	5/8 (63%)	1/8 (13%)	0.237
Power abnormality	35/51 (69%)	10/22 (46%)	6/12 (50%)	5/8 (63%)	8/8 (100%)	**0.044**
Coma (GCS ≤8)	25/83 (30%)	10/37 (27%)	1/12 (8%)	1/9 (11%)	0/8 (0%)	0.192
Laboratory results						
HIV-positive	32/75 (43%)	12/35 (34%)	12/12 (100%)	5/9 (56%)	0/3 (0%)	**<0.001**
Blood leucocyte count (x 10^9/L)	9.7 (6.5,15.3) [n=82]	10.1 (8.5,15.4) [n=37]	6.5 (4.5,9.9) [n=12]	8.4 (7.3,16.5) [n=9]	11.9 (7.3,15.4) [n=8]	0.057
Blood neutrophil (%)	83 (71,87) [n=81]	81 (73,89) [n=36]	77 (55,87) [n=11]	86 (64,88) [n=9]	83 (71,87) [n=7]	0.741
CSF opening pressure (cm CSF)	11 (8,16) [n=42]	23 (13,32) [n=31]	15 (7,19) [n=9]	22 (16,29) [n=5]	10.5 (5.5,21) [n=7]	**<0.001**
CSF pleocytosis[†]	25/51 (49%)	37/37 (100%)	7/11 (64%)	5/7 (71%)	4/7 (57%)	**<0.001**
CSF leucocyte count (cells/µL)	4 (1,22) [n=51]	100 (18,311) [n=37]	7 (1,18) [n=11]	8 (1,30) [n=7]	12 (1,25) [n=7]	**<0.001**
CSF neutrophil (%)	14 (0,35) [n=50]	20 (8,51) [n=37]	15 (4,60) [n=11]	12 (8,40) [n=7]	35 (7,64) [n=7]	0.411
CSF protein (mg/dL)	60 (30,150) [n=51]	100 (70,210) [n=37]	70 (40,110) [n=11]	80 (50,320) [n=7]	70 (60,300) [n=7]	0.122
CSF glucose (mg/dL)	70 (57,88) [n=51]	55 (43.5,77.5) [n=37]	60 (52,76) [n=11]	52 (43,107) [n=7]	74 (72,110) [n=7]	**0.044**
CSF:blood glucose ratio	0.5 (0.4,0.6) [n=49]	0.4 (0.3,0.5) [n=37]	0.5 (0.4,0.6) [n=11]	0.4 (0.3,0.5) [n=7]	0.7 (0.6,0.8) [n=6]	**<0.001**
Neuroimaging						
Abnormal CT scan result	28/36 (78%)	11/11 (100%)	9/9 (100%)	7/7 (100%)	0/2 (0%)	**0.009**
Abnormal MRI result	12/14 (86%)	10/10 (100%)	4/4 (100%)	5/5 (100%)	4/4 (100%)	0.790

Values are median (IQR) for continuous data and n/number evaluable (%) for categorical data. [¥]Includes confusion and decreased consciousness. [§]Includes coryzal symptoms, sore throat, and chronic cough. [¶]Fever at the time of admission. [‡]Includes nuchal rigidity, Kernig's sign and Brudzinski's signs. [†]CSF leucocyte count >4 cells/µL (for patients ≥6 weeks of age) or >14 cells/µL (for patients <6 weeks of age).

CNS – central nervous system; vs – versus; GCS – Glasgow Coma Scale; CSF – cerebrospinal fluid; CT – computerised tomography; MRI – magnetic resonance imaging.

Within the adult CNS infection group, there were a few remarkable differences in the clinical signs among the major CNS infection sub-types, including fever at admission, positive meningeal sign(s), and power abnormality (table 3.10). Fever at admission was more common in adults with meningoencephalitis (43%) and encephalitis (39%) than in brain abscess (9%), presumptive cerebral toxoplasmosis (8%), and myelitis (0%, p=0.014). As expected, meningeal sign(s) was more frequently found in adults with meningoencephalitis (81%) than in those with encephalitis (40%), presumptive cerebral toxoplasmosis (25%), brain abscess (0%), and myelitis (0%, p<0.001). Power abnormality was the only clinical sign associated with myelitis.

3.3.3.3 Comparison of laboratory findings

The proportion of CSF pleocytosis among children who underwent LP/EVD with encephalitis (48/141 (34%)) was far less than those with meningoencephalitis (42/42 (100%)), definite shunt infection (9/10 (90%)), and meningitis (7/7 (100%), p<0.001). Furthermore, the CSF leucocyte count in children with encephalitis (median 2 cell/µL) was much lower than in those with meningoencephalitis (median 92 cells/µL), definite shunt infection (median 113 cells/µL), and meningitis (median 166 cells/µL, p<0.001). Additionally, the blood neutrophil percentage, the CSF neutrophil percentage, and the CSF protein level greatly varied among the major paediatric CNS sub-types (table 3.9).

Looking into pathogen detection by the standard hospital diagnostic testing, all cases with definite shunt infection (10/10 (100%)) had a causative pathogen detected. Other cases which had a pathogen detected included 8/43 (19%) cases of meningoencephalitis, 1/6 (16.7%) cases of brain abscess, 1/7 (14.3%) cases of meningitis, and 6/175 (3.4%) cases of encephalitis (table 3.11).

Table 3.19 Proportion of paediatric syndromic CNS infection cases in which a causative pathogen was identified by the standard hospital diagnostic testing

Clinical syndromic diagnosis	Number of cases (n=247)	Number of cases with causative pathogen detected (i.e. known cause)
Encephalitis*	175 (70.8%)	6/175 (3.4%)
Meningoencephalitis	43 (17.4%)	8/43 (19%)
Definite shunt infection	10 (4%)	10/10 (100%)
Meningitis	7 (2.8%)	1/7 (14.3%)
Brain abscess	6 (2.4%)	1/6 (16.7%)
CNS infection - unclassified	4 (1.6%)	-
Encephalomyelitis	2 (0.8%)	-
Myelitis	-	-
Presumptive cerebral toxoplasmosis	-	-
Total cases with causative pathogen detected [n (%)]		**26/247 (11%)**

*Include 1 case of post-neurosurgical encephalitis.

CSF culture was the most informative diagnostic test in children, where it detected the causative pathogens in 18/247 (7.2%) of the syndromic CNS infection cases. These included 8/10 (80%) cases of definite shunt infection, 1/7 (14.3%) case of meningitis, 5/43 (11.6%) cases of meningoencephalitis, and 4/175 (2.3%) cases of encephalitis.

The most common causative pathogen identified in children was *E. coli* (n=4, table 3.3), which was detected in three cases of encephalitis and one case of meningoencephalitis. The second most common aetiology identified were *Salmonella* spp., *Staphylococcus aureus*, *Staphylococcus haemolyticus*, and Gram-negative bacilli, accounting for two cases each (table 3.3). *Salmonella spp.* was detected in a case of meningitis and a case of meningoencephalitis, whereas *Staphylococcus aureus* was identified in two cases of meningoencephalitis. Additionally, *Staphylococcus haemolyticus* was found in two cases of definite shunt infection, whilst Gram-negative bacilli were detected in two cases of encephalitis.

Among the major CNS infection sub-types in adults, there was notable variations in the proportion of HIV-positive patients, the CSF opening pressure, the proportion of CSF

pleocytosis, the CSF glucose level, and the CSF:blood glucose ratio (table 3.10). Moreover, the CSF leucocyte count in adults with meningoencephalitis (median 100 cells/μL) was significantly higher compared to that with myelitis (median 12 cells/μL), brain abscess (median 8 cells/μL), presumptive cerebral toxoplasmosis (median 7 cells/μL), and encephalitis (median 4 cells/μL, $p<0.001$).

The standard hospital diagnostic testing detected a causative pathogen in 1/7 (14.3%) case of unclassified CNS infection (in this case cerebral malaria), 1/9 (11.1%) case of brain abscess, 3/37 (8.1%) cases of meningoencephalitis, and 5/83 (6%) cases of encephalitis (table 3.12).

Table 3.20 Proportion of adult syndromic CNS infection cases in which a causative pathogen was identified by the standard hospital diagnostic testing

Clinical syndromic diagnosis	Number of cases (n=168)	Number of cases with causative pathogen detected (i.e. known cause)
Encephalitis	83 (49.4%)	5/83 (6.0%)
Meningoencephalitis	37 (22%)	3/37 (8.1%)
Presumptive cerebral toxoplasmosis	12 (7.1%)	-
Brain abscess	9 (5.3%)	1/9 (11.1%)
Myelitis	8 (4.8%)	-
Encephalomyelitis	7 (4.2%)	-
CNS infection - unclassified	7 (4.2%)	1/7 (14.3%)
Meningitis	5 (3%)	-
Definite shunt infection	-	-
Total cases with causative pathogen detected [n (%)]		**10/168 (6%)**

CSF culture results were only informative in 5/168 (3.6%), including 3/37 (8.1%) cases of meningoencephalitis, 1/9 (11.1%) cases of brain abscess, and 1/83 (1.2%) cases of encephalitis.

Comparison of steroid treatment

Among the major paediatric CNS infection sub-types, meningitis (7/7 (100%)) and brain abscess (5/6 (83%)) were the two most common diagnoses treated with steroids. Within

the adult CNS infection group, the two most common diagnoses treated with steroids were brain abscess (9/9 (100%)) and presumptive cerebral toxoplasmosis (11/12 (92%)). Similar to the paediatric group, all adult patients with meningitis (5/5 (100%)) received steroids.

3.3.3.4 Comparison of outcome

Hospital stay

Within the paediatric CNS infection group, children with definite shunt infection had the longest hospital stay (median 54 days), followed by those with meningoencephalitis (median 24 days), meningitis (median 15 days) and encephalitis (median 16 days).

The length of hospital stay among the major adult CNS infection sub-types varied widely. Adults with brain abscesses had the longest hospital stay (median 17 days), whilst those with meningoencephalitis had the shortest hospital stay (median 9 days). The median length of hospital stay in adults with myelitis, presumptive cerebral toxoplasmosis, and encephalitis were 16 days, 14 days, and 12 days, respectively.

Critical care requirement

Within the paediatric CNS infection group, encephalitis (91/117 (78%)) and meningoencephalitis (20/117 (17%)) were the two most common diagnoses which required critical care.

Similar to the paediatric group, the two most common diagnoses in adults requiring critical care were encephalitis (11/20 (55%)) and meningoencephalitis (6/20 (30%)).

Mortality at hospitalisation

Mortality among children with meningoencephalitis (15/43 (35%)) was significantly higher compared to those with encephalitis (20/175 (11%)), definite shunt infection (1/10 (10%)), and meningitis (1/7 (14%), p=0.004).

In the adult syndromic CNS infection group, the highest mortality was seen in patients with meningoencephalitis (22/37 (60%)), whereas the lowest mortality was seen in those with presumptive cerebral toxoplasmosis (0/12 (0%)). Additionally, mortality in patients with encephalitis, brain abscess, and myelitis were 41/83 (49%), 4/9 (44%), and 2/8 (25%), respectively.

3.4 Discussion

This study is the largest cohort in Indonesia to date, undertaking a comprehensive analysis of all patients aged ≥1 month presenting to the hospital with clinical features suggestive of CNS infection. Previous studies on CNS infections in Indonesia involved children or adult participants only. They focused on a smaller scope of CNS infections, such as Japanese encephalitis, meningitis, brain infections, tuberculous meningitis, and viral CNS infections. To my knowledge, there has been no such study in Indonesia that includes spinal cord infection. Therefore, this study provides valuable knowledge on the causes, clinical features, and outcomes of CNS infections in both children and adults in Indonesia.

In the present study, 108/355 (30%) children and 27/195 (14%) adults enrolling in the study were found not to have CNS infection. These proportions are similar to those reported by Trung *et al*. from a CNS infection study in Vietnam, of which 247/914 (27%) children and 157/826 (19%) adults who met the inclusion criteria did not have CNS infection.[3] Moreover, a recent study from Jakarta, another province in Indonesia, also reported that 56/340 (16%) adults suspected of having a CNS infection were found to have

alternative diagnoses.[6] The high proportion of cases found not to have a CNS infection is because many conditions mimic CNS infection, of which the two most common are febrile seizure (particularly in children) and encephalopathy.[3] On the contrary, a study from the Netherlands showed that only 89/363 (25%) of suspected CNS infection cases had a confirmed CNS infection, whilst the remaining cases were identified as other neurological disorders (33%), systemic infection (31%), CNS inflammatory disease (19%) and other systemic disorders (2%).[271]

3.4.1 Clinical presentation

Adult patients presented late to Dr Sardjito Hospital. The median time to presentation (i.e. median symptom duration prior to attendance at Dr Sardjito Hospital) was 14 days (IQR 7-28 days). This finding was consistent with the result from a similar study in Jakarta where adult patients presented to the hospital after a median of 14 days (IQR 7-30 days) of neurological symptoms.[6] Furthermore, 69% of adults with suspected CNS infection in my study went to other hospitals before being referred to Dr Sardjito Hospital. Thus, patients were likely to receive medical treatment prior to recruitment into my study. Pre-treatment prior to LP is likely to have impaired the pathogen detection rate by both standard hospital testing and the PCR tests introduced during this study. However, pathogen-specific PCR has been reported to have a better pathogen detection rate than microbiological culture even when the patients have received antibiotics.[222-226] Thus, PCR implementation still offers a significant opportunity to improve pathogen detection rates in my study setting.

Among the most notable symptoms and signs found in adults were a history of confusion (79%), headache (68%), power abnormality (62%), and cranial nerve palsy (41%). This finding was also consistent with the Jakarta study finding where adults commonly

presented to the hospital with altered mental status (70%), motor abnormalities (42%), and cranial nerve palsy (39%).[6]

In the present study, children with suspected CNS infection presented to the hospital much earlier than adults (median 5 days (IQR 2-10 days), p=0.004). This might be because parents often worry when their children are ill; thus, they sought medical help as soon as possible. In contrast, adults usually neglect their illness until they feel really suffering before seeking medical help. The majority of children in this study had a history of confusion (90%), a history of seizures (73%), fever at admission (54%), and power abnormality (34%). This finding was similar to the result from the Vietnam study, where children with acute encephalitis syndrome presented to the hospital with a history of fever (93%), history of seizures (74%), fever at admission (73%), and power abnormality (31%).[230]

There were only a few differences in clinical features between patients with CNS infection and those without CNS infection among children and adults. These include confusion, seizures, neck stiffness, vomiting, positive meningeal signs, power abnormality, and GCS ≤8, which proportion was significantly higher in children with CNS infection than in those with no CNS infection. The differences were even fewer in adults, limited to confusion, seizures, headache, and vomiting, with no significant differences in clinical signs between those who had CNS infection and those who did not. These findings are in accordance with a previous study from England, which reported a few differences in clinical features, including confusion, sore throat, myalgia, and seizures, between patients with meningitis and not meningitis.[2] These findings highlight that the differences in clinical features were insufficient to distinguish CNS infection from not CNS infection cases. Further investigations are required to establish a diagnosis of CNS infection.

3.4.2 Investigations performed

The rates of LP/EVD in children and adults with suspected CNS infection were similar (71% and 73%, respectively); however, it was performed earlier in children than in adults (median time to LP one day versus three days). The LP/EVD rates were relatively high compared to other studies in Liverpool (LP rate was 53% in children) and Jakarta (LP rate was 67% in adults).[6, 272] In the former study, the findings from LP provided essential information for the clinical management of 72% of patients by enabling pathogen detection or ruling out bacterial meningitis. [272]

Current guidelines strongly recommend LP be performed in all patients with suspected CNS infection as soon as possible unless a contraindication is present.[79, 138, 273] In my study, although Dr Sardjito Hospital is the top referral hospital in the province and is a renowned teaching hospital in Indonesia, these guidelines were poorly adhered to, particularly in the adult setting. In adults, most of the time, LP was performed only during the standard hospital working hours (i.e. Monday to Friday, 8 am – 4 pm). Therefore, if a patient is admitted to the hospital on the weekend, the LP would be delayed at least for one day. Moreover, before LP, adult patients are referred to the neuro-ophthalmology division to be examined for papilloedema, which likely is another cause for delayed LPs.

In contrast, the LP in children was not restricted by the working hours. Nonetheless, there remained a delay in the performance of LPs in children, particularly in the emergency unit and the ward. This is because children are first referred to the Anaesthetic Department to examine whether the LP can be done safely. Moreover, during the LP procedure, the anaesthetic should be administered under the observation of an anaesthetic trainee. This often caused an LP delay due to the strict schedule of the anaesthetic trainees at the hospital, who also took care of many other patients undertaking surgery.

The finding that none of the adult patients enrolled in the study had dengue serology testing is interesting. This may be partly due to the fact that adults with dengue fever are not allowed to be referred to Dr Sardjito Hospital, except for those with dengue haemorrhagic fever. Furthermore, this may be because none of the adult patients had classic symptoms and signs of dengue fever or dengue haemorrhagic fever. The serology testing is only performed on selected cases based on the clinician's decision. This explains why patients, either children or adults, with suspected CNS infection were not routinely screened for dengue infection, although dengue is endemic in the country.

Another interesting finding was that HIV tests were performed much more frequently in adults than in children. This was partly due to the fact that HIV is a highly sensitive matter in Indonesia, and as such, patients (or their parents if in children) are required to receive counseling before HIV testing can be performed. HIV testing is particularly sensitive in children because of the stigma associated with it. Therefore, in children, HIV testing was only performed on those who were highly suspected to have HIV and those who were at a high risk of getting HIV infection based on their history and clinical features. Similar to HIV testing, Indian ink stain and *Toxoplasma* serology tests were performed much more often in adults than children. This may be because cryptococcal and *Toxoplasma* CNS infections are more likely to occur in immunocompromised individuals. In the present study, this condition was more prevalent in adults than in children.

Neuroimaging is indicated prior to LP if the patient has focal neurological signs, papilloedema, continuous or uncontrolled seizures, or GCS ≤12.[138] When prior neuroimaging is required, LP should be performed as soon as possible following the neuroimaging unless a significant brain shift is evident from the neuroimaging, an alternative diagnosis is established, or the patient had continuous or uncontrolled seizures, rapidly declining GCS or decreased cardiac/respiratory function. In the present

study, 20/80 (25%) children and 10/38 (26%) adults did not have a justified indication for undergoing neuroimaging before LP. Nevertheless, 2/10 (20%) adults who did not have the recognised indications were immunocompromised, a condition where neuroimaging prior to LP is recommended according to the Infectious Diseases Society of America guideline.[273]

3.4.3 Results of investigations

The only parameters which I have shown to be useful in distinguishing between CNS infection and the mimics were CSF parameters. There were no significant differences in blood parameters between adults with CNS infection and those who presented with similar symptoms and signs. McGill *et al*. also reported similar findings in a study of meningitis in England, where CSF parameters were more informative than clinical symptoms and signs in distinguishing meningitis from not meningitis cases.[2] These findings highlight the importance of LP or EVD in the diagnosis of CNS infection.

Eighteen percent of children and 5% of adults with syndromic CNS infection did not have any leucocyte found in their CSF. This might be because of the case definition for some types of CNS infection allow patients with no cells in CSF to be classified as such (table 3.1). However, there is also a possibility of false CSF leucocyte count. The procedure of CSF leucocyte counting in Dr Sardjito Hospital included the addition of lysis buffer, which aimed to lyse erythrocytes after the erythrocytes were counted. The addition of lysis buffer, therefore, could make the leucocyte count easier. However, it is also possible that the lysis buffer lysed the leucocytes as well, which in turn made false zero leucocyte count, or the overall leucocyte count became falsely low. If this is the case, the actual burden of CNS infection could be underestimated.

The proportion of syndromic CNS infection cases with a pathogen detected was very low in children, where only 11% (26/247) cases had a pathogen detected. This proportion decreased to 6% (10/168) in adult cases. This low proportion of CNS infection cases with pathogen detected might be because 84% of children and 69% of adults had sought medical help in other hospitals (section 3.3.1.2). In such cases, patients were more likely to have received broad-spectrum antibiotics, which could reduce a load of pathogens if they were sensitive to broad-spectrum antibiotics. Moreover, 92% of children and 95% of adults received antibiotics prior to LP with a median of three and four doses, respectively (section 3.3.1.5). This antibiotic treatment prior to LP might have affected the CSF culture results. Furthermore, the diagnostic tests routinely performed at Dr Sardjito Hospital were limited to Gram stain and culture. Although serology testing had been attempted at the hospital, these were applied to selected cases based on a clinician's decision. These tests were only performed on serum samples but not in CSF samples which therefore could not confirm the definite aetiology of CNS infection. Additionally, the serology tests were often performed on single rather than paired serum samples, making the results less informative in defining the cause of CNS infection.

Standard hospital diagnostics in adults yielded a result in just 6% of cases. The further delay in LP in adults compared to in children might have contributed to the poor performance of the CSF culture in adults, as the more delayed the LP, the longer the patient received antibiotics and steroids, which might nullify the culture result. Ninety-two percent of children and 78% of adults received antibiotics prior to LP/EVD with a median of three and four doses, respectively, and 58% of children and 67% of adults also received steroids prior to LP/EVD. This explains why the positivity rate of CSF culture is only 8.7% in children and 4.1% in adults with syndromic CNS infection (sections 3.3.1.4 and 3.3.3.3). Blood culture was informative, especially when CSF culture was negative, or

LP/EVD was not done. Nevertheless, it was only performed in 78% of children and 60% of adults overall, which again showed low compliance with the guidelines. The recent guidelines strongly recommend blood culture in all patients with suspected CNS infection, ideally before the first antibiotic is administered, to obtain an optimum result.[79, 138, 273] It was difficult to apply in my study setting as the vast majority of the patients had sought medical help prior to Dr Sardjito Hospital admission and were thus more likely to receive antibiotics before their referral.

Unlike other studies from West Java and Jakarta where CSF Ziehl-Neelsen stain could identify acid-fast bacilli in 23/227 (10%) and 7/274 (3%) adults with meningitis / CNS infection, respectively,[6, 274] it completely failed to identify the pathogen in any of the syndromic CNS infection cases in my study. This might be due to different protocols used for the Ziehl-Neelsen stain in the West Java and the Jakarta studies. In those studies, the CSF sample was concentrated by centrifugation prior to the stain, whilst in my study, the CSF was directly stained. Furthermore, the positivity rate of CSF Ziehl-Neelsen stain is lower in HIV-negative than in HIV-positive patients.[274]

Of 11 adult patients who had positive Indian ink stain results, only one patient had positive CSF culture for *C. neoformans*, while another patient had positive CSF culture for *C. albicans*. The latter patient was further diagnosed with brain tumour instead of CNS infection based on a pathology anatomy investigation. This fact indicated that the positive Indian ink stain result was a false positive, and the *C. albicans* growth from the CSF culture was most likely a contaminant. False positives in Indian ink stain tests have been previously reported as a result of artefacts; thus it has been suggested that the result should be interpreted by an experienced technician.[275] Moreover, a positive result should be further confirmed with a cryptococcal antigen (CrAg) assay or a CSF fungal culture.

E. coli was the most common causative pathogen detected in children (n=4, table 3.3). This finding differed from that in Vietnam, where Japanese encephalitis virus (JEV) was the most common aetiology of CNS infection.[3] The overall results from the Vietnam study showed that viruses more commonly caused CNS infection than bacteria. This might be because the detection of viruses, particularly in the CSF samples, was not routinely performed in patients with suspected CNS infection in Dr Sardjito Hospital.

In the adult group, *K. pneumoniae* and *Acinetobacter baumannii* were the two most common aetiology detected by the standard hospital diagnostic tests. This finding was not consistent with the result from studies in West Java[276] and Jakarta[6], which found that the majority of CNS infections were caused by *M. tuberculosis*. This might be due to a more comprehensive *M. tuberculosis* testing performed in the latter two studies, which included CSF Ziehl-Nielsen stain, CSF *M. tuberculosis* culture using Löwenstein–Jensen medium and/or *Mycobacteria* Growth Indicator Tube (MGIT), CSF MTB/RIF GeneXpert, and CSF polymerase chain reaction (PCR). The finding in the present study also differed from the Vietnam study finding, where *Streptococcus suis* and *S. pneumoniae* were the two most common aetiology of adult CNS infection.[3] Moreover, global data showed that *S. pneumoniae* is the most commonly reported cause of bacterial meningitis worldwide.[45] Therefore, the findings of the present study and the two previous Indonesian studies differed from the overall global data. This might be because all Indonesian studies were conducted in top-referral hospitals where most of the patients were referred from lesser health care facilities. Therefore, they might have received broad-spectrum antibiotics, which could improve pneumococcal CNS infection. Tuberculous CNS infection treatment requires a specific antibiotic regimen, thus the disease cannot be treated by broad spectrum antibiotics. Consequently, patients with pneumococcal CNS infection could have been treated well in lesser health care facilities and not referred to the top-referral

hospitals, or if the patients were referred to the top-referral hospitals then they have been partially treated with broad-spectrum antibiotics which might cause the pathogen become undetected by culture and/or molecular testing.

The proportion of CNS infection cases with no causative pathogen detected by standard hospital diagnostics in this cohort (221/247 (89%) in children and 158/168 (94%) in adults) was much higher than that described in the Jakarta study where only 95/274 (35%) cases had an unknown aetiology. It should be noted that the median time to presentation in adults in both Yogyakarta and Jakarta studies was 12 days, but the Jakarta study applied molecular testing targeting a panel of pathogens as well as a more comprehensive *M. tuberculosis* testing.[6] This finding underlines the low diagnostic capability of the standard hospital testing in identifying the aetiology of CNS infection and the need for its improvement at Dr Sardjito Hospital.

3.4.4 Treatment

Ninety-seven percent of children and 95% of adults with suspected CNS infection received at least one dose of antibiotics, of whom 69% of children and 53% of adults received more than one type of antibiotics. These proportions are exceptionally high. Moreover, the massive use of antibiotics might be unnecessary if the yields of the hospital diagnostic testing were good enough to detect pathogens causing CNS infection. The use of unnecessary antibiotics may potentially increase antimicrobial resistance. According to 2014 WHO report, the overall resistance rate of *E. coli* – an important aetiological agent of paediatric CNS infection in my study setting – to third-generation cephalosporins in Indonesia ranged from 10-13.8%, whilst the resistance rate to fluoroquinolones was 17.3%.[277] A more recent study from Fatmawati Hospital in Jakarta showed that those rates increased to 46.2% for both third-generation cephalosporins and fluoroquinolones.[278]

Taken together, these findings highlight the importance of prompt diagnosis to avoid the administration of unnecessary antibiotics and to a greater extent, unnecessary treatment.

Twelve children and 40 adults with suspected CNS infection, including three children and seven adults who were later categorised as having no CNS infection, received anti-tuberculosis treatment. The treatment for CNS tuberculosis in the study setting follows guidelines from WHO for severe forms of extrapulmonary tuberculosis (i.e. WHO tuberculosis diagnostic category I).[279] The regimens include 2 months of a standard dose of rifampicin combined with other anti-tuberculosis drugs, followed by 7-10 months of a standard dose of rifampicin and isoniazid. Therefore, the difference between these regimens with the standard regimens for pulmonary tuberculosis is only in the duration of treatment. Consequently, it is difficult to distinguish whether the anti-tuberculosis treatment was for pulmonary or extrapulmonary (including CNS) tuberculosis.

Not only antibiotics, but steroids were also used excessively in my study setting. Fifty-eight percent (58%) of children and 67% of adults received steroids before LP/EVD, and 46% of children and 38% of adults received steroids before neuroimaging. The use of steroids, such as dexamethasone, for the treatment of CNS infection, has been under debate for a number of years. Despite its excellent CNS penetration, which may be beneficial in reducing inflammation of the brain, the meninges, and/or the spinal cord; its long-term use has been associated with some adverse events.[280] Steroids have been shown to be useful as an adjunctive treatment for bacterial meningitis, tuberculous meningitis, and HSV encephalitis;[198, 281-283] although some other studies have reported conflicting results.[284-286] A recent meta-analysis investigating the benefit of adjunctive dexamethasone in bacterial meningitis cases showed that the treatment did not significantly reduce mortality and neurological sequelae following bacterial meningitis.[287]

3.4.5 Outcome

Despite the frequent use of antibiotics and steroids, mortality remains high, particularly in adult patients. This might be because some patients presented to the hospital in the late phase of the disease. Moreover, almost half of the adult patients had an immunocompromised condition which posed a higher risk for a fatal outcome. Such minimal diagnostic tests performed at the hospital could cause further delays in diagnosis and treatment. This finding underlines the need for advanced and rapid diagnostic tests to identify pathogens causing CNS infection at Dr Sardjito Hospital.

3.4.6 Syndromic clinical classification

Encephalitis was the most common syndromic CNS infection in children and adults, accounting for 175/247 (70.8%) and 83/168 (49.4%) cases, respectively. These proportions were much higher than those of meningoencephalitis, the second most common syndromic CNS infection in both age groups, constituting 43/247 (17.4%) cases in children and 37/168 (22%) cases in adults. The exceptionally high proportion of encephalitis cases in this study is interesting. As shown in tables 3.6 and 3.7, all patients with encephalitis had altered consciousness and fever at admission or a history of fever during the recent illness. Altered consciousness was the main criterion for encephalitis (table 3.1), whilst fever at admission or history of fever during the recent illness were parts of the inclusion criteria for the study; therefore, all of the patients enrolled in the study had those features.

In addition to altered consciousness and fever (or a history of fever), other criteria for encephalitis include any of the following: seizures or focal neurological signs (with evidence of brain parenchyma involvement), CSF leucocyte count >4 cells/µL (for any age), EEG findings suggesting encephalitis, and CT or MRI suggestive of encephalitis. This

definition allows patients with CSF leucocyte count ≤4 cells/μL to be classified as having encephalitis. Nevertheless, it remains interesting that the proportions of patients in the encephalitis group who had a CSF leucocyte count >4 cells/μL were as low as 48/175 (27%) in children and 25/83 (30%) in adults (or 48/141 (34%) in children and 25/51 (49%) in adults who underwent LP/EVD). These proportions are much lower than the proportion of patients with pleocytosis in an English study by Granerod *et al.* from which the encephalitis case definition was referred. This study reported that 159/198 (80%) patients with encephalitis who underwent LP had CSF pleocytosis.[78]

According to the consensus statement of the International Encephalitis Consortium, the absence of CSF pleocytosis does not rule out encephalitis as patients with an immunocompromised condition may have cell depletion and those who have LP in the early course of the disease may have not yet have developed CSF pleocytosis.[126] Immunodeficiency may explain the lack of pleocytosis for the adult patients with encephalitis in the present study, as 43% of them were HIV-positive. In the paediatric group, however, only three of four children with encephalitis tested for HIV were HIV-positive, while the remaining children were assumed to be immunocompetent. This finding narrowed the possibility that the low proportion of CSF pleocytosis in the paediatric group was due to immunodeficiency. It is possible, however, that some children might have the LP done in the early course of their disease, where an inflammatory response in the CNS has not been produced. If this is the case, a second LP may be necessary to evaluate the CSF findings, particularly if the patients did not improve.

Interestingly, among the notable criteria apart from altered consciousness and fever (or history of fever) by which the patients were classified as having encephalitis included abnormal neuroimaging findings (95%%) and seizures (84%) in children as well as abnormal neuroimaging findings (81%) and focal neurological signs (49%) in adults. CT and

MRI result interpretation is subjective by nature. Additionally, detailed descriptions of the CT or MRI results were unavailable for some patients; instead, there were only conclusions by the radiologists. Therefore, there might be a misinterpretation for a few of the neuroimaging results, resulting in a higher than anticipated proportion of CT or MRI abnormalities in the present study. Another possible explanation is that as CSF pleocytosis is not necessary to include encephalitis, some of these patients might actually have had encephalopathy rather than encephalitis.

Another interesting finding was the relatively high proportion of patients with classic symptoms and signs of meningitis, including headache, neck stiffness, and positive meningeal signs in both children and adults with encephalitis. These patients did not have CSF pleocytosis or a pathogen detected in their CSF or blood by the standard hospital testing, therefore could not be classified as having meningoencephalitis. The definition of meningitis which requires CSF pleocytosis or detection of an appropriate pathogen in CSF or blood, may better distinguish true meningitis cases from mimics, particularly in settings with an excellent diagnostic capacity. However, this definition might be too strict to implement in a limited resource setting where the diagnostic capacity is low or in a setting where HIV is common, such as in this study. As a result, patients with encephalitis that did not have a CSF pleocytosis (particularly the adult patients) might be those with clinical meningoencephalitis and HIV. Another possibility is that those patients underwent LP too early during disease progression, such that the CSF pleocytosis had not developed, or too late, such that the CSF leucocyte count could have normalised as the patient recovered. This could occur in both viral and bacterial cases where antibiotics and steroids had been given extensively. Due to the low capacity of the diagnostic testing, it was not possible to detect the pathogen in CSF or blood. It is also possible, however, that those cases were meningism and encephalopathy rather than encephalitis.

The diagnosis of brain abscess mainly was determined based on neuroimaging, and a causative pathogen was only identified in 1/9 (11.1%) cases (table 3.12). Furthermore, despite advances in neuroimaging techniques which may aid in distinguishing pathogens causing brain abscess, only a few of the cases had a detailed description in the neuroimaging results, which made it difficult to determine the causative pathogen. Therefore, the brain abscess cases described in this study might be caused by either bacteria, *M. tuberculosis*, fungi, or parasites. Additionally, they might also be cerebral toxoplasmosis cases that occurred in HIV-negative patients or those that occurred in HIV-positive patients but did not response to anti-toxoplasmosis treatment, which subsequently were not classified as presumptive cerebral toxoplasmosis.

Presumptive cerebral toxoplasmosis was the joint second most prevalent syndromic CNS infection in immunocompromised adults, specifically in those with HIV-positive status, along with meningoencephalitis, accounting for 12/67 (17.9%) cases each. Cerebral toxoplasmosis very rarely occurs in HIV-negative individuals with normal immunity.[288-290] Imran *et al*. reported a higher proportion of cerebral toxoplasmosis from a similar study in adults in Jakarta, the capital city of Indonesia, which accounted for 48/147 (33%) cases, almost double the proportion in the present study.[6] They also found that cerebral toxoplasmosis was the most common cause of CNS infection in HIV-positive adults. Although this was partly due to the case definition of definite and presumptive cerebral toxoplasmosis applied in both studies, these findings were also in accordance with a report from a multicentre study from the Asia Pacific region where cerebral toxoplasmosis was one of the most common neurological opportunistic infections seen in HIV-positive inpatients, in addition to cryptococcal meningitis, which accounted for 31% of the cases each.[291]

The criteria for various types of syndromic classification in this study might differ from those used by the treating clinicians. For instance, the clinicians in the study setting would classify patients with clinical signs and symptoms of meningitis as having meningitis (or meningoencephalitis if the patients also had decreased consciousness), although the patients did not have CSF pleocytosis and did not have any pathogen detected in their CSF. Meanwhile, the criteria for meningitis that I used in this study required CSF pleocytosis and/or pathogen detection in the CSF and/or blood (if LP was not performed). Consequently, patients diagnosed with meningoencephalitis by the treating clinicians but did not have any leucocyte and/or any pathogen detected in the CSF would be reported as having encephalitis in this study. Furthermore, in adults, the diagnostic criteria for cerebral toxoplasmosis used by the treating clinicians did not require an immunocompromised condition and a documented response to anti-toxoplasmosis treatment. These criteria were different from the case definition I used in the present study for presumptive cerebral toxoplasmosis, which required HIV-positive status and a documented clinical response to anti-toxoplasmosis treatment (table 3.1). Thus, the number of adults with syndromic CNS infection who received anti-toxoplasmosis treatment (n=32) was much higher than the number of adults whom I classified as having such a diagnosis (n=12).

3.5 Conclusion

The proportion of patients with CNS infection who did not have a pathogen detected was exceptionally high and unusual compared with other existing studies, including those conducted at the other tertiary referral hospitals in Indonesia. The standard hospital diagnostic testing showed a very poor performance in identifying a causative pathogen. Delays in LP might have contributed to this poor performance. Classic microbiological

testing applied at the study site could have yielded better results and provided better information if the implementation of LPs followed the recent guidelines as strictly as possible. The positivity rate of blood culture in identifying the pathogen causing CNS infection ranges from 50-80% in paediatric and adult cases if applied promptly; however, it decreases by 20% if the culture is done after the administration of antibiotics.[292-294] The yield of blood cultures in the present study remains much lower than the previously reported rate, indicating that its application remains sub-optimal. Patient outcomes (section 3.3.1.6) underline the impact of such poor diagnostic testing on the patients at the study site. Previous studies have shown that molecular diagnostics such as PCR can improve the detection of pathogens causing CNS infection even when the patients have received antibiotics prior to LP.[222-226] In the next chapter, I therefore want to assess if the same was true for the patients in my study.

Chapter 4 – Improving the detection of pathogens causing central nervous system infections in Yogyakarta using PCR technique

4.1 Background

I have shown in the previous chapter that most patients with CNS infection had an unknown aetiology. Limitations in the available diagnostic techniques that were routinely used at the hospital and delays in LP severely limited the pathogen detection rate to 11% in children and 6% in adults. In this chapter, I ask whether employing real-time PCR for a comprehensive panel of pathogens would increase the pathogen detection rate.

PCR has an essential role in the diagnosis of bacterial or viral CNS infections. It is much quicker than the traditional bacterial or viral cultures and provides more sensitive evidence of a current infection than serological tests. Although commercial multiplex PCR assays for the diagnosis of CNS infection are currently available, the target pathogens may be different from the pathogens which cause CNS infection in Indonesia. For example, the Fast Track Diagnostic (FTD) Bacterial Meningitis multiplex PCR assay targets *Neisseria meningitidis*, *Streptococcus pneumoniae,* and *Haemophilus influenza.* However, it does not include *Mycobacterium tuberculosis* in the panel, a pathogen reported as the most common aetiology of adult CNS infection in West Java and Jakarta, Indonesia.[6, 274]

Moreover, to my knowledge, none of the commercially available multiplex PCR kits has been previously used in Indonesia. Furthermore, multiplex PCR assays are generally more expensive but are less sensitive than singleplex PCR assays.[295] Despite their ability to detect multiple pathogens in one assay, the multiplex PCR assays need a specialised multi-channel thermocycler which may not be available in many laboratories in Indonesia. Even

if the multi-channel thermocycler is available, it requires a special installation and calibration for all channels to enable its multiplex function, which may cost more. With those limitations, it may be hard to translate the multiplex PCR assays into routine investigations at the hospital in the future. Moreover, if there are problems with the assays, it would be difficult to address. Although it is possible to develop in-house multiplex PCR assays as an alternative to the commercially available ones, designing primers and probes for a multiplex assay is more complex than designing those for a singleplex assay. Taking these into account, I decided to develop in-house singleplex PCR assays for my study.

My specific aims for this chapter are:

1) To describe the selection of specific primers and probes
2) To assess the sensitivity and specificity of the PCR primers and probes
3) To describe the challenges in transferring PCR methods to Indonesia
4) To describe the results of pathogen-specific real-time PCR in clinical samples
5) To compare in-house PCR and standard hospital diagnostic testing results
6) To compare in-house *M. tuberculosis* PCR and GeneXpert results

4.2 Methods

The specimens tested to obtain the data for this result chapter were provided from the UNITY-NeuroID project as described in chapter 2 unless otherwise stated.

4.2.1 Determining target pathogens for PCR

When I first set up this study, there were only a few publications on the aetiology of CNS infections in Indonesia which provided limited references on what pathogens to test for.[4, 5, 8, 10, 12, 29, 221, 274, 296] The reported aetiology included Japanese encephalitis virus (JEV), *H. influenzae, N. meningitidis, Escherichia coli, Staphylococcus aureus, Klebsiella ozaenae,*

and poliovirus in children and *M. tuberculosis, Cryptococcus neoformans, Toxoplasma gondii,* and *S. pneumoniae* in adults. These bacteria were primarily detected by CSF culture, latex agglutination test, or CSF Gram stain. Additionally, *H. influenzae* was also detected by PCR assay. *M. tuberculosis* was detected by in-house PCR assay, CSF Ziehl-Nielsen stain, or CSF culture. Detection of *C. neoformans* was performed using CSF Indian ink stain and antigen testing, whilst an in-house PCR assay was used to detect *T. gondii*. Serology testing on CSF or serum samples was used to detect anti-JEV IgM. The technique used to detect poliovirus in Indonesia was not reported in the literature. In summary, the only pathogens associated with CNS infection in Indonesia which PCR assays had successfully detected were *M. tuberculosis*, *H. influenza,* and *T. gondii*.

Using PCR assays, I aimed to screen for a larger scope of potential pathogens causing CNS infection in my study setting. Therefore, I searched for similar studies in neighbouring countries in the Southeast Asia region that used the same techniques as these countries might have similar CNS infection aetiology. I found two published studies, including those by Trung *et al*. and Tan *et al*., which investigated the aetiology of CNS infection in Vietnam using PCR and serology testing.[3, 230] The findings of these two studies helped inform the selection of target pathogens in the current study. The target pathogens for my PCR included enterovirus, herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2), CMV, varicella zoster virus (VZV), *S. pneumoniae, H. influenzae*, *N. meningitidis, M. tuberculosis, Streptococcus suis, E. coli,* Group B *Streptococcus* / *Streptococcus agalactiae, Klebsiella pneumoniae,* and *Salmonella* species (*Salmonella* spp.).

4.2.2 Selection of primers and probes sequences

Oligonucleotide primer and probe sequences used in the study were sought from published papers. Initially, I reviewed publications on pathogen detection, particularly

those related to CNS infection, which documented PCR primer and probe sequences in the reports. In developing pathogen-specific PCR assays, it is essential to use primer and probe sets with high specificity and sensitivity to obtain optimum PCR results. The specificity and sensitivity of each PCR primer and probe set were assessed to ensure that it only detected the target pathogen it was specifically designed to detect and not detect other pathogens I was screening for. This is a crucial step to avoid false-positive and false-negative results.

Initially, each primer and probe sequence was assessed *in silico* for their alignment with the pathogen sequence. A good primer/probe sequence should have high alignment with the target pathogen sequence it is designed to detect and low alignment with the other pathogens' sequence. Once the potential primer and probe set for each pathogen were selected based on the *in silico* assessment, its specificity and sensitivity were tested *in vitro*.

The above-mentioned methods were applied for all target pathogens except HSV-1, *M. tuberculosis, K. pneumoniae,* and *Salmonella* spp. *M. tuberculosis* primer and probe set used in this study were based on a PCR assay developed by Chaidir *et al*.[274] in Hasan Sadikin Hospital in Bandung, West Java, Indonesia; whilst primer and probe sets used for the detection of HSV-1, *K. pneumoniae,* and *Salmonella* spp. have been previously optimised by the other members of my research group (unpublished). Nevertheless, these primer and probe sets remained tested for their specificity *in vitro*.

4.2.2.1 Primers and probes assessment *in silico*

The primers and probes assessment *in silico* was performed by checking the similarity (matches) between the primers and probes sequence with particular viral and bacterial genomes using the Basic Local Alignment Search Tool (BLAST). This tool analyses the

similarity between query and subject sequences available in genome database, representing the results in scores. The bit score, also designated as the alignment score, which indicates whether the alignment is good or not, is calculated based on the number of matches, misses and gaps as well as the length of the gap; a higher score is regarded as a good alignment. On the other hand, the expect value (E-value) indicates the significance of the alignment calculated from the actual matches between the primer/probe sequence and the pathogen sequence as compared to the matches that may occur coincidentally between the primer/probe sequence with random sequences. Therefore, a lower E-value is associated with a more significant alignment.[297]

Each primer and probe sequence was blasted against the pathogen it was specifically designed to detect and against the other target pathogens I was screening for. Primers and probes with a higher alignment score and a lower E-value when tested against the pathogen it was designed to detect, and had a lower alignment score and a higher E-value when tested against the other target pathogens were selected.

4.2.2.2 Assessment of primer and probe specificity and sensitivity *in vitro*

From the BLAST results, I identified primer and probe sets that had the highest alignment with the target pathogen and the lowest alignment with the other pathogens. To confirm whether the primer and probe sets had high specificity and sensitivity, they were further assessed by *in vitro* testing. Initially, the custom primer and probe sets were ordered from Sigma Aldrich Corporation (UK). The specificity of each selected primer and probe set was then assessed by testing it against a series of positive controls. These included the target pathogen it was specifically designed to detect and the other pathogens I screened for. The primer's and probe's sensitivity was assessed by testing it against a serial dilution of the target pathogen to determine the lowest pathogen unit or nucleic acid concentration

it could detect, also known as the limit of detection (LoD). Due to time constraints, the LoD assays were only performed on several primer and probe sets including CMV, *S. suis*, *H. influenzae,* and *S. pneumonia*.

Enterovirus 71 (EV71) positive control was generated from clinical isolates owned by my research group, whilst echovirus, HSV-1, HSV-2, CMV, VZV, *S. pneumoniae*, *H. influenzae*, *K. pneumoniae,* and *Salmonella* spp. positive controls were recovered from laboratory strains isolates. *M. tuberculosis* control was donated from Biodefense and Emerging Infections Research Resources Repository, USA; whereas *S. suis*, *E. coli,* and *S. agalactiae* controls were attained from Public Health England culture collections. Additionally, *N. meningitidis* control was extracted directly from clinical specimens owned by my research group.

Initially, the *in vitro* specificity testing was performed using the standard PCR protocol established in my research group. The standard protocol was as follows: 50 °C for 2 minutes, then 95 °C for 10 minutes to allow *Taq* polymerase activation, followed by 55 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. If the PCR assay using the selected primer and probe set did not show optimum results when run using the standard protocol, the assay was then optimised by modifying the annealing temperature. This was done by testing the primer and probe set using an annealing temperature gradient and selecting the optimum temperature where the assay only amplified the target pathogen it was designed to detect (i.e. the positive control for the pathogen-specific PCR assay) and not amplified the other target pathogens (i.e. the negative controls for the pathogen-specific PCR assay). If the latter step failed, another set of primer and probe was sought based on the BLAST results, and the new set would be tested *in vitro* as described above. Details on the PCR reaction are described in chapter 2. The CFX Connect™ or CFX 96™ PCR machines (Bio-Rad) were used for all PCR reactions.

4.2.3 Pathogen-specific real-time PCR

4.2.3.1 General laboratory practice for performing molecular procedures

In order to ensure good laboratory practice and reduce the risk of cross-contamination when performing molecular procedures, the following precautions were taken:

- Avoiding contamination

  RNA/DNA extraction, master mix preparation, and PCR set-up were performed in separate areas. Surfaces and pipettes were sprayed with 70% ethanol before working, and gloves were changed frequently during experimental work. Any water used was nuclease-free. The nuclease-free water was divided into aliquots, and a new aliquot was used in each experiment. All plastic consumables, such as pipette tips and tubes, were also nuclease-free. Filtered pipette tips were used at all times.

- Avoiding sample and reagent degradation

  All samples and reagents were kept at appropriate temperatures until use to avoid degradation. All samples thawed for the first time were always divided into multiple aliquots to prevent unnecessary repeated freeze-thaw cycles.

- Adequate use of PCR controls

  At least two negative controls were included in each PCR run when testing clinical samples: (1) no template control / NTC [PCR master mix with an appropriate volume of nuclease free water instead of a clinical sample] and (2) negative water control [nuclease free water that had been extracted together with the same batch of samples]. The NTC was always included in duplicates. One positive control (specific to the pathogen being screened for) was included in each PCR batch. No reverse transcription / NRT [enterovirus RNA] control was included for the enterovirus PCR assay when I developed the assay at the Liverpool laboratory. However, as RNA is easily degraded when not stored at -80 ^{o}C, it was not possible to transfer the

enterovirus RNA from Liverpool to Indonesia. Consequently, the NRT control was not included in the enterovirus PCR assay performed in Indonesia.

- General practice

  Each reagent and sample was mixed thoroughly with a plate spinner, vortex, or by pipetting where appropriate. Samples were run in duplicates when necessary.

4.2.3.2 RNA/DNA extraction

RNA/DNA extraction was carried out using the QIAamp® MinElute® Virus Spin kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with a minor modification in the washing steps. Briefly, 200 µL of well-mixed sample was added to 25 µL of re-suspended protease. Two hundred microlitres (200 µL) of AVL buffer (containing 2.8 µL of carrier RNA) was then added before mixing and incubating at 56 °C for 15 minutes. Following the incubation, 250 µL of ethanol (96–100%) was added to the mixture and further incubated for 5 minutes at room temperature. The entire mixture was then transferred to the QIAamp spin column and centrifuged at 6000 *x g* for 1 minute. The column was then washed with buffers AW1 and AW2 (500 µL each) and centrifuged at 6000 *x g* for 1 minute after each wash. A modification was made from the kit protocol in the latter step where each washing step was performed twice instead of once. Following the final wash, a further 500 µL of ethanol was added, and the column was centrifuged at 6000 *x g* for 1 minute. To dry the membrane, the column was then centrifuged for 3 minutes before incubating at 56 °C for 3 minutes to allow any remaining liquid to evaporate. Finally, the RNA/DNA was eluted with 25-30 µL of buffer AVE. The modified extraction protocol was based on the optimisation performed by another member of my research group.

4.2.3.3 Reverse transcription

Reverse transcription (RT) was performed using the Ambion RETROscript™ kit (Thermo Fisher Scientific, Massachusetts, US). One microgram (1 µg) of RNA was made up to a volume of 10 µL with nuclease-free water. If 10 µL of RNA contained less than 1 µg of RNA, the full 10 µL of eluted RNA was used. RT master mix, which contained 2 µL random decamers, 2 µL of 10x RT buffer, 4 µL of dNTP mix, 1 µL of RNase inhibitor, and 1 µL of M-MLV reverse transcriptase enzyme, was then added. RT was carried out at 44 °C for 1 hour, followed by 92 °C for 10 minutes.

4.2.3.4 Real-time PCR assay

The reaction mix for TaqMan-based real-time PCR included 1X TaqMan® Universal Master Mix (Thermo Fisher Scientific, Massachusetts, US), 0.3 µM of each oligonucleotide primer, 0.2 µM of probe, 1.6 µl of template/sample, and nuclease-free water with a final volume of 25 µL. This reaction mix was applied to all target pathogens except *K. pneumoniae* and *Salmonella,* where 2 µl of template/sample was used, and the water volume was adjusted accordingly. All primers and probes were obtained from Sigma Aldrich (Missouri, US). A no-template control (NTC), positive control, and negative extraction control were included in every run. The PCR conditions were as follows: 50 °C for 2 minutes, then 95 °C for 10 minutes to allow *Taq* polymerase activation, followed by 55 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. This was applied to all target pathogens except *S. suis, H. influenzae*, *E. coli,* and *K. pneumoniae* where modified PCR protocols were applied. The modified PCR protocols were obtained after performing assay optimisation using an annealing temperature gradient. All samples were tested singly, and the quantification cycle (Cq) values were recorded.

4.2.4 Transfer of PCR methods from the University of Liverpool to Indonesia

The initial primer and probe assessment were performed at the University of Liverpool laboratory. Once the PCR protocol for each primer and probe set had been optimised, the assessment was repeated at Yogyakarta laboratory using the same reagents and positive controls brought from Liverpool. The reagents that were brought from Liverpool included the PCR master mix, the oligonucleotide primers and probes, as well as the nuclease-free water. All positive controls were tested in duplicate except for *N. meningitidis* due to limited volume. The PCR assays at the local laboratory were also performed using the Bio-rad CFX Connect™ PCR machine and the same PCR protocol. Other reagents, including RNA extraction and reverse transcription kits, were also brought from Liverpool to Indonesia.

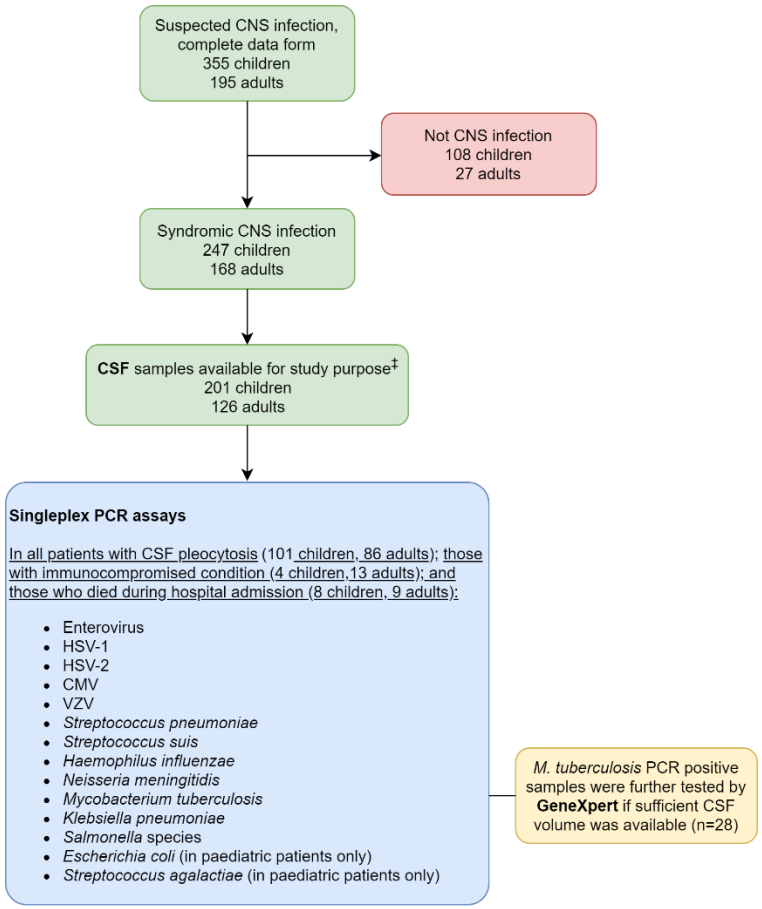
4.2.5 Determining which clinical samples to test for

Once the pathogen-specific (singleplex) PCR methods were successfully transferred to the local Yogyakarta laboratory, I began to test CSF samples obtained from patients with syndromic (clinical) CNS infection as classified in chapter 3 using these assays. Of the 208 children and 107 adults with syndromic CNS infection who underwent LP/EVD, only 201 (97%) children and 126 (99%) adults had CSF samples available for study purposes (figure 4.1).

In this chapter, patients with syndromic CNS infection were further classified into two sub-groups. These included the following:

1) Patients with non-neurosurgical CNS infection;
2) Patients with neurosurgical infection, including those with definite shunt infection (n=10 children) and other post-neurosurgical infection (n=1 child).

Figure 4.7 Singleplex PCR testing algorithm



CSF pleocytosis is defined as a CSF leucocyte count of >4 cells/μL for patients ≥6 weeks of age or >14 cells/μL for patients <6 weeks of age. ‡CSF samples were obtained from 201/208 (97%) children and 126/127 (99%) adults with syndromic CNS infection who underwent LP/EVD.
CNS – central nervous system; CSF – cerebrospinal fluid; PCR – polymerase chain reaction; HSV – herpes simplex virus; CMV – cytomegalovirus; VZV – varicella-zoster virus.

Due to limited reagent availability and time constraints, the clinical samples were tested in batches based on priority. The first priority was to test samples with CSF pleocytosis as this parameter indicates CNS inflammation which may be due to infection, a reason why it is often used as a criterion for CNS infection. Moreover, the high CSF leucocyte count might also indicate that the patients had the LP done before receiving appropriate treatment. Therefore, the chance for successful pathogen detection in these samples could be higher than in samples with low CSF leucocyte count. The numbers of patients with CSF pleocytosis whose CSF sample was available for study purposes were as follows:

- One-hundred-and-one (101) children, including 92 children with non-neurosurgical CNS infection and nine children with neurosurgical infection;
- Eighty-six (86) adults, all of whom had non-neurosurgical CNS infections.

The second priority was to test CSF samples obtained from immunocompromised patients who did not have CSF pleocytosis. Immunocompromised patients may be unable to develop an inflammatory response within the CNS against pathogens causing CNS infection, or this inflammatory response may be delayed.[298] Moreover, immunocompromised patients are at a higher risk for having any infections, including CNS infection, due to their low immunity. In the present study, 4 children and 13 adults with the immunocompromised condition did not have CSF pleocytosis, all of whom had non-neurosurgical CNS infection.

The next priority was to test CSF samples obtained from patients without CSF pleocytosis who were immunocompetent but died during hospital admission. These included 8 children and 9 adults, all of whom had non-neurosurgical CNS infection.

All the CSF samples mentioned above were tested for the following PCR target pathogens: enterovirus, HSV-1 and HSV-2, CMV, VZV, *S. pneumoniae, H. influenzae*, *N. meningitidis, M. tuberculosis, S. suis, K. pneumoniae,* and *Salmonella* spp*.* Additionally, for paediatric samples only, they were also tested for *E. coli* and *S. agalactiae* (figure 4.1).

In summary, PCR assays were performed on the following clinical samples (figure 4.1):

- One-hundred-and-thirteen (113) paediatric CSF samples, including 101 samples with CSF pleocytosis, 4 samples from immunocompromised children, and 8 samples from children who died during hospital admission. These samples were tested for enterovirus, HSV-1, HSV-2, CMV, VZV, *S. pneumoniae, H. influenzae*, *N. meningitidis, M. tuberculosis, S. suis, K. pneumoniae, Salmonella* spp., *E. coli,* and *S. agalactiae*.

- One-hundred-and-eight (108) adult CSF samples, including 86 samples with CSF pleocytosis, 13 samples from immunocompromised adults, and 9 samples from adults who died at the hospital. These samples were tested for enterovirus, HSV-1 and HSV-2, CMV, VZV, *S. pneumoniae, H. influenzae*, *N. meningitidis, M. tuberculosis, S. suis, K. pneumoniae,* and *Salmonella* spp.

4.2.6 Statistical analysis

Continuous data were checked for normality, and a *t*-test was used to analyse normally distributed data, whilst non-parametric data were analysed using Mann Whitney U or Kruskal-Wallis tests. Chi-Square (or Fisher's exact if any of the expected counts were less than five) test was used to analyse categorical data. Results were recorded as number per number available (n/N), as some patients had missing data, and therefore, the number available (N) did not always represent the total number of patients. SPSS v24 and GraphPad Prism v6 were used to analyse the data; graphs in this chapter were created using draw.io (www.draw.io) and GraphPad Prism v6.

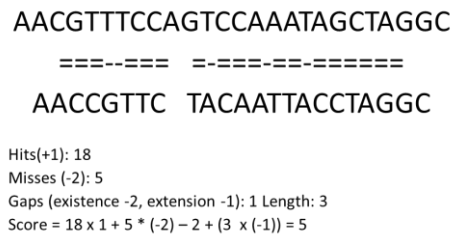
4.3 Results

4.3.1 Assessing the alignment of the primer and probe sequence with the pathogen sequence *in silico*

For each target pathogen, each potential primer and probe sequence was blasted against the target pathogen it was designed to detect and against other target pathogens I was screening for. BLAST tool calculates the E-value and bit score of each primer/probe sequence against the specified pathogen sequence. As described above, the bit score (or alignment score) indicates how well the primer/probe sequence aligns with the target pathogen sequence, and a higher score is regarded as a good alignment. It is calculated based on the number of matches, misses and gaps as well as the length of the gap.

A simplified example to explain the principle of bit score calculation is shown in figure 4.2. A “hit” is scored by +1, a “miss” is scored by -2, a “gap existence” is scored by -2, a “gap extension” is scored by -1, and the “length of gap” is scored by -1. In the example below, the reference sequence (top) length is 26 bases, whereas the query sequence (bottom) is 23. Therefore, if the query sequence is 100% matched with the reference sequence without any misses and gaps (i.e. there are 23 hits), the maximum bit score will be (23 x 1), equal to 23. However, comparing both sequences, the query sequence has 18 hits, 5 misses, and 1 gap existence with a gap length of 3 bases. Therefore, the bit score is calculated as follows: (18 x 1) + (5 x (-2)) – 2 + (3 x (-1)), which is equal to 5.

Figure 4.8 Example of bit score calculation



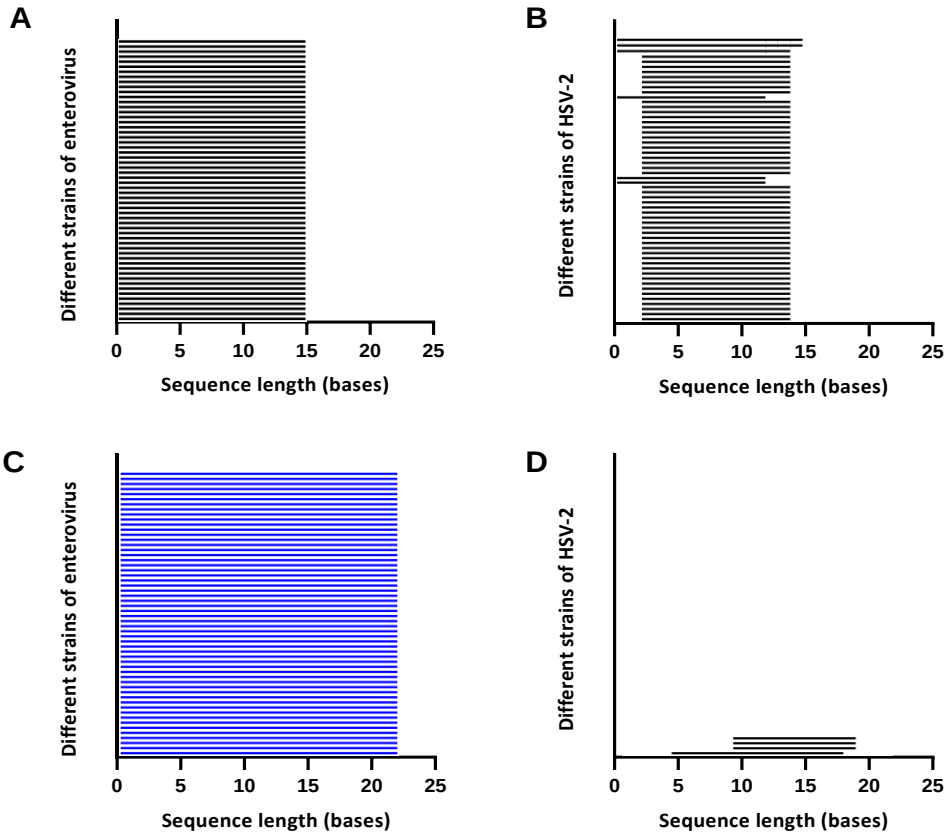
The above example is a simplified example whereby the formula differs slightly from the formula used by the BLAST tool. The actual BLAST results, including the E-value and the bit score, for enterovirus primers and probes sequence adapted from Nijhuis *et al*.[299] and Rotbart *et al*.[300] are presented in table 4.1. Figure 4.2 depicts the example of BLAST results for enterovirus forward primer sequence adapted from both publications. The Nijhuis’ forward primer sequence is shorter than Rotbart’s sequence (15 bases versus 22 bases). Therefore, when blasted against the enterovirus genome, although both sequences show 100% alignment with the enterovirus genome (figures 4.2 A and 4.2 C), the bit score of the former sequence is lower than the latter one (30.2 versus 44.1) (table 4.1). Nevertheless, the E-value of the Nijhuis’ sequence is higher than Rotbart’s sequence (0.14

versus 3e-05) (table 4.1), suggesting that the latter sequence had a more significant alignment to the enterovirus genome compared to the former sequence.

Table 4.21 E-value and bit score of enterovirus primers and probe from two different publications when blasted against enterovirus and HSV-2

Target	Sequence reference	Primers and probes	Sequence (5' to 3')	Sequence length	Against enterovirus		Against HSV-2	
					E-value	Bit score	E-value	Bit score
Enterovirus	Nijhuis *et al.*[299]	Forward primer	TCCTCCGGCCCCTGA	15	0.14	30.2	5.2	24.3
		Reverse primer	AATTGTCACCATAAGCAGCCA	21	1e-04	42.1	248	20.3
		Probe	CGGAACCGACTACTTTGGGTGTCCGT	26	2e-07	52	449	20.3
	Rotbart *et al.*[300]	Forward primer	CAATTGTCACCATAAGCAGCCA	22	3e-05	44.1	311	20.3
		Reverse primer	GGCCCCTGAATGCGGCTAAT	20	4e-04	40.1	61	22.3
		Probe	GAAACACGGACACCCAAAGTA	21	1e-04	42.1	71	22.3

Figure 4.9 BLAST results of enterovirus forward primer sequence adapted from Nijhuis *et al.*[299] (15 bases) when blasted against enterovirus genome (A) and HSV-2 genome (B), and sequence from Rotbart *et al.*[300] (22 bases) when blasted against enterovirus genome (C) and HSV-2 genome (D). Both Nijhuis' and Rotbart's primer sequences show 100% alignment with the enterovirus sequence (figures 2A and 2C), suggesting that both primers have a high sensitivity. However, Rotbart's sequence shows a lower alignment with the HSV-2 sequence and fewer matches with HSV-2 strains (figure 2D) compared to the Nijhuis' sequence (figure 2B), indicating the Rotbart's sequence is more specific for enterovirus.



When the Nijhuis' primer sequence was blasted against the HSV-2 genome (figure 4.2 B), most of the primer sequence matched with the HSV-2 genome with a bit score of 24.3 and E-value of 5.2 (table 4.1), indicating this enterovirus primer sequence also has a good alignment with HSV-2. In contrast, when Rotbart's primer sequence was blasted against the HSV-2 genome (figure 4.2 D), there were many "gaps" between the primer sequence and the HSV-2 genome. Moreover, Rotbart's primer sequence only matches with 4 HSV-2 strains, much less than Nijhuis's (figures 4.2 D and 4.2 A). The bit score and E-value for the Rotbart's sequence, when blasted against the HSV-2 genome, are 20.3 and 311, respectively (table 4.1), indicating this enterovirus primer has a low and less significant alignment with HSV-2. Compared with the Nijhuis' sequence, the Rotbart's sequence has a more significant alignment with the enterovirus genome and a lower and lesser significant alignment with the HSV-2 genome.

4.3.2 Assessing the specificity and sensitivity of the selected primers and probes *in vitro*

4.3.2.1 Assessing the specificity of the selected primers and probes *in vitro*

The selected primers and probe sets were then tested against a series of positive controls, including enteroviruses, HSV-1, HSV-2, CMV, VZV, DENV, *S. pneumoniae*, *S. suis*, *H. influenzae*, *N. meningitidis*, *M. tuberculosis*, *E. coli*, *S. agalactiae, K. pneumoniae,* and *Salmonella* spp. Most of the selected primer and probe sets work optimally when tested using the standard PCR protocol, including those targeting enteroviruses, HSV-1, HSV-2, CMV, VZV, DENV, *S. pneumoniae*, *S. suis*, *N. meningitidis*, *M. tuberculosis*, *S. agalactiae* and *Salmonella* spp. The results of the *in vitro* specificity testing for these primer and probe sets are shown in table 4.5.

Interestingly, primer and probe sets targeting *H. influenzae, S. suis, E. coli* and *K. pneumoniae* did not work straight away when tested using the standard protocol. In these cases, the protocol was modified by adjusting the annealing temperature and, if required, adjusting the number of cycles.

Below is an example of *H. influenzae* PCR assay optimisation. The optimisation was performed in the early stage of my study, where I had not had positive controls for *M. tuberculosis*, *E. coli*, *S. agalactiae, K. pneumoniae,* and *Salmonella* spp. Therefore, the primers and probes were tested *in vitro* only against the positive controls for *H. influenza,* enteroviruses, HSV-1, HSV-2, CMV, VZV, DENV, *S. pneumoniae*, *S. suis*, and *N. meningitidis*. The primers and probes used to detect *H. influenza* were adapted from a previous study conducted by Wang *et al*.[301] In their study, two real-time PCR assays targeting protein D-encoding gene (*hpd*) were developed, including *hpd* #1 and *hpd* #3 assays for detection of both typeable and non-typeable *H. influenzae*. Based on the BLAST results, primer and probe sets used for *hpd* #1 and *hpd* #3 assays had the highest sensitivity and specificity among the other published primer and probe sequences (data not shown). Moreover, the *hpd* #3 primer and probe set had slightly higher sensitivity and specificity than the *hpd* #1 and therefore became my first preference.

The *hpd* #3 primer and probe set was initially assessed using the standard PCR conditions with an annealing temperature of 60 ^{o}C. However, not only were *H. influenzae* positive controls amplified, but also *N. meningitidis* and CMV controls (data not shown). Assay optimisation was then performed by running a gradient PCR with annealing temperatures set in a range of 60 ^{o}C to 68 ^{o}C. The *H. influenzae* positive control in this experiment was consistently amplified in each annealing temperature set. Nevertheless, the CMV and *S. pneumoniae* controls were also amplified at almost all annealing temperatures, not to mention the EV71 control, which also amplified at 60 ^{o}C, 60.6 ^{o}C, 61.6 ^{o}C, and 63.2 ^{o}C

(table 4.2). These results suggest that this primer and probe set is not specific for *H. influenzae*. These also indicate that although the primer and probe set showed high sensitivity and specificity *in silico* (i.e. when the sequences were assessed using BLAST), it might not be the case *in vitro.*

The *hpd* #1 primer and probe set was then preferred as the alternative. This set was initially assessed using the standard PCR conditions. Unexpectedly, the results were similar to those of the *hpd* #3 where some negative controls, including CMV, *S. pneumoniae*, and EV71, were amplified in addition to the *H. influenzae* positive controls. Following my assay optimisation protocol, a gradient PCR was performed with annealing temperatures set at 60 ^{o}C to 68 ^{o}C.

Table 4.22 Optimisation of hpd #3 *Haemophilus influenzae* primer and probe set using an annealing temperature gradient

Sample	Cq value at specific annealing temperature							
	60 ^{o}C	60.6 ^{o}C	61.6 ^{o}C	63.2 ^{o}C	65.1 ^{o}C	66.7 ^{o}C	67.6 ^{o}C	68 ^{o}C
H. influenzae	**16.87**	**16.68**	**16.59**	**16.44**	**17.07**	**21.22**	**27.98**	**33.53**
EV71	40.83	40.87	40.87	40.30	ND	ND	ND	ND
Echovirus	ND	ND	ND	ND	ND	ND	ND	ND
HSV-1	ND	ND	ND	ND	ND	ND	ND	ND
HSV-2	ND	ND	ND	ND	ND	ND	ND	ND
VZV	ND	ND	ND	ND	ND	ND	ND	ND
CMV	40.03	38.50	ND	38.61	39.72	41.53	49.90	ND
DENV	ND	ND	ND	ND	ND	ND	ND	ND
S. pneumoniae	37.58	41.25	39.03	38.32	40.09	43.68	51.63	ND
S. suis	ND	ND	ND	ND	ND	ND	ND	ND
N. meningitidis	ND	ND	ND	ND	ND	ND	ND	ND
NTC	ND	ND	ND	ND	ND	ND	ND	ND

Cq – quantification cycle; EV71 – enterovirus 71; HSV-1 – herpes simplex virus type 1; HSV-2 – herpes simplex virus type 2; CMV – cytomegalovirus; VZV – varicella-zoster virus; DENV – dengue virus; *S. pneumoniae* – *Streptococcus pneumoniae; S. suis* – *Streptococcus suis; H. influenza – Haemophilus influenza; N. meningitidis – Neisseria meningitidis*; NTC – no template control; ND – not detected.

As described in table 4.3, the *H. influenzae* positive control was optimally amplified at the four lowest annealing temperature sets, including 60 ^{o}C, 60.6 ^{o}C, 61.6 ^{o}C, and 63.2 ^{o}C, where the Cq values of the *H. influenzae* positive control were relatively low. Although

the *H. influenzae* positive control was also amplified in the annealing temperature of 65.1 ^{o}C, its Cq value was too high. Nevertheless, the *S. pneumoniae* control was also consistently amplified at the annealing temperatures of 60 ^{o}C, 60.6 ^{o}C, 61.6 ^{o}C, and 63.2 ^{o}C. The fact that EV71 and VZV were also amplified at 60.6 ^{o}C and 61.6 ^{o}C narrowed down the preferences of the optimum annealing temperature. As the difference in Cq values between the *H. influenzae* and the *S. pneumoniae* controls at the annealing temperature of 63.2 ^{o}C was bigger than that of 60 ^{o}C (i.e. 24.62 versus 11.31 cycles), the former temperature was established as the optimum annealing temperature. Moreover, to avoid the formation of a nonspecific PCR product which may lead to a false positive result, the PCR assay for the detection of *H. influenzae* was run for 40 cycles instead of the standard 55 cycles.

Table 4.23 Optimisation of *hpd* #1 *Haemophilus influenzae* primers and probe using an annealing temperature gradient

Sample	Cq value at specific annealing temperature							
	60 ^{o}C	60.6 ^{o}C	61.6 ^{o}C	*63.2 ^{o}C*	65.1 ^{o}C	66.7 ^{o}C	67.6 ^{o}C	68 ^{o}C
H. influenzae	**17.11**	**17.65**	**18.17**	***21.87***	**42.56**	**ND**	**ND**	**ND**
EV71	ND	ND	42.52	*ND*	ND	ND	ND	ND
Echovirus	ND	ND	ND	*ND*	ND	ND	ND	ND
HSV-1	ND	ND	ND	*ND*	ND	ND	ND	ND
HSV-2	ND	ND	ND	*ND*	ND	ND	ND	ND
VZV	ND	39.73	40.24	*ND*	ND	ND	ND	ND
CMV	ND	ND	ND	*ND*	ND	ND	ND	ND
DENV	ND	ND	ND	*ND*	ND	ND	ND	ND
S. pneumoniae	38.42	38.71	37.63	*46.49*	ND	ND	51.63	ND
S. suis	ND	ND	ND	*ND*	ND	ND	ND	ND
N. meningitidis	ND	ND	ND	*ND*	ND	ND	ND	ND
NTC	ND	ND	ND	*ND*	ND	ND	ND	ND

The optimum annealing temperature and the Cq values at which the series of controls were amplified are highlighted in *italic.*
Cq – quantification cycle; EV71 – enterovirus 71; HSV-1 – herpes simplex virus type 1; HSV-2 – herpes simplex virus type 2; CMV – cytomegalovirus; VZV – varicella zoster virus; DENV – dengue virus; *S. pneumoniae – Streptococcus pneumoniae; S. suis – Streptococcus suis; H. influenza – Haemophilus influenza; N. meningitidis – Neisseria meningitidis*; NTC – no template control; ND – not detected.

Following the primers and probes assessment *in silico* and *in vitro* and the assay optimisation for each pathogen-specific PCR assay, a decision was made to select the final sets of pathogen-specific primers and probes. The sequences of the selected primers and probes are shown in table 4.4, whilst the PCR protocols for each pathogen-specific PCR assay are outlined in table 4.5.

The results of the *in vitro* specificity testing of the selected primer and probe sets are shown in table 4.6. The primers and probes for enteroviruses, HSV-1, HSV-2, CMV, *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, *M. tuberculosis*, *E. coli*, and *S. agalactiae* were tested against each other, VZV, *S. suis*, and dengue virus (DENV). Primers and probes for VZV and *S. suis* were tested against all of the pathogens above, excluding *M. tuberculosis*, *E. coli*, and GBS. This was because the VZV and *S. suis* PCR assays had been optimised and tested at Liverpool and Yogyakarta laboratories before the positive controls of the three latter pathogens were available.

The decision to include *K. pneumoniae* and *Salmonella spp.* as target pathogens for my systematic PCR testing was made in the later stage of my PhD after a few cases in my study with blood and/or CSF culture positive for those pathogens were found in routine clinical testing in the hospital laboratory. Therefore, the primers and probes targeting the other pathogens optimised prior to this were not tested against both *K. pneumoniae* and *Salmonella spp*. The optimisation for these two PCR assays was done by the other members of my research group. Not all of the target pathogens in my study were included in their optimisation (table 4.6).

Table 4.24 Oligonucleotide sequences of the primers and probes used in pathogen-specific PCR assays

Target pathogen	Gene encoded	Oligonucleotide sequence (5' to 3')					
		Forward primer	Reverse primer	Probe	Tm °C- forward	Tm°C - reverse	Reference
Enterovirus	*polyprotein*	CAATTGTCACCATAAGCAGCCA	GGCCCCTGAATGCGGCTAAT	GAAACACGGACACCCAAAGTA	66.8	71.0	Rotbart *et al.*[300]
HSV1	*gB-1*	GCAGTTTACGTACAACCACATACAG	AGCTTGCGGGCCTCGTT	CGGCCCAACATATCGCGTTGACATGGC	66.2	68.4	Nyström *et al.*[302]
HSV2	*gG2*	CAAGCTCCCGCTAAGGACAT	GGTGCTGATGATAAAGAGGATATCTAGA	ACACATCCCCCTGTTCTGGTTCCTAACG	65.4	64.4	Pevenstain *et al.*[303]
CMV	*MCP*	CATTCCCACTGACTTTCTGACGCACGT	TGAGGTCGTGGAACTTGATGGCGT	GGTCATCGCCGTAGTAGATGCGTAAGGCCT	75.0	74.4	Atkins *et al.*[304]
VZV	*DNA polymerase*	GATGGTGCATACAGAGAACATTCC	CCGTTAAATGAGGCGTGACTAA	AAAGTTCCGCGCTGCAGGTTCCAGTAATGCTCTA	65.5	64.8	Bøving *et al.*[305]
S. pneumoniae	*psaA*	GCCCTAATAAATTGGAGGATCTAATGA	GACCAGAAGTTGTATCTTTTTTTCCG	CTAGCACATGCTACAAGAATGATTGCAGAAAGAAA	65.6	65.5	Maria da Gloria *et al.*[306]
S. suis	*cps2J*	GGTTACTTGCTACTTTTGATGGAAATT	CGCACCTCTTTTATCTCTTCCAA	TCAAGAATCTGAGCTGCAAAAGTGTCAAATTGA	64.1	65.2	Thi Hoang Mai *et al.*[307]
H. influenzae	*hpd*	AGATTGGAAAGAAACACAAGAAAAAGA	CACCATCGGCATATTTAACCACT	AAACATCCAATCGTAATTATAGTTTACCCAATAACCC	64.8	65.5	Wang *et al.*[301]
N. meningitidis	*ctrA*	GCTGCGGTAGGTGGTTCAA	TTGTCGCGGATTTGCAACTA	CATTGCCACGTGTCAGCTGCACAT	66.2	66.4	Corless *et al.*[308]
M. tuberculosis	*IS6110*	GGGTAGCAGACCTCACCTATGTG	TAGGCGTCGGTGACAAAGG	TCGCCTACGTGGCCTTT	65.7	66.2	Chaidir *et al.*[274]
E. coli	*16S rDNA*	CATGCCGCGTGTATGAAGAA	CGGGTAACGTCAATGAGCAAA	TATTAACTTTACTCCCTTCCTCCCCGCTGAA	67	66.8	Huijsdens *et al.*[309]
S. agalactiae	*dltS*	AGGAATACCAGGCGATGAAC	AGGCCCTACGATAAATCGAG	ATTTGGCTAGTTATGAAGTCCCTTATGC	62.8	61.9	Chiba *et al.*[310]
K. pneumoniae	*phoE*	CCTGGATCTGACCCTGCAGTA	CCGTCGCCGTTCTGTTTC	CAGGGTAAAAACGAAGGC	66.4	66.9	Shannon *et al.*[311]
***Salmonella* spp.**	*ttrC/A*	CTCACCAGGAGATTACAACATGG	AGCTCAGACCAAAAGTGACCATC	CACCGACGGCGAGACCGACTTT	64.4	65.5	Malorny *et al.*[312]

HSV – herpes simplex virus; CMV – cytomegalovirus; VZV – varicella-zoster virus; *S. pneumoniae – Streptococcus pneumonia; S. suis – Streptococcus suis; H. influenzae – Haemophilus influenzea; N. meningitidis – Neisseria meningitidis; M. tuberculosis – Mycobacterium tuberculosis; E. coli – Escherichia coli; S. agalactiae – Streptococcus agalactiae; K. pneumoniae – Klebsiella pneumoniae; Salmonella* spp. *– Salmonella species*; Tm – melting temperature.

Table 4.25 Thermocycler protocols for pathogen-specific PCR reactions showing different annealing temperatures and number of cycles

Protocol	Start temperature	Duration	Activation temperature	Duration	Denaturing temperature*	Duration	Annealing temperature*	Duration	Number of cycles
Standard protocol	50 °C	2 minutes	95 °C	10 minutes	95 °C	15 seconds	60 °C	1 minute	55
S. suis	50 °C	2 minutes	95 °C	10 minutes	95 °C	15 seconds	60.4 °C	1 minute	55
H. influenzae	50 °C	2 minutes	95 °C	10 minutes	95 °C	15 seconds	63.2°C	1 minute	40
E. coli	50 °C	2 minutes	95 °C	10 minutes	95 °C	15 seconds	66.1 °C	1 minute	40
K. pneumoniae	50 °C	2 minutes	95 °C	10 minutes	95 °C	15 seconds	60 °C	1 minute	40

*Denaturing and annealing steps are repeated the number of times as indicated in column "number of cycles".

Table 4.26 Assessment of the selected primers and probes against the positive control of all target pathogens

Primer and probe set	Target (Cq value)															
	EV71	**Echovirus**	**HSV-1**	**HSV-2**	**CMV**	**VZV**	**DENV**	***S. pneumoniae***	***S. suis***	***H. influenzae***	***N. meningitidis***	***M. tuberculosis***	***E. coli***	**GBS**	***K. pneumoniae***	***Salmonella* spp.**
Enteroviruses	14.76	16.70	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	x	x
HSV-1	ND	ND	17.78	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	x	x
HSV-2	ND	ND	ND	16.47	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	x	x
CMV	ND	ND	ND	ND	19.33	ND	ND	ND	ND	ND	ND	ND	ND	ND	x	x
VZV	ND	ND	ND	ND	ND	20.13	ND	ND	ND	ND	ND	x	x	x	x	x
S. pneumoniae	ND	ND	ND	ND	ND	ND	ND	18.75	ND	ND	ND	ND	ND	ND	x	x
S. suis	ND	ND	ND	ND	ND	ND	ND	ND	20.30	ND	ND	x	x	x	x	x
H. influenzae	ND	ND	ND	ND	ND	ND	ND	ND	ND	21.87	ND	ND	ND	ND	x	x
N. meningitidis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	28.15	ND	ND	ND	x	x
M. tuberculosis	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.66	ND	ND	x	x
E. coli	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	19.53	ND	x	x
S. agalactiae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	27.38	x	x
K. pneumoniae	x	x	ND	ND	ND	ND	x	ND	ND	ND	ND	ND	ND	ND	21.45	ND
***Salmonella* spp.**	x	x	ND	ND	ND	ND	x	ND	ND	ND	x	ND	ND	ND	x	32.31

Values are mean values of duplicates amplified.

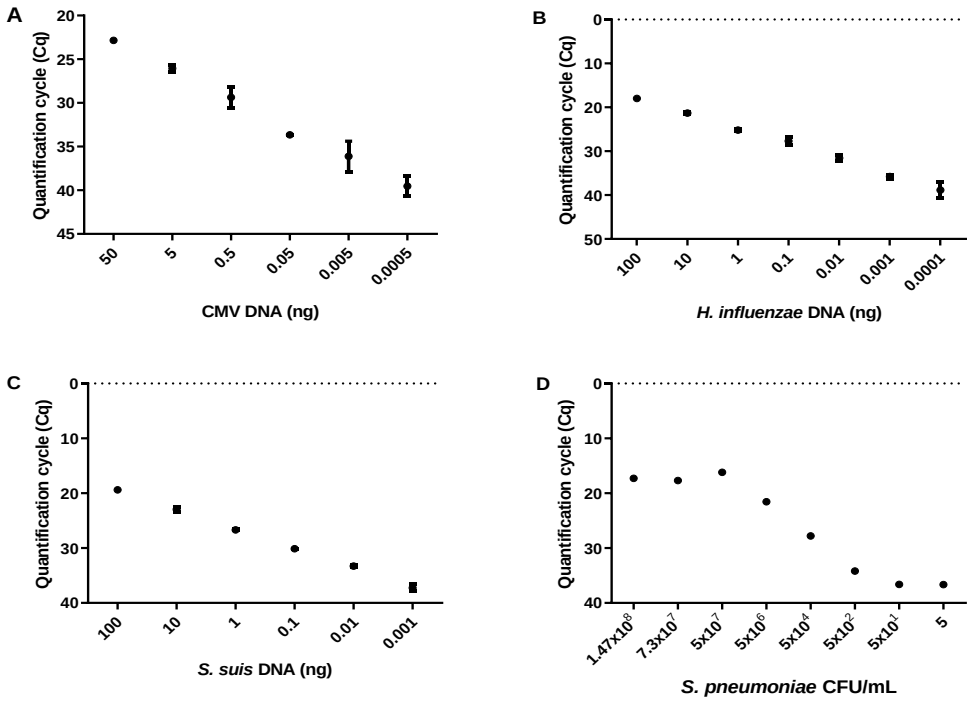
Cq – Quantification cycle; EV71 – enterovirus 71; HSV – herpes simplex virus; CMV – cytomegalovirus; VZV – varicella-zoster virus; DENV – dengue virus*; S. pneumoniae – Streptococcus pneumoniae; S. suis – Streptococcus suis; H. influenzae – Haemophilus influenzae; N. meningitidis – Neisseria meningitidis; M. tuberculosis – Mycobacterium tuberculosis; E. coli – Escherichia coli; S. agalactiae – Streptococcus agalactiae; K. pneumoniae – Klebsiella pneumoniae; Salmonella* spp. *– Salmonella* species; ND – not detected; x – not done

Each primer and probe set only detected the target pathogen(s) it was designed for and did not detect any other target pathogens (table 4.5. The quantification cycle (Cq) value of the positive controls ranged from 11.66 (for *M. tuberculosis*) to 32.31 (for *Salmonella spp.*), with a median of 19.83. All positive controls were detected at Cq values of <22 except for *N. meningitidis* (28.15), GBS (27.38), and *Salmonella spp.* (32.31). The positive control for *N. meningitidis* was DNA extracted from a clinical sample with proven meningococcal meningitis; thus, a slightly higher Cq value was expected. The mean Cq value of the *Salmonella spp*. positive control was obtained from the Yogyakarta experiment, where its primer and probe set was tested against more target pathogens than in the experiments performed in Liverpool.

4.3.2.2 Assessing the sensitivity (limit of detection) of the selected primers and probes *in vitro*

Primers and probes targeting CMV, *H. influenzae, S. suis,* and *S. pneumonia* were assessed for their limit of detection. CMV primer and probe set was tested on a serial dilution of CMV DNA starting from 50 ng down to 0.005 pg, and the lowest amount of DNA detected was 0.5 pg with a mean (± SD) Cq value of 39.54 (± 1.17) (figure 4.4 A). *H. influenzae* primer and probe set was assessed on a serial dilution of *H. influenzae* DNA starting from 100 ng to 0.01 pg, and the lowest detection was seen at a DNA concentration of 1 pg with a mean Cq value of 38.86 (± 1.91) (figure 4.4 B). *S. suis* primer and probe set was tested on a serial dilution of *S. suis* DNA starting from 100 ng to 0.01 pg, the lowest detection was observed at a DNA concentration of 0.1 pg with a mean Cq value of 37.24 (± 0.66) (figure 4.4 C).

Figure 4.10 Assessment of the limit of detection of the primers and probes specific for cytomegalovirus (CMV) [A], *Haemophilus influenzae* (*H. influenzae)* [B], *Streptococcus suis* (*S. suis*) [C], and *Streptococcus pneumoniae* (*S. pneumoniae*) [D]. All experiments were done in duplicate except for experiment D, which was done singly. Quantification cycle (Cq) values are mean values of duplicates amplified ± SD.



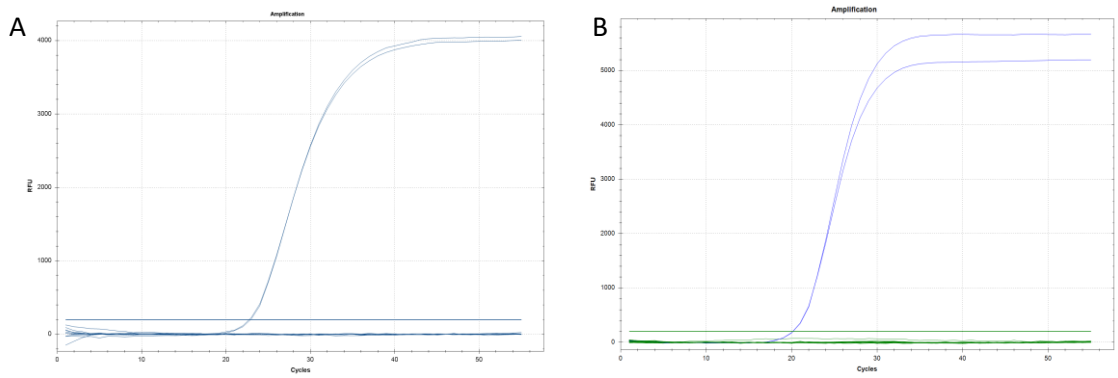
For S*. pneumoniae* primer and probe set, the limit of detection was assessed on a serial dilution of *S. pneumoniae* D39 lab strain serotype 2 starting at $1.47x10^8$ colony forming units per millilitre (CFU/mL) down to 5 CFU/mL. Amplification of the target sequence still occurred in the lowest dilution; therefore, the limit of detection for this assay was <5 CFU/mL (figure 4.4 D). Due to time constraints, the other selected primer and probe sets were not tested for their limit of detection.

4.3.3 Problems in transferring PCR methods to Indonesia

Most of the pathogen-specific PCR assays worked optimally when tested in the local laboratory in Yogyakarta, Indonesia. These included PCR targeting HSV-1, CMV, VZV, *S. pneumoniae, S. suis, N. meningitidis, M. tuberculosis, E. coli, S. agalactiae, K. pneumoniae* and *Salmonella* spp. Below is an example of successful replication of VZV PCR assay in

Yogyakarta. The VZV PCR assay was performed using standard PCR conditions with an annealing temperature of 60 ^{o}C. Figure 4.5 A shows PCR amplification curves obtained in Liverpool, where the positive controls were amplified at Cq values of 22.91 and 22.74. Similarly, both positive controls were amplified at Cq values of 20.15 and 20.11 in the experiment performed in Yogykarta. The negative controls were not amplified in either experiment.

Figure 4.11 Amplification curves generated from VZV PCR assays performed at the Liverpool laboratory (A) and at the Yogyakarta laboratory (B).



Interestingly, three pathogen-specific PCR assays were not successfully replicated at the Yogyakarta laboratory in the first experiment, including those targeting enterovirus, HSV-1 and *H. influenzae*. The following is an example of the problems occurred when transferring the enterovirus PCR assay to Yogyakarta. Enterovirus primer and probe set was initially assessed at the Liverpool laboratory using the standard PCR protocol with an annealing temperature of 60 ^{o}C. As described in table 4.7 (i.e. experiment 1), both enterovirus 71 (EV71) and echovirus positive controls were amplified, and none of the negative controls was amplified. These results suggest that this primer and probe set is sensitive and specific for enterovirus.

The assessment was then repeated at the Yogyakarta laboratory. The results of the first enterovirus PCR assay in Yogyakarta (experiment 2) are outlined in table 4.7. Although both EV71 and echovirus positive controls were amplified, one of the DENV controls was also amplified. The assay was then repeated using the same aliquots of positive and negative controls, as well as the same aliquots of primers and probe (experiment 3). However, the results were unexpectedly worse than the previous ones, where one of each HSV-1, HSV-2, CMV, DENV, and *S. pneumoniae* control was also amplified (table 4.7).

Table 4.27 Results of Enterovirus primers and probe assessment at the Liverpool and the Yogyakarta laboratories

Sample	Experiment 1 - Liverpool		Experiment 2 - Yogyakarta		Experiment 3 - Yogyakarta		Experiment 4 - Yogyakarta	
	Cq 1	Cq 2	Cq 1	Cq 2	Cq 1	Cq 2	Cq 1	Cq 2
EV71	**14.90**	**13.72**	**13.82**	**13.06**	**13.46**	**13.36**	**13.46**	**13.39**
Echovirus	**15.89**	**16.60**	**15.98**	**16.12**	**17.27**	**16.48**	**16.31**	**16.70**
NRT-EV71	ND	ND	ND	ND	ND	ND	ND	ND
NRT-echovirus	ND	ND	ND	ND	ND	ND	ND	ND
HSV-1	ND	ND	ND	ND	40.49	ND	38.82	37.90
HSV-2	ND	ND	ND	ND	ND	41.65	ND	ND
CMV	ND	ND	ND	ND	41.79	ND	39.13	ND
VZV	ND	ND	ND	ND	ND	ND	ND	ND
DENV	ND	ND	ND	41.75	41.23	ND	ND	ND
S. pneumoniae	ND	ND	ND	ND	42.46	ND	ND	ND
S. suis	ND	ND	ND	ND	ND	ND	ND	40.94
H. influenzae	ND	ND	ND	ND	ND	ND	ND	ND
N. meningitidis	ND	ND	ND	ND	ND	ND	ND	ND
NTC	ND	ND	ND	ND	ND	ND	ND	ND

Cq – quantification cycle; EV71 – enterovirus 71; HSV-1 – herpes simplex virus type 1; HSV-2 – herpes simplex virus type 2; CMV – cytomegalovirus; VZV – varicella-zoster virus; DENV – dengue virus; NRT – no reverse transcription control; NTC – no template control; ND – not detected.

To investigate whether the aliquots of primers, probe, and nuclease-free water were contaminated, another experiment was conducted using new aliquots of primers, probe, and RNase-free water (table 4.7, experiment 4). In this experiment, the source of the positive and negative controls was similar to those used in experiment 3. As shown in table 4.7, the results became more unexpected where HSV-1, CMV, and *S. suis* controls

were amplified. These results suggested that there might be contamination in the aliquots of control samples.

All control sample aliquots used in the previous experiments were transferred back to Liverpool to further investigate the problem. At the Liverpool laboratory, two separate experiments, including experiments 5 and 6, were conducted. In experiment 5, the source of the positive and negative controls was the control samples' aliquots transferred from Yogyakarta. As described in table 4.8, most of the negative controls were amplified with lower Cq values compared to the Yogyakarta results.

Table 4.28 Results of repeated Enterovirus primers and probe assessment at the Liverpool laboratory

Sample	Experiment 5 – controls transferred back from Yogyakarta		Experiment 6 – controls remained staying in Liverpool	
	Cq 1	Cq 2	Cq 1	Cq 2
EV71	**13.05**	**13.30**	**13.15**	**13.05**
Echovirus	**15.26**	**15.36**	**13.59**	**13.94**
NRT-EV71	ND	ND	ND	ND
NRT-echovirus	ND	ND	ND	ND
HSV-1	35.82	36.26	ND	ND
HSV-2	ND	37.99	ND	ND
CMV	38.56	40.14	ND	ND
VZV	ND	ND	ND	ND
DENV	34.66	35.20	ND	ND
S. pneumoniae	35.22	36.55	ND	ND
S. suis	37.86	39.10	ND	ND
H. influenzae	38.92	38.64	ND	ND
N. meningitidis	ND	ND	ND	ND
NTC	ND	ND	ND	ND

Cq – quantification cycle; EV71 – enterovirus 71; HSV-1 – herpes simplex virus type 1; HSV-2 – herpes simplex virus type 2; CMV – cytomegalovirus; VZV – varicella-zoster virus; DENV – dengue virus; NRT – no reverse transcription control; NTC – no template control; ND – not detected.

In experiment 6, different aliquots of controls were applied. These aliquots of control samples were the ones used in the initial enterovirus primers and probe assessment at the Liverpool Laboratory (i.e. experiment 1). As shown in table 4.8, none of the negative

controls was amplified in this experiment. These results indicated that there might be widespread contamination of enterovirus across the majority of the control samples aliquots used in Yogyakarta.

4.3.4 Results of pathogen-specific real-time PCR in clinical samples

4.3.4.1 Results of pathogen-specific real-time PCR in paediatric samples

Paediatric samples were tested for all target pathogens, including enterovirus, HSV-1, HSV-2, CMV, VZV, *S. pneumoniae, S. suis, H. influenzae, N. meningitidis, M. tuberculosis, E. coli, S. agalactiae*, *K. pneumoniae*, and *Salmonella* spp. Among all target pathogens, *M. tuberculosis* was the most common pathogen identified by PCR; it was detected in 9 paediatric CSF samples. The second most common pathogen identified in CSF was *E. coli* (n = 6), followed by CMV (n = 4), *S. pneumoniae* (n = 3), *S. agalactiae* (n = 2), S*almonella* spp. (n=1), *H. influenzae* (n = 1), *N. meningitidis* (n = 1) and *K. pneumoniae* (n=1). None of the paediatric CSF samples tested positive for enterovirus, HSV-1, HSV-2, VZV and *S. suis*.

Table 4.29 Results of pathogen-specific PCR in paediatric CSF samples

Pathogen	Number of paediatric cases with pathogen detected by PCR
Mycobacterium tuberculosis	9
Escherichia coli	6
CMV	4
Streptococcus pneumoniae	3
Streptococcus agalactiae	2
Salmonella spp.	1
Haemophilus influenzae	1
Neisseria meningitidis	1
Klebsiella pneumoniae	1
Enterovirus	0
HSV-1	0
HSV-2	0
VZV	0
Streptococcus suis	0

PCR – polymerase chain reaction; HSV – herpes simplex virus; CMV – cytomegalovirus; VZV – varicella-zoster virus.

All paediatric CSF samples with pathogens identified by PCR had a Cq value of < 41, except for 1 sample from subject P-229 that was positive for *S. agalactiae* where the Cq value was 44.55. To confirm this PCR result, the PCR assay was repeated on the CSF sample in duplicate. *S. agalactiae* was detected in both P-229 CSF duplicates with Cq values of 45.58 and 45.15. Therefore, I am confident that this result is a true positive.

The demographics and laboratory features of paediatric patients who had positive PCR results are shown in table 4.10. In total, 24 children had pathogens detected by PCR, including 20 children with a single pathogen and four children with two pathogens.

Characteristics of children with a positive *Mycobacterium tuberculosis* PCR result

Nine children had a positive *M. tuberculosis* PCR result. Eight were detected by in-house PCR TB testing alone. One was positive for in-house testing and for GeneXpert. All had a negative CSF Ziehl-Neelsen stain result. One child was tested for HIV with a negative result. The remaining cases were not tested. Two children had a positive CSF culture for *Staphylococcus haemolyticus* (table 4.10). However, this pathogen is very unlike to cause non-neurosurgical CNS infection outside neonatal period in children with normal immunity.

All nine cases had two or more of the following CSF characteristics for tuberculous meningitis (TBM): CSF pleocytosis, CSF lymphocytic predominance (>50%), CSF:blood glucose ratio of <0.5, and CSF protein of >100 mg/dL.

Five children were treated in the hospital for TBM. Four were treated based on clinical judgement. In one case, anti-tuberculous treatment was prompted by CSF GeneXpert result. Anti-tuberculous treatment was started at a range of intervals from 5 days before to 8 days after LP. Three died in the hospital and two survived. However, several children had attended previous hospitals before being referred. For example, P-296 was ill for eight

days before the first hospital admission. He received intracranial ceftriaxone and hospitalised for six days before referral to our hospital. Anti-TB drugs were started on day 5 (GeneXpert CSF positive). Ceftriaxone was continued until day 19 before switching to high-dose meropenem. The patient died on day 23 in Dr Sardjito hospital (day 37 of illness).

Four children were not treated for TBM (despite clinical features supportive of TBM based on retrospective note review). Three died in the hospital. One child (P-125) was reported alive and without neurological sequelae 6 months later (as part of study follow-up), indicating the in-house TB PCR result was likely to have been a false positive result.

Characteristics of children with a positive bacterial PCR result from CSF

E. coli was detected by PCR in six children. Three children also had positive CSF cultures (two of which also had a positive Gram stain in CSF - table 4.10). All were under one year of age. All six patients had an increased CSF leucocyte count, CSF protein level of >80 mg/dL and low CSF glucose level, supportive for bacterial CNS infection (table 4.10).

All six patients were treated with cefotaxime within a day following LP (prior to culture results). Four patients received meropenem. Timing of meropenem ranged from 1 to 22 days after LP. Four patients were alive at discharge and two died (P-216 [PCR positive only] and P-155 [PCR and culture positive]). P-216 received cefotaxime followed by meropenem (on day 3 post-LP). P155 received cefotaxime and ampicillin for the first 24 hours, then was switched to meropenem. Antibiotic sensitivity for this culture showed resistance to ampicillin but sensitivity to meropenem.

All three cases with positive blood cultures for *E. coli* were culture positive for additional pathogens; Enterobacteriaceae, Shigella group and Staphylococcus epidermidis. All three patients were treated with cefotaxime, amikacin and additional antibiotics (table 4.10).

Table 4.30 Demographics and laboratory features of paediatric patients who had positive PCR results

Subject ID	Age (year)	Sex	PCR results	CSF leucocyte count (cells/µL)	CSF lympho -cytes (%)	CSF glucose (mg/dL)	CSF/ blood glucose ratio	CSF protein (mg/dL)	CSF Gram stain result	CSF ZN stain result	CSF culture results	Blood culture results	HIV status	Treatment received by patients	Outcome at discharge
P-003	17	F	*M. tuberculosis*	14	45	38	0.2	30	ND	ND	*S. haemolyticus*	ND	negative	Anti-TB drugs, ceftriaxone	alive
P-018	12	F	*M. tuberculosis*	500	90	27	0.2	180	ND	ND	*S. haemolyticus*	ND	unknown	Anti-TB drugs, cefotaxime	died
P-026	3	F	*M. tuberculosis*	9	67	38	0.3	30	ND	ND	ND	ND	unknown	Anti-TB drugs, ceftriaxone, metronidazole	alive
P-058	15	M	*M. tuberculosis*	130	64	31	0.3	300	X	x	ND	ND	unknown	Anti-TB drugs, cefotaxime, ceftazidime, ampicillin, meropenem	died
P-069	12	M	*M. tuberculosis*	1580	9	4	0.0	250	ND	ND	ND	ND	unknown	Cefotaxime, metronidazole, meropenem, amikacin, ciprofloxacin	died
P-125	0.1	F	*M. tuberculosis*	7	70	60	0.8	30	ND	x	ND	ND	unknown	Cefotaxime, ceftriaxone, ciprofloxacin, clindamycin	alive
P-178	0.3	M	*M. tuberculosis*	450	18	84	0.9	130	ND	ND	ND	ND	unknown	Anti-TB drugs, cefotaxime, ampicillin, meropenem, azithromycin, clindamycin	died
P-296	15	M	*M. tuberculosis*	134	26	15	0.2	40	ND	ND	ND	ND	unknown	Anti-TB drugs, ceftriaxone, meropenem, ciprofloxacin	died
P-347	16	F	*M. tuberculosis*	144	93	17	0.2	200	ND	ND	ND	ND	unknown	Anti-TB drugs*, ceftriaxone, ciprofloxacin	died
P-071	0.3	M	*E. coli*	490	65	11	0.1	110	ND	x	ND	x	unknown	Cefotaxime	alive
P-216	0.1	F	*E. coli*, CMV	130	72	1	0.0	200	ND	ND	ND	ND	unknown	Cefotaxime, meropenem, amikacin	died
P-322	0.3	M	*E. coli*	6400	10	11	x	3730	ND	x	ND	ND	unknown	Cefotaxime, metronidazole, meropenem, cefixime	alive
P-155	0.3	M	*E. coli*, CMV	141000	5	3	0.0	2050	X	x	*E. coli*	*Shigella* group	unknown	Cefotaxime, ampicillin, meropenem	died
P-185	0.2	M	*E. coli*, CMV	12640	30	1	0.0	550	Gram-negative bacilli	x	*E. coli*	*S. epidermidis*	unknown	Cefotaxime, ampicillin, meropenem, cotrimoxazole	alive
P-323	0.3	M	*E. coli*	780	21	75	x	50	Gram-negative bacilli	x	*E. coli*	*Enterobacteriaceae*	unknown	Cefotaxime, ampicillin, ceftazidime, gentamycin	alive
P-008	7	M	*S. pneumoniae*	7200	10	59	0.4	160	ND	ND	ND	ND	unknown	Cefotaxime, ampicillin, amoxicillin-clavulanic	alive

Table 4.10 (Continued)

Subject ID	Age (year)	Sex	PCR results	CSF leucocyte count (cells/µL)	CSF lympho -cytes (%)	CSF glucose (mg/dL)	CSF/ blood glucose ratio	CSF protein (mg/dL)	CSF Gram stain result	CSF ZN stain result	CSF culture results	Blood culture results	HIV status	Treatment received by patients	Outcome at discharge
P-118	2	M	*S. pneumoniae*	92	36	43	0.4	100	ND	x	ND	ND	unknown	Cefotaxime, ampicillin, amikacin, imipenem	alive
P-150	9	F	*S. pneumoniae*	337	28	17	0.1	160	ND	x	ND	ND	unknown	Anti-TB drugs, cefotaxime, ceftriaxone, meropenem	died
P-154	0.2	F	*S. agalactiae,* CMV	8	34	17	0.2	160	ND	x	ND	x	unknown	Cefotaxime, ampicillin	alive
P-229	0.1	M	*S. agalactiae*	398	40	28	0.3	70	ND	x	ND	x	unknown	Cefotaxime, ampicillin, cefixime	alive
P-270	0.8	M	*Salmonella* spp.	166	66	14	0.1	80	Gram-negative bacilli	x	*Salmonella* spp.	*Salmonella paratyphi B*	unknown	Cefotaxime, ceftriaxone, ampicillin, meropenem	alive
P-258	0.3	F	*H. influenzae*	325	26	9	0.1	100	ND	x	*Pasteurella canis*	*Pasteurella canis*	unknown	Cefotaxime, meropenem	alive
P-093	13.1	M	*N. meningitidis*	1968	4	71	x	70	ND	NR	ND	ND	unknown	Cefotaxime	alive
P-021	0.3	F	*K. pneumoniae*	1500	32	18	x	1430	Gram-negative bacilli	x	*K. pneumoniae*	ND	unknown	Cefotaxime, ampicillin, meropenem	alive

*Anti-TB drugs were prescribed by the clinician, but the patient died before receiving them.

PCR – polymerase chain reaction; CSF – cerebrospinal fluid; ZN – Ziehl-Neelsen; HIV – human immunodeficiency virus; *M. tuberculosis – Mycobacterium tuberculosis; E. coli – Escherichia coli*; CMV – cytomegalovirus*; S. pneumoniae – Streptococcus pneumoniae; S. agalactiae – Streptococcus agalactiae; Salmonella* spp. *– Salmonella* species*; H. influenzae – Haemophilus influenzae; K. pneumoniae – Klebsiella pneumoniae, S. haemolyticus – Staphylococcus haemolyticus; S. epidermidis – Staphylococcus epidermidis*; TB- tuberculosis; F – female; M – male; ND – not detected; x – not done.

Among children with a positive PCR test for *S. pneumoniae* (n=3) or *S. agalactiae* (n=2), all had a CSF pleocytosis with a neutrophil predominance (>50%) and a CSF:blood glucose ratio of <0.5 (table 4.10). All children *S. pneumoniae*-positive and one child *S. agalactiae*-positive had CSF protein level of >80 mg/dL. The two children positive for *S. agalactiae* were under one year of age (table 4.10). Four children were discharged alive. One patient died (P-150). All children received multiple antibiotics. Four received cefotaxime prior to LP (except patient P-008). The patient who died tested positive for *S. pneumoniae*. She underwent LP on the sixth day of admission. She was treated with cefotaxime, followed by ceftriaxone, meropenem, and anti-tuberculous drugs.

One child, subject P-270 who had a positive *Salmonella* spp. PCR result, also had positive blood culture and positive CSF culture results for *Salmonella* (table 4.10). All of the children who tested positive for *H. influenzae* (n=1), *N. meningitidis* (n=1), and *K. pneumoniae* (n=1) had either CSF pleocytosis, CSF:blood glucose ratio of <0.5 or CSF protein concentration of >80 mg/dL. Subject P-258, whose CSF sample tested positive for *H. influenzae,* had a positive culture result for *Pasteurella canis* from both blood and CSF samples. *Pasteurella canis* is not known to cause CNS infection; therefore, it is presumed to be a contaminant (chapter 3, table 3.3). All four children received cefotaxime as the first-line antibiotic before or on the same day of LP, except for patient P-021 who received it five days after LP. All were discharged alive.

Characteristics of children with a positive viral PCR result

CMV was detected by PCR in four cases; however, all of these cases also had another pathogen detected by PCR. These included three cases positive for *E. coli* and one case positive for *S. agalactiae* (table 4.10). None of these children had CMV serology and HIV tests performed at the hospital. Furthermore, all these children had CSF characteristics of

bacterial infection, including CSF:blood glucose of <0.5 and CSF protein level of >80 mg/dL. None of these children received antiviral treatment.

Outcomes of children with a positive PCR result from CSF

Nine of 24 (38%) children with positive PCR results died during hospitalisation. Of those, two children had the same pathogen identified via standard hospital testing (P-269 - positive for *M. tuberculosis* by CSF GeneXpert; P-155 positive for *E. coli* by CSF culture). The remaining seven children who died received the following management: five tested positive for *M. tuberculosis* but only four received anti-TB drugs; one tested positive for both *E. coli* and CMV – she was treated with cefotaxime, meropenem plus further antibiotics; one tested positive for *S. pneumoniae* – she received cefotaxime, ceftriaxone and anti-TB drugs (table 4.10).

The others (n=15) were alive at discharge. This included one patient (P-125) with a probable false-positive PCR result for MTB.

4.3.4.2 Results of pathogen-specific real-time PCR in adult samples

Adult samples were tested for enteroviruses, HSV-1, HSV-2, CMV, VZV, *S. pneumoniae, S. suis, H. influenzae, N. meningitidis, M. tuberculosis*, *K. pneumoniae*, and *Salmonella* spp. *M. tuberculosis* was the most common pathogen identified by PCR; it was detected in 23 adult CSF samples (table 4.11).

Table 4.31 Results of pathogen-specific PCR in adult samples

Pathogen	Number of adult cases with pathogen detected by PCR
Mycobacterium tuberculosis	23
Streptococcus pneumoniae	3
HSV-2	2
VZV	1
Enterovirus	0
HSV-1	0
CMV	0
Streptococcus suis	0
Haemophilus influenzae	0
Neisseria meningitidis	0
Klebsiella pneumoniae	0
Salmonella spp	0
Escherichia coli	x
Streptococcus agalactiae	x

PCR – polymerase chain reaction; HSV – herpes simplex virus; CMV – cytomegalovirus; VZV – varicella-zoster virus; x – not done.

The second most common pathogen identified in adults was *S. pneumoniae* which was detected in 3 CSF samples, followed by HSV-2 and VZV which were detected in 2 and 1 CSF samples, respectively. Interestingly, none of the adult patients had a positive PCR result for enteroviruses, HSV-1, *H. influenzae* and *N. meningitidis*. Additionally, none of the adult patients tested positive for CMV, *S. suis*, *K. pneumoniae* and *Salmonella spp*.

The demographics and laboratory features of 29 adult patients who had positive PCR results are presented in table 4.12. All these patients had a single pathogen detected by PCR in their CSF samples.

Characteristics of adults with a positive *Mycobacterium tuberculosis* PCR result

Twenty-three adults tested positive for *M. tuberculosis* (MTB) by PCR. None had MTB detected by ZN stain for acid-fast bacilli in the CSF. One had MTB detected via GenXpert testing of sputum.

All had at least two of the following CSF characteristics suggestive of tuberculous CNS infection: CSF pleocytosis, CSF lymphocytic predominance, CSF absolute glucose of <40 mg/dL, CSF:blood glucose ratio of <0.5 and CSF protein of >100 mg/dL. Six adults (26%) were HIV-positive, and the remaining were HIV-negative. Four cases had a positive bacterial CSF culture: *Kocuria varians, Staphylococcus epidermidis, Enterobacter aerogenes* and *Staphylococcus haemolyticus*. These pathogens are unusual to cause CNS infection in adults and thus are presumed to be contaminants (chapter 3, table 3.10).

Seven received anti-TB drugs and the remainder did not (n=16). All those (100%) who did not receive anti-tuberculous treatment had a fatal outcome.

Among those who received anti-tuberculous treatment, four (57%) were alive at discharge (table 4.12). The latter received anti-TB drugs within three days following LP. The patients (n=3) who died in the hospital despite receiving anti-TB drugs received anti-tuberculous drugs on days 3, 4 and 21 post-admission. The latter patient (N-154) received anti-TB drugs following the detection of *M. tuberculosis* by GeneXpert from sputum. LP was performed on day 10, but his CSF was not tested by GeneXpert.

Among the 16 adults who tested positive for *M. tuberculosis* but did not receive anti-TB drugs, nine patients met the criteria for probable TBM and seven met the criteria for possible TBM based on their clinical, laboratory, and/or neuroimaging features. All received antibiotics, of whom six patients received multiple antibiotics. Four patients also received acyclovir.

Characteristics of adults with a positive bacterial PCR result

Three adults had a positive *S. pneumoniae* PCR result, including one (N-157) who had *S. pneumoniae* detected via CSF culture. The two other patients (N-142 and N-167) did not have any pathogen detected by the standard hospital testing. Patients N-142 and N-167

received a single antibiotic regimen with third-generation cephalosporin and were discharged alive. Patient N-157 died.

All three patients had a syndromic clinical diagnosis of meningoencephalitis. Patient N-142 had a CSF neutrophil predominance, CSF:blood glucose ratio of <0.5 and CSF protein of >80 mg/dL, again characteristic of bacterial CNS infection (table 4.12). She was treated with high-dose ceftriaxone from day 1 of admission and was discharged alive on day 7. Patient N-167 was HIV-positive. He had atypical CSF characteristics with lymphocytic predominance, CSF:blood glucose ratio of 0.5 and normal protein level. He developed symptoms two days before admission to his first hospital, where he was kept for five days prior to referral to Dr Sardjito hospital. He was given high-dose ceftazidime and dexamethasone since day 2 of admission at our hospital. Chest X-ray on admission showed pneumonia. The patient underwent a brain MRI on day 3, and the result showed signs of meningoencephalitis in bilateral fronto-temporo-parietal lobes and grade 4 cerebritis in the posterior left parietal lobe. Toxoplasma serology was performed on day 4, the result showed normal anti-toxoplasma IgM and increased IgG (table 4.17, see below). Following the serology result, pyrimethamine was administered since day 5. LP was delayed until day 10, the reason was not documented in the study case report form. The patient was discharged alive on day 15.

CSF investigation of patient N-157 showed marked pleocytosis, neutrophil predominance, low CSF:blood glucose ratio and increased CSF protein (table 4.12). She developed symptoms three days before being admitted to Dr Sardjito hospital. A Head CT scan was performed on the day of admission, and the result showed diffuse cerebral oedema with suspected cerebritis in the right basal ganglia. LP was performed on day 2 of admission. Antibiotic sensitivity from culture showed sensitivity to ceftazidime but was not tested for

meropenem. She received ceftazidime for the first two days, then switched to meropenem for a day. The patient died on the fourth day of hospitalisation.

Characteristics of adults with a positive viral PCR result

Three adults had positive PCR results for viruses. Two patients were HSV-2 PCR positive and one was VZV positive. All three were HIV positive on serological testing.

The two HSV2 patients had a CSF pleocytosis, CSF blood:glucose ratio of ≥0.5, CSF protein of >80 mg/dL (table 4.12). One patient, N-140, had a CSF neutrophil predominance. He had a genital ulcer. He did not have detectable HSV-2 IgM in serum. LP was performed on day 2 of admission. HSV-2 serology was tested the following day. He was already on routine ARV treatment prior to hospitalisation. He was given acyclovir on day 16. Additionally, he also received ceftriaxone, meropenem and anti-TB drugs. Despite the intense treatment, the patient died on day 26. The other patient, N-044, did not have any genital ulcers. He was not tested for HSV-2 serology. His HIV status was not recorded in the study case report form. He did not receive antiviral therapy and was discharged alive.

The patient who tested VZV-positive by PCR had a normal CSF leucocyte count, normal CSF:blood glucose ratio, and normal CSF protein level; but had a HIV-positive status and was on ARV treatment (table 4.12). He had a markedly increased anti-toxoplasma IgG (>300) but normal IgM and had an abnormal head CT scan result. He was clinically diagnosed as having cerebral toxoplasmosis by the treating clinician, was given anti-toxoplasmosis treatment, and was discharged alive. In the present study, he was defined as having presumptive cerebral toxoplasmosis (chapter 3). He did not receive specific antiviral treatment aside from the ARV during hospitalisation.

Table 4.32 Clinical and laboratory features of adult patients who had positive PCR results

Subject ID	Age (year)	Sex	PCR results	CSF leucocyte count (cells/µL)	CSF lympho-cytes (%)	CSF glucose (mg/dL)	CSF/ blood glucose ratio	CSF protein (mg/dL)	CSF Gram stain result	CSF ZN stain result	CSF culture results	Blood culture results	HIV status	Treatment received by patients	Outcome at discharge
N-013	20	F	*M. tuberculosis*	4	82	76	0.4	150	ND	ND	ND	ND	negative	Ceftazidime, acyclovir	died
N-021	25	F	*M. tuberculosis*	3300	90	67	0.5	70	NR	NR	x	ND	positive	Ceftazidime	died
N-022	46	M	*M. tuberculosis*	5	100	48	0.6	10	ND	ND	ND	x	positive	Anti-TB drugs, ARV	alive
N-028	46	M	*M. tuberculosis*	690	80	145	0.2	6290	ND	x	ND	x	positive	Ceftazidime	died
N-029	37	F	*M. tuberculosis*	190	90	21	0.2	110	ND	ND	ND	ND	negative	Anti-TB drugs, ceftriaxone, pyrimethamine	alive
N-033	26	M	*M. tuberculosis*	8	85	51	0.4	30	ND	ND	*Kocuria varians*	ND	positive	Ceftazidime, acyclovir, ARV, pyrimethamine	died
N-039	21	F	*M. tuberculosis*	140	85	18	0.2	700	ND	ND	ND	x	negative	Ceftriaxone	died
N-049	30	M	*M. tuberculosis*	78	92	66	0.5	4440	ND	ND	ND	ND	positive	Anti-TB drugs, meropenem, levofloxacin, cotrimoxazole, co-amoxiclav	died
N-054	37	M	*M. tuberculosis*	427	90	84	0.6	470	ND	ND	ND	x	negative	Ceftriaxone, acyclovir	died
N-061	57	M	*M. tuberculosis*	1	88	25	-	200	ND	ND	*S. epidermidis*	x	negative	Ceftriaxone	died
N-088	41	F	*M. tuberculosis*	192	94	28	0.2	60	ND	ND	*Enterobacter aerogenes*	ND	negative	Ceftazidime	died
N-089	45	M	*M. tuberculosis*	75	49	49	0.2	220	diplococci	ND	*S. haemolyticus*	ND	negative	Anti-TB drugs, ceftriaxone, ceftazidime, levofloxacin	died
N-131	35	M	*M. tuberculosis*	22	100	41	0.3	500	ND	ND	ND	x	negative	Anti-TB drugs, ceftazidime, ciprofloxacin	alive
N-139	21	M	*M. tuberculosis*	177	71	55	0.5	70	ND	ND	ND	ND	negative	Ceftriaxone, vancomycin	died
N-149	34	M	*M. tuberculosis*	322	92	70	0.3	160	ND	ND	ND	ND	negative	Anti-TB drugs, ceftazidime	alive
N-150	23	M	*M. tuberculosis*	17	24	6	0.1	170	ND	ND	ND	x	negative	Ceftriaxone, gentamycin	died
N-153	42	F	*M. tuberculosis*	70	6	46	0.2	100	ND	x	ND	ND	negative	Cefotaxime, meropenem	died
N-154	34	M	*M. tuberculosis*	197	84	38	0.3	470	ND	ND	ND	ND	positive	Anti-TB drugs, ceftriaxone, meropenem, cotrimoxazole, pyrimethamine	died
N-168	26	M	*M. tuberculosis*	493	82	70	0.5	1300	ND	ND	ND	ND	negative	Ceftazidime, meropenem	died
N-169	23	M	*M. tuberculosis*	208	88	16	0.1	190	ND	ND	ND	ND	negative	Meropenem	died
N-170	69	F	*M. tuberculosis*	73	86	65	0.3	80	ND	ND	ND	x	negative	Ceftriaxone, levofloxacin	died
N-175	21	F	*M. tuberculosis*	270	8	13	0.1	120	ND	ND	ND	ND	negative	Cefotaxime, ceftriaxone, amikacin, acyclovir	died
N-179	25	M	*M. tuberculosis*	178	42	39	0.4	2780	x	x	x	ND	negative	Ceftazidime, meropenem, vancomycin	died
N-142	47	F	*S. pneumoniae*	1170	12	74	0.4	90	ND	ND	ND	ND	negative	Ceftriaxone	alive
N-157	58	F	*S. pneumoniae*	13200	2	54	0.3	5300	ND	ND	*S. pneumoniae*	ND	negative	Ceftazidime, meropenem	died

Table 4.12 (Continued)

Subject ID	Age (year)	Sex	PCR results	CSF leucocyte count (cells/µL)	CSF lympho-cytes (%)	CSF glucose (mg/dL)	CSF/ blood glucose ratio	CSF protein (mg/dL)	CSF Gram stain result	CSF ZN stain result	CSF culture results	Blood culture results	HIV status	Treatment received by patients	Outcome at discharge
N-167	33	M	*S. pneumoniae*	25	92	48	0.5	30	ND	ND	ND	ND	positive	Ceftazidime, pyrimethamine	alive
N-044	32	M	HSV-2	79	90	50	0.5	90	ND	ND	ND	ND	positive	Ceftazidime, levofloxacin, amikacin, imipenem	alive
N-140	34	M	HSV-2	92	42	93	0.8	360	ND	ND	ND	x	positive	Ceftriaxone, meropenem, anti-TB drugs, acyclovir, ARV	died
N-084	33	M	VZV	0	29	64	0.5	60	ND	ND	ND	ND	positive	Pyrimethamine, clindamycin, ARV	alive

PCR – polymerase chain reaction; CSF – cerebrospinal fluid; ZN – Ziehl-Neelsen; HIV – human immunodeficiency virus; ARV – antiretroviral; *M. tuberculosis – Mycobacterium tuberculosis; S. pneumoniae – Streptococcus pneumoniae;* HSV-2 – herpes simplex virus type-2; VZV – varicella-zoster virus*; S. haemolyticus – Staphylococcus haemolyticus; S. epidermidis – Staphylococcus epidermidis*; F – female; M – male; ND – not detected; x – not done.

Outcomes of adults with a positive PCR result

Twenty-one of 29 (72%) adults with a positive PCR result died during their hospital stay. Twenty of these cases (95%) did not have a causative pathogen identified via standard hospital testing (table 4.12). This group included 19 positive cases for *M. tuberculosis* and one positive for HSV-2. Of the 19 positive *M. tuberculosis* PCR cases, 16 did not receive anti-TB drugs. The patient who tested positive for HSV-2 received acyclovir, ARV, and multiple antibiotics (table 4.12). Seven patients were alive at discharge.

4.3.5 Comparison of PCR and standard hospital diagnostic testing results

4.3.5.1 Comparison of PCR and standard hospital diagnostic testing results in children

As described previously in the methods section, the PCR assays were performed on 113 paediatric CSF samples. These included 101 CSF samples with pleocytosis, four without CSF pleocytosis but had an immunocompromised condition, and eight without CSF pleocytosis who died during hospital admission (table 4.13). To assess whether the PCR assays improve pathogen detection in CSF compared to the standard hospital CSF testing, the results were compared in each diagnosis sub-groups, including non-neurosurgical and neurosurgical infection.

Comparison of CSF PCR and standard hospital testing results in children with non-neurosurgical CNS infection

The PCR assays detected the target pathogen in the CSF from 23 of the 92 children with non-neurosurgical CNS infection who had CSF pleocytosis (table 4.13). Twenty-one of the 92 children in this sub-group had a pathogen detected by CSF culture. Of these, eight children had a pathogen consistent with the aetiology of CNS infection detected. These pathogens included *E. coli* (n=3), *Staphylococcus aureus* (n=2), *Salmonella* spp. (n=1), *Enterococcus faecium* (n=1), and coagulase-positive *Staphylococcus* (n=1). The remaining

pathogens, including *Staphylococcus haemolyticus* (n=6), *Staphylococcus hominis* (n=4), *Leuconostoc mesenteroides* (n=1), *Acitenobacter lwoffii* (n=1), and *Pasteurella canis* (n=1), are unlikely to cause non-neurosurgical CNS infection in these cases and are regarded as contaminants (chapter 3, table 3.8).

Nine of the 92 children in this sub-group had a positive CSF Gram stain result, six of whom had a causative pathogen detected by the standard hospital testing. Among these, three children tested positive for Gram-negative bacilli and had a positive CSF culture result for *E. coli* (n=2) and *Salmonella* spp. (n=1). Two other children tested positive for Gram-negative bacilli, but CSF culture grew no pathogen. Additionally, one child tested positive for chained cocci and had a positive CSF culture for *Enterococcus faecium*. None of the children in the sub-group had a positive CSF Ziehl-Neelsen stain result; however, one child had *M. tuberculosis* detected by CSF GeneXpert.

Twenty of these 92 children had a positive blood culture result, including patient P-155 whose CSF culture grew *E. coli* (but blood culture grew *Shigella* group) and patient P-337 whose blood culture grew *Salmonella* sp. (table 4.13). In the latter case, *Salmonella* sp. was regarded as the aetiology of CNS infection, although his CSF tested negative for the pathogen by PCR (see more information in the following sub-section). Another child, patient P-212, had a positive blood culture for *K. pneumoniae,* but his CSF sample tested negative for the pathogen by PCR. In this case, the pathogen was not regarded as the causative pathogen (see more information in the following sub-section). The remaining pathogens detected by blood culture, including *Sphingomonas paucimobilis* (n=3), *Staphylococcus epidermidis* (n=2), *Staphylococcus hominis* (n=2), *Enterobacteriaceae* (n=2), *Staphylococcus haemolyticus* (n=1), *coagulase-negative Staphylococcus* (n=1), *Pseudomonas aeruginosa* (n=1), *Pasteurella canis* (n=1), *Micrococcus luteus* (n=1),

Cronobacter saazakii (n=1), and *Kocuria kristinae* (n=1), were not regarded as the aetiology of CNS infection.

Taking into account the standard hospital testing results consistent with CNS infection aetiology (see chapter 3, table 3.8 for the full list), 12 children had a pathogen identified by the standard hospital CSF testing. These included four cases in which pathogens were detected by CSF culture test only, four by both CSF culture and Gram stain tests, two by CSF Gram stain test only, one by blood culture, and one by CSF GeneXpert assay only.

In children without CSF pleocytosis who had an immunocompromised condition (n=4), all had non-neurosurgical CNS infection, and none had a pathogen detected either by the PCR assays, CSF culture, CSF Gram stain or CSF Ziehl-Neelsen stain tests (table 4.13). None of the eight children with non-neurosurgical CNS infection who did not have a CSF pleocytosis and died during hospital admission had a pathogen detected by the PCR assays (table 4.13). Moreover, in this sub-group, a child had a pathogen (*Rhizobium radiobacter*) identified by both CSF culture and CSF Gram stain tests, and another child had *Acinetobacter baumannii* detected by CSF culture test only. Nevertheless, as both children did not have a CSF pleocytosis, these pathogens are unlikely to cause their CNS infection (chapter 3, table 3.8).

Table 4.33 Comparison of PCR and standard hospital diagnostic testing results in children

Paediatric patient sub-group (n)	Samples with positive CSF PCR result (n)	Samples with positive CSF culture results (n)	Samples with positive CSF Gram stain results (n)	Samples with positive CSF ZN stain results (n)	Samples with positive blood culture results (n)
Children with non-neurosurgical CNS infection (n=236)					
Children with CSF pleocytosis (n=92)	22	21	9	0	20
Children without CSF pleocytosis who had an immunocompromised condition (n=4)	0	0	0	0	1
Children without CSF pleocytosis who died during hospitalisation (n=8)	0	2	1	0	1
Children whose CSF was not tested for PCR (n=93)*	N/A	10	3	0	12
Children who did not undergo LP/EVD (n= 39)	N/A	N/A	N/A	N/A	4
Children with neurosurgical CNS infection (n=11)					
Children with CSF samples available for PCR testing (n=9)	1	7	4	0	1
Children with CSF samples unavailable for PCR testing (n=2)	N/A	2	0	0	2

*Included six children with CSF pleocytosis and one immunocompromised child without CSF pleocytosis whose CSF was unavailable for study purposes, as well as 86 children without CSF pleocytosis who were discharged alive

CSF – cerebrospinal fluid; CNS – central nervous system; PCR – polymerase chain reaction; ZN – Ziehl-Neelsen; LP – lumbar puncture; EVD – extra ventricle drainage

Table 4.34 Discrepancies between PCR and culture results in children

Subject ID	Date of onset	Date of hospital admission	Date of the first antibiotic administered	CSF investigation results				Date of blood collection for culture	Blood culture results
				Date of CSF collection	CSF leucocyte count (cells/µL)	CSF PCR results	CSF culture results		
P-337	15/01/2018	20/01/2018	20/01/2018	05/02/2018	97	ND	ND	20/01/2018	*Salmonella* spp.
P-212	06/01/2016	07/01/2016	08/01/2016	14/01/2016	7	ND	x	13/01/2016 28/01/2016	ND *K. pneumoniae*

PCR – polymerase chain reaction; CSF – cerebrospinal fluid; *Salmonella* spp. – Salmonella species; *K. pneumoniae – Klebsiella pneumoniae;* ND – not detected; x – not done.

Table 4.35 Clinical and laboratory features of paediatric patients who had causative pathogen(s) detected by standard hospital testing and/or PCR testing

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results				Initial diagnosis	CSF PCR result	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investigations						
Non-neurosurgical CNS infection														
P-003	17	F	14	negative	ND	*S. haemolyticus*	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ceftriaxone	alive	yes
P-018	12	F	500	unknown	ND	*S. haemolyticus*	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, cefotaxime	died	yes
P-026	3	F	9	unknown	ND	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ceftriaxone, metronidazole	alive	yes
P-058	15	M	130	unknown	X	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM, dengue CNS infection	Anti-TB drugs, cefotaxime, ceftazidime, ampicillin, meropenem	died	yes
P-069	12	M	1580	unknown	ND	ND	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Cefotaxime, metronidazole, meropenem, amikacin, ciprofloxacin	died	no
P-178	0.3	M	450	unknown	ND	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, cefotaxime, ampicillin, meropenem, azithromycin, clindamycin	died	yes
P-296	15	M	134	unknown	ND	ND	ND	CSF Xpert: *M. tuberculosis* (+)	Definite TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ceftriaxone, meropenem, ciprofloxacin	died	yes
P-347	16	F	144	unknown	ND	ND	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs*, ceftriaxone, ciprofloxacin	died	no
P-155	0.3	M	141000	unknown	X	*E. coli*	*Shigella* group	-	*E. coli* CNS infection	*E. coli*, CMV	*E. coli* CNS infection, latent CMV infection	Cefotaxime, ampicillin, meropenem	died	yes

Table 4.15 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results				Initial diagnosis	CSF PCR result	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investigations						
P-185	0.2	M	12640	unknown	Gram-negative bacilli	*E. coli*	*Staphylococcus epidermidis*	-	*E. coli* CNS infection	*E. coli*, CMV	*E. coli* CNS infection, latent CMV infection	Cefotaxime, ampicillin, meropenem, cotrimoxazole	alive	yes
P-216	0.1	F	130	unknown	ND	ND	ND	-	Unknown aetiology	*E. coli*, CMV	*E. coli* CNS infection, latent CMV infection	Cefotaxime, meropenem, amikacin	died	yes
P-071	0.3	M	490	unknown	ND	ND	x	-	Unknown aetiology	*E. coli*	*E. coli* CNS infection	Cefotaxime	alive	yes
P-322	0.3	M	6400	unknown	ND	ND	ND	-	Unknown aetiology	*E. coli*	*E. coli* CNS infection	Cefotaxime, metronidazole, meropenem, cefixime	alive	yes
P-323	0.2	M	780	unknown	Gram-negative bacilli	*E. coli*	*Enterobacteriaceae*	-	*E. coli* CNS infection	*E. coli*	*E. coli* CNS infection	Cefotaxime, ampicillin, ceftazidime, gentamycin	alive	yes
P-112	6	F	N/A	unknown	X	x	*E. coli*	-	*E. coli* CNS infection	X	*E. coli* CNS infection	Cefotaxime, ampicillin	died	yes
P-008	7	M	7200	unknown	ND	ND	ND	-	Unknown aetiology	*S. pneumoniae*	*S. pneumoniae* CNS infection	Cefotaxime, ampicillin, amoxicillin-clavulanic	alive	yes
P-118	2	M	92	unknown	ND	ND	ND	-	Unknown aetiology	*S. pneumoniae*	*S. pneumoniae* CNS infection	Cefotaxime, ampicillin, amikacin, imipenem	alive	yes
P-150	9	F	337	unknown	ND	ND	ND	-	Unknown aetiology	*S. pneumoniae*	*S. pneumoniae* CNS infection	Anti-TB drugs, cefotaxime, ceftriaxone, meropenem	died	yes
P-154	0.2	F	8	unknown	ND	ND	x	-	Unknown aetiology	*S. agalactiae,* CMV	*S. agalactiae* CNS infection, latent CMV infection	Cefotaxime, ampicillin	alive	yes
P-229	0.1	M	398	unknown	ND	ND	x	-	Unknown aetiology	*S. agalactiae*	*S. agalactiae* CNS infection	Cefotaxime, ampicillin, cefixime	alive	yes
P-270	0.8	M	166	unknown	Gram-negative bacilli	*Salmonella spp.*	*Salmonella paratyphi B*	-	*Salmonella* CNS infection	*Salmonella* spp.	*Salmonella* CNS infection	Cefotaxime, ceftriaxone, ampicillin, meropenem	alive	yes

Table 4.15 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results				Initial diagnosis	CSF PCR result	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investigations						
P-337	0.8	M	97	unknown	ND	ND	*Salmonella spp.*	-	*Salmonella* CNS infection	ND	*Salmonella* CNS infection	Cefotaxime, ceftriaxone, cefixime, ciprofloxacin	alive	yes
P-258	0.3	F	325	unknown	ND	*Pasteurella canis*	*Pasteurella canis*	-	Unknown aetiology	*H. influenzae*	*H. influenzae* CNS infection	Cefotaxime, meropenem	alive	yes
P-093	13.1	M	1968	unknown	ND	ND	ND	-	Unknown aetiology	*N. meningitidis*	*N. meningitidis* CNS infection	Cefotaxime	alive	yes
P-016	2	F	35	unknown	ND	*Staphylococcus aureus*	ND	-	*S. aureus* CNS infection	ND	*S. aureus* CNS infection	Ceftriaxone, ceftazidime, cefixime	alive	yes
P-055	0.3	F	110	unknown	ND	*Staphylococcus aureus*	ND	-	*S. aureus* CNS infection	ND	*S. aureus* CNS infection	Cefotaxime	alive	yes
P-064	0.8	M	100	unknown	Gram-negative bacilli	ND	ND	-	Gram-negative bacilli CNS infection	ND	Gram-negative bacilli CNS infection	Cefotaxime, ampicillin, metronidazole, meropenem, chloramphenicol, acyclovir	alive	yes
P-262	0.4	M	19	unknown	Gram-negative bacilli	ND	ND	-	Gram-negative bacilli CNS infection	ND	Gram-negative bacilli CNS infection	Cefotaxime, ampicillin, cefixime	alive	yes
P-237	9	M	345	unknown	ND	*Bacillus anthracis*	*Cupriavidus pauculus*	-	*Bacillus anthracis* CNS infection	X	Anthrax CNS infection	Cefotaxime, metronidazole	died	no
P-302	10	M	7	unknown	Chains of cocci	*Enterococcus faecium*	ND	-	*Enterococcus* CNS infection	ND	*Enterococcus* CNS infection	Cefotaxime, ceftriaxone, ceftazidime, metronidazole, meropenem	died	yes
P-345	17	M	38	unknown	ND	Coagulase-positive *Staphylococcus*	ND	-	*Staphylococcus* CNS infection	ND	*Staphylococcus* CNS infection	Ceftriaxone	died	yes
P-149	4	F	N/A	unknown	X	x	*Sphingomonas paucimobilis*	-	*Sphingomonas paucimobilis* CNS infection	X	*Sphingomonas paucimobilis* CNS infection	Cefotaxime, ceftriaxone, ampicillin, metronidazole	alive	yes

Table 4.15 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results				Initial diagnosis	CSF PCR result	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investigations						
Neurosurgical CNS infection														
P-013	4	F	52	unknown	ND	ND	ND	*Staphylococcus haemolyticus* positive (pus culture)	Definite shunt infection	ND	Definite shunt infection	Cefotaxime, ceftriaxone, ampicillin	alive	yes
P-201	0.4	F	5	unknown	X	*S. haemolyticus*	ND	-	Definite shunt infection	ND	Definite shunt infection	Cefotaxime, ceftriaxone, ampicillin, cefixime, cefepime, levofloxacin, ciprofloxacin	alive	yes
P-007	11	M	610	unknown	Gram-negative bacilli	*Enterobacter cloacae*	ND	-	Definite shunt infection	ND	Definite shunt infection	Ceftriaxone, metronidazole	alive	yes
P-021	0.3	F	1500	unknown	Gram-negative bacilli	*K. pneumoniae*	ND	-	Definite shunt infection	*K. pneumoniae*	Definite shunt infection	Cefotaxime, ampicillin, meropenem	alive	yes
P-039	0.4	F	1750	unknown	ND	*Staphylococcus warneri*	*Streptococcus viridans*	-	Definite shunt infection	ND	Definite shunt infection	Cefotaxime, ceftriaxone, imipenem, ciprofloxacin	alive	yes
P-078	0.4	M	170	unknown	ND	*Kocuria kristiane*	x	-	Definite shunt infection	ND	Definite shunt infection	Cefotaxime, gentamycin	alive	yes
P-105	0.1	F	818	unknown	X	*Acinetobacter baumannii*	ND	-	Definite shunt infection	ND	Definite shunt infection	Cefotaxime, ampicillin, cefepime, cefixime, valganciclovir	alive	yes
P-238	1	M	1	unknown	X	*Pseudomonas aeruginosa*	*Cupriavidus pauculus*	-	Definite shunt infection	X	Definite shunt infection	Cefotaxime, ceftazidime, ampicillin, gentamycin, amikacin	alive	yes

Table 4.15 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results				Initial diagnosis	CSF PCR result	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investigations						
P-285	2.1	M	55	unknown	Gram-negative cocci	ND	ND	-	Definite shunt infection	ND	Definite shunt infection	Cefotaxime, ceftriaxone	died	yes
P-286	0.4	M	69	unknown	Gram-negative bacilli	*Pseudomonas stutzeri*	ND	-	Post-neurosurgical encephalitis	ND	Post-neurosurgical encephalitis	Cefotaxime, ceftazidime, cefixime, ciprofloxacin	alive	yes
P-344	0.6	F	42	unknown	ND	*S. epidermidis*	*Candida albicans*	-	Definite shunt infection	X	Definite shunt infection	Cefotaxime, ampicillin, azithromycin, chloramphenicol	alive	yes

PCR – polymerase chain reaction; CSF – cerebrospinal fluid; ZN – Ziehl-Neelsen; TB – tuberculosis; HIV – human immunodeficiency virus; *M. tuberculosis – Mycobacterium tuberculosis; E. coli – Escherichia coli*; CMV – cytomegalovirus*; S. pneumoniae – Streptococcus pneumoniae; S. agalactiae – Streptococcus agalactiae; Salmonella* spp. – *Salmonella* species*; H. influenzae – Haemophilus influenzae; K. pneumoniae – Klebsiella pneumoniae, S. haemolyticus – Staphylococcus haemolyticus; S. epidermidis – Staphylococcus epidermidis*; F – female; M – male; ND – not detected; N/A - not applicable; x – not done

The CSF PCR assays were not performed in 93 children with non-neurosurgical CNS infection. These included six children with CSF pleocytosis, one immunocompromised child without CSF pleocytosis whose specimens were not available/sufficient for study purposes, and 86 children without CSF pleocytosis who were discharged alive. Ten of these 93 children had a positive CSF culture result; however, only one was consistent with the aetiology of CNS infection (i.e. *Bacillus anthracis*; see chapter 3, table 3.8 for the full list of the remaining pathogens).

Thirty-nine children did not undergo LP/EVD and had no CSF samples available. Of these, four children had a positive blood culture result; however, only two children had a pathogen consistent with the aetiology of CNS infection (chapter 3, table 3.8).

In summary, among 104 children with non-neurosurgical CNS infection whose CSF was tested by PCR, the PCR assays detected a causative pathogen in 22 children. These included 17 children whose pathogen was detected by the PCR assays only and five children whose pathogen was detected by the PCR and the standard hospital diagnostic testing.

Taking all children with non-neurosurgical CNS infection into account (n=236), the standard hospital testing identified a causative pathogen in 15 (6%) cases. The CSF PCR assays detected a causative pathogen in 22 cases, five of which were also identified by the standard hospital testing. Therefore, the CSF PCR assays improved pathogen detection by 17 (7%) cases overall.

Comparison of CSF PCR and standard hospital CSF testing results in children with neurosurgical infection

Of the nine children with neurosurgical infection whose CSF was tested by the in-house PCR assays, eight had a definite shunt infection, and one had post-neurosurgical encephalitis. The PCR assays detected *K. pneumoniae* in a child (P-021) whose CSF Gram stain and CSF culture results were consistent with the PCR result. Four other children with neurosurgical infections had a positive CSF culture for *Staphylococcus haemolyticus, Staphylococcus warnerii, Kocuria kristiane* and *Acinetobacter baumanii*; where each pathogen accounted for a single case (table 4.15). Moreover, two children had a positive Gram stain result for Gram-negative bacilli and a positive CSF culture result for *Enterobacter cloacae* (n=1) and *Pseudomonas stutzeri* (n=1). Another child had a positive CSF Gram stain result for Gram-negative cocci which was not detected by both CSF culture and PCR assays. Another child with shunt infection did not have a pathogen detected by either CSF culture, CSF Gram stain, CSF Ziehl-Neelsen stain or PCR assays. Instead, the pathogen (*Staphylococcus epidermidis*) was detected by culture from pus taken from the overlying shunt wound.

In summary, the CSF PCR assays detected a causative pathogen in 1/9 (11%) children with neurosurgical infection, whilst the standard hospital testing detected a pathogen in 9/9 (100%) children. The child with a positive CSF PCR result already had a pathogen detected by the standard hospital; therefore, the CSF PCR assays did not improve but confirmed the pathogen detection.

The PCR assays were not performed in CSF from two other children with shunt infection due to insufficient CSF volume. These patients had a causative pathogen, *Pseudomonas*

aeruginosa and *Staphylococcus epidermidis*, detected by CSF culture. All of the pathogens mentioned above are known to cause neurosurgical infections.

Improvement of pathogen detection by PCR assays in children

Taking both the non-neurosurgical and neurosurgical CNS infection into account (n=247), the PCR assays detected a causative pathogen in 23 cases, including 17 cases of a newly identified pathogen and six cases where the pathogen was also detected by the standard hospital testing. The standard hospital testing alone identified a causative pathogen in 26/247 (11%) cases (chapter 3, section 3.3.3.1). Therefore, the CSF PCR assays improve pathogen detection by 17/247 (7%) cases overall. In total, 43/247 (17%) children had a causative pathogen detected by the CSF PCR assays and/or the standard hospital testing. A full list of the subjects and the pathogens identified is shown in table 4.15.

Forty of 43 children whose pathogen was detected by either the standard hospital testing or the pathogen-specific PCR assay received appropriate antimicrobial treatment. However, 11 children remained to have a fatal outcome. These included four children with *M. tuberculosis*, two with *E. coli*, two with dual infection of *E. coli* and CMV, and three with *S. pneumoniae*, *Staphylococcus,* and Gram-negative cocci CNS infection (n=1 each). Three children did not receive appropriate treatment, including two (P-069 and P-347) with tuberculous CNS infection and one (P-237) with anthrax CNS infection (table 4.15). All these three children died during their hospital stay.

Discrepancies between PCR and standard hospital diagnostic testing results in children with syndromic CNS infection

A paediatric patient, subject P-337, had a CSF pleocytosis and a positive blood culture result for *Salmonella* spp. (table 4.14). This patient, however, did not have any pathogen

detected in his CSF sample either by PCR or culture tests. The blood sample for the culture test was taken on the same day as his hospital admission, whereas his CSF sample was collected 16 days later. This patient received antibiotics from day two of his hospital admission. The antibiotics administered before LP included cefotaxime for five days, followed by ceftriaxone for ten days.

Another paediatric patient, subject P-212, had a CSF pleocytosis and a positive blood culture result for *K. pneumoniae* (table 4.14). Similarly, this patient did not have any pathogen detected in his CSF sample by PCR assays. His blood culture sample that was positive for *K. pneumoniae* was collected on day 22 of his hospital stay, whilst his CSF sample was taken on day eight. Interestingly, an earlier blood culture test on this patient, from which the sample was taken on day 7 showed no growth.

4.3.5.2 Comparison of PCR, culture and Gram stain results in adults

In the adult group, the PCR assays were performed on 108 CSF samples. These included 86 CSF samples with CSF pleocytosis, 13 without pleocytosis but had an immunocompromised condition, and 9 without CSF pleocytosis who died during hospital admission (table 4.16). All these patients had non-neurosurgical CNS infections.

Of 86 adults with CSF pleocytosis, the PCR assays detected the causative pathogens in 28 adults (table 4.16). Twenty-one of the 86 adults in this sub-group had a pathogen detected by CSF culture, but only five were consistent with the aetiology of CNS infection. These pathogens included *Acinetobacter baumanii* (n=2), *S. pneumoniae* (n=1), *Cryptococcus neoformans* (n=1), and *Pseudomonas* species (n=1). The remaining pathogens were *Staphylococcus haemolyticus* (n=5), *Brevundimonas diminuta/vesicularis* (n=2), *Staphylococcus epidermidis* (n=1), *Staphylococcus capitis* (n=1), *Staphylococcus arlettae* (n=1), *Staphylococcus sciuri* (n=1), *Acinetobacter lwoffii* (n=1), *Enterobacter aerogenes*

(n=1), *Enterococcus faecium* (n=1), *Kocuria varians* (n=1), and *Micrococcus tetragenus* (n=1). These pathogens are unlikely to cause CNS infection, thus presumed as contaminants (chapter 3, table 3.10). Seven of the 86 adults in this sub-group had a pathogen detected by CSF Gram stain, but only one was consistent with CNS infection aetiology in adults. None of the adults in this sub-group had a positive CSF Ziehl-Neelsen stain result. Four adults in this sub-group had a positive blood culture result for *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Staphylococcus hominis*, and *Staphylococcus sciurii* (n=1 each), but none of them had a pathogen identified in CSF.

Among 13 immunocompromised adults who did not have CSF pleocytosis, none had a pathogen detected by either CSF PCR or CSF Ziehl-Neelsen stain tests (table 4.16). One of the 13 adults in this sub-group had a pathogen (*P. stutzeri*) identified by both CSF culture and CSF Gram stain tests, whereas two other adults had a pathogen detected by CSF culture only. In the latter cases, the pathogens identified included *Staphylococcus arlettae* and *Pantoea* species. These pathogens were regarded as contaminants, as described in chapter 3, section 3.3.3.1. One adult in this subgroup had a positive blood culture result for *Acinetobacter junii*; however, no pathogen was detected from his CSF, and the patient was clinically diagnosed as having presumptive cerebral toxoplasmosis.

Table 4.36 Comparison of PCR and standard hospital diagnostic testing results in adults

Adult patient sub-group (n)	Samples with positive CSF PCR result (n)	Samples with positive CSF culture results (n)	Samples with positive CSF Gram stain results (n)	Samples with positive CSF ZN stain results (n)	Samples with positive blood culture results (n)
Adults with non-neurosurgical CNS infection (n=168)					
Adults with CSF pleocytosis (n=86)	28	21	7	0	4
Adults without CSF pleocytosis who had an immunocompromised condition (n=13)	0	3	1	0	1
Adults without CSF pleocytosis who died during hospitalisation (n=9)	1	2	0	0	0
Adults whose CSF was not tested for PCR (n=19)*	N/A	5	2	0	0
Adults who did not undergo LP (n=41)¥	N/A	N/A	N/A	N/A	5
Adults with neurosurgical CNS infection (n=0)					

*Included one patient without CSF pleocytosis who died during hospitalisation whose CSF was not available for study purposes and 18 patients without CSF pleocytosis who were discharged alive
¥One patient had a causative pathogen detected by a blood smear
CSF – cerebrospinal fluid; CNS – central nervous system; PCR – polymerase chain reaction; ZN – Ziehl-Neelsen; N/A – not applicable.

Table 4.37 Clinical and laboratory features of adult patients who had causative pathogen(s) detected by standard hospital testing and/or PCR testing

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results					Initial diagnosis	CSF PCR	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations						
N-013	20	F	4	negative	ND	x	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Ceftazidime, acyclovir	died	no
N-021	25	F	3300	positive	NR	x	x	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Ceftazidime	died	no
N-022	46	M	5	positive	ND	x	ND	x	Anti-toxoplasma IgM 0.07 (normal), IgG >300 (markedly increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ARV	alive	yes
N-028	46	M	690	positive	ND	x	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Ceftazidime	died	no
N-029	37	F	190	negative	ND	x	ND	ND	Anti-toxoplasma IgM 0.05 (normal), IgG 176 (markedly increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ceftriaxone, pyrimethamine	alive	yes
N-033	26	M	8	positive	ND	x	*Kocuria varians*	ND	Anti-toxoplasma IgM 0.25 (normal), IgG >300 (markedly increased)	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Ceftazidime, acyclovir, ARV, pyrimethamine	died	no
N-039	21	F	140	negative	ND	x	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Ceftriaxone	died	no
N-049	30	M	78	positive	ND	negative	ND	ND	Anti-toxoplasma IgM 0.05 (normal), IgG 61 (increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, meropenem, levofloxacin, cotrimoxazole, co-amoxiclav	died	yes
N-054	37	M	427	negative	ND	negative	ND	x	-	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Ceftriaxone, acyclovir	died	no
N-061	57	M	1	negative	ND	negative	*S. epidermidis*	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Ceftriaxone	died	no

Table 4.17 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results					Initial diagnosis	CSF PCR	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations						
N-088	41	F	192	negative	ND	negative	*Enterobacter aerogenes*	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Ceftazidime	died	no
N-089	45	M	75	negative	diplo cocci	negative	*S. haemolyticus*	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ceftriaxone, ceftazidime, levofloxacin	died	yes
N-131	35	M	22	negative	ND	negative	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ceftazidime, ciprofloxacin	alive	yes
N-139	21	M	177	negative	ND	negative	ND	ND	Anti-toxoplasma IgM 0.08 (normal), IgG 128 (markedly increased)	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Ceftriaxone, vancomycin	died	no
N-149	34	M	322	negative	ND	negative	ND	ND	Anti-toxoplasma IgM 0.03 (normal), IgG 46 (increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ceftazidime	alive	yes
N-150	23	M	17	negative	ND	positive	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Ceftriaxone, gentamycin	died	no
N-153	42	F	70	negative	ND	negative	ND	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Cefotaxime, meropenem	died	no
N-154	34	M	197	positive	ND	negative	ND	ND	Anti-toxoplasma IgM 0.06 (normal), IgG 171 (markedly increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Anti-TB drugs, ceftriaxone, meropenem, cotrimoxazole, pyrimethamine	died	yes
N-168	26	M	493	negative	ND	negative	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Ceftazidime, meropenem	died	no
N-169	23	M	208	negative	ND	negative	ND	-	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Meropenem	died	no

Table 4.17 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results					Initial diagnosis	CSF PCR	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations						
N-170	69	F	73	negative	ND	positive	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Ceftriaxone, levofloxacin	died	no
N-175	21	F	270	negative	ND	negative	ND	ND	Anti-toxoplasma IgM 0.22 (normal), IgG 258 (markedly increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	Definite TBM	Cefotaxime, ceftriaxone, amikacin, acyclovir	died	no
N-179	25	M	178	negative	x	x	x	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	Definite TBM	Ceftazidime, meropenem, vancomycin	died	no
N-142	47	F	1170	negative	ND	negative	ND	ND	-	Unknown aetiology	*S. pneumoniae*	*S. pneumoniae* CNS infection	Ceftriaxone	alive	yes
N-157	58	F	13200	negative	ND	negative	*S. pneumoniae*	ND	-	*S. pneumoniae* CNS infection	*S. pneumoniae*	*S. pneumoniae* CNS infection	Ceftazidime, meropenem	died	yes
N-167	33	M	25	positive	ND	negative	ND	ND	Anti-toxoplasma IgM 0.02 (normal), IgG 44 (increased)	Unknown aetiology	*S. pneumoniae*	*S. pneumoniae* CNS infection	Ceftazidime	alive	yes
N-044	32	M	79	positive	ND	x	ND	ND	-	Unknown aetiology	HSV-2	HSV-2 CNS infection	Ceftazidime, levofloxacin, amikacin, imipenem	alive	no
N-140	34	M	92	positive	ND	negative	ND	x	Anti-HSV-1 IgM 0.7, IgG 4.8 (normal) Anti-HSV-2 IgM 0.69, IgG 4.5 (normal) Anti-toxoplasma IgM 0.1 (normal), IgG 53 (increased)	Unknown aetiology	HSV-2	HSV-2 CNS infection	Ceftriaxone, meropenem, anti-TB drugs, acyclovir, ARV	died	no
N-084	33	M	0	positive	ND	negative	ND	ND	Anti-toxoplasma IgM 0.47 (normal), IgG >300 (markedly increased)	Presumptive cerebral toxoplasmosis	VZV	Presumptive cerebral toxoplasmosis, VZV CNS infection	Pyrimethamine, clindamycin, ARV	alive	yes

Table 4.17 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results					Initial diagnosis	CSF PCR	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations						
N-100	23	F	77	negative	ND	positive	*Pseudomonas sp.*	x	Anti-toxoplasma IgM 0.11 (normal), IgG 166 (markedly increased)	*Pseudomonas* CNS infection	ND	*Pseudomonas* CNS infection	Ceftazidime, clindamycin, anti-TB drugs	alive	yes
N-120	34	M	10	positive	Solitary Gram-negative, Gram-positive cocci	negative	*Acinetobacter baumannii*	ND	-	*Acinetobacter baumannii* CNS infection	ND	*Acinetobacter baumannii* CNS infection	Ceftazidime	died	yes
N-143	37	M	32	positive	ND	positive	*C. neoformans*	ND	Anti-toxoplasma IgM 0.04 (normal), IgG >300 (markedly increased)	Cryptococcal CNS infection	ND	Cryptococcal CNS infection	Ceftriaxone	died	no
N-147	27	F	5	positive	x	negative	*Acinetobacter baumannii*	ND	-	*Acinetobacter baumannii* CNS infection	ND	*Acinetobacter baumannii* CNS infection	Cefotaxime, ceftazidime, meropenem, imipenem, anti-TB drugs	alive	yes
N-063	47	M	N/A	negative	N/A	N/A	N/A	*K. pneumoniae*	Anti-toxoplasma IgM 0.14 (normal), IgG 169 (markedly increased)	*K. pneumoniae* CNS infection	N/A	*K. pneumoniae* CNS infection	Ceftazidime, meropenem	alive	yes
N-070	33	M	N/A	positive	N/A	N/A	N/A	*K. pneumoniae*	-	*K. pneumoniae* CNS infection	N/A	*K. pneumoniae* CNS infection	Meropenem, metronidazole, gentamycin, clindamycin, anti-TB drugs	alive	yes
N-073	34	M	N/A	x	N/A	N/A	N/A	ND	*P. falciparum* positive (blood smear)	Cerebral malaria	N/A	Cerebral malaria	Ceftriaxone	died	no

Table 4.17 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results					Initial diagnosis	CSF PCR	Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations						
N-080	40	M	N/A	positive	N/A	N/A	N/A	*Staphylo-coccus hominis*	Anti-toxoplasma IgM 0.16 (normal), IgG >300 (markedly increased)	*Staphylo-coccus* CNS infection	N/A	*Staphylo-coccus* CNS infection	Ceftriaxone, meropenem, pyrimethamine	died	yes
N-186	52	M	N/A	positive	N/A	N/A	N/A	*Shigella* group	-	*Shigella* CNS infection	N/A	*Shigella* CNS infection	Ceftazidime	died	yes

PCR – polymerase chain reaction; CSF – cerebrospinal fluid; ZN – Ziehl-Neelsen; HIV – human immunodeficiency virus; TB – tuberculosis; TBM – tuberculous meningitis; HIV – human immunodeficiency virus; ARV – antiretroviral; CNS – central nervous system; *M. tuberculosis – Mycobacterium tuberculosis; S. pneumoniae – Streptococcus pneumoniae;* HSV-2 – herpes simplex virus type-2; VZV – varicella-zoster virus*; K. pneumoniae – Klebsiella pneumoniae; S. haemolyticus – Staphylococcus haemolyticus; S. epidermidis – Staphylococcus epidermidis; P. falciparum – Plasmodium falciparum*; F – female; M – male; ND – not detected; N/A - not applicable; x – not done.

The PCR assays detected a causative pathogen in 1 of 9 immunocompetent adults with non-neurosurgical CNS infection who did not have a CSF pleocytosis and died during hospital admission (table 4.16). In this sub-group, two adults had a positive CSF culture result for *Staphylococcus epidermidis* and *Pseudomonas fluorecens*. Both pathogens are unlikely to cause CNS infection and are considered contaminants (chapter 3, table 3.10). None of the adults in this sub-group had a pathogen detected by CSF Gram stain, CSF Ziehl-Neelsen stain, or blood culture.

The CSF PCR assays were not performed in 19 adults with non-neurosurgical CNS infection. All were immunocompetent and did not have CSF pleocytosis: 18 were discharged alive, and one died but did not have CSF available for study purposes. Among these, three had a positive CSF culture for *Staphylococcus warneri, Staphylococcus hominis ssp hominis, and Kocuria rose*a (n=1 each); two had a positive CSF culture and a consistent CSF Gram stain result for *Staphylococcus hominis* and *Acinetobacter baumannii* (n=1 each). These pathogens were considered contaminants, as described in chapter 3, section 3.3.3.1.

Forty-one adults did not undergo LP and therefore had no CSF samples available. Among these, five adults had a positive blood culture result. However, only four had a pathogen consistent with the aetiology of CNS infection, as described in chapter 3, section 3.3.3.1. One adult in this subgroup had *Plasmodium falcipar*um, a known cause of cerebral malaria, from a blood smear.

In summary, the PCR assays detected the causative pathogen in 29/108 (27%) adults with non-neurosurgical CNS infection whose CSF was available for study purposes. These included 28 adults whose pathogens were detected by the PCR assays only and one adult whose pathogen was identified by both the PCR assays and CSF culture.

Improvement of pathogen detection by PCR assays in adults

All of the adult patients with syndromic CNS infection had non-neurosurgical infections. Taking all adults with syndromic CNS infection into account (n=168), the standard hospital testing detected a causative pathogen in 10 (6%) cases (chapter 3, section 3.3.3.1). The CSF PCR assays detected a causative pathogen in 29 patients, one of which was also identified by the standard hospital testing. Therefore, the CSF PCR assays improved pathogen detection by 28/168 (17%) cases overall. Taking both the standard hospital testing and the PCR assays results, 38/168 (23%) adults had a causative pathogen identified. The cases are listed in table 4.17.

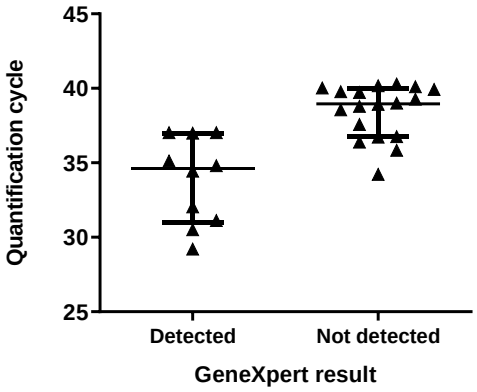
Eighteen of 38 adults with pathogens identified by the standard hospital testing or the PCR assays received appropriate antimicrobial treatment. The remaining 20 adults did not receive appropriate treatment. These included 16 adults with tuberculous CNS infection who did not receive anti-TB drugs, two with HSV-2 CNS infection who received antibiotics, and two with cryptococcal CNS infection and cerebral malaria (n=1 each) who did not receive pathogen-specific treatment (table 4.17). Nineteen adults had a fatal outcome, including those with tuberculous CNS infection (n=16), HSV-2 (n=1), cryptococcal CNS infection (n=1), and cerebral malaria (n=1). Interestingly, among 18 adults who received appropriate treatment, seven remained to have a fatal outcome. These included three adults with tuberculous CNS infection and four with *S. pneumoniae*, *Acinetobacter, Staphylococcus,* and *Shigella* CNS infection (n=1 each) [table 4.17].

4.3.6 Comparison of in-house *Mycobacterium tuberculosis* PCR and GeneXpert results

In order to confirm the in-house *M. tuberculosis* PCR results, 28 CSF samples with *M. tuberculosis* positive were transferred to Dr Sardjito Hospital laboratory for further *M.*

tuberculosis testing using the GeneXpert MTB/RIF assay. The GeneXpert assay has only been available at the hospital laboratory since July 2017, and its use is mainly for *M. tuberculosis* detection in sputum samples. None of the 32 CSF samples with positive *M. tuberculosis* detected by PCR had previously been tested using the GeneXpert assay, except for sample P-296. Due to limited sample volume, four samples were not included in the GeneXpert assay.

Figure 4.12 Comparison of in-house *Mycobacterium tuberculosis* PCR quantification cycle values between CSF samples with positive (detected) and negative (not detected) GeneXpert results



Twenty-eight CSF samples had sufficient surplus volume (>1ml) to be re-tested on GeneXpert. Ten yielded a positive result when the same CSF sample was tested using the GeneXpert MTB/RIF assay. These included samples from four children (i.e. patients P-003, P-018, P-058, and P-296) and six adults (i.e. patients N-061, N-150, N-153, N-154, N-168, and N-169). Of these ten samples with *M. tuberculosis* identified by GeneXpert, one adult patient, N-154, had a mutation associated with possible rifampicin resistant detected (data not shown). Patients with a positive CSF GeneXpert result had a significantly lower PCR Cq value than those with a negative CSF GeneXpert result (median Cq 34.6 versus Cq 39, $p<0.001$) (figure 4.6).

4.4 Discussion

In this chapter, I have successfully developed and transferred the single-plex PCR assays from the University of Liverpool to the local laboratory in Yogyakarta, Indonesia. Learning from the contamination problem which occurred when I introduced the enterovirus PCR assay at the local laboratory, special precautions were taken to minimise this risk. The first attempt was to have a set of personal PCR equipment, including pipettes, tips, tubes, PCR plates and seals, and all of the equipment was stored in a private storage box that other individuals could not access except me. The second attempt was to divide the control samples into many aliquots; therefore, if one aliquot was contaminated, then there would be other stocks of control samples aliquots available. Moreover, each control sample's aliquots were packed in separate seal bags during the transfer from Liverpool to Yogyakarta. Lastly, all standard procedures of PCR were cautiously completed.

4.4.1 Improvement in bacteria detection by CSF PCR assays

The PCR assays detected pyogenic bacteria in CSF from 15 children (table 4.10). All of these children had non-neurosurgical CNS infections, except for subject P-021. Four of the 14 children with non-neurosurgical CNS infections had the pathogen detected by the standard hospital testing: three by both CSF culture and CSF Gram stain and one by CSF culture only (table 4.10). Therefore, compared to the CSF culture and CSF Gram stain tests, the CSF PCR assays improve the detection of pyogenic bacteria in children with non-neurosurgical CNS infection from four cases to 14 cases. Furthermore, the present study has successfully identified *S. pneumoniae*, *S. agalactiae, N. meningitidis,* and *H. influenzae* which are rarely reported as the aetiology of paediatric CNS infection among patients admitted to Dr Sardjito Hospital.

Within the adult group, pyogenic bacteria were detected by the PCR assays in three adults (table 4.12), all of whom had non-neurosurgical CNS infections. Only one of these three adults had a positive CSF culture for the corresponding pathogen, and none had a corresponding pathogen detected by the CSF Gram stain. Therefore, the use of CSF PCR assays improves the detection of pyogenic bacteria in adults with non-neurosurgical CNS infection from one to three cases.

The above findings indicate that the CSF PCR assays targeting pyogenic bacteria are more sensitive than the CSF culture and CSF Gram stain tests in patients with non-neurosurgical CNS infection.

All of the children (15/15 (100%)) and all of the adults (3/3 (100%)) with syndromic CNS infection who had a positive PCR result for pyogenic bacteria had CSF pleocytosis. Among these, 13 (87%) children and 2 (67%) adults had a CSF leucocyte count of >100 cells/μL (tables 4.10 and 4.12). This finding suggests pathogen nucleic acid is more likely to be detected by PCR amplification in patients with a higher CSF leucocyte count. In the present study, among the patients with non-neurosurgical CNS infection, the detection of pyogenic bacteria by PCR in children was much higher than in adults. This might be partly due to the fact that the time to LP in children was shorter than in adults (1 day versus 3 days, chapter 3). Delays in LP, which consequently prolong the treatment duration prior to LP, might decrease pathogen load in CSF to a level below the PCR limit of detection.

The fact that no *S. suis* CNS infection case was identified among the patients enrolled in my study is not unexpected. *S. suis* is a zoonotic pathogen causing infection mainly in pigs. *S. suis* infection in humans occurs particularly in those frequently exposed to pigs or pork. Therefore, the absence of *S. suis* infection in the study population is not surprising because most of the patients are Muslim and pig-rearing in the region is not common.

4.4.2 Improvement of *M. tuberculosis* detection by PCR assay

The in-house *M. tuberculosis* PCR assay detected the pathogen in nine children, including eight cases of assumed true-positive and one case of false-positive. Among the eight true-positive cases, CSF GeneXpert was only performed in one child during hospital admission, where the pathogen was detected by this assay. CSF Ziehl-Neelsen stain and CSF culture tests were performed on six and eight children, respectively. However, none had a positive result for *M. tuberculosis* (table 4.10). Therefore, the in-house PCR assay improves *M. tuberculosis* detection in children from one to eight cases.

Within the adult group, the in-house *M. tuberculosis* PCR assay detected the pathogen in 23 patients. CSF Ziehl-Neelsen stain and CSF culture tests were performed on 19 and 21 of these patients, respectively. However, none tested positive for *M. tuberculosis* (table 4.12). None of the adult patients had a CSF GeneXpert performed at the hospital during admission. Therefore, the in-house PCR assay improves M. tuberculosis detection from zero to 23 cases in the adult group.

The GeneXpert became available at the hospital in July 2017 and was not routinely used to test CSF samples. Moreover, to date, there is no standard methodology for CSF testing on GeneXpert. As I have described in chapter 3, the detection of *M. tuberculosis* in CSF at the hospital was performed by the Ziehl-Neelsen stain test. However, this test was not frequently performed in children. The fact that none of the patients with a positive CSF PCR result for *M. tuberculosis* had a positive CSF Ziehl-Neelsen stain result showed that the latter test had extremely poor sensitivity. Moreover, an *M. tuberculosis* culture was not performed. These factors explain why tuberculous CNS infection is underdiagnosed in the study setting.

I have shown in the result section that among the patients whose CSF sample was tested by both the in-house PCR and the GeneXpert assays, those with positive GeneXpert results had a significantly lower PCR Cq value than those with negative GeneXpert results (median 34.6 versus 38.9, p=0.001, figure 4.6). A lower PCR Cq value correlates with a higher starting DNA concentration in the sample. Therefore, this result indicates that the in-house *M. tuberculosis* PCR assay potentially detects a lower bacterial load than GeneXpert. Moreover, the CSF GeneXpert assay required a much larger CSF volume (1-2 mL versus 200 µL) but showed a poorer yield than the PCR assay. These results suggest that the in-house *M. tuberculosis* PCR assay had a lower limit of detection and thus had a higher sensitivity than the GeneXpert.

The sensitivity of the *M. tuberculosis* PCR assay was not assessed in the present study. However, a study in Bandung, West Java, where the in-house *M. tuberculosis* PCR assay was initially developed in the country, reported a sensitivity of 64% compared to 42% for GeneXpert when the clinical diagnosis was used as a reference standard.[31] A more recent study by Heemskerk *et al*.[313] compared the sensitivities and specificities of CSF GeneXpert for diagnosing tuberculous meningitis in adults across Indonesia, Vietnam, and South Africa. When the clinical diagnosis of tuberculous meningitis was used as the gold standard, the overall sensitivity and specificity of the CSF GeneXpert were 25.1% (95% CI 21.0-29.7%) and 100%, respectively. Comparing the sensitivities each site, the sensitivities of the CSF GeneXpert in Indonesia, Vietnam, and South Africa were 37.5% (95% CI 29.4-46.4%), 19.9% (95% CI 14.8-26.2%), and 17.8% (95% CI 10.7-28.1%); whereas the specificities were 100% for each site.

Patients with a positive *M. tuberculosis* CSF PCR result had clinical and laboratory features consistent with tuberculous CNS infection based on the classification used [chapter 3, table 3.1].[231] However, clinical classifications, particularly “possible TBM”, are not specific.

In addition, although child data were used, the classifications were developed largely based on data from adults.[314-316] One patient, P-125, who was three months old, only had the following characteristics from the scoring system: altered consciousness (score 1) and CSF lymphocytic predominance (score 1) [total score: 2]. Thus, this patient did not reach the minimum score to be classified as having possible TBM (i.e. score 6-9 when neuroimaging is unavailable or score 6-11 when neuroimaging is available). This patient did not receive tuberculosis treatment and was alive, without neurological sequelae, six months post-discharge. An intact outcome is unusual in TBM without appropriate anti-tuberculous treatment. Therefore, it is likely that this patient did not have a tuberculous CNS infection, and the positive *M. tuberculosis* PCR result was a false-positive. There are no studies on GeneXpert accuracy for TBM in infants. Studies on pulmonary TB report a reduced specificity for GeneXpert Ultra in children.[317] Compared to adult studies, our in-house *M. tuberculosis* PCR assay results indicate potentially higher sensitivity than GeneXpert, as also reported by Chaidir *et al*. from the West Java study,[31] but with lower specificity. Ideally, to fully assess our in-house PCR assay accuracy and test CSF from patients fulfilling the clinical definitions for TBM, I would test CSF samples that are culture positive.

4.4.3 Improvement in virus detection by PCR assays

The PCR assays help detect viral pathogens in my study setting, including two HSV-2 and one VZV infection detected in adults and four CMV infections detected in children. Only one of the two adults with a positive PCR result for HSV-2 had HSV-2 serology testing done at the hospital, of which the HSV-2 IgM was not detected in serum. This serology testing was performed only on a single serum sample and not CSF samples. None of the children with a positive CMV PCR result underwent CMV serology testing at the hospital. As I have

described in chapter 3, routine virology culture and viral-specific PCR testing were unavailable at the hospital. These factors explain why the standard hospital diagnostic testing yield in detecting viral pathogens was poor.

Among the four children who tested CMV-positive by PCR, all were aged three months or younger and had a co-positivity with other pathogens, including *E. coli* (n=3) and *S. agalactiae* (n=1). None of them were tested for HIV during hospitalisation, but no immunocompromised condition was reported in their medical records. Furthermore, none of them had clinical characteristics of congenital CMV infection, and their CSF characteristics were more of a bacterial rather than a viral infection pattern. This finding is of interest as CMV infection, if it happens beyond the neonatal period, mainly occurs in immunocompromised individuals. Therefore, it is likely that the CMV infection was a latent infection rather than an active infection. Nevertheless, there is increasing evidence that CMV infection, including that involving the CNS, also occurs in immunocompetent individuals.[318-320] A recent study by Mallewa *et al*. on Malawian children showed that only 2/12 (7%) children with CMV CNS infection were HIV-positive.[321]

None of the CSF samples tested by the PCR assays had a positive result for either enterovirus or HSV-1. This finding is of interest as enterovirus is known as one of the most common causes of viral CNS infection, particularly in countries where flaviviruses are not endemic.[2, 3, 322, 323] A recent study from North Sulawesi, Indonesia reported two possible enteroviral infections out of 74 suspected CNS infection cases enrolled in the study, of which the pathogens were detected by PCR from a throat swab.[47] This finding may suggest that enterovirus is not a common cause of CNS infection in Indonesia.

Enterovirus is an RNA virus in which nucleic acids are easily degraded if the samples are not immediately stored at an appropriate temperature. In the present study, there was a

delay in storing samples in a -80 °C freezer. Initially, I had limited access to any of the -80 °C freezers in the hospital laboratory. Consequently, samples collected in the early stage of this study were stored in a -20 °C freezer for a few months until access to a -80 °C freezer in the hospital laboratory was granted. However, access remained limited. Therefore, the samples were usually stored in a -20 °C freezer for a few weeks before being transferred to a -80 °C freezer. Consequently, this delay could cause RNA degradation, which could explain why no enterovirus cases were found in the study.

4.4.4 Discrepancies among PCR, culture, and Gram stain results in patients with syndromic CNS infection

The *K. pneumoniae* PCR assay failed to detect the pathogen in the CSF sample from subject P-212 who had a positive blood culture result for *K. pneumoniae* (table 4.14). In this case, the positive blood culture result was found later in his hospital stay, and an earlier blood culture test showed no organisms had grown. His onset of illness was one day prior to his hospital admission; thus, it is less likely that *K. pneumoniae* was the cause of his CNS infection. The late positive blood culture result might also indicate that the patient was co-infected with *K. pneumoniae* during their stay at the hospital. *K. pneumoniae* is a common aetiology of nosocomial infection in children and adults.[324, 325]

The *Salmonella* PCR assay failed to detect the pathogen in the CSF sample from subject P-337 who had a positive blood culture for *Salmonella* (table 4.14). In this case, the patient had not received any antibiotics when his blood was taken for culture but had received antibiotics for 15 days when he underwent LP. Therefore, the negative PCR result might be due to the long course of antibiotic treatment before LP. Alternatively, it is also possible that the pathogen DNA on the CSF sample had degraded due to sub-optimal storage prior to the PCR assays.

In children with neurosurgical infection, the CSF PCR assays only detected the causative pathogen in 1/9 (11%) children, whilst the standard hospital CSF testing detected the pathogen in 8/9 (89%) children. This is because the PCR assays were not designed to detect pathogens causing neurosurgical infection.

Two causative pathogens, including Gram-negative bacilli and Gram-negative diplococci, were detected by the CSF Gram stain only. None of these pathogens was detected by the PCR assays. The Gram-negative bacilli were found in two children with encephalitis, whilst the Gram-negative diplococci were found in a child with shunt infection. Gram-negative bacilli organisms that cause CNS infection include *H. influenzae*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Salmonella* spp., *Acinetobacter* spp., *Enterobacter* species, and *Proteus* species. Some of these pathogens are associated with nosocomial/neurosurgical infection.[241, 325, 326] In the present study, both children who tested positive for Gram-negative bacilli were under one year of age. Thus, the CNS infection in these children might be caused by either *H. influenzae*, *E. coli*, *K. pneumoniae,* or *Salmonella* spp., but the PCR assays failed to detect the pathogen. This might happen if the pathogen DNA in the CSF sample degraded due to sub-optimal storage prior to PCR. Alternatively, the CNS infection might be due to other Gram-negative bacilli organisms not targeted by the PCR assays.

Gram-negative diplococci include *Moraxella* and *Neisseria* organisms, of which the most notable pathogen associated with CNS infection is *N. meningitidis*.[326] Nevertheless, *N. meningitidis* is not a typical cause of shunt infection. Other pathogens frequently linked with shunt infection, *Acinetobacter species*, are Gram-negative coccobacilli which often appear as diplococci in Gram stain.[327] Therefore, the Gram-negative diplococci found in this study were more likely to be *Acinetobacter* spp. This also explains why the pathogen was not detected by the *N. meningitidis* PCR assay.

4.4.5 Potential implications for patient management

The application of pathogen-specific PCR, in addition to the standard hospital testing, improved the detection of CNS infection aetiology by 17/247 (7%) cases in children and 28/168 (17%) cases in adults.

The turn-around time of PCR assays is usually two days if samples are processed immediately following collection. In the present study, samples were initially pooled and then tested later in divided batches. However, during the sample testing, it took approximately 2-3 days to finish the process from nucleic acid extraction to PCR run for a single pathogen. Similarly, the turn-around time of the GeneXpert assay conducted at the hospital laboratory was 2-3 days from sample collection until result validation, as samples were also pooled and tested in batches. The turn-around time of both the PCR and the GeneXpert assays is twofold quicker than the traditional culture, where the results are usually obtained in our hospital within five days.

In terms of tuberculous CNS infection, eighteen patients with a positive *M. tuberculosis* CSF PCR result did not receive anti-TB treatment and had a fatal outcome (sections 4.3.4.1 and 4.3.4.2). If the *M. tuberculosis* PCR assay and/or the GeneXpert were routinely performed on CSF, results could have been available within 2-3 days. The results could have prompted clinicians to give anti-TB treatment, and patient outcome may have been better. Among the *M. tuberculosis*-positive patients where anti-tuberculous medication was started, the results could have prompted clinicians to start the treatment sooner.

The in-house *M. tuberculosis* PCR assay exhibited dramatically higher sensitivity than the Ziehl-Neelsen stain on CSF (no patients were detected by stain). My analysis of *M. tuberculosis* samples also demonstrated that the in-house PCR assay showed better sensitivity and required a lower CSF volume than the GeneXpert. Therefore, routine use

of the in-house *M. tuberculosis* PCR at Dr Sardjito Hospital could be recommended from my results. However, accepting the *M. tuberculosis* GeneXpert test is available and the Indonesian Government fully supports its consumables, the inclusion of GeneXpert testing into routine CSF investigation should also be considered.

The proportion of tuberculous CNS infection in the study setting was markedly higher than other known pathogens. Moreover, the mortality in tuberculous CNS infection cases was also high. Therefore, my study findings indicate early initiation of anti-tuberculosis treatment in cases with clinically suspected tuberculous CNS infection should also be recommended.

The use of PCR testing could enable more pathogens to be detected or detected earlier. In turn, earlier results can enable the clinician to give more targeted treatment. Downstream, this may lead to improved patient outcome.

Six paediatric cases of *E. coli* CNS infection were detected by CSF PCR assay. Three cases were also detected by culture (CSF n=2; blood n=1). All six cases were infants. All were treated with cefotaxime. They also received other antibiotics, including meropenem (n=4), ampicillin, and ciprofloxacin. Meropenem treatment is valuable because previous reports indicate an increasing incidence of meningitis due to *E. coli* with extended-spectrum β-lactamases (ESBL) (44, 45). However, in our study, additional antibiotics were given late (e.g. one patient received meropenem seven days after LP) or as an incomplete course (e.g. one patient received only two doses of meropenem). The PCR results, being provided in real-time (i.e. within 2-3 days), could have prompted clinicians to give appropriate treatment for *E. coli* meningitis in infants, including prompt and full-course treatment with cefotaxime and early consideration for adding in meropenem. Of course, not all cases of *E. coli* are ESBL-producing. Therefore, ESBL testing in patients diagnosed

with *E. coli* or other ESBL-producing pathogens may also be a useful addition in our hospital setting.

The *S. pneumoniae* PCR assay has successfully identified three paediatric and two adult cases that were not detected by the standard hospital testing (tables 4.10 and 4.12). All of them received first-line antibiotics for suspected bacterial infection (cefotaxime/ceftriaxone/ceftazidime) and were discharged alive, except for patient P-150. The latter patient underwent LP on day 6 and received multiple antibiotics as well as anti-TB drugs. The latter patient's fatal outcome may have been due to disease severity. Detection of *S. pneumoniae* by PCR in the hospital setting would have provided diagnostic information (beyond the negative culture results) to the treating clinician. In turn, this information could help them promote appropriate and rationalised antibiotic treatment and avoid unnecessary treatments, such as fixed-dose combination prescriptions of anti-TB drugs.

Four children who had a positive CSF PCR result for *S. agalactiae* (n=2, P-154 and P-229), *H. influenzae* (n=1, P-258), and *N. meningitidis* (n=1, P-093) did not have the corresponding pathogen detected by the standard hospital testing (table 4.10). All of them received appropriate treatment (i.e. first-line antibiotic) prior to LP and were discharged alive. Two children had a causative pathogen detected by both CSF culture and PCR assay, including patients P-270 who tested positive for *Salmonella spp.* and P-021 who tested positive for *K. pneumoniae*. The former patient received the appropriate antibiotic before LP, while the latter received it five days after LP. Both of them were discharged alive. In the latter case, if both CSF culture and PCR assay had been performed simultaneously, the CSF PCR assay result would have been available sooner than the culture result. Consequently, the appropriate antibiotic could have been administered earlier.

In the present study, I found a case of *H. influenzae* CNS infection that was not detected by standard hospital testing. Currently, the vaccine against *H. influenzae type b* (Hib) is widely available. However, this vaccine was not included in the national immunisation programme in Indonesia before 2013. Therefore, it is very likely that the above patient had not received the Hib vaccine. Although there is a national Hib immunisation programme from the government, its coverage in 2018 was only 61%.[24] Therefore, the data obtained from the present study could be used to encourage more parents to vaccinate their children.

Two adults had a final diagnosis of HSV-2 CNS infection by the CSF PCR assay, including patients N-044 and N-140. The former patient received multiple antibiotics and was discharged alive. The latter case was an HIV-positive patient with a current genital ulcer, and thus was treated with acyclovir and ARV. Additionally, he also received multiple antibiotics and anti-TB drugs. In both cases, the HSV-2 CSF PCR assay is essential to guide the clinicians to stop giving unnecessary antibiotics.

Another adult tested positive for VZV by the CSF PCR assay but not by the standard hospital testing. He was HIV-positive, received ARV but not acyclovir, and was discharged alive. The advantage of acyclovir in viral CNS infection remains unclear, and to date, no randomised controlled trials have been conducted to help guide clinicians.

In summary, among 43 children and 38 adults who had pathogens detected by either the PCR or the standard hospital testing, three children and 20 adults did not receive appropriate treatment during their hospital stay; all of whom, except for one adult, had a fatal outcome. Of those, the majority (i.e. two children and 16 adults) had a final diagnosis of tuberculous CNS infection. If the pathogen had been correctly identified at the hospital, these patients would have received anti-TB treatment more promptly, and they may have

had a chance of a better outcome. These findings emphasise the importance of molecular techniques for pathogen detection, particularly those targeting *M. tuberculosis*, which could guide patient treatment and subsequently improve patient outcome.

4.5 Conclusion

The introduction of in-house single-plex CSF PCR assays has increased the detection of pathogens causing CNS infection by 7% in children and 17% in adults.

Given many cases had already received antibiotics prior to LP, I have also confirmed the PCR assays can detect pathogens in cases where antibiotics have already been given.

M. tuberculosis was the most common pathogen detected by PCR among both children and adults. The pathogen caused high mortality compared to other identified pathogens in the study. The potential positive impact on clinical care from these test results could be dramatic. Sixteen out of 23 adults (70%) with a positive PCR test never received anti-tuberculous drugs. Among the seven adult cases that did receive anti-tuberculous drugs, initiation was often delayed (up to 21 days post LP). The PCR results could have prompted clinicians either to start anti-TB treatment or start it earlier. In turn, patient outcome may have improved. These findings emphasise the critical need for the inclusion of *M. tuberculosis* PCR assay and/or GeneXpert into routine CSF testing in the study setting.

The identification of *S. pneumoniae*, *S. agalactiae, N. meningitidis,* and *H. influenzae* as the cause of CNS infection among children admitted to Dr Sardjito Hospital by PCR strengthens local microbial knowledge. These pathogens have been infrequently reported by the standard diagnostic testing in the hospital prior to this study. The potential influence on clinical care from these test results would be less dramatic than for TBM, as many patients were already on appropriate first-line antibiotics. However, many patients were on multiple antibiotics/treatments. Again, PCR results may enable the clinician to

rationalise treatment and reduce or avoid the prescription of unnecessary treatments, which in turn may shorten the length of hospital stay.

Although an increased diagnosis has been achieved by the use of PCR assays, diagnostic yield may have been increased further if the LP/EVD was not delayed. Therefore, strict implementation of the recent guidelines for diagnosis and management of CNS infection, where LP should be performed as early as possible prior to initial antibiotic administration, remains essential to obtain optimum diagnostic yield.

It is noteworthy that when bacterial culture and/or antibiotic sensitivity results were available to clinicians, these results did not always influence clinical treatment. Therefore, any plan to introduce routine hospital PCR testing may benefit from close communication and discussion with the clinical teams ahead of implementation.

Taking these factors into account, I believe routine PCR testing for CNS infection in Dr Sardjito Hospital should be considered.

In this chapter, I have shown that the PCR assays increased the detection of pathogen causing CNS infection in Dr Sardjito Hospital. In the next chapter, I will assess the usefulness of antibody-mediated assays, including ELISA and *Cryptococcus* antigen lateral flow assay, in diagnosing CNS infection at the study site.

Chapter 5 – Improving the detection of pathogens causing central nervous system infections in Yogyakarta using antibody techniques

5.1 Background

I have shown in the previous chapter that using PCR techniques increases the pathogen detection rate in the CSF of patients with syndromic CNS infection. In addition to PCR techniques, antibody techniques have shown to be useful in identifying certain aetiology of CNS infection such as flaviviruses, rickettsiae, and *Cryptococcus*.[154, 166, 232, 328-330] Therefore, in this chapter, I ask whether this is also the case in my study setting, particularly for identifying dengue virus, Japanese encephalitis (JE) virus, *Orientia tsutsugamushi* and *Cryptococcus*.

My specific aims for this chapter are:

1) To assess selected commercial ELISA kits' performance in clinical samples
2) To describe the results of dengue and JE antibody tests
3) To describe the results of *Orientia tsutsugamushi* antibody test
4) To describe the results of *Cryptococcus* antigen test
5) To describe the clinical and laboratory features of patients with positive antibody testing results

5.2 Methods

5.2.1 Determining which pathogens to test using antibody and antigen techniques

As described in the previous chapter, a few publications highlighted the aetiology of CNS infection in Indonesia when I set up this study in 2014. Among the pathogens reported causing CNS infection in Indonesia were Japanese encephalitis virus (JEV) and Cryptococcus neoformans, both tested by antibody techniques.[8, 10] The previous Japanese encephalitis (JE) studies in the country did not include Yogyakarta as a study site. Thus, whether the JE cases were present in the Yogyakarta region was unknown. Approximately one-sixth of Yogyakarta area is wetland – mainly used as rice paddies – which is one of the main breeding sites for *Culex* mosquitoes, the JE vector.[14, 80] Therefore, I decided to include JEV as one of the target pathogens in my study.

Another flavivirus which has been associated with CNS infection is dengue virus. Dengue and dengue haemorrhagic fever are epidemics in Indonesia.[85] The incidence rate of dengue haemorrhagic fever (DHF) in the country was 44 per 100,000 in 2013, with a case fatality rate of 0.73%.[331] Yogyakarta had the fourth highest incidence rate of DHF in the country, with 96 cases per 100,000 persons in the same year.[331] Previous reports have highlighted paediatric dengue infection cases with neurological manifestations in Jakarta, Indonesia. However, none of the cases had confirmed dengue virus or antibody detected in the CSF.[332-335] Therefore, it was therefore interesting to see if I could find confirmed cases of dengue CNS infection in Yogyakarta.

As described above, *C. neoformans* has been reported as a CNS infection aetiology in another province in Indonesia.[10] Cryptococcal meningitis is one of the most common opportunistic infections in HIV-positive individuals, contributing to high mortality.[59, 336, 337]

The HIV epidemic in Indonesia is the second fastest growing in Asia,[338] suggesting the burden of cryptococcal meningitis is also likely to increase. As *Cryptococcus* detection was not routinely performed in patients with suspected CNS infection at Dr Sardjito Hospital when I started this study, I decided to include it in my pathogen detection panel.

Scrub typhus is known to have neurological manifestations such as meningitis, meningoencephalitis and encephalopathy.[339] It is endemic in the Asia Pacific region, including Indonesia.[340] Interestingly, there are limited reports on scrub typhus cases in Indonesia and none linked with CNS infection.[341-345] Additionally, to date, there is no scrub typhus diagnostic test available at Dr Sardjito Hospital, suggesting that the disease is somewhat underestimated by the clinicians. Therefore, I wanted to investigate whether such cases were present at the study site.

5.2.2 Determining which antibody and antigen techniques to use

Antibody tests have been widely used to diagnose flaviviral infections, including dengue and Japanese encephalitis. Rapid immunochromatographic (ICT) assays are more commonly used than enzyme-linked immunosorbent assays (ELISA) at hospitals or other healthcare facilities, as the ICT assays are quicker and simpler to perform. However, the ELISA has a higher sensitivity than the ICT assay in general;[346, 347] therefore, it is often used in study settings, including this study.

For the diagnosis of dengue CNS infection in the present study, detection of dengue IgM was preferred to dengue viral genome as the former technique is recommended if the specimen is collected either before or after seven days post-symptom onset.[154] Dengue virus has a short viraemia period (i.e. up to five days after onset of illness which typically concurs with the fever period) – this reduces the chance for viral genome detection via real-time PCR if the samples are taken after that period.[348] In primary dengue infection

(i.e. when dengue virus is the first flavivirus to infect a person), the dengue IgM typically increases slowly and is detectable in 50% of patients by days 3-5 following the onset of illness, in 80% of patients by day five, and in 93-99% of patients by day 6-10.[348] Dengue IgG typically develops at day seven, increases slowly, and remains detectable in serum after several months or even for life.[85, 348] Therefore, a positive dengue IgG in a single sample cannot confirm a diagnosis of acute dengue infection, for which reason the detection of dengue IgM is preferred over dengue IgG in the present study.

For JE diagnostics, detection of JE IgM via ELISA was preferred to detection of JE viral genome via real-time PCR due to the nature of JE infection in humans where viraemia occurs for a short duration and is typically over by the time encephalitis has developed.[349] Following the primary JE virus infection, JE antibody titres increase rapidly in serum and CSF; by day seven, most patients have raised IgM titres.[330] The IgM levels remain high until day 30 when it begins to decrease, and most patients have raised IgG titres in serum and CSF by this time. JE IgM may increase in serum but not in CSF in individuals with asymptomatic JE infection. Therefore, the detection of JE-specific IgM in CSF is key for establishing the diagnosis of definite JE.

The downside of serological tests for diagnosing dengue and JE infection is their relatively low specificity in differentiating between the two, as the antibodies may cross-react, particularly during secondary infection.[350] The cross-reactivity problem can be partially solved by performing a neutralisation assay such as plaque reduction neutralisation test (PRNT). PRNT allows better differentiation between dengue and JE viruses, and to a further extent, is able to distinguish the neutralising antibodies to the four dengue virus serotypes.[85] PRNT measures the functional antibodies that bind and neutralise the virus, thus regarded as the most specific and gold standard for flaviviral serological diagnostics.[351] Therefore, in the present study, patients who tested positive for dengue

IgM and/or tested positive or equivocal for JE IgM were further tested for dengue and JE neutralising antibodies using the PRNT assay.

Similar to dengue and JE diagnostics, the molecular detection of *O. tsutsugamushi* is limited to the short rickettsaemia period.[352] Moreover, previous reports have shown that PCR targeting *O. tsutsugamushi* has a higher sensitivity when performed on eschar samples compared to blood or buffy coat.[353] However, the presence of eschar in scrub typhus patients varies widely.[232, 354, 355] Therefore, antibody techniques are preferred for the diagnosis of scrub typhus. These techniques include Weil-Felix test, immunofluorescence antibody assay (IFA), immunochromatographic test (ICT) and ELISA.[352] Although IFA is the current reference test for scrub typhus, it requires an immunofluorescent microscope which is often unavailable in hospitals. Among the remaining antibody techniques, ELISA offers the highest sensitivity and specificity,[356] for which it is used in the present study.

In terms of *Cryptococcus* detection, the cryptococcal antigen (CrAg) lateral flow assay (LFA) has the highest sensitivity and specificity compared to other assays, including culture, Indian ink microscopy, and latex agglutination assays;[166] thus, it was used in the present study.

5.2.3 Determining which antibody and antigen testing kits to use

Commercially available testing kits were preferred for dengue, JE and scrub typhus IgM ELISA as well as CrAg LFA in the present study as developing in-house assays would take more time, cost and effort. Moreover, it will be easier to obtain the commercial kits than reagents for the in-house assays if the antibody tests are to be applied for routine investigations at the study site.

The Panbio Dengue IgM Capture ELISA (Alere [now Abbot], Massachusetts, USA) and the Inbios DENV Detect IgM Capture ELISA (Inbios International Inc., Seattle, USA) are two commercially available dengue IgM ELISA kits which have been evaluated by previous studies. Both kits showed a relatively comparable sensitivity and specificity – the sensitivity and specificity for the Panbio Dengue IgM Capture ELISA range from 83.2-96.8% and 87.8-99.4%,[346, 357-360] whereas for the Inbios DENV Detect IgM Capture ELISA it ranges from 88.7-92% and 93.1-94%,[361, 362] respectively. The Inbios dengue IgM ELISA kit has been approved by the Food and Drug Administration (FDA. However, it has to be imported from the USA, which is not practical for my study if the expected end-point is to apply the test as part of routine investigations in the future. The Panbio dengue IgM ELISA kit is available in both the UK and Indonesia and therefore was the preferred kit to be used in the present study.

For the JE IgM ELISA test, three commercially available kits have been used in previous studies.[363-366] These included the Panbio JE-Dengue IgM combo ELISA (Panbio, Brisbane, Australia), the JEV CheX (Xyton Diagnostics Ltd., Bangalore, India), and the Inbios JE Detect IgM Antibody Capture ELISA (Inbios International Inc., Seattle, USA). Previous reports have shown that both the Panbio JE-Dengue IgM combo ELISA and the Inbios JE Detect IgM Antibody Capture ELISA have a better sensitivity and specificity compared to the JEV CheX.[366, 367] However, when I started testing my laboratory work, the Panbio JE-Dengue IgM combo ELISA kit was no longer produced, making the Inbios JE Detect IgM Antibody Capture ELISA kit my preference.

$PRNT_{90}$ was used as the gold standard for dengue and JE antibody tests. $PRNT_{90}$ is defined as a PRNT in which samples repressing 90% of the challenge virus are considered to have positive neutralising antibodies against the challenge virus. The lowest sample dilution used was 1:10; thus, the lowest limit of detection was a $PRNT_{90}$ titre of 10 and a $PRNT_{90}$

titre of <10 was defined to have negative neutralising antibodies. Samples that tested positive for DENV neutralising antibody and negative for JEV neutralising antibody were classified as DENV seropositive and vice versa. As dengue and JE antibodies could cross-react, when samples tested positive for both DENV and JEV neutralising antibodies, the $PRNT_{90}$ titre for DENV should be ≥4-fold greater than the titre for JEV to be classified as DENV seropositive and vice versa. If the $PRNT_{90}$ titre for DENV was <4-fold greater than the titre for JEV or vice versa (i.e. equivocal DENV and JEV PRNT result), the samples were classified as indeterminate. The PRNT assay was performed by colleagues at the Eijkman Institute for Molecular Biology, one of the reference laboratories in Indonesia renowned for its experience in performing confirmatory diagnostic testing.

The Scrub Typhus Detect IgM ELISA (Inbios International Inc., Seattle, USA) is the only commercially available kit for detecting scrub typhus IgM. This kit has been extensively used in previous studies;[368-374] thus I decided to use it in my study. For the detection of cryptococcal antigen, the CrAg® LFA (Immuno-Mycologics [IMMY], Norman, USA) was used as it is a rapid, FDA-approved test and has consistently shown a high sensitivity and specificity across studies.[166, 375-378]

5.2.4 Procedures of antibody and antigen testing

5.2.4.1 General laboratory practice for performing antibody testing

When conducting antibody testing, several precautions were taken to ensure good laboratory practice. All samples and reagents were stored at appropriate temperatures until use to avoid degradation. Multiple aliquots were made and labelled appropriately when samples were thawed for the first time. This helped to avoid repeated freeze-thaw cycles which could affect the test result. Filter tips were always used in making aliquots,

and surfaces and pipettes were sprayed with 70% ethanol prior to working. All reagents and samples were mixed thoroughly by vortexing or pipetting where appropriate.

5.2.4.2 ELISA for detection of dengue IgM

Panbio Dengue IgM Capture ELISA kit (Alere [now Abbot], Massachusetts, USA) was used for the qualitative detection of dengue IgM antibodies in serum and CSF. The dengue ELISA was performed according to the manufacturer's instructions. Patient serum or CSF was diluted 1:100 in sample diluent, and 100 µL of this was used in the assay. Three calibrators, a positive and a negative control were added to the anti-human IgM-coated microwells and incubated at 37 °C for 1 hour. For 1:2 dilution CSF testing, 100 µL of 1:2 diluted patient CSF, 1x positive, 1x negative control and 3x calibrator were added to the anti-human IgM-coated microwells and incubated as described above. The lyophilised dengue antigen (DENV 1-4) was reconstituted in buffer (as described in the manufacturer's manual) and mixed well with an equal volume of HRP-conjugated monoclonal antibody (mAb) tracer to make antigen-mAb complex. The antigen-mAb complex was incubated at room temperature for 1 hour alongside the samples' incubation. Following the incubation, the assay plate was washed 6 times with 1x washing buffer manually to remove unbound serum or CSF. 100 µL of the antigen-mAb complex was added into each well and incubated at 37 °C for 1 hour to detect the captured dengue-specific IgM. The assay plate was washed 6 times and 100 µL of TMB substrate was added to each well. Following this, the assay plate was incubated at room temperature for 15 minutes, and then 100 µL of stop solution was added. The sample absorbance was read at 450 nm using a microtitre plate reader with a reference filter (655 nm) within 30 minutes. The absorbance reading (i.e. optical density / OD) was then related to the

absence or presence of anti-dengue IgM antibodies in the patient sample. Interpretation of the OD results will be further described in chapter 5.

Initially, dengue IgM ELISA was performed on the patient's acute serum. If samples tested positive, the result was further confirmed using plaque reduction neutralisation tests (PRNT). Due to high cross-reactivity between antibodies against dengue and JE viruses, sera with detectable dengue IgM were further tested for both dengue (all serotypes) and JE PRNT. In addition to PRNT, a further confirmatory test was performed by conducting dengue IgM ELISA on CSF patients with detectable dengue IgM in serum.

5.2.4.3 ELISA for detection of Japanese encephalitis IgM

Qualitative detection of IgM antibodies against the JE virus in serum was carried out using JE Detect IgM Antibody Capture ELISA kit (Inbios International Inc., Seattle, USA). The JE IgM ELISA was conducted following the manufacturer's instructions. Briefly, 50 µL of diluted patient serum or CSF, two positive and two negative controls (1:100 when testing serum and 1:2 when testing CSF) were added to the anti-human IgM-coated microwells and incubated at 37 °C for 1 hour. The assay plate was then manually washed 6 times with 1x washing buffer to remove unbound serum. Following this, 50 µL of JE antigen (JERA) for IgM was added into rows A-D of the assay plate, and 50 µL normal cell antigen (NCA) for IgM was added into rows E-H, then incubated at 37 °C for 1 hour. The assay plate was washed 6 times and 50 µL of enzyme-HRP conjugate was added to each well. The assay plate was incubated at 37 °C for 1 hour and then washed 6 times. Following this, 150 µL of EnWash buffer was added into all wells, incubated at room temperature for 5 minutes, and then washed 6 times. After the final wash, 75 µL of TMB substrate was added to each well, and the assay plate was incubated in a dark place at room temperature for 10 minutes. 50 µL of stop solution was added, and the assay plate was further incubated at

room temperature for 1 minute. Following the final incubation, the sample absorbance was read at 450 nm with a microtitre plate reader.

The results were interpreted according to the manufacturer's recommendation, with patients being classified as positive (i.e. having detectable JE IgM) if Immune Status Ratio (ISR) >10, equivocal if ISR ≥6 and ≤10, and negative (i.e. not having detectable JE IgM) if ISR <6. Similar to dengue ELISA, positive results will be further confirmed using PRNT for both dengue and JE.

5.2.4.4 PRNT for detection of dengue and Japanese encephalitis IgM

The $PRNT_{90}$ (PRNT with neutralisation defined as ≥90% reduction in challenge virus PFUs) assays were conducted by Dr Khin Shaw Aye Myint's team in Eijkman Institute for Molecular Biology, Jakarta, Indonesia. The protocol used is described below.

BHK-21 cells were seeded at 2.5 x 10^5 cells/well in 12-well tissue culture plates (Corning, NY, USA) and incubated for 2 days in a 37 °C incubator with 5% CO_2. In each batch of samples tested, anti-DENV-1–4 and anti-JE virus–positive, as well as anti-DENV-1–4 and anti-JE virus–negative serum samples were included as controls. Medium only was added to control wells. Serum diluent for serum samples, controls, and challenge viruses consisted of RPMI medium supplemented with 2% FBS, 2 mM L-glutamine, and 1x antimicrobial-antimycotic drug solution. Serum samples were initially diluted 1:5 in the first wells of 96-well plates, followed by six 2-fold serial dilutions. Suspensions of DENV-1, DENV-2, DENV-3, DENV-4 and JE virus adjusted to 60 PFUs/60 µL were prepared and mixed 1:1 with the diluted serum samples and incubated for 1 hour at 37°C to enable neutralisation to occur. Additionally, virus-only controls and two 10-fold serially diluted (1:10 and 1:100) virus-only controls were included in determining the $PRNT_{90}$ cut-off.

Following the 1-hour neutralisation step, the culture medium was aspirated from wells of 12-well plates containing BHK-21 cell monolayers. Without delay, the serum-virus suspensions were then inoculated onto BHK-21 cell monolayers in 12-well culture plates. The plates were incubated for 1 hour at 37 °C with agitation every 20 minutes to enable non-neutralised viruses to infect BHK-21 cell monolayers. Inoculums were aspirated. Each well was overlaid with 1 mL of 1% carboxymethylcellulose (CMC) RPMI medium supplemented with 2% FBS 1 x antimicrobial-antimycotic drug solution 0.4% $NaHCO_3$, 2.5 mM HEPES, and 0.5% dimethyl sulfoxide. The plates were incubated at 37°C with 5% CO_2 for 5 days, followed by the CMC overlay medium removal. The cell monolayers were fixed with 3.7% formaldehyde solution for 30 minutes. The plates were then washed with tap water and stained with 1% crystal violet staining solution for 5 minutes. The plates were finally rinsed with tap water and air-dried.

The presence of DENV and JE virus-infected cells was indicated by the formation of viral plaques marked by a clear area of detached cells. The neutralisation titre ($PRNT_{90}$ titre) of the serum samples was defined as the reciprocal of the highest test serum dilution for which the virus infectivity was reduced by 90% compared to the average plaque count of the challenge virus controls. Plaques for all 6 serial dilutions of the serum samples were counted to ensure a dose-response reduction. The dilution factor of 2 was included in the final calculation of the neutralisation titres (i.e. 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320). Therefore, the lower limit of quantitation of the assay is a $PRNT_{90}$ titre of 10 (reciprocal of the dilution). Serum samples that neutralised any DENV serotype only or had a DENV $PRNT_{90}$ titre ≥4-fold greater than the JE virus $PRNT_{90}$ titre were considered confirmed DENV seropositive, and vice versa. Serum samples that neutralised any DENV and JE viruses and had a JE virus $PRNT_{90}$ titre <4-fold greater than the $PRNT_{90}$ titre of any DENV were considered flavivirus seropositive.

5.2.4.5 ELISA for detection of scrub typhus IgM

Scrub Typhus Detect™ IgM ELISA kit (Inbios International Inc., Seattle, USA) was used for the qualitative detection of IgM antibodies to *Orientia tsutsugamushi* in serum. The scrub typhus ELISA was performed according to the manufacturer's manual. One-hundred microlitre (100 µL) of diluted patient serum, two positive and two negative controls (1:100) were added to the anti-human IgM-coated microwells and incubated at 37 °C for 30 minutes. After the incubation, the assay plate was manually washed 6 times with 1x washing buffer. One-hundred microlitre (100 µL) of enzyme-HRP conjugate for scrub typhus IgM was added to each well, and the assay plate was further incubated at 37 °C for 30 minutes. The plate was washed 6 times and 150 µL of EnWash buffer was added per well, followed by incubation at room temperature for 5 minutes. The plate was then washed again, and 100 µL of TMB substrate was added to each well. A further incubation was performed at room temperature in a dark place for 10 minutes, and then 50 µL of stop solution was added per well. After 1 minute of incubation at room temperature, the sample OD was read at 450 nm with a microtitre plate reader.

The manufacturer does not provide a fixed cut-off value as it varies depending on the disease prevalence and recommends determining the cut-off value using geographically relevant specimens. Only one previous study examined scrub typhus IgM among Indonesian patients as part of a multi-country study. However, this study used a different ELISA kit from the one I used in this study.[342] Furthermore, there were no confirmed cases/samples of scrub typhus positive in Yogyakarta which I could use to establish the cut-off. Therefore, I used an alternative approach in determining this assay's cut-off value, which will be further described below in the result section.

5.2.4.6 Lateral flow assay for detection of *Cryptococcus* antigen

The detection of *Cryptococcus* antigen in CSF was first introduced in Dr Sardjito Hospital in late March 2016 using the Indian ink staining method. Therefore, patients admitted to the hospital prior to this did not have their CSF tested for Indian ink stains. Following this introduction, Indian ink stain was routinely performed on adult CSF samples regardless patient's human immunodeficiency virus (HIV) status unless limited CSF volume was available. Unfortunately, this practice was only applied in adults and not in paediatric CSF samples.

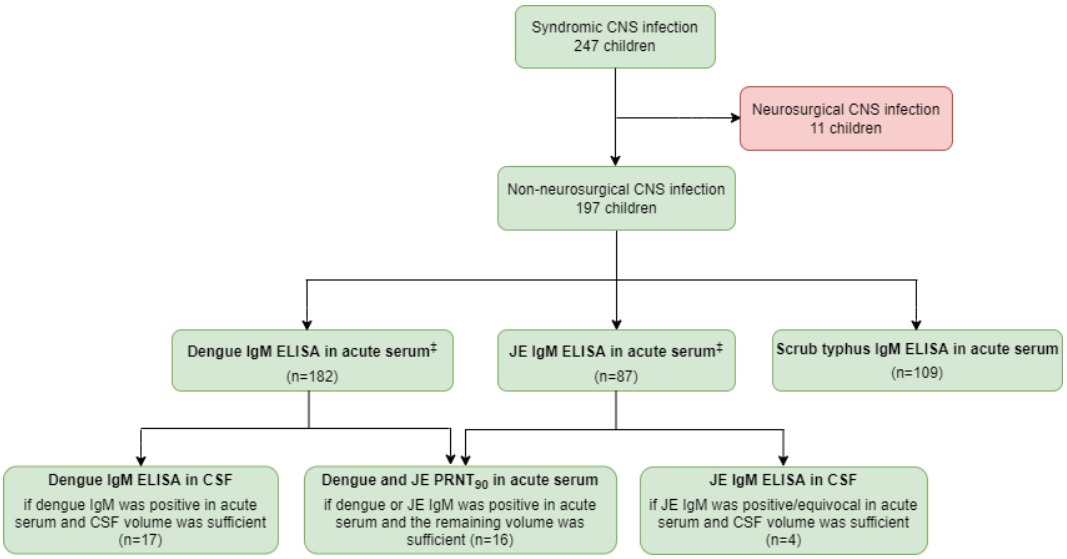
In order to confirm the Indian ink stain results and to retrospectively test the CSF samples obtained before March 2016, samples with positive Indian ink results and those obtained from HIV-positive patients were further tested for *Cryptococcu*s antigen using a Cryptococcal Antigen® Lateral Flow Assay (CrAg® LFA) [Immuno-Mycologics (IMMY), Norman, USA). The assay was performed according to the manufacturer's instructions.

The sample pad of the CrAg LFA strip was submerged into a mixture of 40 µL of CSF and 40 µL of specimen diluent, allowing the specimen to flow through the strip, and then the result was read after 10 minutes. The presence of both test and control lines, regardless of the intensity of the test line, indicated a positive result; whilst the presence of the control line only indicated a negative result. The absence of the control line indicated an invalid result where the test should be repeated.

5.2.5 Determining which clinical samples to test for using antibody and antigen techniques

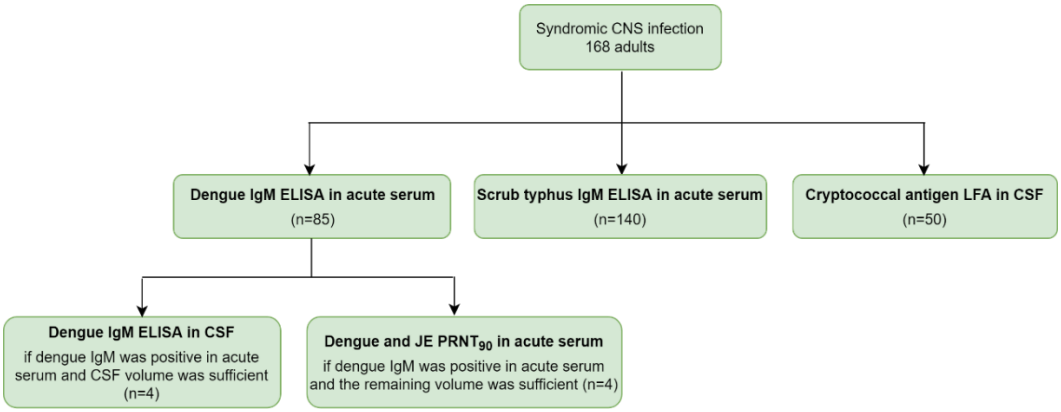
Similar to the sample investigations in the previous chapter, the investigations in this chapter were only performed on samples obtained from children (figure 5.1) and adults (figure 5.2) with syndromic CNS infection.

Figure 5.13 Antibody-mediated testing algorithm in children



‡Dengue and JE IgM ELISA tests were only performed in children with non-neurosurgical CNS infection who underwent LP and had both CSF and acute serum samples available for study purposes. The dengue IgM ELISA test was performed on 182 paediatric acute serum samples. The JE IgM ELISA test was only performed on 87 paediatric acute serum samples due to reagents and time limitations. Children whose acute serum tested positive for dengue IgM and had sufficient CSF volume were further tested on their CSF samples. Similarly, children whose acute serum tested positive or equivocal for JE IgM were further tested on their CSF samples. Furthermore, dengue and JE $PRNT_{90}$ were performed on acute serum samples from children whose acute serum tested positive for dengue IgM or positive/equivocal for JE IgM.
Scrub typhus IgM ELISA test was done in 102 children who underwent LP and 7 children who did not undergo LP but died at the hospital.
CNS – central nervous system; ELISA – enzyme-linked immunosorbent assay; JE – Japanese encephalitis; CSF – cerebrospinal fluid; PRNT – plaque reduction neutralisation test; LP – lumbar puncture.

Figure 5.14 Antibody-mediated testing algorithm in adults



All of the adults with syndromic CNS infection had non-neurosurgical CNS infection. Dengue IgM ELISA test was done in 85 adults who underwent LP and had both CSF and acute serum samples available for study purposes. Adults whose acute serum tested positive for dengue IgM and had sufficient CSF were further tested on their CSF samples. Additionally, when they had sufficient acute serum, the sample was further tested for dengue and JE-neutralising antibodies using $PRNT_{90}$. Cryptococcal antigen lateral flow assay was only performed in adults with positive HIV status and those with negative HIV status but had positive CSF Indian ink staining results (total n=50). Scrub typhus IgM ELISA test was performed in 106 adults who underwent LP and 34 adults who did not undergo LP but had immunocompromised conditions or died within 2 weeks post-discharge (total n=140).
CNS – central nervous system; ELISA – enzyme-linked immunosorbent assay; CSF – cerebrospinal fluid; JE – Japanese encephalitis; PRNT – plaque reduction neutralisation test; LFA – lateral flow assay; LP – lumbar puncture; HIV – human immunodeficiency virus.

In the present study, none of the target pathogens for antibody/antigen tests are associated with neurosurgical infection. Therefore, samples from patients with neurosurgical infection, including 10 children with definite shunt infection and a child with post-neurosurgical encephalitis, were not included in the investigations (figure 5.1).

5.2.5.1 Clinical samples to test for dengue and Japanese encephalitis IgM

Detection of dengue and JE IgM was initially performed using the ELISA technique with the commercial kits mentioned above. Referring to the case definition described in chapter 3, viral CNS infection is defined as syndromic CNS infection and detection of an appropriate viral pathogen by PCR or detection of viral-specific IgM by antibody test in CSF. Therefore, when antibody techniques are used, detection of JE or dengue IgM in CSF is required to establish a diagnosis of JE or dengue CNS infection. The presence of JE or dengue IgM in CSF indicates the inflammatory response against those viruses in the CNS, thus confirming that the viruses are the definitive aetiology of the CNS infection. Consequently, in the present study, dengue and JE IgM tests were only performed in children and adults who had CSF samples available for study purposes (figures 5.1 and 5.2). The dengue and JE ELISA kits are principally designed for serological tests (i.e. antibody detection in serum samples). As CSF is a more precious specimen than serum (i.e. more difficult to obtain), both dengue and JE IgM ELISA tests were initially performed on acute serum samples. Patients with positive dengue IgM and/or positive or equivocal JE IgM in their acute serum were further investigated for dengue and/or JE IgM on their CSF samples. Additionally, if they had sufficient acute serum, the acute serum was further tested for dengue and JE neutralising antibody using $PRNT_{90}$ (figures 5.1 and 5.2).

Before using the dengue IgM ELISA kits on patient samples from the main study, the kit's performance was initially assessed on serum samples from 10 subjects whose dengue and

JE serological status were known. These samples were obtained from a colleague's cohort. Similarly, the JE IgM ELISA kit's performance was initially assessed on the above samples and four additional serum samples from my primary supervisor's cohort in Nepal. The latter four samples had previously tested positive for JE IgM by antibody capture ELISA (MAC-ELISA) and PRNT. The kit pre-testing aimed to assess whether the threshold/cut-offs provided by the kit's manufacturer could detect the samples with known dengue and JE serological status accurately. Furthermore, as I was new to the ELISA test, the kit pre-testing allowed me to practice before applying it to the clinical samples from the main study.

Clinical samples to test for dengue IgM

The clinical samples that were tested for dengue IgM were as follows:

- Acute serum samples

  The dengue IgM ELISA test was performed on 182 paediatric and 85 adult acute serum samples (figures 5.1 and 5.2). These samples were tested in batches based on priorities 1-4 as follows:

  1. Samples from patients with CSF pleocytosis, regardless of their immune status and outcome at discharge. These were chosen as the first priority as CSF pleocytosis is the main CSF parameter indicating CNS infection.
  2. Samples from patients without CSF pleocytosis but had an immunocompromised condition, regardless of their outcome at discharge. Immunocompromised patients may have CNS infection without CSF pleocytosis as their repressed immune system may fail to develop an inflammatory response within the CNS or the response is delayed. Their repressed immune system also places them at a higher risk for CNS infection.
  3. Samples from patients without CSF pleocytosis who died at the hospital.

4. Samples from patients without CSF pleocytosis who were alive at discharge.

- CSF samples

  Patients with a positive dengue IgM in acute serum who had sufficient CSF volume available were further tested for dengue IgM on their CSF. These included 17 children (figure 5.1) and four adults (figure 5.2).

Clinical samples to test for Japanese encephalitis IgM

JE typically occurs in children rather than adults with a ratio of 7:1 among unvaccinated populations in endemic areas.[81] Therefore, in the present study, the JE IgM ELISA test was initially performed in paediatric samples. The JE IgM ELISA kits were shipped from the United States; thus, reagents were limited in quantity. Taking this into account, in addition to time constraints in performing the laboratory work in Indonesia, the JE IgM ELISA test was performed in 87 paediatric acute serum samples (figure 5.1). These samples were selected based on the same priorities as the samples tested for dengue IgM.

Similarly, when the children had a positive or an equivocal JE IgM in acute serum and had sufficient CSF volume available, the JE IgM ELISA test was further performed on their CSF. This was performed on four children (figure 5.1). Due to the low positivity rates for the JE IgM ELISA results in children, I decided not to test the adult samples.

Clinical samples to test for dengue and JE neutralising antibody

Dengue and JE $PRNT_{90}$ assays were performed on the following samples:

- Sixteen paediatric acute serum samples previously tested positive for dengue IgM or tested positive or equivocal for JE IgM by ELISA in the present study (figure 5.1);
- Four adult acute serum samples previously tested positive for dengue IgM by ELISA in the present study (figure 5.2).

5.2.5.2 Clinical samples to test for scrub typhus IgM

The scrub typhus IgM ELISA kit is designed and intended for serum testing only, which is why the diagnosis of scrub typhus meningitis/encephalitis remains reliant on the detection of scrub typhus IgM in serum. Following the case definition from a previously published paper, presumptive scrub typhus meningitis/encephalitis was defined as meningitis/encephalitis and detection scrub typhus IgM in serum with no other aetiological agents found in CSF.[232] In the present study, neurosurgical infection cases were excluded. Similarly, as with the JE IgM ELISA kit, the scrub typhus IgM ELISA kit was also shipped from the United States; therefore, reagents were limited in quantity. The test was performed on 109 paediatric and 140 adult acute serum samples (figures 5.1 and 5.2). These samples were selected based on the following priorities 1-3:

1. Samples from patients with CSF pleocytosis, regardless of their immune status and outcome at discharge;
2. Samples from patients without CSF pleocytosis but had an immunocompromised condition, regardless of their outcome at discharge;
3. Samples from patients without CSF pleocytosis who died at the hospital (for children and adults) and who died within two weeks following hospital discharge (for adults only).

5.2.5.3 Clinical samples to test for *Cryptococcus* antigen

Cryptococcal antigen lateral flow assay was only performed in adult patients, particularly HIV-positive patients. Additionally, as the standard hospital diagnostic test for *Cryptococcus* detection during the study period was CSF Indian ink staining, patients with positive CSF Indian ink staining results were also tested for *Cryptococcus* antigen,

regardless of their HIV status. There were 50 CSF samples overall that were tested (figure 5.2), including:

- Forty-four samples from HIV-positive adults, three of whom had positive CSF Indian ink staining results;
- Six samples from HIV-negative adults with positive CSF Indian ink staining results.

5.2.6 Statistical analysis

Results were recorded as number per number available (n/N). Some patients had missing data; therefore, the number available (N) did not always represent the total number of patients. Data were analysed using GraphPad Prism v6. Graphs in this chapter were created using draw.io (www.draw.io) and GraphPad Prism v6.

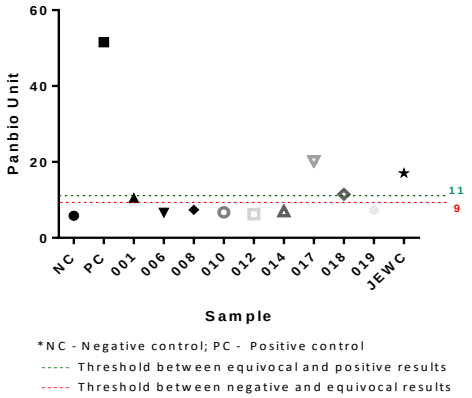
5.3 Results

5.3.1 Results of dengue IgM ELISA

Results of the dengue IgM ELISA kit pre-testing are shown in figure 5.3. Three samples, including samples 017, 018, and JEWC, had detectable dengue IgM, whilst sample 001 had an equivocal result. Sample 017 was obtained from a person who had a history of dengue infection in the past three months and had tested positive for dengue by PRNT. Therefore, the ELISA result is consistent with the history and the PRNT result. Sample 018 was obtained from a person who had never left Europe and thus was unlikely to have previous dengue or JE exposure. Sample JEWC was obtained from a person with a history of Japanese encephalitis in the past month. Therefore, the positive dengue IgM result is more likely due to cross-reactivity between dengue and JE IgM. Sample 001 was obtained from a person with a history of dengue infection in 2002 and was thus less likely to have detectable dengue IgM. In summary, the initial kit testing showed one true positive (sample 017), two false positives (samples JEWC and 018), and one false equivocal

(sample 001). These results suggest that the kit's cut-offs might not apply to every case or setting.

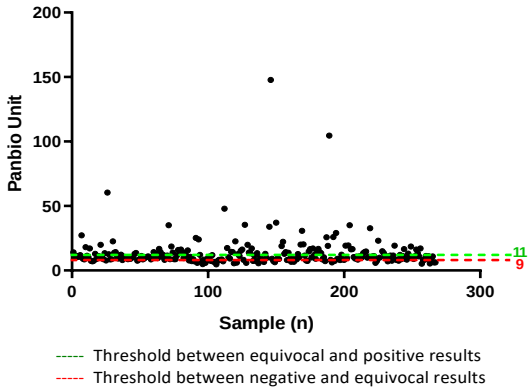
Figure 5.15 Results of the dengue IgM ELISA kit pre-testing on 10 samples from a colleague's cohort



Note: A different symbol is used for each patient sample

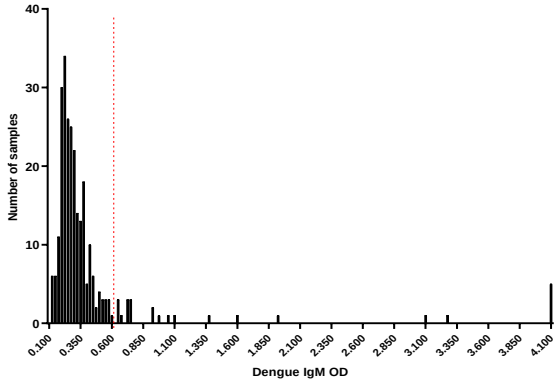
A total of 267 patients' acute serum samples collected in the present study were tested for dengue IgM, including 182 paediatric and 85 adult samples. The median time of serum collection from the onset of illness was 10 days (IQR 5-20 days). The cut-offs mentioned above were applied to these serum samples, and 121 (45%) samples gave a positive result for dengue IgM (figure 5.4). The high number of cases with positive dengue IgM suggests that the threshold provided by the manufacturers of the ELISA kit is likely to be very low. These results were consistent with the previous result (figure 5.3). Therefore, the cut-offs provided by the kit might not be accurate in the local Indonesian population in this study.

Figure 5.16 Dengue IgM level among paediatric and adult patients assessed using the kit's cut-offs



With the above issue, in the absence of confirmed positive and negative dengue cases, I then used an alternative approach to determine a new cut-off. This was done by plotting the optical density (OD) value for all samples in a histogram and defining the cut-off as the first gap between the majority of cases (i.e. where the OD of most samples lied) and the rest of the cases (figure 5.5). This approach assumed that for samples which should be classified as negative, there would be an approximately normal distribution of OD values, and any values above this normal distribution were likely to represent true positives. In the graph below (figure 5.5), the first gap was seen at OD >0.60 (as represented by the dashed line). Therefore, all samples with OD >0.60 are defined as positive for dengue IgM. With this cut-off, 25 patients had detectable dengue IgM, including 21 children and four adults.

Figure 5.17 Histogram representing the distribution of dengue IgM titres



A further ELISA was performed on the CSF samples from 21 patients (17 children and four adults) who tested positive for dengue IgM in serum. The CSF dengue IgM ELISA was not performed in the remaining four patients due to CSF volume limitation (n=1) and time constraints (n=3). Initially, the dengue ELISA test was performed using the sample dilution suggested for serum in the kit's manual (i.e. 1:100) because there was no indication of what dilution to use for CSF. The same cut-off of OD >0.6 was applied to this assay. Dengue IgM was positive in four CSF paediatric samples, including P-058, P-095, P-117, and P-204 (figure 5.6).

Figure 5.18 Dengue IgM level in CSF samples assessed with 1:100 sample dilution

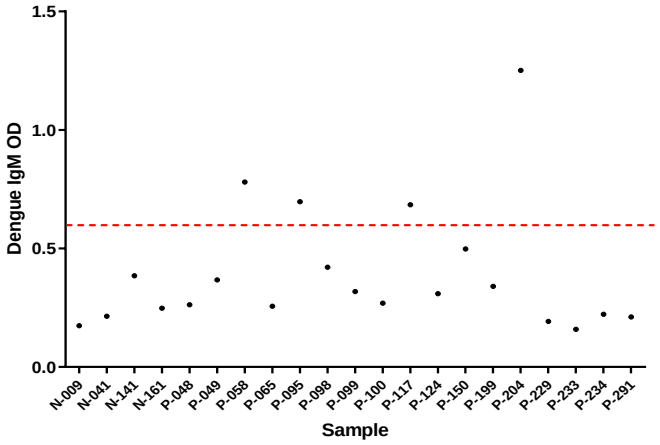
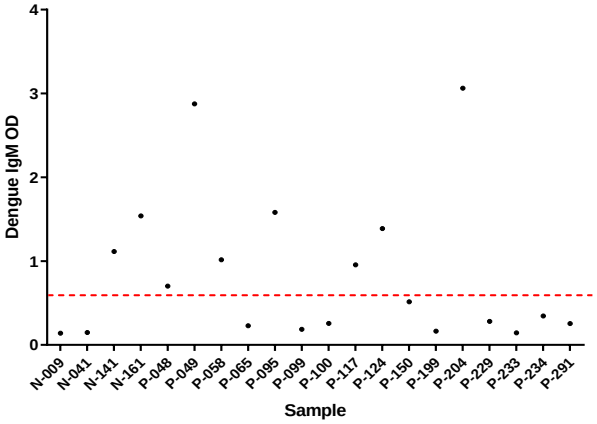


Figure 5.19 Dengue IgM level in CSF samples assessed with 1:2 sample dilution

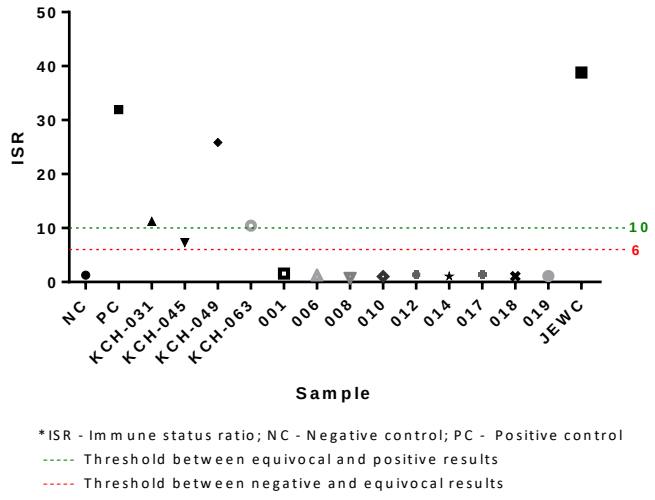


The manufacturer's protocol for the dengue ELISA has been validated for use in serum.[357] No protocol is established by the manufacturer for CSF. Therefore, samples were also tested using a more concentrated 1:2 sample dilution. The latter dilution was based on a previous study using the same kit with CSF.[379] Sample P-098 was not tested further due to its limited volume. Dengue IgM was positive in nine CSF samples from two adults (i.e. subjects N-141 and N-161) and seven children (i.e. subjects P-048, P-049, P-058, P-095, P-117, P-124 and P-204) (figure 5.7). All four paediatric CSF samples that were positive at the 1:100 dilution in the previous assay were also positive in this latter assay.

5.3.2 Results of Japanese encephalitis IgM ELISA

Similar to the dengue IgM ELISA test, the JE IgM ELISA kit's performance was initially assessed on serum samples from 10 subjects mentioned previously. Additionally, serum samples from four other subjects, including subjects KCH-031, KCH-045, KCH-049 and KCH-063, were also used to test the JE IgM ELISA kit's performance.

Figure 5.20 Results of JE IgM ELISA kit pre-testing on 14 samples from other cohorts



Note: A different symbol is used for each patient sample

JE IgM was detected in four samples, including samples KCH-031, KCH-049, KCH-063, and JEWC (figure 5.8); all of these were obtained from subjects with a recent history of JE. One

subject with a recent history of JE, KCH-045, had an equivocal (borderline) result. One subject who had a recent JE vaccination, subject 014, did not have a detectable JE IgM. No information was available on what day after vaccination the sample was taken; therefore, the negative result might be because blood was collected from this individual before or after JE IgM developed in the blood. Alternatively, this individual may have failed to seroconvert following vaccination. The results from the initial assessment suggest that the kit's cut-offs give appropriate specificity and thus will be applied for the remaining JE ELISA test.

JE IgM ELISA test was then performed on 87 paediatric acute serum samples. The serum samples were collected in the median of 8 days (IQR 4-18 days) from the onset of illness. Two acute serum samples, including samples P-098 and P-204, had a positive JE IgM; whilst two other samples, including samples P-008 and P-095, had an equivocal result (figure 5.9).

Figure 5.21 JE IgM titres in paediatric acute serum samples

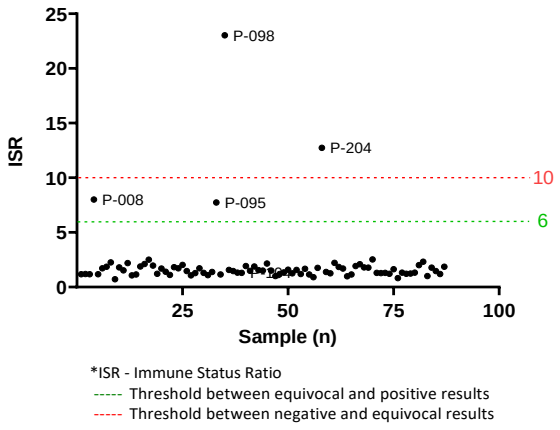
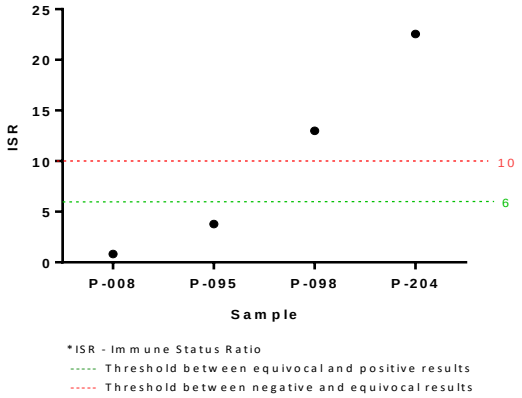


Figure 5.22 JE IgM titres in paediatric CSF samples



A further JE IgM ELISA test was performed on CSF samples from the four subjects whose acute serum tested positive or equivocal for JE IgM. JE IgM was detected in CSF samples from subjects P-098 and P-204 (figure 5.10).

5.3.3 Comparison of dengue and Japanese encephalitis ELISA and $PRNT_{90}$ results

5.3.3.1 Dengue and Japanese encephalitis IgM ELISA and $PRNT_{90}$ results in children

Twenty-two paediatric sera were categorised as positive (or equivocal) by dengue (n=21) or JE (n=4) IgM ELISA testing. Among these samples, 16 samples had sufficient sera to undergo PRNT. Using the PRNT assay as the gold standard for serological diagnosis, seven paediatric samples were categorised as DENV positive, four as indeterminate, and five as negative (table 5.1). Therefore, the dengue IgM ELISA had a 44% (7/16) agreement for correctly detecting DENV. Among the four cases categorised as JE IgM ELISA positive (n=2) or equivocal (n=2), three cases had sera available for PRNT. Among these, two cases were classified as DENV positive and one as negative by PRNT. No cases were identified as JEV positive by PRNT. The JE ELISA had a 0% (0/3) agreement with PRNT.

Table 5.38 Summary of dengue and JE antibody-mediated testing in children

Subject ID	Dengue investigation results at hospital	Dengue IgM ELISA result in this study*		JE IgM ELISA result in this study		PRNT$_{90}$ titre in acute serum					PRNT$_{90}$ result resume
		Acute serum	CSF (1:2 dilution)	Acute serum	CSF	DENV-1	DENV-2	DENV-3	DENV-4	JE virus	
P-008	nd	-	n/a	+/-	-	nd	nd	nd	nd	nd	n/a
P-012	nd	+	n/a	-	n/a	nd	nd	nd	nd	nd	n/a
P-048	nd	+	+	-	n/a	nd	nd	nd	nd	nd	n/a
P-049	IgM +, IgG +	+	+	-	n/a	10	20	<10	20	<10	Indeterminate
P-058	nd	+	+	-	n/a	>320	80	80	80	<10	DENV-1
P-065	nd	+	-	nd	nd	<10	<10	20	<10	<10	Indeterminate
P-095	IgM +, IgG +	+	+	+/-	-	40	40	40	320	20	DENV-4
P-098	nd	+	n/a	+	+	<10	<10	<10	<10	<10	-
P-099	nd	+	-	-	n/a	nd	nd	nd	nd	nd	n/a
P-100	nd	+	-	-	n/a	<10	<10	<10	20	<10	Indeterminate
P-117	nd	+	+	-	n/a	<10	160	<10	<10	<10	DENV-2
P-124	nd	+	+	nd	nd	nd	nd	nd	nd	nd	n/a
P-150	nd	+	-	-	n/a	80	10	<10	<10	<10	DENV-1
P-199	nd	+	-	nd	nd	<10	<10	40	<10	<10	DENV-3
P-204	IgM +, IgG +	+	+	+	+	>320	160	160	<10	40	DENV
P-214	nd	+	n/a	-	n/a	<10	<10	<10	<10	<10	-
P-229	nd	+	-	nd	nd	10	10	<10	<10	<10	Indeterminate
P-233	nd	+	-	nd	nd	<10	<10	<10	<10	<10	-
P-234	nd	+	-	nd	nd	nd	nd	nd	nd	nd	n/a
P-242	IgM -, IgG +	+	n/a	nd	nd	160	<10	<10	<10	<10	DENV-1
P-289	nd	+	n/a	nd	nd	<10	<10	<10	<10	<10	-
P-291	IgM +, NS1 +	+	-	nd	nd	<10	<10	<10	<10	<10	-

*Dengue IgM ELISA result was based on the modified cut-offs.

CSF – cerebrospinal fluid; JE – Japanese encephalitis; ELISA – enzyme-linked immunosorbent assay; PNRT – plaque reduction neutralisation test; DENV – dengue virus; NS1 – non-structural protein 1; n/a – not available; nd – not done; (+) – positive; (-) – negative; **(**+/-) – equivocal; indeterminate – equivocal PRNT result.

Table 5.39 Summary of dengue and JE IgM ELISA and $PRNT_{90}$ results in adults

Subject ID	Age (years)	Dengue investigation results at hospital	Dengue IgM ELISA result in this study*		JE IgM ELISA result in this study		$PRNT_{90}$ titre in acute serum					$PRNT_{90}$ result resume
			Acute serum	CSF (1:2 dilution)	Acute serum	CSF	DENV-1	DENV-2	DENV-3	DENV-4	JE	
N-009	51	nd	+	-	nd	nd	20	20	40	20	10	Indeterminate
N-041	31	nd	+	-	nd	nd	<10	<10	<10	<10	<10	-
N-141	58	nd	+	+	nd	nd	<10	<10	>320	20	20	DENV3
N-161	57	nd	+	+	nd	nd	<10	80	80	80	10	DENV

*Dengue IgM ELISA result was based on the modified cut-offs.

CSF – cerebrospinal fluid; JE – Japanese encephalitis; ELISA – enzyme-linked immunosorbent assay; PNRT – plaque reduction neutralisation test; DENV – dengue virus; NS1 – non-structural protein 1; N/A – not applicable; nd – not done; (+) – positive; (-) – negative; **(**+/-) – equivocal; indeterminate – equivocal PRNT result.

Examining sera tested by PRNT and the hospital rapid dengue IgM test, the rapid test was positive for five samples. Among these samples, two were positive for DENV, one was indeterminate, and two were negative when tested by PRNT (table 5.1). Therefore, taking PRNT as the gold standard, the rapid dengue test had a 40% (2/5) agreement for correctly detecting DENV. Four of the seven paediatric cases categorised as DENV positive by PRNT in sera had a positive dengue IgM in CSF by ELISA testing.

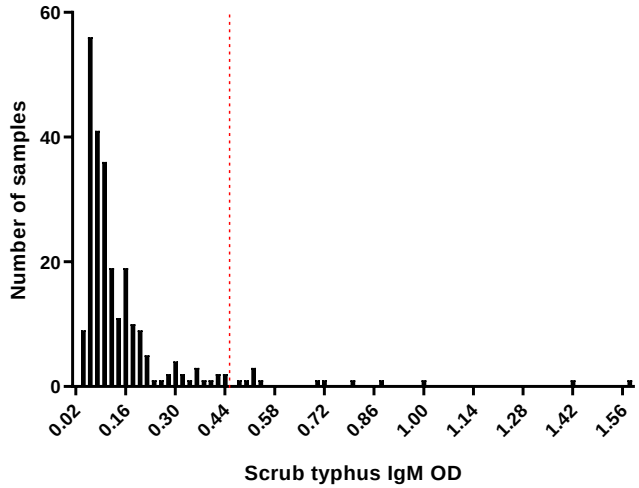
5.3.3.2 Dengue and Japanese encephalitis IgM ELISA and $PRNT_{90}$ results in adults

The dengue IgM ELISA categorised four adult sera as DENV positive. All had sufficient sera to undergo PRNT. When using the PRNT assay as the gold standard, two of these sera were categorised as DENV positive, one as indeterminate, and one as negative. Therefore, the dengue IgM ELISA exhibited a specificity of 50% (2/4). Both patients, confirmed to have DENV via PRNT, also exhibited a positive dengue IgM ELISA result from CSF. None of the patients identified as DENV positive by either ELISA or PRNT had been tested in the hospital using the rapid test.

5.3.4 Results of scrub typhus IgM ELISA

Scrub typhus IgM ELISA was performed in 249 acute sera, including 140 adult and 109 paediatric patients. The median time of serum collection from the onset of illness was 15 days (IQR 7-24 days). The cut-off was determined using the same method as with the dengue IgM ELISA. As depicted in figure 4.10, it lies at OD >0.44. Therefore, all samples with OD >0.44 are defined to have positive scrub typhus IgM. Scrub typhus IgM was positive in 14/250 (5.6%) patients, including 6/140 (4.2%) adults and 8/110 (7.3%) children.

Figure 5.23 Histogram representing the distribution of scrub typhus IgM titres



5.3.5 Results of Cryptococcal antigen lateral flow assay

Cryptococcal antigen lateral flow assay (CrAg LFA) was performed on 50 adults with suspected CNS infection. Forty-four cases were HIV-positive (by hospital testing), four of whom exhibited positive Indian ink staining. An additional six cases were HIV-negative but had a positive Indian ink result via hospital testing. One case was *Cryptococcus neoformans*-positive in CSF by hospital culture. CrAg was positive in three cases (N-033, N-138, and N-143), including the culture-positive case (N-143). All three CrAg-positive cases were HIV-positive. Twenty-nine suspected CNS infection cases (including 23 HIV-positive) undertook Indian ink staining via hospital testing. Nine were positive. Two of these cases were CrAg positive (N-138 and N-143). Two other patients who tested Indian ink positive in CSF also had positive CSF cultures for *Pseudomonas* species and *Brevundimonas vesicularis*.

5.3.6 Clinical and laboratory features of patients with positive antibody and antigen testing results

5.3.6.1 Children with positive antibody testing results

Seven children had DENV positive in sera (confirmed by PRNT). Among these, four had a positive dengue IgM in CSF by ELISA testing. Eight children tested positive for scrub typhus in sera. CrAg testing was not performed in children.

Among the seven DENV cases, five had CSF pleocytosis and three had another pathogen identified in CSF by PCR or hospital culture (table 5.3). All four DENV cases with positive dengue IgM in CSF exhibited a CSF pleocytosis. Two of them had other pathogens identified in CSF/serum, including subject P-058 who tested *M. tuberculosis* positive in CSF and scrub typhus positive in serum, and subject P-204 who had CSF culture positive for *Acinetobacter lwoffii* and *Staphylococcus saprophyticus*. Additionally, subjects P-095 and P-204 had positive dengue IgM and IgG detected in serum by rapid test at the hospital, but the test was not performed in CSF. Thus, the CNS infection aetiology was not established by the hospital testing. In terms of treatment, all four DENV cases with positive dengue IgM in CSF received antibiotics for five or more days.

Among the eight paediatric scrub typhus cases, all had CSF pleocytosis. Five had another pathogen identified in CSF by PCR or hospital culture. These included subjects P-058, as described above, P-154 who tested *S. agalactiae* and CMV positive by PCR, P-322 who tested *E. coli* positive by PCR, P-347 who tested *M. tuberculosis* positive by PCR, and P-325 who had positive CSF culture for *Staphylococcus hominis.*

Table 5.40 Clinical and laboratory features of children with positive antibody-mediated testing results

Subject ID	Age (year)	Sex	Pathogens identified by antibody-mediated testing[¥]	Pathogens identified by PCR	CSF leucocyte count (cells/μL)	CSF lympho -cytes (%)	CSF glucose (mg/dL)	CSF/ blood glucose ratio	CSF protein (mg/dL)	CSF Gram staining results	CSF culture results	HIV status	Treatment received by patients	Outcome at discharge
P-058	15	M	DENV*, *O. tsutsugamushi*	*M. tuberculosis*	130	64	31	0.3	300	ND	ND	unknown	Anti-TB drugs, cefotaxime, ceftazidime, ampicillin, meropenem	died
P-095	8	M	DENV*	ND	26	13	57	0.6	90	ND	ND	unknown	Cefotaxime, ceftazidime, meropenem, amikacin, fluconazole	alive
P-117	5	F	DENV*	ND	261	70	64	0.6	500	ND	ND	unknown	Cefotaxime, ceftriaxone, meropenem, imipenem, gentamycin, fluconazole	alive
P-150	9	F	DENV	*S. pneumoniae*	337	28	17	0.1	160	ND	ND	unknown	Anti-TB drugs, cefotaxime, ceftriaxone, meropenem	died
P-199	0.8	F	DENV	ND	0	0	72	0.8	20	x	ND	unknown	Cefotaxime, gentamycin	alive
P-204	11	M	DENV*	ND	90	92	63	0.6	150	Gram-positive cocci	*Acinetobacter lwoffii, Staphylococcus saprophyticus*	unknown	Cefotaxime, cefftriaxone	alive
P-242	2	F	DENV	x	0	0	74	0.7	20	ND	ND	unknown	Cefotaxime	alive
P-012	15	F	*O. tsutsugamushi*	ND	575	45	37	0.2	1830	ND	x	unknown	Ceftriaxone, anti-TB drugs	alive
P-132	3	M	*O. tsutsugamushi*	ND	10	92	131	0.6	90	ND	ND	unknown	Cefotaxime, ampicillin, meropenem	alive
P-154	0.2	F	*O. tsutsugamushi*	*S. agalactiae*, CMV	8	34	17	0.2	160	ND	ND	unknown	Cefotaxime, ampicillin	alive
P-297	3	M	*O. tsutsugamushi*	ND	150	31	140	1.8	50	ND	ND	unknown	Cefotaxime	alive
P-322	0.3	M	*O. tsutsugamushi*	*E. coli*	6400	10	11	nd	3730	ND	ND	unknown	Cefotaxime, metronidazole, meropenem, cefixime	alive
P-325	4	M	*O. tsutsugamushi*	ND	6	94	70	0.9	200	ND	*Staphylococcus hominis*	unknown	Cefotaxime, azithromycin, gentamycin, acyclovir	alive
P-347	16	F	*O. tsutsugamushi*	*M. tuberculosis*	144	93	17	0.2	200	ND	ND	unknown	Anti-TB drugs[‡], ceftriaxone, ciprofloxacin	died

[¥]Antibody-mediated testing results in acute serum and/or CSF for dengue and Japanese encephalitis virus identification and in acute serum for *O. tsutsugamushi* identification. *DENV positive in both acute serum and CSF samples. [‡]Anti-TB drugs were prescribed by the clinician, but the patient died before receiving them.

PCR – polymerase chain reaction; CSF – cerebrospinal fluid; HIV – human immunodeficiency virus; DENV – dengue virus; TB – tuberculosis; *O. tsutsugamushi – Orientia tsutsugamushi*; *M. tuberculosis – Mycobacterium tuberculosis; S. pneumoniae – Streptococcus pneumoniae; S. agalactiae – Streptococcus agalactiae; E. coli – Escherichia coli;* CMV – cytomegalovirus; F – female; M – male; x – not done; ND – no pathogen detected.

Subject P-325 had a non-neurosurgical CNS infection and *Staphylococcus hominis* is unlikely to cause it; thus, the pathogen was regarded as a contaminant (chapter 3, table 3.8). The remaining three paediatric scrub typhus cases, including subjects P-012, P-132, and P-297, did not have any other pathogen detected in their CSF. Based on the case definition described in chapter 3, the last four cases were defined to have presumptive scrub typhus CNS infection. None of them had a reported presence of eschars, a characteristic skin lesion of scrub typhus disease. Subject P-012 received anti-TB drugs, including rifampicin, an antibiotic that is effective for treating scrub typhus. Subject P-325 received azithromycin, which is also recommended for scrub typhus treatment. Both subjects were discharged alive after 24 and 19 days of hospitalisation, respectively. Both subjects P-132 and P-297 did not receive any specific antibiotics for scrub typhus (i.e. doxycycline, azithromycin, quinolones, chloramphenicol and rifampicin) and were discharged alive after 8 days and 15 days of hospitalisation.

Taking these results into account, the antibody-mediated testing improves the detection of dengue CNS infection from zero to four cases (i.e. patients P-058, P-095, P-117, and P-204) and presumptive scrub typhus from zero to four cases (i.e. patients P-012, P-132, P-297, and P-325) (table 5.3).

5.3.6.2 Adults with positive antibody and antigen testing results

Two adults were DENV-positive in sera (confirmed by PRNT) – both had a positive dengue IgM in CSF by ELISA, six were scrub typhus positive in sera, and three were CrAg positive in CSF. All cases exhibited CSF pleocytosis. Three adults had another pathogen identified in CSF. These included subject N-138 who tested positive for both scrub typhus in serum and CrAg in CSF, subject N-139 who tested positive for both *M. tuberculosis* (by PCR) and

CrAg in CSF, and subject N-033 who tested positive for both *Kocuria varians* and CrAg in CSF (table 5.4).

Both subjects N-141 and N-161 who tested positive for DENV had at least three of the following CSF characteristics of viral CNS infection: CSF pleocytosis, CSF lymphocytic predominance, CSF protein of ≥50 mg/dL, and CSF:blood glucose ratio of ≤0.66 (table 5.2). Both subjects received antibiotics for more than 5 days during their hospital stay.

Subjects N-006, N-032, and N-171 had a positive scrub typhus IgM without any other pathogens detected in their CSF either by the standard hospital diagnostic techniques or by the systematic testing used in my study (table 5.4). Therefore, these three adults were presumed to have scrub typhus CNS infection. None of them had a reported presence of eschar and received specific antibiotic treatment for scrub typhus. Subject N-032 had a fatal outcome, whilst subjects N-006 and N-171 were discharged alive after hospitalised for 21 days and 30 days, respectively.

Two adults who tested positive for scrub typhus in sera (N-138 and N-139) had another pathogen detected in CSF; thus, they were not defined to have presumed scrub typhus meningitis/encephalitis (based on the case definition described in chapter 3). Subject N-185 had a clinical presentation of encephalitis and a positive scrub typhus IgM in serum but did not undergo an LP. Without any CSF analysis, it is unknown whether she had any pathogens in the CSF. Therefore, she potentially had systemic scrub typhus infection with an unconfirmed cause of encephalitis.

Table 5.41 Clinical and laboratory features of adults with positive antibody and antigen testing results

Subject ID	Age (year)	Sex	Pathogens identified by antibody/antigen testing[¥]	Pathogens identified by PCR	CSF opening pressure (cm CSF)	CSF leucocyte count (cells/μL)	CSF lympho-cytes (%)	CSF glucose (mg/dL)	CSF/ blood glucose ratio	CSF protein (mg/dL)	CSF Indian ink staining results	CSF Gram staining results	CSF culture results	HIV status	Treatment received by patients	Outcome at discharge
N-141	58	F	DENV*	ND	x	15	78	127	0.5	50	negative	ND	ND	negative	Ceftriaxone, ceftazidime, levofloxacin	died
N-161	57	M	DENV*	ND	x	50	27	111	0.7	170	negative	ND	ND	unknown	Cefotaxime	alive
N-006	45	M	*O. tsutsugamushi*	ND	x	8	100	102	nd	70	x	x	ND	unknown	Cefotaxime, ceftazidime	alive
N-032	63	M	*O. tsutsugamushi*	ND	x	8	20	78	0.6	10	x	ND	ND	unknown	Ceftriaxone	died
N-138	40	M	*O. tsutsugamushi, C. neoformans*	ND	40	65	83	42	0.3	40	positive	ND	ND	positive	Ceftriaxone	died
N-139	21	M	*O. tsutsugamushi*	*M. tuberculosis*	30	178	71	55	0.5	70	negative	ND	ND	negative	Ceftriaxone, vancomycin	died
N-171	40	M	*O. tsutsugamushi*	ND	30	8	60	91	0.4	10	negative	ND	ND	negative	Cefotaxime, ceftazidime, gentamycin, meropenem	alive
N-185	29	F	*O. tsutsugamushi*	x	x	x	x	x	x	x	x	x	x	negative	Ceftriaxone, ceftazidime, levofloxacin, anti-TB drugs	died
N-033	26	M	*C. neoformans*	ND	17	8	85	51	0.4	30	x	ND	*Kocuria varians*	positive	Ceftazidime, acyclovir, pyrimethamine, ARV	died
N-143	37	M	*C. neoformans*	ND	>40	32	100	31	0.3	50	positive	ND	*C. neoformans*	positive	Ceftriaxone	died

[¥]Antibody/antigen testing results in CSF for dengue virus and *C. neoformans* identification or in acute serum for *O. tsutsugamushi* identification. *DENV was detected in both acute serum and CSF samples.
PCR – polymerase chain reaction; CSF – cerebrospinal fluid; HIV – human immunodeficiency virus; DENV – dengue virus; *O. tsutsugamushi – Orientia tsutsugamushi; C. neoformans – Cryptococcus neoformans*; *M. tuberculosis – Mycobacterium tuberculosis;* F – female; M – male; x – not done; ND – no pathogen detected.

As one would expect for cryptococcal CNS infection, all three CrAg-positive cases exhibited a lymphocyte predominance in CSF cell count and a low CSF:blood glucose ratio (<0.5). Two also had a raised opening CSF pressure (i.e. ≥20 cmCSF). In terms of treatment, all three adults with cryptococcal meningitis mentioned above received at least one dose of antibiotics, one of whom received antibiotics for >5 days. None received antifungal treatment during their hospital stay, whereas one of them also received antiviral treatment. All of those three patients died at the hospital.

Taking all of the antibody and antigen testing results into account, the techniques improve the detection of CNS infection aetiology by seven cases in adults. These included cryptococcal CNS infection from one to three cases (i.e. patients N-143, N-033, and N-138); dengue CNS infection from zero to two cases (i.e. patients N-141 and N-161); and presumptive scrub typhus from zero to three cases (i.e. patients N-006, N-032, and N-171) (table 5.4).

5.3.7 Final results of standard hospital testing, PCR, and antibody/antigen testing

5.3.7.1 Final results of standard hospital testing, PCR, and antibody-mediated testing in children

Pathogen-specific PCR and antibody-mediated testing in child samples improved the detection of CNS infection aetiology by 24 cases. Accounting for standard hospital testing in all children with syndromic CNS infection, overall detection of CNS infection aetiology increased from 26/247 (11%) to 50/247 (20%) child cases (9% improvement - table 5.5). These include 45 cases with single infection and five cases with dual infection. The latter cases consist of one case of tuberculous and dengue CNS co-infection, three *E. coli* and CMV co-infection, and one *S. agalactiae* and CMV co-infection.

Table 5.42 Clinical and laboratory features of paediatric patients who had causative pathogen(s) detected by the standard hospital and the advanced diagnostic testing

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results				Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investi-gations		CSF PCR	Antibody/ antigen testing				
Non-neurosurgical CNS infection															
P-003	17	F	14	negative	ND	*S. haemolyticus*	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ceftriaxone	alive	yes
P-018	12	F	500	unknown	ND	*S. haemolyticus*	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, cefotaxime	died	yes
P-026	3	F	9	unknown	ND	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ceftriaxone, metronidazole	alive	yes
P-058	15	M	130	unknown	x	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	DENV*, *O. tsutsugamushi*	Definite TBM, dengue CNS infection	Anti-TB drugs, cefotaxime, ceftazidime, ampicillin, meropenem	died	yes
P-069	12	M	1580	unknown	ND	ND	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	ND	Definite TBM	Cefotaxime, metronidazole, meropenem, amikacin, ciprofloxacin	died	no
P-178	0.3	M	450	unknown	ND	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, cefotaxime, ampicillin, meropenem, azithromycin, clindamycin	died	yes
P-296	15	M	134	unknown	ND	ND	ND	CSF Xpert: *M. tuberculosis* (+)	Definite TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ceftriaxone, meropenem, ciprofloxacin	died	yes
P-347	16	F	144	unknown	ND	ND	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	*O. tsutsugamushi*	Definite TBM	Anti-TB drugs*, ceftriaxone, ciprofloxacin	died	no

Table 5.5 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results				Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investiga-tions		CSF PCR	Antibody/ antigen testing				
P-155	0.3	M	141000	unknown	x	*E. coli*	*Shigella* group	-	*E. coli* CNS infection	*E. coli*, CMV	ND	*E. coli* CNS infection, latent CMV infection	Cefotaxime, ampicillin, meropenem	died	yes
P-185	0.2	M	12640	unknown	Gram-negative bacilli	*E. coli*	*Staphylococcus epidermidis*	-	*E. coli* CNS infection	*E. coli*, CMV	ND	*E. coli* CNS infection, latent CMV infection	Cefotaxime, ampicillin, meropenem, cotrimoxazole	alive	yes
P-216	0.1	F	130	unknown	ND	ND	ND	-	Unknown aetiology	*E. coli*, CMV	ND	*E. coli* CNS infection, latent CMV infection	Cefotaxime, meropenem, amikacin	died	yes
P-071	0.3	M	490	unknown	ND	ND	x	-	Unknown aetiology	*E. coli*	ND	*E. coli* CNS infection	Cefotaxime	alive	yes
P-322	0.3	M	6400	unknown	ND	ND	ND	-	Unknown aetiology	*E. coli*	*O. tsutsugamushi*	*E. coli* CNS infection	Cefotaxime, metronidazole, meropenem, cefixime	alive	yes
P-323	0.2	M	780	unknown	Gram-negative bacilli	*E. coli*	*Enterobacteriaceae*	-	*E. coli* CNS infection	*E. coli*	ND	*E. coli* CNS infection	Cefotaxime, ampicillin, ceftazidime, gentamycin	alive	yes
P-112	6	F	N/A	unknown	x	x	*E. coli*	-	*E. coli* CNS infection	x	x	*E. coli* CNS infection	Cefotaxime, ampicillin	died	yes
P-008	7	M	7200	unknown	ND	ND	ND	-	Unknown aetiology	*S. pneumoniae*	ND	*S. pneumoniae* CNS infection	Cefotaxime, ampicillin, amoxicillin-clavulanic	alive	yes
P-118	2	M	92	unknown	ND	ND	ND	-	Unknown aetiology	*S. pneumoniae*	ND	*S. pneumoniae* CNS infection	Cefotaxime, ampicillin, amikacin, imipenem	alive	yes
P-150	9	F	337	unknown	ND	ND	ND	-	Unknown aetiology	*S. pneumoniae*	DENV	*S. pneumoniae* CNS infection	Anti-TB drugs, cefotaxime, ceftriaxone, meropenem	died	yes
P-154	0.2	F	8	unknown	ND	ND	x	-	Unknown aetiology	*S. agalactiae,* CMV	*O. tsutsugamushi*	*S. agalactiae* CNS infection, latent CMV infection	Cefotaxime, ampicillin	alive	yes

Table 5.5 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results				Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investi-gations		CSF PCR	Antibody/ antigen testing				
P-229	0.1	M	398	unknown	ND	ND	x	-	Unknown aetiology	*S. agalactiae*	ND	*S. agalactiae* CNS infection	Cefotaxime, ampicillin, cefixime	alive	yes
P-270	0.8	M	166	unknown	Gram-negative bacilli	*Salmonella spp.*	*Salmonella paratyphi B*	-	*Salmonella* CNS infection	*Salmonella* spp.	ND	*Salmonella* CNS infection	Cefotaxime, ceftriaxone, ampicillin, meropenem	alive	yes
P-337		M	97	unknown	ND	ND	*Salmonella spp.*	-	*Salmonella* CNS infection	ND	ND	*Salmonella* CNS infection	Cefotaxime, ceftriaxone, cefixime, ciprofloxacin	alive	yes
P-258	0.3	F	325	unknown	ND	*Pasteurella canis*	*Pasteurella canis*	-	Unknown aetiology	*H. influenzae*	ND	*H. influenzae* CNS infection	Cefotaxime, meropenem	alive	yes
P-093	13.1	M	1968	unknown	ND	ND	ND	-	Unknown aetiology	*N. meningitidis*	ND	*N. meningitidis* CNS infection	Cefotaxime	alive	yes
P-016	2	F	35	unknown	ND	*Staphylococcus aureus*	ND	-	*S. aureus* CNS infection	ND	ND	*S. aureus* CNS infection	Ceftriaxone, ceftazidime, cefixime	alive	yes
P-055	0.3	F	110	unknown	ND	*Staphylococcus aureus*	ND	-	*S. aureus* CNS infection	ND	ND	*S. aureus* CNS infection	Cefotaxime	alive	yes
P-064	0.8	M	100	unknown	Gram-negative bacilli	ND	ND	-	Gram-negative bacilli CNS infection	ND	ND	Gram-negative bacilli CNS infection	Cefotaxime, ampicillin, metronidazole, meropenem, chloramphenicol, acyclovir	alive	yes
P-262	0.4	M	19	unknown	Gram-negative bacilli	ND	ND	-	Gram-negative bacilli CNS infection	ND	ND	Gram-negative bacilli CNS infection	Cefotaxime, ampicillin, cefixime	alive	yes
P-237	9	M	345	unknown	ND	*Bacillus anthracis*	*Cupriavidus pauculus*	-	*Bacillus anthracis* CNS infection	X	x	Anthrax CNS infection	Cefotaxime, metronidazole	died	no
P-302	10	M	7	unknown	Chains of cocci	*Enterococcus faecium*	ND	-	*Enterococcus* CNS infection	ND	ND	*Enterococcus* CNS infection	Cefotaxime, ceftriaxone, ceftazidime, metronidazole, meropenem	died	yes

Table 5.5 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results				Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investiga-tions		CSF PCR	Antibody/ antigen testing				
P-345	17	M	38	unknown	ND	Coagulase-positive *Staphylococcus*	ND	-	*Staphylococcus* CNS infection	ND	ND	*Staphylococcus* CNS infection	Ceftriaxone	died	yes
P-149	4	F	N/A	unknown	x	x	*Sphingomonas paucimobilis*	-	*Sphingomonas paucimobilis* CNS infection	x	x	*Sphingomonas paucimobilis* CNS infection	Cefotaxime, ceftriaxone, ampicillin, metronidazole	alive	yes
P-095	8	M	26	unknown	ND	ND	ND	DENV IgM (+), DENV IgG (+) [using rapid test]	Unknown aetiology, systemic dengue infection	ND	DENV*	Dengue CNS infection	Cefotaxime, ceftazidime, meropenem, amikacin, fluconazole	alive	no
P-117	5	F	261	unknown	ND	ND	ND	-	Unknown aetiology	ND	DENV*	Dengue CNS infection	Cefotaxime, ceftriaxone, meropenem, imipenem, gentamycin, fluconazole	alive	no
P-204	11	M	90	unknown	Gram-positive cocci	*Acinetobacter lwoffii, Staphylococcus saprophyticus*		DENV IgM (+), DENV IgG (+) [using rapid test]	Unknown aetiology, systemic dengue infection	ND	DENV*	Dengue CNS infection	Cefotaxime, ceftriaxone	alive	no
P-012	15	F	575	unknown	ND	x	x	-	Unknown aetiology	ND	*O. tsutsugamushi*	Presumptive scrub typhus	Ceftriaxone, anti-TB drugs	alive	yes
P-132	3	M	10	unknown	ND	ND	ND	-	Unknown aetiology	ND	*O. tsutsugamushi*	Presumptive scrub typhus	Cefotaxime, ampicillin, meropenem	alive	no
P-297	3	M	150	unknown	ND	ND	ND	-	Unknown aetiology	ND	*O. tsutsugamushi*	Presumptive scrub typhus	Cefotaxime	alive	no
P-325	4	M	6	unknown	ND	*Staphylococcus hominis*	ND	-	Unknown aetiology	ND	*O. tsutsugamushi*	Presumptive scrub typhus	Cefotaxime, azithromycin, gentamycin, acyclovir	alive	yes

Table 5.5 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results				Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investigations		CSF PCR	Antibody/ antigen testing				
Neurosurgical CNS infection															
P-013	4	F	52	unknown	ND	ND	ND	*Staphylococcus haemolyticus* (pus culture)	Definite shunt infection	ND	x	Definite shunt infection	Cefotaxime, ceftriaxone, ampicillin	alive	yes
P-201	0.4	F	5	unknown	x	*S. haemolyticus*	ND	-	Definite shunt infection	ND	x	Definite shunt infection	Cefotaxime, ceftriaxone, ampicillin, cefixime, cefepime, levofloxacin, ciprofloxacin	alive	yes
P-007	11	M	610	unknown	Gram-negative bacilli	*Enterobacter cloacae*	ND	-	Definite shunt infection	ND	x	Definite shunt infection	Ceftriaxone, metronidazole	alive	yes
P-021	0.3	F	1500	unknown	Gram-negative bacilli	*K. pneumoniae*	ND	-	Definite shunt infection	*K. pneumoniae*	x	Definite shunt infection	Cefotaxime, ampicillin, meropenem	alive	yes
P-039	0.4	F	1750	unknown	ND	*Staphylococcus warneri*	*Streptococcus viridans*	-	Definite shunt infection	ND	x	Definite shunt infection	Cefotaxime, ceftriaxone, imipenem, ciprofloxacin	alive	yes
P-078	0.4	M	170	unknown	ND	*Kocuria kristiane*	x	-	Definite shunt infection	ND	x	Definite shunt infection	Cefotaxime, gentamycin	alive	yes
P-105	0.1	F	818	unknown	x	*Acinetobacter baumannii*	ND	-	Definite shunt infection	ND	x	Definite shunt infection	Cefotaxime, ampicillin, cefepime, cefixime, valganciclovir	alive	yes
P-238	1	M	1	unknown	x	*Pseudomonas aeruginosa*	*Cupriavidus pauculus*	-	Definite shunt infection	X	x	Definite shunt infection	Cefotaxime, ceftazidime, ampicillin, gentamycin, amikacin	alive	yes

Table 5.5 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results				Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF culture	Blood culture	Other investi-gations		CSF PCR	Antibody/ antigen testing				
P-285	2.1	M	55	unknown	Gram-negative cocci	ND	ND	-	Definite shunt infection	ND	x	Definite shunt infection	Cefotaxime, ceftriaxone	died	yes
P-286	0.4	M	69	unknown	Gram-negative bacilli	*Pseudomonas stutzeri*	ND	-	Post-neurosurgical encephalitis	ND	x	Post-neurosurgical encephalitis	Cefotaxime, ceftazidime, cefixime, ciprofloxacin	alive	yes
P-344	0.6	F	42	unknown	ND	*S. epidermidis*	*Candida albicans*	-	Definite shunt infection	X	x	Definite shunt infection	Cefotaxime, ampicillin, azithromycin, chloramphenicol	alive	yes

*DENV positive in both acute serum and CSF samples; ‡Anti-TB drugs were prescribed by the clinician, but the patient died before receiving them.

PCR – polymerase chain reaction; CSF – cerebrospinal fluid; ZN – Ziehl-Neelsen; TB – tuberculosis; TBM – tuberculous meningitis; HIV – human immunodeficiency virus; DENV – dengue virus; CNS – central nervous system; *M. tuberculosis – Mycobacterium tuberculosis; E. coli – Escherichia coli*; CMV – cytomegalovirus*; S. pneumoniae – Streptococcus pneumoniae; S. agalactiae – Streptococcus agalactiae; Salmonella* spp. – *Salmonella* species*; H. influenzae – Haemophilus influenzae; O. tsutsugamushi – Orientia tsutsugamushi; K. pneumoniae – Klebsiella pneumoniae, S. haemolyticus – Staphylococcus haemolyticus; S. epidermidis – Staphylococcus epidermidis*; F – female; M – male; ND – not detected; N/A - not applicable; x – not done.

Forty-two of 50 children whose pathogen was identified by either the standard hospital testing or the advanced diagnostic testing received appropriate antimicrobial treatment. Nevertheless, 11 children remained to have a fatal outcome. These included four children with *M. tuberculosis*, two with *E. coli*, two with dual infection of *E. coli* and CMV, and three with *S. pneumoniae*, *Staphylococcus,* and Gram-negative cocci CNS infection (n=1 each). Eight children did not receive appropriate treatment, including two (P-069 and P-347) with tuberculous CNS infection, two (P-132 and P-297) with presumptive scrub typhus CNS infection, three (P-095, P-117, and P-204) with dengue CNS infection and one (P-237) with anthrax CNS infection (table 5.5). Interestingly, the latter patient had the pathogen detected at the hospital but did not receive the appropriate first-line treatment for anthrax CNS infection (i.e. ciprofloxacin, doxycycline, or penicillin). Additionally, patient P-347 was planned for anti-TB treatment, but she died before receiving the treatment. All children with a final diagnosis of dengue CNS infection received multiple antibiotics. Three of the eight children who did not receive appropriate treatment had a fatal outcome. These included patients P-069 and P-347 with tuberculous CNS infection and patient P-237 with anthrax CNS infection.

Table 5.43 Clinical and laboratory features of adult patients who had causative pathogen(s) detected by the standard hospital and the advanced diagnostic testing

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results					Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations		CSF PCR	Antibody/ antigen testing				
N-013	20	F	4	negative	ND	x	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Ceftazidime, acyclovir	died	no
N-021	25	F	3300	positive	NR	x	x	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	ND	Definite TBM	Ceftazidime	died	no
N-022	46	M	5	positive	ND	x	ND	x	Anti-toxoplasma IgM 0.07 (normal), IgG >300 (markedly increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ARV	alive	yes
N-028	46	M	690	positive	ND	x	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	x	Definite TBM	Ceftazidime	died	no
N-029	37	F	190	negative	ND	x	ND	ND	Anti-toxoplasma IgM 0.05 (normal), IgG 176 (markedly increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ceftriaxone, pyrimethamine	alive	yes
N-033	26	M	8	positive	ND	x	*Kocuria varians*	ND	Anti-toxoplasma IgM 0.25 (normal), IgG >300 (markedly increased)	Unknown aetiology, possible TBM	*M. tuberculosis*	ND	Definite TBM	Ceftazidime, acyclovir, ARV, pyrimethamine	died	no
N-039	21	F	140	negative	ND	x	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Ceftriaxone	died	no
N-049	30	M	78	positive	ND	negative	ND	ND	Anti-toxoplasma IgM 0.05 (normal), IgG 61 (increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, meropenem, levofloxacin, cotrimoxazole, co-amoxiclav	died	yes

Table 5.6 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results					Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations		CSF PCR	Antibody/ antigen testing				
N-054	37	M	427	negative	ND	negative	ND	x	-	Unknown aetiology, possible TBM	*M. tuberculosis*	ND	Definite TBM	Ceftriaxone, acyclovir	died	no
N-061	57	M	1	negative	ND	negative	*S. epidermidis*	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Ceftriaxone	died	no
N-088	41	F	192	negative	ND	negative	*Enterobacter aerogenes*	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	ND	Definite TBM	Ceftazidime	died	no
N-089	45	M	75	negative	diplococci	negative	*S. haemolyticus*	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ceftriaxone, ceftazidime, levofloxacin	died	yes
N-131	35	M	22	negative	ND	negative	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ceftazidime, ciprofloxacin	alive	yes
N-139	21	M	177	negative	ND	negative	ND	ND	Anti-toxoplasma IgM 0.08 (normal), IgG 128 (markedly increased)	Unknown aetiology, possible TBM	*M. tuberculosis*	*O. tsutsuga-mushi*	Definite TBM	Ceftriaxone, vancomycin	died	no
N-149	34	M	322	negative	ND	negative	ND	ND	Anti-toxoplasma IgM 0.03 (normal), IgG 46 (increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ceftazidime	alive	yes
N-150	23	M	17	negative	ND	positive	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Ceftriaxone, gentamycin	died	no

Table 5.6 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results					Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations		CSF PCR	Antibody/ antigen testing				
N-153	42	F	70	negative	ND	negative	ND	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	ND	Definite TBM	Cefotaxime, meropenem	died	no
N-154	34	M	197	positive	ND	negative	ND	ND	Anti-toxoplasma IgM 0.06 (normal), IgG 171 (markedly increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Anti-TB drugs, ceftriaxone, meropenem, cotrimoxazole, pyrimethamine	died	yes
N-168	26	M	493	negative	ND	negative	ND	ND	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Ceftazidime, meropenem	died	no
N-169	23	M	208	negative	ND	negative	ND	-	-	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Meropenem	died	no
N-170	69	F	73	negative	ND	positive	ND	x	-	Unknown aetiology, probable TBM	*M. tuberculosis*	x	Definite TBM	Ceftriaxone, levofloxacin	died	no
N-175	21	F	270	negative	ND	negative	ND	ND	Anti-toxoplasma IgM 0.22 (normal), IgG 258 (markedly increased)	Unknown aetiology, probable TBM	*M. tuberculosis*	ND	Definite TBM	Cefotaxime, ceftriaxone, amikacin, acyclovir	died	no
N-179	25	M	178	negative	x	x	x	ND	-	Unknown aetiology, possible TBM	*M. tuberculosis*	ND	Definite TBM	Ceftazidime, meropenem, vancomycin	died	no
N-142	47	F	1170	negative	ND	negative	ND	ND	-	Unknown aetiology	*S. pneumoniae*	ND	*S. pneumoniae* CNS infection	Ceftriaxone	alive	yes

Table 5.6 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results					Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations		CSF PCR	Antibody /antigen testing				
N-157	58	F	13200	negative	ND	negative	*S. pneumoniae*	ND	-	*S. pneumoniae* CNS infection	*S. pneumoniae*	ND	*S. pneumoniae* CNS infection	Ceftazidime, meropenem	died	yes
N-167	33	M	25	positive	ND	negative	ND	ND	Anti-toxoplasma IgM 0.02 (normal), IgG 44 (increased)	Unknown aetiology	*S. pneumoniae*	ND	*S. pneumoniae* CNS infection	Ceftazidime	alive	yes
N-044	32	M	79	positive	ND	x	ND	ND	-	Unknown aetiology	HSV-2	ND	HSV-2 CNS infection	Ceftazidime, levofloxacin, amikacin, imipenem	alive	no
N-140	34	M	92	positive	ND	negative	ND	x	Anti-HSV-1 IgM 0.7, IgG 4.8 (normal) Anti-HSV-2 IgM 0.69, IgG 4.5 (normal) Anti-toxoplasma IgM 0.1 (normal), IgG 53 (increased)	Unknown aetiology	HSV-2	ND	HSV-2 CNS infection	Ceftriaxone, meropenem, anti-TB drugs, acyclovir, ARV	died	no
N-084	33	M	0	positive	ND	negative	ND	ND	Anti-toxoplasma IgM 0.47 (normal), IgG >300 (markedly increased)	Presumptive cerebral toxoplasmosis	VZV	ND	Presumptive cerebral toxoplasmosis, VZV CNS infection	Pyrimethamine, clindamycin, ARV	alive	yes
N-100	23	F	77	negative	ND	positive	*Pseudomonas sp.*	x	Anti-toxoplasma IgM 0.11 (normal), IgG 166 (markedly increased)	*Pseudomonas* CNS infection	ND	ND	*Pseudomonas* CNS infection	Ceftazidime, clindamycin, anti-TB drugs	alive	yes

Table 5.6 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results					Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investiga-tions		CSF PCR	Antibody/ antigen testing				
N-120	34	M	10	positive	Solitary Gram-negative, Gram-positive cocci	negative	*Acinetobacter baumannii*	ND	-	*Acinetobacter baumannii* CNS infection	ND	ND	*Acinetobacter baumannii* CNS infection	Ceftazidime	died	yes
N-143	37	M	32	positive	ND	positive	*C. neoformans*	ND	Anti-toxoplasma IgM 0.04 (normal), IgG >300 (markedly increased)	Cryptococcal CNS infection	ND	*C. neoformans*	Cryptococcal CNS infection	Ceftriaxone	died	no
N-147	27	F	5	positive	x	negative	*Acinetobacter baumannii*	ND	-	*Acinetobacter baumannii* CNS infection	ND	ND	*Acinetobacter baumannii* CNS infection	Cefotaxime, ceftazidime, meropenem, imipenem, anti-TB drugs	alive	yes
N-063	47	M	N/A	negative	N/A	N/A	N/A	*K. pneumoniae*	Anti-toxoplasma IgM 0.14 (normal), IgG 169 (markedly increased)	*K. pneumoniae* CNS infection	N/A	x	*K. pneumoniae* CNS infection	Ceftazidime, meropenem	alive	yes
N-070	33	M	N/A	positive	N/A	N/A	N/A	*K. pneumoniae*	-	*K. pneumoniae* CNS infection	N/A	ND	*K. pneumoniae* CNS infection	Meropenem, metronidazole , gentamycin, clindamycin, anti-TB drugs	alive	yes

Table 5.6 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/μL)	HIV status	Standard hospital testing results					Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigation		CSF PCR	Antibody/ antigen testing				
N-073	34	M	N/A	x	N/A	N/A	N/A	ND	*P. falciparum* positive (blood smear)	Cerebral malaria	N/A	ND	Cerebral malaria	Ceftriaxone	died	no
N-080	40	M	N/A	positive	N/A	N/A	N/A	*Staphy-lococ-cus hominis*	Anti-toxoplasma IgM 0.16 (normal), IgG >300 (markedly increased)	*Staphylo-coccus* CNS infection	N/A	ND	*Staphylo-coccus* CNS infection	Ceftriaxone, meropenem, pyrimethamine	died	yes
N-186	52	M	N/A	positive	N/A	N/A	N/A	*Shigella* group	-	*Shigella* CNS infection	N/A	ND	*Shigella* CNS infection	Ceftazidime	died	yes
N-141	58	F	15	negative	ND	negative	ND	x	Anti-toxoplasma IgM 0.08 (normal), IgG 122 (increased)	Unknown aetiology	ND	DENV*	Dengue CNS infection	Ceftriaxone, ceftazidime, levofloxacin	died	no
N-161	57	M	50	unknown	ND	negative	ND	x	-	Unknown aetiology	ND	DENV*	Dengue CNS infection	Cefotaxime	alive	no
N-006	45	M	8	unknown	x	x	ND	ND	-	Unknown aetiology	ND	*O. tsutsugamushi*	Presumptive scrub typhus	Cefotaxime, ceftazidime	alive	no
N-032	63	M	8	unknown	ND	x	ND	ND	-	Unknown aetiology	ND	*O. tsutsugamushi*	Presumptive scrub typhus	Ceftriaxone	died	no
N-138	40	M	65	positive	ND	positive	ND	ND	Anti-toxoplasma IgM 0.02, IgG 0 (normal) Anti-CMV IgM 0.06 (normal), IgG 34 (increased)	Unknown aetiology, suspected cryptococcal CNS infection	ND	*O. tsutsugamushi, C. neoformans*	Cryptococcal CNS infection	Ceftriaxone	died	no
N-171	40	M	8	negative	ND	negative	ND	ND	-	Unknown aetiology	ND	*O. tsutsugamushi*	Presumptive scrub typhus	Cefotaxime, ceftazidime, gentamycin, meropenem	alive	no

Table 5.6 (Continued)

Subject ID	Age (year)	Sex	CSF leucocyte count (cells/µL)	HIV status	Standard hospital testing results					Initial diagnosis	Advanced diagnostic testing		Final diagnosis	Treatment received by patients	Outcome at discharge	Received appropriate treatment at hospital
					CSF Gram stain	CSF Indian ink stain	CSF culture	Blood culture	Other investigations		CSF PCR	Antibody/ antigen testing				
N-033	26	M	8	positive	ND	x	*Kocuria varians*	ND	Anti-toxoplasma IgM 0.25 (normal), IgG >300 (markedly increased) Anti-CMV IgM 0.02 (normal), IgG 54 (increased)	Unknown aetiology	ND	*C. neoformans*	Cryptococcal CNS infection	Ceftazidime, acyclovir, pyrimethamine, ARV	died	no

PCR – polymerase chain reaction; CSF – cerebrospinal fluid; ZN – Ziehl-Neelsen; TB – tuberculosis; TBM – tuberculous meningitis; HIV – human immunodeficiency virus; CNS – central nervous system; *M. tuberculosis – Mycobacterium tuberculosis; E. coli – Escherichia coli*; CMV – cytomegalovirus; VZV – varicella-zoster virus; DENV – dengue virus; HSV – herpes simplex virus; ARV - antiretroviral*; S. pneumoniae – Streptococcus pneumoniae; H. influenzae – Haemophilus influenzae; K. pneumoniae – Klebsiella pneumoniae; C. neoformans – Cryptococcus neoformans; O. tsutsugamushi – Orientia tsutsugamushi; P. falciparum – Plasmodium falciparum; S. haemolyticus – Staphylococcus haemolyticus; S. epidermidis – Staphylococcus epidermidis*; F – female; M – male; ND – not detected; N/A - not applicable; x – not done.

5.3.7.2 Final results of standard hospital testing, PCR, and antibody-mediated testing in adults

Pathogen-specific PCR and antibody-mediated testing in adult samples improved the detection of CNS infection aetiology by 35 cases. Accounting for standard hospital testing, overall detection of CNS infection aetiology increased from 10 cases (10/168 [6%]) to 45 cases (45/168 [27%]). These include 44 patients with a single infection and a patient (N-084) who was categorised as having presumptive cerebral toxoplasmosis based on the case definition defined in chapter 3 (without any pathogen detected by the standard hospital testing) but later found to have VZV detected in CSF by PCR.

Eighteen of 45 adults with pathogens identified by the standard hospital testing or the advanced diagnostic testing received appropriate antimicrobial treatment. The remaining 27 adults did not receive appropriate treatment. These included 16 adults with tuberculous CNS infection who did not receive anti-TB drugs, three with cryptococcal CNS infection who did not receive antifungal treatment, four with viral CNS infection (i.e. two with HSV-2 and two with dengue) who received antibiotics, three with presumptive scrub typhus CNS infection and one with cerebral malaria who did not receive specific treatment for the pathogen (table 5.6). Among these, 23 adults had a fatal outcome. These included 16 with tuberculous CNS infection, three with cryptococcal CNS infection, and four with HSV-2, presumptive scrub typhus, dengue, and cerebral malaria (n=1 each). Interestingly, among 18 adults who received appropriate treatment, seven remained to have a fatal outcome. These included three adults with tuberculous CNS infection and four with *S. pneumoniae*, *Acinetobacter, Staphylococcus* and *Shigella* CNS infection (n=1 each) [table 5.6].

5.4 Discussion

5.4.1 Successful introduction of antibody and antigen tests to Indonesia

I have shown in the results section that I have successfully introduced antibody tests for detecting dengue, scrub typhus and cryptococcal CNS infection in my study setting. The use of commercial antibody/antigen testing kits allowed me to transfer the diagnostic techniques more quickly to the study setting. The dengue IgM ELISA and the CrAg lateral flow assay kits are currently available in Indonesia. Although the JE and scrub typhus IgM ELISA kits are currently not available in Indonesia, it remains possible for the manufacturer to ship them to Indonesia.

Despite the successful introduction of the antibody tests to the Indonesian population, there were challenges in interpreting the ELISA results, particularly for DENV and JEV. Depending on the threshold I applied, I obtained differences in the number of serum samples called positive by the DENV and JEV ELISAs. Depending on the dilution I used, I also obtained differences in the number of CSF called positive by the DENV ELISA. Consequently, all cases identified as positive (or equivocal) by ELISA using the modified (more stringent) thresholds were sent for PRNT testing. PRNT is regarded as the gold standard test for DENV and JEV diagnosis.[351] Taking the PRNT results as the gold standard, the ELISA results had a poor agreement (only 44% (6/17) DENV and 0% (0/3) JEV cases, respectively, were classified as positive by both PRNT and ELISA. Nevertheless, the use of DENV IgM ELISA (as a screen) followed by PRNT in serum and confirmed by DENV IgM ELISA in CSF increased the detection of acute dengue infection from zero to four cases in children (tables 5.1 and 5.3) and zero to two cases in adults (tables 5.2 and 5.4).

Dengue ELISAs have been reported to be more specific than rapid antibody tests.[346, 347] Nevertheless, as ELISAs measure antibody binding, they are still susceptible to non-

specific antibody binding or the presence of cross-reactive with antibody responses in the patient's serum. Both tests are reported as less specific than PRNT.[154, 351] In my study, a high number of positive IgM cases were initially observed following testing via the DENV IgM ELISA when the manufacturer's cut-off was applied. Furthermore, false positive cases had previously been identified when I piloted the kit using a small number of known samples. Therefore, in discussion with my supervisors, I applied a higher threshold. I also re-tested the positive samples using PRNT. Other studies have used the same DENV kit with varying results. For example, Hunsperger *et al.* compared the Panbio dengue IgM ELISA against a 'reference' CDC MAC ELISA.[360] The Panbio ELISA was reported to have high specificity (84.4%) based on testing samples categorised by the reference ELISA. However, in the same paper, they also reported 'concern .. [of] false-positive results .. on sera that were anti-DENV IgM negative but malaria positive, anti-DENV IgG positive, or rheumatoid factor positive'. The Panbio ELISA demonstrated cross-reactivity with 58% of these samples. Consequently, some other unclassified infection or factor may co-exist in the sera I tested that influences the ELISA performance.

Cross-reactivity between JE and dengue IgM has been widely reported by other studies, particularly in countries where both viruses co-circulate.[380-382] Cardosa *et al*. have suggested comparing the OD of dengue and JE IgM to determine which virus is most likely causing the current infection, as the OD is typically much higher for one than the other.[383] However, this should be ideally done by testing both dengue and JE IgM in parallel and specifically using the same ELISA kits. Unfortunately, this could not be applied in the present study as I used two ELISA kits from different manufacturers. Thus, the OD could not be compared in parallel.

I then applied an alternative approach in determining the new cut-off for the dengue IgM ELISA kit, as described in the result section. This approach was based on the assumption

that negative cases would have an approximately normal distribution of OD values and any values beyond the normal distribution were likely to represent true positive cases. The approach was not based on the actual true positive and true negative cases, as there were no such cases when I applied the kit in the study testing. With the new approach, I have shown that changing the cut-offs will greatly change the results. Furthermore, I have shown that changing the CSF dilution factor for the dengue IgM ELISA will also affect the results. The ELISA results can be very variable and it is impossible to define whether the positive cases identified in my study are true positives. This is also the case for the scrub typhus IgM ELISA test, where I used the same approach to determine the cut-off due to the unavailability of the reference cut-off from the local population.

I have shown that the cross-reactivity problem between dengue and JE can be partially solved by performing PRNT. The ideal approach to determine the appropriate cut-off for the dengue IgM ELISA kit would be to initially test the acute serum samples using PRNT assay as the gold standard for serological diagnosis to determine the true positive and true negative cases, then subsequently test those samples using the dengue IgM ELISA kit. Nevertheless, PRNT assay is expensive, time-consuming, and labour-intensive, which is unsuitable for routine practice at hospitals.

Detection of dengue viral genomic RNA via PCR can offer high sensitivity and specificity for diagnosing dengue infection, particularly among acute illnesses (under seven days from symptom onset). I did initially consider using a pan-dengue primer probe to detect DENV cases. I developed and tested probes in Liverpool. However, they exhibited low accuracy against positive (DENV) and negative (other pathogens) controls. Furthermore, ongoing work among colleagues in the Brain Infection Group in Brazil had shown poor detection of DENV using pathogen-specific PCR among samples collected over a week from fever onset. This anecdotal report fitted with previous guidelines[85, 154] that

recommend antibody detection as the first line of testing where the time from illness is over seven days (or not known). For these reasons, I selected an antibody-based DENV ELISA in discussion with my supervisory team.

However, in retrospect, given the limited agreement of my DENV ELISA results with PRNT, if repeating this assessment, I would consider using both PCR and antibody methods. I would also prioritise identifying local samples where DENV exposure had been well characterised and confirmed so that I could determine test accuracy (PCR and ELISA) prior to use in the local patient samples.

5.4.2 Improvement in the detection of scrub typhus infection over the standard hospital diagnostic tests

Previous seroprevalence studies reported the circulation of scrub typhus (*Orientia tsutsugamushi*) in Indonesia. These studies estimated between 1.3-9.4% of the general population were seropositive.[341, 345] Previously, there was only one study looking for scrub typhus among Indonesian patients.[342] It was a multi-country study and only included 14 paediatric and 65 adult cases from Indonesia. To my knowledge, this is the largest study (110 children and 140 adults) measuring the presence of scrub typhus IgM antibodies among hospital patients in Indonesia.

My study identified 4.2% (6/140) of adults and 7.3% (8/110) children presenting with suspected CNS infection being IgM positive for scrub typhus. The proportion of seropositive patients is broadly in line with estimates for the general population (above). Studies from other South Asian countries (e.g. Malaysia, Thailand, Vietnam) have reported a higher seroprevalence of scrub typhus among patients with fever compared to the general population.[340] However, the proportions vary markedly between countries and/or studies. For example, in Malaysia, seroprevalence was 1.5% among the general

population and 24.9% among febrile patients.[384, 385] In Vietnam, seroprevalence was reported as 1.1% among the general population but 3.5% among febrile patients.[386, 387] Reflecting my findings in adults, the study by Limmathurotsakul and colleagues reported 4% of adults with a clinical presentation of acute CNS infection having positive serology for scrub typhus. However, they reported no child cases.

My study supports scrub typhus as a potential cause of CNS infection among adults and children in Indonesia. Scrub typhus CNS infection has been frequently reported from India, Thailand, Korea and Taiwan.[227, 232, 339, 388-394] Of course, my ELISA results have not been validated against another test. The indirect immunofluorescence assay is a reported gold standard test for diagnosing scrub typhus. However, this could not be performed in Indonesia due to limited access to immunofluorescent microscopes and limited experience with the test/equipment.[395, 396] The presence of eschar, a characteristic clinical sign of scrub typhus infection, is only reported in 17.6% of patients with meningitis and 20.8% without meningitis.[397] Unfortunately, it was not actively sought and examined in my study. Accepting these caveats, scrub typhus being a potential cause of CNS infection among both adults and children in Indonesia is of critical clinical relevance.

5.4.3 More specific detection of *Cryptococcus* by the lateral flow assay

CrAg lateral flow testing of CSF among adults with suspected CNS infection identified three cases of cryptococcal meningitis. All three cases (100%) were HIV positive. One patient had *C. neoformans* grown via culture from CSF.

In comparison, Indian ink staining performed by the hospital identified 9 possible cases of *Cryptococcus* infection. Four cases (44% [4/9]) were HIV positive. Two patients were also CrAg positive, including one *C. neoformans* culture-positive case.

Interestingly, another hospital patient (with non-CNS infection - thus not included in the above analysis), reported to have positive Indian ink staining of CSF, was later identified to have *Candida* grown from CSF. This latter culture result indicates false positives can occur during Indian ink staining.

My results are consistent with previous reports of false-positives as a result of artefacts; thus, it is essential to confirm a positive Indian ink result with cryptococcal antigen testing or culture where possible.[275, 398] A multicentre study involving patients with suspected meningitis from South Africa and Uganda reported that the CrAg LFA had sensitivity and specificity of 99.3% and 99.1 %, whilst the Indian ink microscopy were 86.1% and 97.3%.[166] Although the latter assay is useful in poor resource settings and remains recommended by the WHO if antigen testing is unavailable,[399] it should be interpreted carefully and by a person who is experienced to do so. Otherwise, it can lead to a false positive result, as seen in my study.

Studies in Jakarta and West Java have reported a case of cryptococcal meningitis in HIV-negative patients (one each). They both used the same definition, Indian ink staining alone, without confirmation by either culture or CrAg test, as sufficient to define a cryptococcal meningitis case.[6, 276] This raises the question of whether these two cases were false positives. Other studies reported cryptococcal meningitis in immunocompetent patients, particularly among low and middle-income populations.[400-402] In my study, only CSF samples from HIV-positive or those with CSF Indian ink positive results were tested by the CrAg test. There still may have been undiagnosed cryptococcal meningitis among the other CNS infection patients.

5.4.4 No Japanese encephalitis cases were detected in the study setting

Despite the cross-reactivity problem between dengue and JE antibody reaction, which has been partially resolved by the PRNT assay, I found no single case of JE among the patients in my study setting. This may be due to the fact that despite the widespread rice paddies providing breeding sites for *Culex* mosquitoes in Yogyakarta and the surrounding areas, where some of the patients have been referred from, the population are mostly Muslims and pig-rearing in these areas are uncommon. Pigs are known as important hosts for JEV transmission to humans.[80]

The finding contrasts with the previous findings from other regions in Indonesia. Kari *et al*. have reported an annual incidence of 8.2:100,000 for children under 10 years of age in Bali Island, with 86 confirmed and 4 probable JE cases identified throughout July 2001 – December 2003.[29] Unlike most provinces in Indonesia where most of the population are Muslims who generally do not consume pork and breed pigs for a religious reason, 83.5% of Bali residents are Hindus. Moreover, Bali is considered a high-risk area for JE transmission as pig-rearing is common in the community and breeding sites for the JE mosquito vector, such as rice paddies, are widespread in the area.

Ompussunggu *et al*. reported 82 JE cases out of 1,496 (6%) acute encephalitis syndrome (AES) cases over the two years from January 2005 to December 2006 from a larger JE sentinel surveillance involving children aged 1 month to 15 years of age.[8] This surveillance was conducted in hospitals and health centres across 6 provinces/islands, including East Java, West Kalimantan, West Sumatra, Papua, Lombok, and West Timor. The highest proportion was reported from West Kalimantan (18%), followed by West Timor Island (7%). Both were classified as high-risk areas for JE transmission. JE cases were also confirmed in all of the remaining sites, including East Java, West Sumatra, and Lombok Island, where Muslims make up the majority of the population – the proportion of the JE

cases in these sites was 5%, 2% and 4%, respectively. Similar to the previous findings, a more recent study by Konishi *et al*. has highlighted a low prevalence of JE among Surabaya –the capital city of East Java Province– and Jakarta communities (1.8% and 2.2%, respectively), where the pig population are rare.[296]

5.4.5 Dengue encephalitis versus dengue encephalopathy

It remains unclear whether dengue encephalitis is a true entity. The neurological manifestations of dengue infection have been extensively reported in recent years. These include headache, altered consciousness, seizure, behavioural change, abnormal movement, altered sensorium, neck stiffness and cranial nerve.[87, 328, 329, 332, 333, 403-408] These neurological manifestations had been denoted as encephalopathy rather than encephalitis as DENV was thought to be incapable of infecting the nervous system (i.e. non-neurotropic).[409] Therefore, these neurological manifestations were assumed to be attributed to various physiological changes that occurred in extended dengue haemorrhagic fever or dengue shock syndromes, such as cerebral oedema, cerebral haemorrhage, cerebral hypoxia, electrolyte imbalance and severe hepatic failure. [332, 403, 405] Recent reports, however, have shown the possible neurotropic effects of DENV. Animal studies showed that a virus-induced cytokine could break down the blood-brain barrier in mice inoculated by DENV-2 intraperitoneally and intracerebrally. [410] A more recent study revealed that mice inoculated with DENV-3 intracerebrally developed meningoencephalitis and behavioural change, which led to lethality.[411] Other studies reported the discovery of dengue IgM and dengue RNA in CSF,[87, 230, 328, 329, 404, 407] suggesting that the virus can invade the brain and cause encephalitis. Four of the seven cases of dengue infection confirmed by PRNT in this study had dengue IgM in CSF (table 5.1). My finding of IgM antibody against dengue virus in the CSF supports intra-thecal antibody production (although I cannot exclude the passive transfer of IgM across the blood-brain

barrier from the bloodstream).[412] Intra-thecal production is, in turn, supportive of dengue virus in the CSF and dengue being associated with CNS infection.

5.4.6 Potential implications for patient management

All children and adults with dengue CNS infection received antibiotics for five or more days during their hospital stay. If DENV was identified as the cause of their CNS infection by the diagnostic testing, then one could argue extended antibiotic administration in these patients was unnecessary. Antibiotic use in Indonesia is generally high.[413] Providing empiric antibiotic treatment is likely, in part, to be promoted by the low pathogen yield of routine diagnostic testing in health care facilities, including tertiary hospitals such as Dr Sardjito Hospital, to determine the definite causative pathogens. A previous study on antibiotic prescriptions conducted at two tertiary hospitals in East and Central Java Provinces showed that 15-17% of the antibiotic prescriptions were inappropriate in selection, dosage or therapy duration, whilst 34-42% of the prescriptions were given based on no justified indication.[413]

Although there is no specific treatment for dengue CNS infection, it is a devastating disease which can be prevented by vaccination. Dengue vaccination is currently not included in the national immunisation programme in Indonesia. The findings from the present study provide supportive data to clinicians, stakeholders, and the population about the importance of dengue vaccination. Furthermore, the fact that a few of the potential dengue CNS infection cases found in the present study were not identified at the hospital (i.e. the patients did not have any dengue investigations performed at the hospital) suggests that dengue is an under-estimated aetiology of CNS infection. With these findings, dengue testing should be considered in patients with suspected CNS infection.

My study's finding of scrub typhus cases provides clinicians with essential information that such cases exist in Indonesia, particularly in Yogyakarta. As described above, the presence of eschar is characteristic of scrub typhus, but it was not actively examined by the clinicians. With my findings, it is expected that clinicians will consider scrub typhus as one of the differential diagnoses for acute febrile illness, perform a more comprehensive physical examination, and include scrub typhus testing in hospital laboratory investigation. Scrub typhus is easily and rapidly treatable with antibiotics such as doxycycline, azithromycin, quinolones, chloramphenicol, and rifampicin.[414-417] Therefore, identifying scrub typhus cases among Indonesian patients offers a treatable cause of CNS infection.

The finding that none of the three adults with cryptococcal meningitis received antifungal treatment during their hospital stay, and instead, all of them received antibiotics (section 5.3.6.2), is interesting. The fact that all of them died at the hospital suggests that cryptococcal meningitis might cause a high mortality rate if left untreated. The application of a rapid diagnostic test with a high sensitivity and specificity for detecting Cryptococcus, such as the CrAg LFA, is therefore highly necessary in the study setting to guide clinicians in giving prompt and appropriate treatment. If the CrAg lateral flow assay had been performed as a part of routine CSF testing at the hospital, the three patients who tested cryptococcal-positive in this study would have been identified earlier, received antifungal promptly, and potentially had a better outcome.

This study showed that the application of advanced diagnostic testing (i.e. pathogen-specific PCR and antibody/antigen testing) in addition to the standard hospital testing improved the detection of CNS infection aetiology by 24/247 (9%) cases in children and 35/168 (21%) cases in adults. Among 50 children and 45 adults with a pathogen detected by either the advanced or the standard hospital testing, 8 children and 27 adults did not

receive appropriate treatment during their hospital stay; of whom three children and 23 adults had a fatal outcome. Of those, the majority (i.e. 2 children and 16 adults) had a final diagnosis of tuberculous CNS infection. If *Mycobacterium tuberculosis* had been detected at our hospital, these patients could have received appropriate anti-tuberculous treatment earlier and potentially had a better outcome. These findings highlight the importance of advanced diagnostic testing in improving pathogen detection, particularly *M. tuberculosis*.

Despite the increase in pathogen detection, there are some important challenges to improving clinical care as follows:

1) Late patient presentation to Dr Sardjito Hospital, particularly among adults

   Eleven children and seven adults who received appropriate treatment for the pathogens detected (as part of my study testing) had a fatal outcome. This emphasises poor clinical outcome continues to occur despite appropriate treatment. As stated earlier in the thesis, many patients presented late to Dr Sardjito Hospital. Therefore, these patients were already relatively late in their disease course and may have already developed complications (e.g. cerebral infarctions or raised intracranial pressure). The clinical impact of introducing further testing will always be influenced by the time from the patient's initial onset of illness.

2) Treatment prior to attending Dr Sardjito Hospital (before diagnostic testing)

   The additional bacterial pathogens detected by pathogen-specific PCR were relatively low. This might be, in part, due to antibiotic administration prior to LP. Ninety-two percent of children and 95% of adults received antibiotics prior to LP with a median of three and four doses. Moreover, 84% of children and 64% of adults recruited into this study had attended at least one medical centre before admission to Dr Sardjito Hospital, where they might receive antibiotics. Studies have shown that

administration of antibiotics before culture can reduce bacteria detection.[294, 418, 419] To a lesser extent, this practice may affect bacteria detection by PCR.[158, 222, 223, 226]

As previously stated, the impact of testing on clinical care would, in part, be dependent on the time of diagnostic testing relative to the patient starting antibiotics.

3) Delayed LP in Dr Sardjito Hospital, particularly among adults

The LP was performed one or three days (median) after admission in children or adults, respectively. The majority of patients had received antibiotics (92% of children and 95% of adults) and/or steroids (58% of children and 46% of adults) prior to LP. A previous study reported that every hour of delay in LP significantly reduced the pathogen detection rate by 1% in viral meningitis (odds ratio [OR] 0.988 [95% CI 0.982-0.995], p=0.001).[2] In bacterial meningitis, the pathogen detection rate was also reduced by 1% for every hour of delay in LP, although this was not statistically significant (OR 0.995 [95% CI 0.989-1.002], p=0.16).

Again, the impact of testing on clinical care would, in part, be dependent on the time of diagnostic testing relative to the patient starting antibiotics.

4) Clinicians not always giving appropriate treatment despite available diagnostic results

Forty-two out of 50 (84%) children and 18 out of 45 (40%) adults with pathogens detected by either standard hospital testing or study testing received appropriate treatment during their hospital stay. Consequently, 16% of children and 60% of adults did not receive appropriate treatment. Among the latter, 3 out of 8 (38%) children and 23 out of 27 (85%) adults on inappropriate treatment had a fatal outcome. These findings emphasise the need for good communication and/or education between laboratory and clinical teams to highlight the availability of

diagnostic testing and ensure that provision of diagnostic test results promotes consideration of more appropriate treatment.

The clinical impact of testing is subject to clinicians' responding promptly and appropriately to test results. Clinicians will not be able to learn the benefits of appropriate treatment until they have access to routine comprehensive diagnostic testing.

5.5 Conclusion

The introduction of antibody and antigen testing in the present study, in addition to the pathogen-specific PCR and the standard hospital diagnostics, has improved the detection of pathogens causing CNS infection in the study setting. Although it may be expected, the finding of dengue viruses as the most common aetiology of viral CNS infection in children provides new data to the region and the country. Furthermore, the finding of *O. tsutsugamushi* as a potential cause of CNS infection among both children and adults is of great clinical importance because it represents a treatable cause of infection.

In chapters 3 and 4, I have shown the early outcome of patients with suspected CNS infection where mortality is very high, particularly in adults. If not promptly and properly treated, CNS infection is related to high mortality and morbidity. Therefore, in the next chapter, I want to assess whether the latter was true for the patients with suspected CNS infection in the present study. I assessed the functional outcome of the patients with and without CNS infection who were alive at the time of discharge. Furthermore, I followed the patients for up to 6 months and re-assessed their functional outcomes to investigate whether the CNS infection was also related to long-term morbidity and whether mortality occurred during the follow-up period.

Chapter 6 – Functional outcome of patients with central nervous system infection in Yogyakarta

6.1 Background

Central nervous system (CNS) infection has been known to cause functional impairment. Studies on the outcome of CNS infection usually focus on certain types of CNS infection, such as encephalitis and meningitis, in a particular age group (i.e. in children or adults only).[2, 46, 276, 420-428] Some studies only focus on the outcome of CNS infection caused by specific pathogens such as Japanese encephalitis virus,[9, 429-431] West Nile virus,[432, 433] and *Mycobacterium tuberculosis*.[316, 434, 435] There are limited studies comparing the outcome of different types of syndromic CNS infection at discharge and at long-term follow-up in children and adults.[6, 77, 78]

My specific aims for this chapter are as follows:

1) To assess the overall outcome of children and adults with suspected CNS infection;
2) To compare the functional outcome in patients (children and adults) with syndromic CNS infection to those with other conditions mimicking CNS infection;
3) To compare the functional outcome in children with different sub-types of CNS infection, including encephalitis, meningoencephalitis, definite shunt infection, and meningitis at the time of hospital discharge and follow-up;
4) To compare the functional outcome in adults with different sub-types of CNS infection, including encephalitis, meningoencephalitis, presumptive cerebral toxoplasmosis, brain abscess, and myelitis at the time of hospital discharge and follow-up;

5) To describe the types of neurological impairment following CNS infection in children at the time of hospital discharge and follow-up;
6) To describe the types of neurological impairment following CNS infection in adults at the time of hospital discharge and follow-up;
7) To describe the recovery profile following CNS infection in children and adults.

6.2 Methods

6.2.1 Assessing functional outcome in children

Functional outcome in children was assessed using the Liverpool Outcome Score (LOS) questionnaire. The LOS questionnaire is a simple validated tool to assess functional impairment in children following encephalitis.[219] The LOS consists of 15 questions comparing the child's abilities to other children of the same age. Several aspects of the child's function are evaluated by the LOS, such as language function, feeding, behaviour, upper and lower limb function, and episodes of seizures. Each aspect was assessed by a score between 2 and 5, which corresponded to specific interpretations, including severe (2), moderate (3), mild (4), and no (5) functional impairment, respectively. Death was scored as 1. The final LOS measurement was defined by the lowest score for any single question. Children with scores 3-5 were considered able to live independently. Those with a score of 2 were considered to be dependent.[219]

The LOS was assessed at hospital discharge and 1-6 month follow-up. The recruitment period was from February 2015 until January 2018. The follow-up assessment ended in March 2018. Children admitted to Dr Sardjito Hospital before October 2017 were followed-up for six months post-discharge. Those who were hospitalised from October 2017 to January 2018 were followed-up between February and March 2018. The follow-up assessment was undertaken by telephone interview. The Liverpool Brain Infection

group has previously successfully used telephone interviews to complete the outcome questionnaires.[421]

6.2.2 Assessing functional outcome in adults

Adult patients' functional outcome was assessed using the Glasgow Outcome Score Extended (GOSE) questionnaire. The GOSE questionnaire is a validated tool to assess functional impairment in adult patients following brain damage. The GOSE, containing eight-point measurements, is an extended version of the traditional Glasgow Outcome Scale (GOS) tool. The latter only assesses five categories: death, vegetative state, severe disability, moderate disability, and good recovery. The final interpretation of the GOSE was obtained from the lowest score achieved from the structured questionnaire. A score ranging from 1 to 8 corresponds to a specific category, as described in table 6.1.[220]

Table 6.44 Glasgow Outcome Scale Extended (GOSE) classification for functional outcome

Score	Category	Description
1	Death	-
2	Vegetative State	Unable to respond to any commands
3	Lower severe disability	Able to follow commands, unable to do all activities without help, unable to live alone
4	Upper severe disability	Able to follow commands, unable to do most activities without help, unable to live alone
5	Lower moderate disability	Able to live independently, able to do activities with some help, unable to return to work or school
6	Upper moderate disability	Able to live independently, able to do activities with little help, unable to return to work or school
7	Lower good recovery	Able to return to work or school with mild difficulty
8	Upper good recovery	Able to return to normal activities

The GOSE includes questions about whether the patient can return to work and social life. Therefore, the GOSE was assessed within 2 weeks following hospital discharge instead of at the time of hospital discharge. Similar to the paediatric patients, the adult patients

were also followed-up for 1-6 months post-discharge by telephone interview. Adults who were admitted to the hospital before October 2017 were followed-up for 6 months post-discharge, whereas those who were admitted from October 2017 to January 2018 were followed-up between February and March 2018.

6.2.3 Statistical analysis

The chi-square test (or Fisher's exact if any of the expected counts were less than five) was used to analyse binary data. Differences in the outcome between two time points (i.e. at hospital discharge and at follow-up) were analysed using McNemar tests. Mann Whitney U and Kruskal Wallis tests were used to analyse categorical and non-parametric data. Results were recorded as number per number available (n/N), as some patients had missing data; therefore, the number available (N) did not always represent the total number of patients. Data were presented as a proportion (percentage) or median (range). SPSS v24 and Microsoft Excel were used to analyse the data, whilst graphs in this chapter were created using draw.io (www.draw.io) and GraphPad Prism v6.

6.3 Results

6.3.1 Baseline characteristics for patients who were eligible for follow-up

6.3.1.1 Paediatric patient baseline characteristics

Three-hundred-and-four children with suspected CNS infection, including 209 with syndromic CNS infection and 95 with no CNS infection, were discharged alive from Dr Sardjito Hospital, of whom 252 were followed-up for 1-6 months post-discharge (median 6 months) (figure 6.1). Families from 52/304 (17%) children, including 34/208 (16%) with syndromic CNS infection and 18/96 (19%) with no CNS infection, could not be contacted. Sixteen children, including 13 with syndromic CNS infection and 3 with no CNS infection, were reported to have died after hospital discharge but before follow-up.

Figure 6.24 Flow diagram of paediatric patients' follow-up

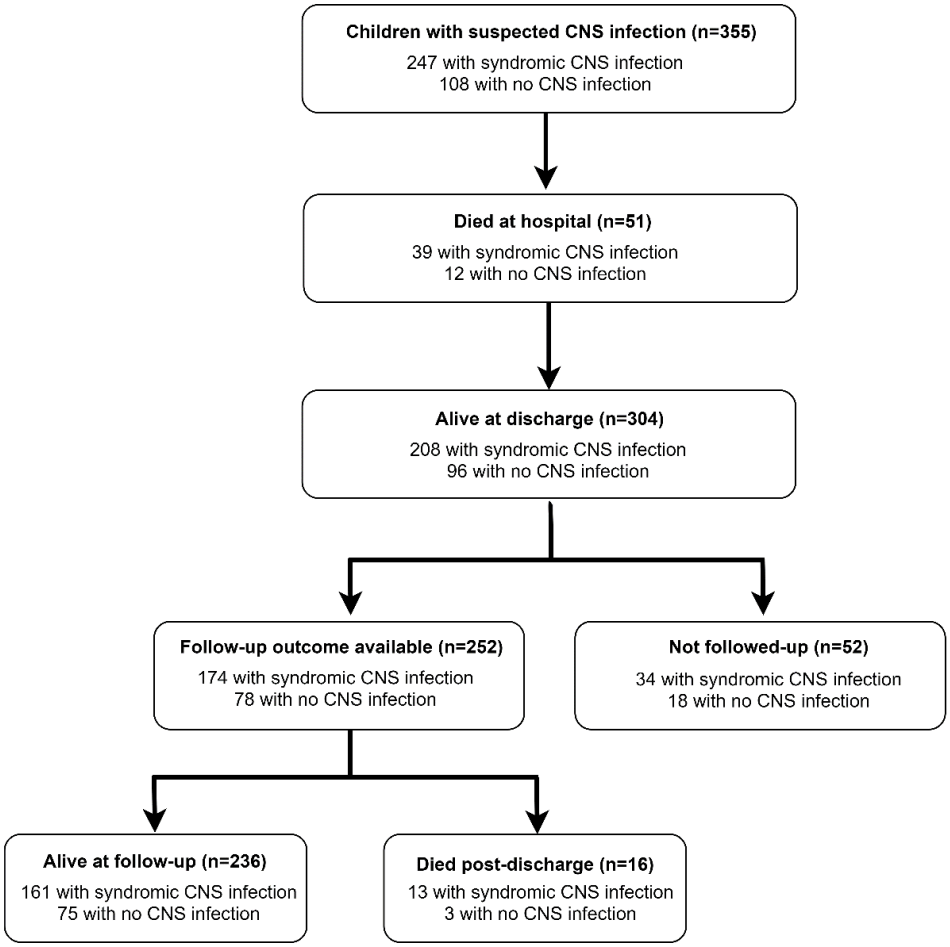


Table 6.45 Baseline characteristics for all children with suspected CNS infection who were eligible for follow-up

	All children with suspected CNS infection (n= 304)	Followed-up (n=252)	Not followed-up (n=52)	P value
Syndromic CNS infection	208 (68%)	174 (69%)	34 (65%)	-
Not CNS infection	96 (32%)	78 (31%)	18 (35%)	-
Male	171 (56%)	138 (55%)	33 (63%)	0.250
Age, years	1.7 (0-17.8)	1.5 (0-17.8)	3.1 (0.3-14.1)	0.146
Discharge GCS¶	15 (4-15)	15 (6-15)	15 (4-15)	0.979
Discharge LOS‡	3 (2-5)	3 (2-5)	3 (2-5)	0.655

Data are presented as number (%) or median (range).
¶Discharge GCS was not available for 2 children who were followed-up.
‡Discharge LOS was not available for 12 children (3 followed-up and 9 not).
CNS – central nervous system; GCS – Glasgow Comma Scale; LOS – Liverpool Outcome Score.

The baseline characteristics for children who were eligible for follow-up (i.e. alive at the time of hospital discharge) are presented in table 6.2. There were no significant differences in the proportion of males (55% versus 63%, p=0.250), median age (1.5 versus 3.1 years, p=0.146), discharge GCS (median 15 for both sub-groups, p=0.979), and discharge LOS (median 3 for both sub-groups, p=0.655) between children who were followed-up and those who were not. The median follow-up interval was similar between those with and without CNS infection (6 [range 1-6] months versus 6 [range 2-6] months, p=0.288).

Table 6.46 Baseline characteristics for all children with syndromic CNS infection who were eligible for follow-up

	All children with syndromic CNS infections (n=208)	**Followed-up (n=174)**	**Not followed-up (n=34)**	**P value**
Sub-types of syndromic CNS infection				
Encephalitis	155 (74.5%)	130 (74.7%)	25 (73.5%)	-
Meningoencephalitis	28 (13.5%)	24 (13.8%)	4 (11.8%)	-
Definite shunt infection	9 (4.3%)	7 (4.0%)	2 (5.9%)	-
Meningitis	6 (2.9%)	5 (2.9%)	1 (2.9%)	-
Others	10 (4.8%)	8 (4.6%)	2 (5.9%)	-
Non-TB vs TB CNS infection				
Non-TB CNS infection	206 (99%)	173 (99.4%)	33 (97.1%)	-
TB CNS infection	2 (1%)	1 (0.6%)	1 (2.9%)	-
Male	111 (53.4%)	90 (51.7%)	21 (61.8%)	0.283
Age, years	1.6 (0-17.8)	1.6 (0-17.8)	1.9 (0.3-14)	0.483
Discharge GCS¶	15 (4-15)	15 (6-15)	15 (4-15)	0.849
Discharge LOS‡	3 (2-5)	3 (2-5)	3 (2-5)	0.651

Data are presented as number (%) or median (range).
¶Discharge GCS was not available for 2 children who were followed-up.
‡Discharge LOS was not available for 9 children (3 followed-up and 6 not).
CNS – central nervous system; TB – tuberculous; GCS – Glasgow Comma Scale; LOS – Liverpool Outcome Score; vs - versus.

Among 208 children with syndromic CNS infection who were alive at the time of hospital discharge, 155 (74.5%) had encephalitis, 28 (13.5%) had meningoencephalitis, 9 (4.3%)

had definite shunt infection, 6 (2.9%) had meningitis, and 10 (4.8%) had other syndromic CNS infection sub-types. Based on the CNS infection aetiology, 206/208 (99%) children had non-tuberculous CNS infection and 2/208 (1%) children had tuberculous CNS infection. There were no significant differences in the proportion of males (p=0.283), median age (p=0.483), discharge GCS (p=0.849), and discharge LOS (p=0.651) between children with syndromic CNS infection who were followed-up and those who were not (table 6.3).

The median follow-up interval was similar among children with encephalitis (6 [range 1-6] months), meningoencephalitis (6 [range 1-6] months), definite shunt infection (6 months [no range]) and meningitis (6 months [no range], p=0.528). Furthermore, there was no significant difference in the median follow-up interval between children with non-tuberculous and tuberculous CNS infection (6 [range 1-6] months versus 6 [no range] months, p=1.000).

6.3.1.2 Adult patient baseline characteristics

In the adult group, 110 patients with suspected CNS infection, including 92 with syndromic CNS infection and 18 with no CNS infection, were discharged alive from the hospital. These patients (or their families) were contacted within 2 weeks post-discharge to assess their functional outcomes. Discharge GOSE was unavailable for 16 adults with syndromic CNS infection and 5 with no CNS infection. Six patients with syndromic CNS infection and 1 without CNS infection died within 2 weeks post-discharge (figure 6.2). A follow-up assessment was performed on 64 adults with syndromic CNS infection and 9 with no CNS infection. Five adults with syndromic CNS infection were reported to have died before follow-up. Thirty of 103 (29%) adults, including 22/86 (26%) with syndromic CNS infection and 8/17 (47%) with no CNS infection, were lost to follow-up.

The baseline characteristics for 103 adults with suspected CNS infection who were eligible for follow-up are shown in table 6.4. There were no significant differences in the proportion of males (64% versus 60%, p=0.675), median age (33 versus 38 years, p=0.156), discharge GCS (median 15 for both groups, p=0.515), and discharge GOSE (median 5 for both groups, p=0.358) between those who were followed-up and those who were not. There was no significant difference in the median follow-up interval between adults with and without CNS infection (6 [range 2-6] months versus 6 [no range] months, p=0.265).

Figure 6.25 Flow diagram of adult patients' follow-up

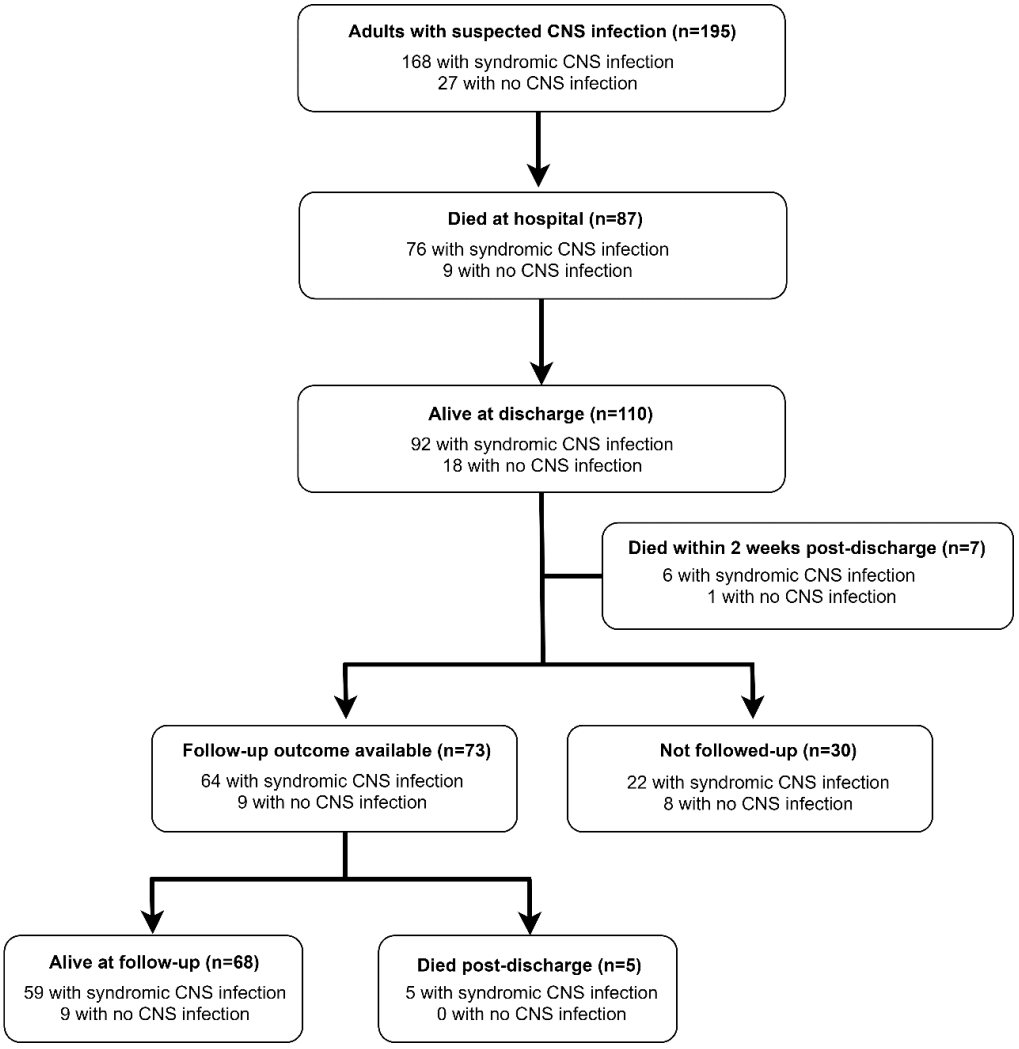


Table 6.47 Baseline characteristics for all adults with suspected CNS infection who were eligible for follow-up

	All adults with suspected CNS infection (n= 103)	Followed-up (n=73)	Not followed-up (n=30)	P value
Syndromic CNS infection	86 (83%)	64 (88%)	22 (73%)	-
Not CNS infection	17 (17%)	9 (22%)	8 (27%)	-
Male	65 (63%)	47 (64%)	18 (60%)	0.675
Age, year	35 (18-81)	33 (19-81)	38 (18-61)	0.156
Discharge GCS¶	15 (3-15)	15 (5-15)	15 (3-15)	0.515
Discharge GOSE‡	5 (2-8)	5 (2-8)	5 (3-7)	0.358

Data are presented as number (%) or median (range).
¶Discharge GCS was not available for four adults (3 followed-up and 3 not).
‡Discharge GOSE was not available for 21 adults (3 followed-up and 18 not).
CNS – central nervous system; GCS – Glasgow Comma Scale; GOSE – Glasgow Outcome Scale Extended.

Table 6.48 Baseline characteristics for all adults with syndromic CNS infection who were eligible for follow-up

	All adults with syndromic CNS infection (n= 86)	Followed-up (n=64)	Not followed-up (n=22)	P value
Sub-types of syndromic CNS infection				
Encephalitis	40 (46.5%)	29 (45.3%)	11 (50.0%)	-
Meningoencephalitis	13 (15.1%)	11 (17.2%)	2 (9.1%)	-
Presumptive cerebral toxoplasmosis	12 (14.0%)	9 (14.1%)	3 (13.6%)	-
Myelitis	6 (7.0%)	4 (6.3%)	2 (9.1%)	-
Brain abscess	5 (5.8%)	4 (6.3%)	1 (4.5%)	-
Others	10 (11.6%)	7 (10.9%)	3 (13.6%)	-
Non-TB vs TB CNS infection				
Non-TB CNS infection	82 (95.3%)	61 (95.6%)	21 (95.5%)	-
TB CNS infection	4 (4.7%)	3 (4.6%)	1 (4.5%)	-
Male	55 (64%)	43 (67%)	12 (55%)	0.287
Age, year	33 (18-68)	32 (19-68)	34 (18-54)	0.403
Discharge GCS¶	15 (3-15)	15 (5-15)	15 (3-15)	0.808
Discharge GOSE‡	5 (2-8)	5 (2-8)	5 (3-7)	0.645

Data are presented as number (%) or median (range).
¶Discharge GCS was not available for four adults (three followed-up and one not).
‡Discharge GOSE was not available for 21 adults (three followed-up and 18 not).
CNS – central nervous system; TB – tuberculous; GCS – Glasgow Comma Scale; GOSE – Glasgow Outcome Scale Extended.

Eighty-six adults with syndromic CNS infection were eligible for follow-up. These included 40 (46.5%) adults with encephalitis, 13 (15.1%) with meningoencephalitis, 12 (14.0%) with presumptive cerebral toxoplasmosis, six (7.0%) with myelitis, five (5.8%) with brain abscess, and ten (11.6%) with other syndromic CNS infection sub-types. Based on the aetiology of CNS infection, 82 (95.3%) adults had non-tuberculous CNS infection and four (4.7%) had tuberculous CNS infection. There were no significant differences in the proportion of males (p=0.287), median age (p=0.403), discharge GCS (p=0.808), and discharge GOSE (p=0.645) between adults with syndromic CNS infection who were followed-up and those who were not (table 6.5). In terms of follow-up interval, there was no difference among adults with encephalitis, meningoencephalitis, presumptive cerebral toxoplasmosis, brain abscess, and myelitis (median six months, p=0.517). Similarly, there was no difference in follow-up interval between those with non-tuberculous and tuberculous CNS infection (median six months, p=0.812).

6.3.2 Functional impairment and recovery following CNS infection in children

6.3.2.1 Functional impairment following CNS infection in children at hospital discharge

Two-hundred-and-fifty-two out of 292 (86%) children with suspected CNS infection had functional impairment (LOS 2-4) at discharge. The proportions of children who experienced functional impairment at discharge were similar between those with syndromic CNS infection and those with no CNS infection (176/200 (88%) versus 76/93 (81.7%), p=0.149, table 6.6). Most children had moderate impairment at discharge, followed by severe impairment, experienced by 39% and 32.5% of children with syndromic CNS infection and 38.7% and 24.7% of children with no CNS infection, respectively (table 6.6).

Table 6.49 Outcome for children with CNS infection at discharge

	Suspected CNS infections	Not CNS infection	Syndromic CNS infection	P value	Sub-types of syndromic CNS infection				P value	Non-TB vs TB CNS infection		P value
					Encephalitis	Meningo-encephalitis	Definite shunt infection	Meningitis		Non-TB CNS infection	TB CNS infection	
No. of children in each group	355	108	247	-	175	43	10	7	-	239	8	-
Died at hospital (LOS 1)	51 (14%)	12 (11.1%)	39 (15.8%)	0.248	20 (11.4%)	15 (35%)	1 (10%)	1 (14.3%)	**0.004**	33 (13.8%)	6 (75%)	**<0.001**
Alive at discharge	304	96	208	-	155	28	9	6	-	206	2	-
Discharge LOS available‡	293	93	200	-	148	27	9	6	-	197	2	-
Any impairment at discharge (LOS 2,3,4)	252 (86%)	76 (81.7%)	176 (88%)	0.149	133 (89.8%)	23 (85.2%)	7 (77.8%)	4 (66.7%)	0.138	174 (88.3%)	1 (50%)	0.227
Severe impairment (LOS 2)	88 (30.1%)	23 (24.7%)	65 (32.5%)	0.177	46 (31.1%)	8 (29.6%)	5 (55.6%)	1 (16.7%)	0.415	64 (32.5%)	1 (50%)	0.548
Moderate impairment (LOS 3)	114 (38.9%)	36 (38.7%)	78 (39%)	0.962	60 (40.5%)	11 (40.7%)	2 (22.2%)	1 (16.7%)	0.548	78 (39.6%)	0 (0%)	0.521
Mild impairment (LOS 4)	50 (17.1%)	17 (18.3%)	33 (16.5%)	0.706	27 (18.2%)	4 (14.8%)	0 (0.0%)	2 (33.3%)	0.351	32 (16.2%)	0 (0%)	1.000
No Impairment (LOS 5)	41 (14%)	17 (18.3%)	24 (12%)	0.149	15 (10.1%)	4 (14.8%)	2 (22.2%)	2 (33.3%)	0.138	23 (11.7%)	1 (50%)	0.227

‡ Discharge LOS was not available for 11 children who were alive at discharge (8 with syndromic CNS infection [non-TB CNS infection] and 3 with no CNS infection).
CNS – central nervous system; TB – tuberculous; LOS – Liverpool Outcome Score; vs - versus.

Within the paediatric CNS infection group, the proportion of patients with meningoencephalitis who died at the hospital (15/43 (35%)) was much higher than those with meningitis (1/7 (14.3%)), encephalitis (20/175 (11.4%)), and definite shunt infection (1/10 (10%), p=0.008, table 6.6). Functional impairment at discharge was reported in 133/148 (89.8%) children with encephalitis, 23/27 (85.2%) with meningoencephalitis, 7/9 (77.8%) with definite shunt infection, and 4/6 (66.7%) with meningitis. Most of the children with definite shunt infection (5/9 (55.6%)) had severe impairment, whereas most of the children with encephalitis (60/148 (40.5%)) and meningoencephalitis (11/27 (40.7%)) had moderate impairment (table 6.6).

At the time of hospital discharge, behavioural disturbance was the most frequently reported neurological impairment in children with syndromic CNS infection, experienced by 125/200 (63%) children. This proportion was significantly higher than that reported in children with no CNS infection (44/93 (47%), p=0.014, figure 6.3). Expressive (78/200 (39%) versus 24/93 (26%), p=0.035) and receptive language impairments (36/200 (18%) versus 8/93 (9%), p=0.036) were also significantly more common in children with syndromic CNS infection than in those with no CNS infection. The second most common neurological problem at discharge was reduced lower limb function, reported in 82/200 (41%) children with syndromic CNS infection and 37/93 (40%) with no CNS infection (figure 6.3).

Figure 6.26 Comparison of neurological impairment experienced by children at discharge classified with syndromic CNS infection and non-CNS infection. The graph shows the proportions (%) of different types of neurological impairment experienced by children with suspected CNS infection who were alive at discharge and had discharge LOS available (n=292). Children were categorised as having syndromic CNS infection (n=199) and non-CNS infection (n=93) based on case definitions described in chapter 3. *Significant difference (p<0.05) between the proportion of children with syndromic CNS infection and children with no CNS infection who experienced a specific type of neurological impairment.

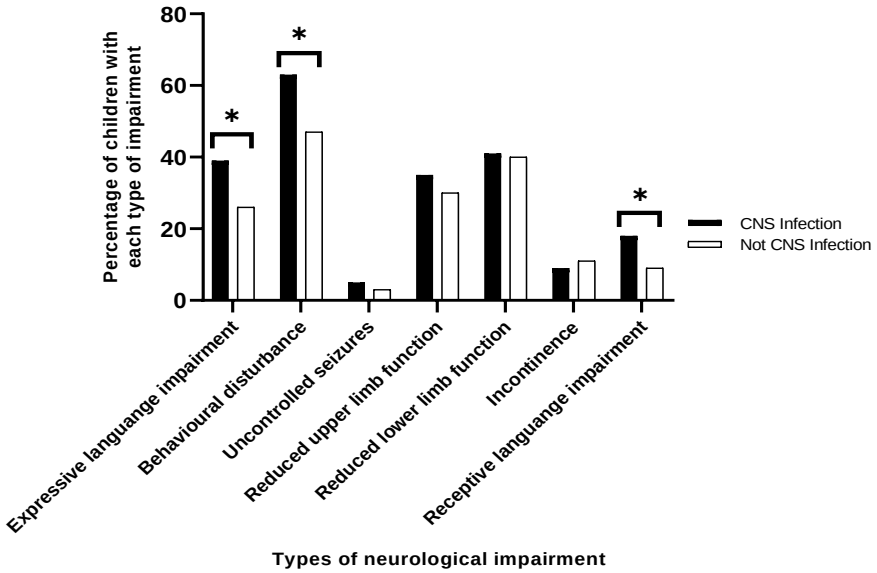
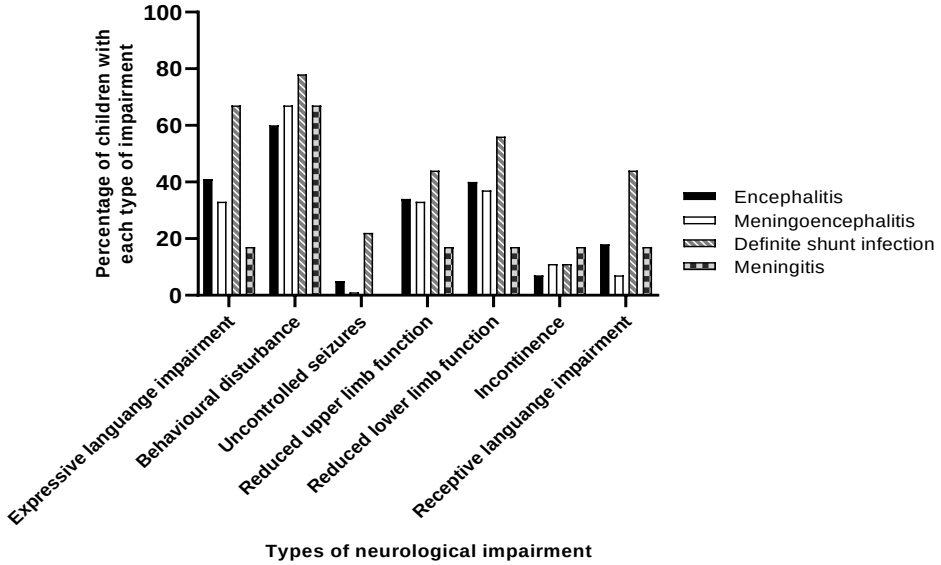


Figure 6.27 Comparison of neurological impairment experienced by children at discharge classified with major sub-types of syndromic CNS infection. The graph shows proportions (%) of different types of neurological impairment experienced by children with encephalitis (n=147), meningoencephalitis (n=27), definite shunt infection (n=9), and meningitis (n=6) who were alive at discharge and had discharge LOS available. *Significant difference (p<0.05) among the proportion of children with the above-mentioned syndromic CNS infection who experienced a specific type of neurological impairment.



Within the syndromic CNS infection group, the proportion of children with definite shunt infection who reported behavioural disturbance (7/9 (78%)), expressive (6/9 (67%)) and receptive (4/9 (44%)) language impairments, reduced lower limb (5/9 (56%)) and upper limb function (4/9 (44%)), and uncontrolled seizures (2/9 (22%)) at the time of hospital discharge was the highest among those with major sub-types of syndromic CNS infection (figure 6.4). Nevertheless, these proportional differences were not statistically significant (p=0.743, p=0.243, p=0.087, p=0.543, p=782, and p=0.203, respectively). The second and third groups commonly reported these neurological sequelae were children with meningoencephalitis and encephalitis.

6.3.2.2 Functional impairment following CNS infection in children at follow up

One-hundred-and-sixty-six of the 236 (70.3%) children with suspected CNS infection who were alive at follow-up had functional impairment. The proportion of children who had functional impairment at follow-up was significantly higher in those with syndromic CNS infection than in those with no CNS infection (122/161 (75.8%) versus 44/75 (58.7%), p=0.007, table 6.7). There were no significant differences in the severity of the impairment between the two groups.

Within the syndromic CNS infection group, children with definite shunt infection (5/6 (83.3%)) and encephalitis (97/120 (80.8%)) were more likely to have functional impairment at follow-up than those with meningoencephalitis (13/24 (56.5%)) and meningitis (2/5 (40.0%), p=0.015, table 6.7). Severe impairment was more commonly reported in children with definite shunt infection (5/6 (83.3%)) than in those with encephalitis (31/120 (25.8%)), meningoencephalitis (6/23 (26.1%)), and meningitis (0/5 (0.0%), p=0.014)).

Table 6.50 Outcome for children with CNS infection at follow up

	Suspected CNS infections	Not CNS infection	Syndromic CNS infection	P value	Sub-types of syndromic CNS infection				P value	Non-TB vs TB CNS infection		P value
					Encephalitis	Meningo-encephalitis	Definite shunt infection	Meningitis		Non-TB CNS infection	TB CNS infection	
No. of children in each group	252	78	174	-	130	24	7	5	-	173	1	
Died post discharge (LOS 1)	16 (6.3%)	3 (9.0%)	13 (7.5%)	0.404	10 (7.7%)	1 (4.2%)	1 (14.3%)	0 (0.0%)	0.764	13 (7.6%)	0 (0%)	1.000
Alive at follow up	236	75	161	-	120	23	6	5	-	160	1	
Any impairment at follow-up (LOS 2,3,4)	166 (70.3%)	44 (58.7%)	122 (75.8%)	**0.007**	97 (80.8%)	13 (56.5%)	5 (83.3%)	2 (40.0%)	**0.015**	122 (76.3%)	0 (0%)	0.242
Severe impairment (LOS 2)	60 (25.4%)	17 (22.7%)	43 (26.7%)	0.507	31 (25.8%)	6 (26.1%)	5 (83.3%)	0 (0.0%)	**0.014**	43 (26.9%)	0 (0%)	1.000
Moderate impairment (LOS 3)	67 (28.4%)	17 (22.7%)	50 (31.1%)	0.183	45 (37.5%)	3 (13.0%)	0 (0.0%)	1 (20.0%)	**0.028**	50 (31.3%)	0 (0%)	1.000
Mild impairment (LOS 4)	39 (16.5%)	10 (13.3%)	29 (18.0%)	0.368	21 (17.5%)	4 (17.4%)	0 (0.0%)	1 (20.0%)	0.852	29 (18.1%)	0 (0%)	1.000
No Impairment (LOS 5)	70 (29.7%)	31 (41.3%)	39 (24.2%)	**0.007**	23 (19.2%)	10 (43.5%)	1 (16.7%)	3 (60.0%)	**0.015**	38 (23.8%)	1 (100%)	0.242

Data presented as number (%) or median (range).

CNS – central nervous system; TB – tuberculous; LOS – Liverpool Outcome Score; vs - versus.

There was a notable difference in the proportion of children with encephalitis (45/120 (37.5%)), meningitis (1/5 (20.0%)), meningoencephalitis (3/23 (13.0%)), and definite shunt infection (0/6 (0.0%)) who experienced moderate impairment (p=0.028, table 6.7). Full recovery at follow-up was more common in children with meningitis (3/5 (60.0%)) and meningoencephalitis (10/23 (43.5%)) than in those with encephalitis (23/120 (19.2%)) and definite shunt infection (1/6 (16.7%), p=0.015).

Among the children with syndromic CNS infection who were followed-up, 122/160 (76.3%) children with non-tuberculous CNS infection had a functional impairment (table 6.7). In contrast, the single child treated for tuberculous CNS infection who was followed-up was fully recovered (LOS 5). Among the 160 children with non-tuberculous CNS infection, 50 (31.3%) had a severe impairment, whilst 43 (26.9%) had a moderate impairment.

Sixteen children with suspected CNS infection died prior to follow-up, 13 of whom had syndromic CNS infection (table 6.7). Taking into account 51 children with suspected CNS infection who died during hospitalisation (table 6.6), a total of 64/355 (18%) children died within 6 months. Looking specifically among children with syndromic CNS infection, the overall mortality within 6 months was 52/247 (21%).

Similar to the neurological impairment experienced by children at the time of discharge, behavioural disturbance and reduced lower limb function were the two most frequent impairments experienced by children at follow-up (figure 6.5). Behavioural disturbance was reported in 78/161 (48%) children with syndromic CNS infection and 27/75 (36%) children with no CNS infection, whilst reduced lower limb function was reported in 60/161 (37%) children with syndromic CNS infection and 21/75 (28%) children with no CNS infection.

Figure 6.28 Comparison of neurological impairment experienced by children at follow-up classified with syndromic CNS infection and non-CNS infection. The graph shows the proportions (%) of different types of neurological impairment experienced by children with suspected CNS infection who were alive at discharge and had follow-up LOS available (n=236). Children were categorised as having syndromic CNS infection (n=161) and non-CNS infection (n=75) based on case definitions described in chapter 3.

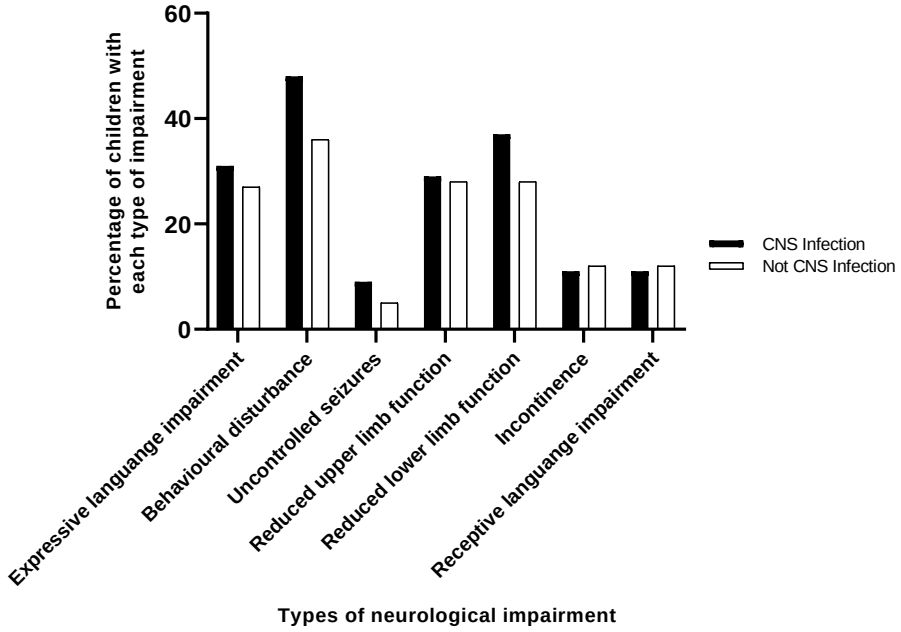
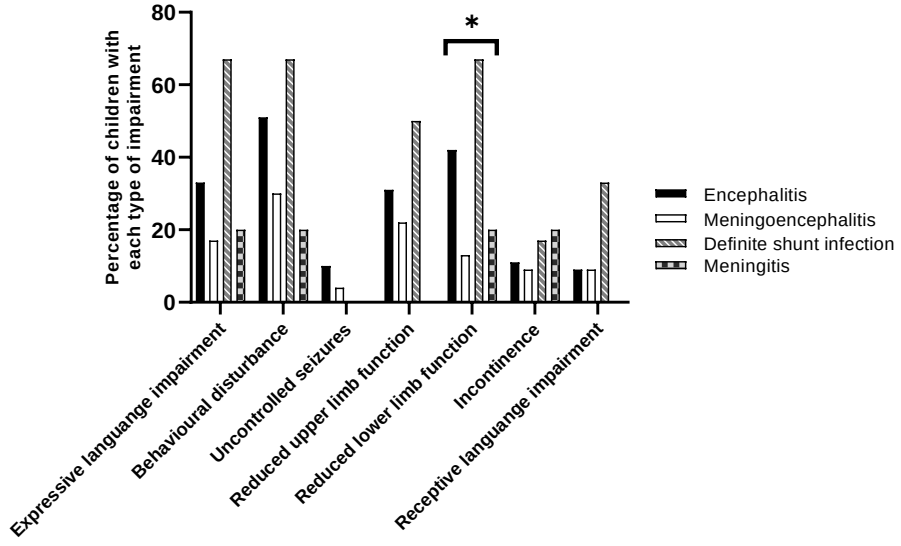


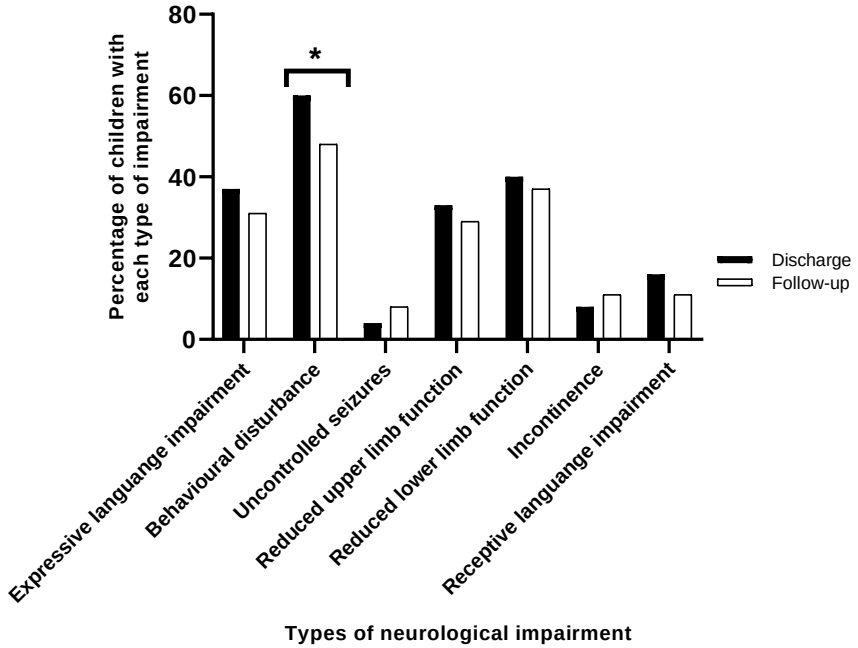
Figure 6.29 Comparison of neurological impairment experienced by children at follow-up classified with major sub-types of syndromic CNS infection. The graph shows the proportions (%) of different types of neurological impairment experienced by children with encephalitis (n=120), meningoencephalitis (n=23), definite shunt infection (n=6), and meningitis (n=5) who were alive at discharge and had follow-up LOS available. *Significant difference ($p<0.05$) among the proportion of children with the above-mentioned syndromic CNS infection who experienced a specific type of neurological impairment.



6.3.2.3 Recovery profile following syndromic CNS infection in children

Among 172 children with syndromic CNS infection who had both discharge and follow-up LOS available, 63 (37%) had a better LOS at follow-up than at discharge, with 29 (17%) improved from having a severe functional impairment (LOS 2) to having a moderate functional impairment or better (LOS 3-5). Of these, 5 (3%) children had a full recovery (LOS 5). Fifty-seven (33%) children had the same LOS at discharge and follow-up. The remaining 51 (30%) children had a worse LOS at follow-up than discharge, of whom 13 (7.6%) had died.

Figure 6.30 Comparison of neurological impairment experienced by children with syndromic CNS infection at discharge and at follow-up. The graph shows the proportions (%) of different types of neurological impairment experienced by children with syndromic CNS infection who were alive at follow-up and had both discharge and follow-up LOS available (n=159). *Significant difference ($p<0.05$) between the proportions of children who experienced a specific type of neurological impairment at discharge and at follow-up.



In children with syndromic CNS infection, most neurological impairments were less frequently reported at follow-up than at hospital discharge. The behavioural disturbance was significantly less common at follow-up compared to at discharge (76/159 (48%) versus 95/159 (60%), p=0.025) (figure 6.7). Uncontrolled seizures were more frequently

reported at follow-up than at discharge; however, the difference was not statistically significant (13/159 (8.2%) versus 6/159 (3.8%), p=0.143) (figure 6.7). The 13 children with uncontrolled seizures at follow-up included a child who continued to have uncontrolled seizures following discharge, 9 who had controlled seizures at the time of discharge, and 3 with no seizures documented during their hospital stay.

6.3.3 Functional impairment and recovery following CNS infection in adults

6.3.3.1 Functional impairment following CNS infection in adults within 2 weeks post-discharge

Fifty-six (62.9%) of the 89 adults with suspected CNS infection assessed within two weeks post-hospital-discharge had functional impairment (GOSE 2-6). The impairment was more common in adults with no CNS infection (9/13 (69.3%)) compared to those with syndromic CNS infection (47/76 (61.8%)); however, this difference was not statistically significant (p=0.760, table 6.8). It should be noted that the mortality at discharge was higher in those with syndromic CNS infection than in those with no CNS infection, although this difference was also not statistically significant (76/168 (45%) versus 9/27 (33%), p=0.247).

Among the adults with syndromic CNS infection, as may be expected, mortality at hospital was higher in patients with meningoencephalitis (59%), encephalitis (49%), and brain abscess (44%) than in those with myelitis (25%) and presumptive cerebral toxoplasmosis (0%) (p=0.002, table 6.8). Two more patients with encephalitis and 2 more with meningoencephalitis were reported to have died within 2 weeks post-discharge. Furthermore, almost 70% of patients with encephalitis and 50% of patients with meningoencephalitis who were alive at the time of hospital discharge continued to have functional impairment within 2 weeks post-discharge, most of whom had severe disability.

Table 6.51 Outcome for adults with CNS infection at discharge

	Suspected CNS infections	Not CNS infection	Syndromic CNS infection	P value	Sub-types of syndromic CNS infection					P value	Non-TB vs TB CNS infection		P value
					Encephalitis	Meningo-encephalitis	Presumptive cerebral toxoplasmosis	Brain abscess	Myelitis		Non-TB CNS infection	TB CNS infection	
No. of adults in each group	195	27	168	-	83	37	12	9	8	-	145	23	-
Died at hospital (GOSE 1)	85 (44%)	9 (33%)	76 (45%)	0.247	41 (49%)	22 (59%)	0 (0%)	4 (44%)	2 (25%)	**0.002**	57 (39%)	19 (83%)	**<0.001**
Alive at discharge	110	18	92	-	42	15	12	5	6	-	88	4	-
Discharge GCS¶	15 (3-15)	15 (5-15)	15 (3-15)	0.793	15 (3-15)	15 (9-15)	15 (6-15)	15 (5-15)	15 (15-15)	0.832	15 (3-15)	15 (15-15)	0.547
Discharge GOSE available‡	89	13	76	-	33	14	9	5	5	-	73	3	-
Died within 2 weeks post-discharge (GOSE 1)	7 (7.9%)	1 (7.7%)	6 (7.9%)	1.000	2 (6.1%)	2 (14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.734	6 (8.2%)	0 (0.0%)	1.000
Any impairment within 2 weeks post-discharge (GOSE 2-6)	56 (62.9%)	9 (69.2%)	47 (61.8%)	0.760	23 (69.7%)	7 (50.0%)	7 (77.8%)	2 (40.0%)	3 (60.0%)	0.472	45 (61.6%)	2 (66.7%)	1.000
Vegetative state (GOSE 2)	1 (1.1%)	0 (0.0%)	1 (1.3%)	1.000	1 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000	1 (1.4%)	0 (0.0%)	1.000
Severe disability (GOSE 3-4)	38 (42.7%)	7 (53.8%)	31 (40.8%)	0.379	15 (45.5%)	4 (28.6%)	6 (66.7%)	0 (0.0%)	2 (40.0%)	0.130	30 (41.1%)	1 (33.3%)	1.000
Moderate disability (GOSE 5-6)	17 (19.1%)	2 (15.4%)	15 (19.7%)	1.000	7 (21.2%)	3 (21.4%)	1 (11.1%)	2 (40.0%)	1 (20.0%)	0.833	14 (19.1%)	1 (33.3%)	0.521
Good recovery (GOSE 7-8)	26 (29.2%)	3 (23.1%)	23 (30.3%)	0.748	8 (24.2%)	5 (35.7%)	2 (22.2%)	3 (60.0%)	2 (40.0%)	0.477	22 (30.1%)	1 (33.3%)	1.000

Data are presented as number (%) or median (range).

‡Discharge GOSE was not available for 21 adults who were alive at discharge (16 with syndromic CNS infection [15 with non-TB CNS infection and 1 with TB CNS infection] and 5 with no CNS infection).

¶Discharge GCS was assessed in adults who were alive at discharge, not available for 5 adults with syndromic CNS infection (all with non-TB CNS infection).

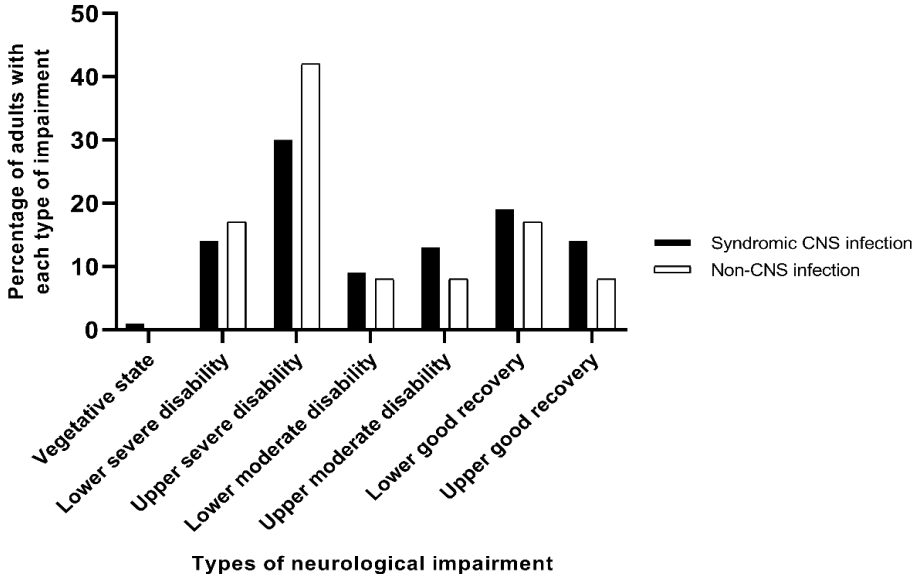
CNS – central nervous system; TB – tuberculous; GCS – Glasgow Comma Scale; GOSE – Glasgow Outcome Scale Extended; vs - versus.

Although none of the patients with presumptive cerebral toxoplasmosis died at the hospital, 7/9 (77.8%) reported functional impairment within 2 weeks post-discharge, 6 (66.7%) of whom experienced severe disability (table 6.8). Similarly, although mortality in adults with myelitis was not as high as in those with meningoencephalitis and encephalitis, 60% of them continued to have functional impairment, mostly with severe disability, within 2 weeks post-discharge. Most adults with brain abscesses who were alive at the time of hospital discharge (3/5 (60%)) had good recovery within 2 weeks post-discharge.

Mortality at the hospital was significantly higher in adults with tuberculous CNS infection compared to those with non-tuberculous CNS infection (19/23 (83%) versus 57/145 (39%), $p<0.001$, table 6.8). Forty-five of 73 (61.6%) adults with non-tuberculous CNS infection and 2/3 adults (66.7%) with tuberculous CNS infection who were assessed within two weeks post-discharge had functional impairment. Most of the adults with non-tuberculous CNS infection (30/73 (41.1%)) had a severe disability, whereas 22/73 (30.1%) had good recovery (table 6.8).

In the adult group, upper-grade severe disability was the most common impairment reported within two weeks post-discharge in both syndromic CNS infection and no CNS infection sub-groups, experienced by 21/70 (30%) and 5/12 (42%) patients, respectively (figure 6.8). The second most frequently reported impairments in the adult group include lower-grade good recovery (reported in 19% of adults with syndromic CNS infection and 17% of adults with no CNS infection) and lower-grade severe disability (reported in 14% of adults with syndromic CNS infection and 17% of adults with no CNS infection).

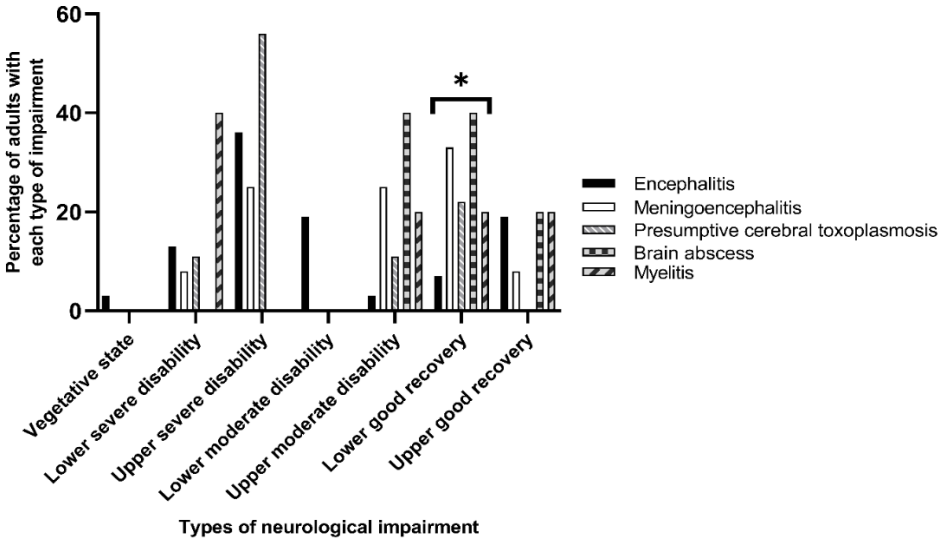
Figure 6.31 Comparison of neurological impairment experienced by adults within 2 weeks post-discharge classified with syndromic CNS infection and non-CNS infection. The graph shows the proportions (%) of different types of neurological impairment experienced by adults with suspected CNS infection who were alive within 2 weeks post-discharge and had discharge GOSE available (n=82). Adults were categorised as having syndromic CNS infection (n=70) and non-CNS infection (n=12) based on case definitions described in chapter 3.



Within 2 weeks post-discharge, severe disability, including the upper-grade and the lower-grade, was more commonly experienced by adults with no CNS infection (figure 6.8). In contrast, good recovery and moderate disability (both including the upper-grade and the lower-grade) were more commonly reported in adults with syndromic CNS infection. Nevertheless, none of these proportional differences was statistically significant.

Within the syndromic CNS infection group, there was a significant difference in the proportion of adults who had lower-grade good recovery within 2 weeks post-discharge, where it was reported in 2/5 (40%) adults with brain abscess, 4/12 (33%) adults with meningoencephalitis, 2/9 (22%) adults with presumptive cerebral toxoplasmosis, 1/5 (20%) adults with myelitis, and 2/31 (7%) adults with encephalitis (p=0.046, figure 6.8).

Figure 6.32 Comparison of neurological impairment experienced by adults within 2 weeks post-discharge classified with major sub-types of syndromic CNS infection. The graph shows the proportions (%) of different types of neurological impairment experienced by adults with encephalitis (n=31), meningoencephalitis (n=12), presumptive cerebral toxoplasmosis (n=9), brain abscess (n=5), and myelitis (n=5) who were alive within 2 weeks post-discharge and had discharge GOSE available. *Significant difference ($p<0.05$) among the proportion of adults with the above-mentioned syndromic CNS infection who experienced a specific type of neurological impairment.



Upper severe disability was the most commonly reported impairment in adults with presumptive cerebral toxoplasmosis (5/9 (56%)) and encephalitis (11/31 (36%)). In contrast, lower severe disability was the most commonly reported neurological problem in adults with myelitis (2/5 (40%)) within 2 weeks post-discharge. Most adults with meningoencephalitis experienced lower-grade good recovery as mentioned above, followed by upper-grade severe disability and upper-grade moderate disability, which was reported in 3/12 (25%) adults each (figure 6.8). The worst impairment, vegetative state, was only experienced by one adult (1/31 (3%)) with encephalitis.

6.3.3.2 Functional impairment following CNS infection in adults at follow-up

Among 68 adults with suspected CNS infection alive at follow-up, 27 (39.7%) had functional impairment. The impairment at follow-up was more common in adults with syndromic CNS infection than in those with no CNS infection (25/59 (42.4%) versus 2/9

(22.2%)), although this difference was not statistically significant (p=0.330, table 6.9). Twelve (20.3%) of the 59 adults with syndromic CNS infection experienced severe disability at follow-up, whereas none of the adults with no CNS infection experienced it.

Among the adults whose functional impairment remained at follow-up, most of the patients with presumptive cerebral toxoplasmosis and a patient with brain abscess had severe disability, whereas most of the patients with encephalitis and meningoencephalitis had moderate disability (table 6.9). None of the patients with myelitis had functional impairment at follow-up. Nevertheless, there was no significant difference in the proportion of adults with encephalitis, meningoencephalitis, presumptive cerebral toxoplasmosis, brain abscess, and myelitis who had severe and moderate disability at follow-up (p=0.169 and p=0.601, respectively).

Within the adult syndromic CNS infection group, 22/56 (39.3%) adults with non-tuberculous CNS infection and 3/3 (100%) adults with tuberculous CNS infection had functional impairment at follow-up. The proportion of patients who had a moderate disability at follow-up was significantly higher in those with tuberculous CNS infection compared to those with non-tuberculous CNS infection (3/3 (100%) versus 10/56 (16.9%), p=0.009, table 6.9).

Five adults with suspected CNS infection died prior to follow-up (table 6.9). Taking into account 85 adults who died at the hospital and 7 adults who died within 2 weeks post-discharge (table 6.8), the overall 6-months mortality in adults with suspected CNS infection was 97/195 (50%). Of the five adults who died before follow-up, they had encephalitis (n=2), brain abscess (n=1), myelitis (n=1), and another type of syndromic CNS infection (n=1) (table 6.9). The overall 6-months mortality in adults with syndromic CNS infection was 87/168 (52%).

Table 6.52 Outcome for adults with CNS infection at follow-up

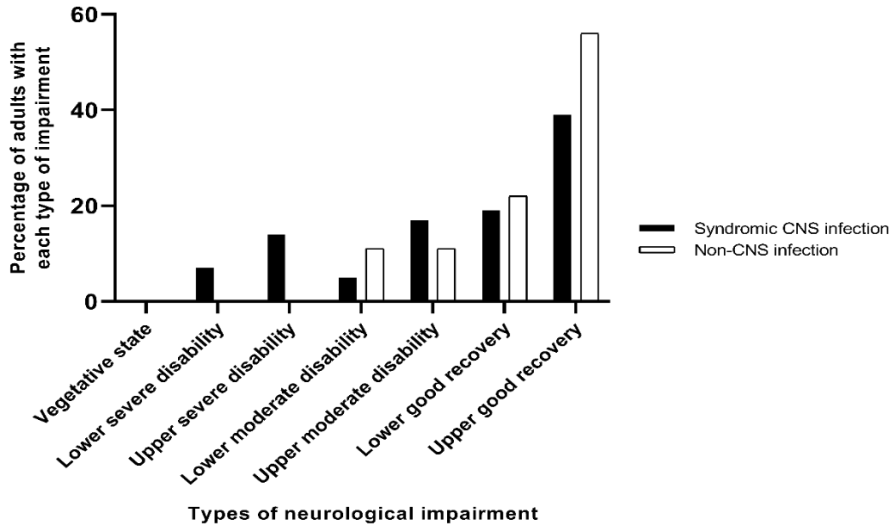
	Suspected CNS infections	Not CNS infection	Syndromic CNS infection	P value	Sub-types of syndromic CNS infection					P value	Non-TB vs TB CNS infection		p value
					Encephalitis	Meningo-encephalitis	Presumptive cerebral toxoplasmosis	Brain abscess	Myelitis		Non-TB CNS infection	TB CNS infection	
No. of adults in each group	73	9	64	-	29	11	9	4	4	-	61	3	
Died after 2 weeks post-discharge (GOSE 1)	5 (6.8%)	0 (0.0%)	5 (7.8%)	1.000	2 (6.9%)	0 (0.0%)	0 (0.0%)	1 (25%)	1 (25%)	0.198	5 (8.2%)	0 (0.0%)	1.000
Alive at follow up	68	9	59	-	27	11	9	3	3	-	56	3	
Any impairment at follow-up (GOSE 2-6)	27 (39.7%)	2 (22.2%)	25 (42.4%)	0.330	12 (44.4%)	5 (45.5%)	5 (55.6%)	1 (33.3%)	0 (0.0%)	0.667	22 (39.3%)	3 (100%)	0.071
Vegetative state (GOSE 2)	0 (0.0%)	0 (0.0%)	0 (0.0%)	N/A	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	N/A	0 (0.0%)	0 (0.0%)	N/A
Severe disability (GOSE 3-4)	12 (17.6%)	0 (0.0%)	12 (20.3%)	0.343	3 (11.1%)	2 (18.2%)	4 (44.4%)	1 (33.3%)	0 (0.0%)	0.169	12 (21.4%)	0 (0.0%)	1.000
Moderate disability (GOSE 5-6)	15 (22.1%)	2 (22.2%)	13 (22.1%)	1.000	9 (33.3%)	3 (27.3%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0.601	10 (16.9%)	3 (100%)	**0.009**
Good recovery (GOSE 7-8)	41 (60.3%)	7 (87.8%)	34 (57.6%)	0.300	15 (55.6%)	6 (54.5%)	4 (44.4%)	2 (66.7%)	3 (100%)	0.670	34 (60.7%)	0 (0.0%)	0.071

Data are presented as number (%) or median (range).

CNS – central nervous system; TB – tuberculous; GCS – Glasgow Comma Scale; GOSE – Glasgow Outcome Scale Extended; vs - versus.

As may be expected, a small proportion of adults with CNS infection continued to have a severe disability at follow-up, where 8/59 (14%) adults reported upper-grade severe disability and 4/59 (7%) adults reported lower-grade severe disability (figure 6.10). In contrast, none of the adults with no CNS infection reported severe disability at follow-up. Ten of 59 (17%) adults with syndromic CNS infection and 1/9 (11%) adults with no CNS infection continued to have upper-grade moderate disability, whereas 3/59 (5%) adults with syndromic CNS infection and 1/9 (11%) adults with no CNS infection reported lower-grade moderate disability. Five of 9 (56%) adults with no CNS infection and 23/59 (39%) adults with syndromic CNS infection had upper-grade good recovery, whilst 2/9 (22%) adults with no CNS infection and 11/59 (19%) adults with syndromic CNS infection had lower-grade good recovery (figure 6.10).

Figure 6.33 Comparison of neurological impairment experienced by adults at follow-up classified with syndromic CNS infection and non-CNS infection. The graph shows the proportions (%) of different types of neurological impairment experienced by adults with suspected CNS infection who were alive at follow-up and had follow-up GOSE available (n=68). Adults were categorised as having syndromic CNS infection (n=59) and non-CNS infection (n=9) based on case definitions described in chapter 3.



Within the syndromic CNS infection group, the highest proportion of adults who reported upper-grade good recovery at the time of follow-up were those with myelitis (2/3 (67%)), followed by those with presumptive cerebral toxoplasmosis (4/9 (44%)) and encephalitis (10/27 (37%)) (figure 6.11). Nevertheless, almost half of the adults with presumptive cerebral toxoplasmosis had severe disability, where 2/9 (22%) reported lower-grade severe disability and 2/9 (22%) reported upper-grade severe disability. Other syndromic CNS infection sub-groups who had upper-grade severe disability at follow-up included 1/3 (33%) adults with brain abscess, 2/11 (18%) with meningoencephalitis, and 2/27 (7%) with encephalitis (figure 6.11). Additionally, an adult (1/27 (4%)) with encephalitis had lower-grade severe disability at follow-up.

Figure 6.34 Comparison of neurological impairment experienced by adults at follow-up classified with major sub-types of syndromic CNS infection. The graph shows the proportions (%) of different types of neurological impairment experienced by adults with encephalitis (n=27), meningoencephalitis (n=11), presumptive cerebral toxoplasmosis (n=9), brain abscess (n=3), and myelitis (n=3) who were alive at follow-up and had follow-up GOSE available.

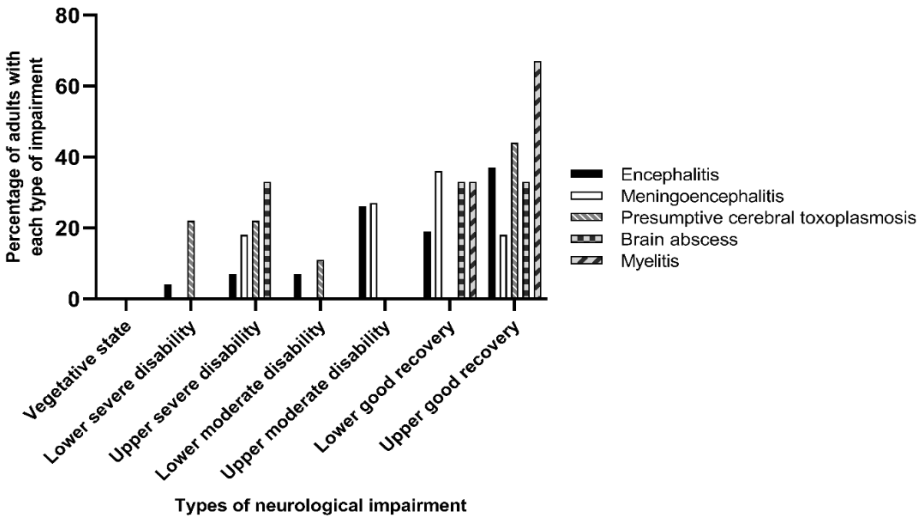
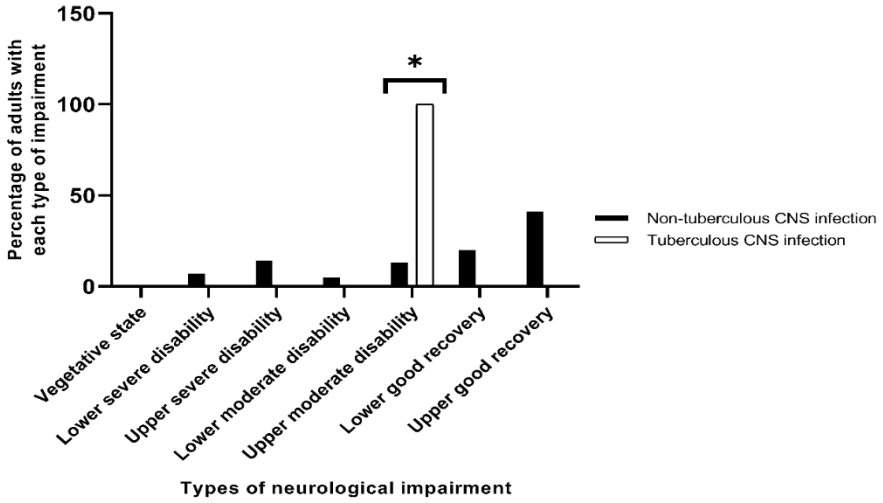


Figure 6.35 Comparison of neurological impairment experienced by adults with syndromic CNS infection at follow-up classified with non-tuberculous and tuberculous CNS infection. The graph shows the proportions (%) of different types of neurological impairment experienced by adults with non-tuberculous CNS infection (n=56) and tuberculous CNS infection (n=3) who were alive at follow-up and had follow-up GOSE available. *Significant difference ($p<0.05$) between the proportions of adults with non-tuberculous and tuberculous CNS infection who experienced a specific type of neurological impairment.



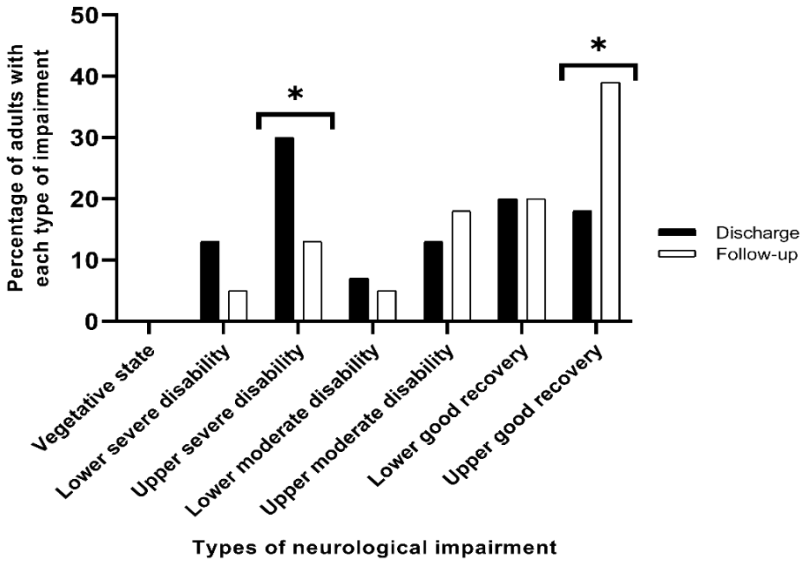
Within the syndromic CNS infection group, all adults (3/3 (100%)) with tuberculous CNS infection reported upper-grade moderate disability at follow-up. This proportion is much higher than that reported by adults with non-tuberculous CNS infection (7/56 (13%), p=0.004, figure 6.12). The majority of adults with non-tuberculous CNS infection (23/56 (41%)) had upper-grade good recovery, followed by lower-grade good recovery (11/56 (20%)). Eight (14%) and four (7%) of 56 adults with non-tuberculous CNS infection, however, continued to have an upper-grade and lower-grade severe disability (figure 6.12).

6.3.3.3 Recovery following syndromic CNS infection in adults

Thirty-two of 61 (52%) adults with syndromic CNS infection had a better GOSE at follow-up than at discharge, with 9 (15%) adults moving from the severe disability category (GOSE 3-4) into the moderate disability category (GOSE 5-6) and 4 (2.3%) adults having a good recovery (GOSE 7-8). Twelve (20%) adults had the same GOSE at discharge and

follow-up. Nine (15%) adults had a worse GOSE at follow-up than at discharge, 5 (8.2%) of whom had died.

Figure 6.36 Comparison of neurological impairment experienced by adults with syndromic CNS infection at discharge and at follow-up. The graph shows the proportions (%) of different types of neurological impairment experienced by adults with syndromic CNS infection who were alive at follow-up and had both discharge and follow-up GOSE available (n=56). *Significant difference ($p<0.05$) between proportions of adults who experienced a specific type of neurological impairment at discharge and at follow-up.



In adults with syndromic CNS infection, most types of disability were less common at follow-up compared to hospital discharge. The proportion of adults with syndromic CNS infection who experienced upper-grade severe disability at follow-up decreased significantly compared to those who experienced the same level of disability at discharge (7/56 (13%) versus 17/56 (30%), p=0.021, figure 6.13). Furthermore, the proportion of adults with syndromic CNS infection who reported upper-grade good recovery at follow-up increased significantly compared to those who reported the same at discharge (22/56 (39%) versus 10/56 (18%), p=0.002). The only level of disability which was increasingly reported at follow-up compared to discharge was upper-grade moderate disability.

6.4 Discussion

6.4.1 Functional outcome following CNS infection in children

I have shown a substantial burden of functional impairment among children admitted to Dr Sardjito Hospital with suspected CNS infection. At the time of hospital discharge, there was no significant difference in the proportion of functional impairment experienced by children with syndromic CNS infection compared to that experienced by those with no CNS infection (table 6.6). This finding suggests that all children can have a reduction in their ability to perform daily activities following hospital admission. This has been reported before in children leaving paediatric intensive care unit.[436-438] This finding also suggests that the children might have had underlying impairments prior to their hospital admission. This, in part, may explain the higher rate of impairments, including behavioural problems, expressive and receptive language impairments, and reduced lower and upper limb function, reported among children with shunt infection (figure 6.4). Nine of 10 (90%) children with shunt infection in the present study had hydrocephalus as the underlying condition for shunt insertion, and hydrocephalus is associated with cerebral palsy.[439] The above-mentioned impairments, particularly the reduction in lower and upper limbs function, are characteristic of cerebral palsy.[440] Therefore, it is possible that the functional impairments at discharge were not only due to the current illness but also affected by the pre-existing neurological condition.

Another explanation for why there was no notable difference in the proportion of functional impairment at hospital discharge between children with and without CNS infection is that non-infective patients may also have neurological sequelae. In this study, the two most common diagnoses of children with no CNS infection who had functional impairment at discharge were complex febrile seizures and septic encephalopathy.

Children with complex febrile seizures may still have attacks at the time of hospital discharge. Moreover, complex febrile seizures may be recurrent and are associated with an increased risk of epilepsy.[441, 442] Encephalopathy is a common feature in patients with severe sepsis. A previous study on the outcome of severe sepsis has shown that 34% of children with severe sepsis had declined function within 28 days following hospital discharge, and 18% had a poor functional outcome.[443]

Further analysis showed that the proportion of children who had reduced lower limb function at hospital discharge was similar between those with no CNS infection and those with syndromic CNS infection (40% versus 41%, figure 6.3). Moreover, the proportion of children with no CNS infection who had reduced upper limb function was not significantly different from that of children with syndromic CNS infection (figure 6.3). In the LOS questionnaire, lower limb function was assessed by observing the child's ability to sit, stand up and walk compared to his/her ability to do so prior to the current illness or other children's ability at the same age. However, in the present study, discharge LOS was mainly assessed in the ward, and direct observation by the interviewer was not always possible. In such cases, the assessment was made based on the parent's observation on the day of discharge but before the LOS assessment/interview. Therefore, the assessment was mainly based on the parent's perceptions.

The proportion of functional impairment at follow-up was significantly higher in children with syndromic CNS infection than in those with no CNS infection (table 6.7). Moreover, children with no CNS infections were more likely to have full recovery (LOS 5) at the follow-up time than those with syndromic CNS infection (table 6.7). These findings suggest children with CNS infection may experience neurological sequelae for longer than children with other conditions mimicking CNS infection. Furthermore, there was a reduction in the proportion of functional impairment reported at the follow-up time compared to that

reported at hospital discharge among both syndromic CNS infection and no CNS infection groups. This finding, in addition to the previous results, suggests parents are reasonably accurate in reporting their child's function despite the subjective nature of the LOS assessment.

Almost 88% of children with syndromic CNS infection had functional impairment at hospital discharge, and almost 76% continued to have functional impairment at follow-up. These proportions were much higher than that reported from previous studies in Nepal and Australia, where only 48.5% and 45.8% of children with encephalitis had functional impairment at follow-up.[420, 421] The proportion of functional impairment in the present study was also higher than that reported by previous studies from Indonesia, Malaysia, and Vietnam, where functional impairment was found in 60%, 58%, and 58% of children with Japanese encephalitis at the time of follow-up.[9, 429, 430] Interestingly, Hills *et al*. reported a higher proportion of long-term functional impairment following childhood Japanese encephalitis in Cambodia, where 95% of the children remained having the impairment 4-20 months after the illness.[430] All studies mentioned above used the LOS questionnaire to assess patient's functional outcomes except for the Malaysian one.

The finding of behavioural disturbance as the most common type of impairment reported in children is consistent with a previous study of outcomes for Japanese encephalitis among Indonesian, Vietnamese, Cambodian and Indian children.[9, 430, 444] Additionally, Griffiths *et al*. reported from a similar study in Nepal that expressive language impairment and reduced lower limb function were the two most common types of neurological impairment reported at discharge in children with acute encephalitis syndrome, whereas behavioural disturbance and expressive language impairment were the two most common types of neurological impairment reported at follow-up.[421] Other types of long-term neurological impairment commonly reported in children with encephalitis include

seizure or epilepsy, learning problems, cognitive impairment, and developmental delay.[420, 427, 430, 431, 445, 446]

Among children with syndromic CNS infection, expressive and receptive language impairments were reported in 39% and 18% of children at discharge as well as 31.1% and 10.6% of children at follow-up. Both expressive and receptive language functions are associated with hearing function. Thus, hearing impairment will result in both language functions, particularly the receptive one. Hearing impairment may also affect the child's behaviour, and behavioural disturbance was the most common impairment reported in children at both discharge and follow-up. This may, in part, explain the poor outcome in children.

Among the children with syndromic CNS infection, functional outcome improved at follow-up in 37%, deteriorated in 30%, and was similar in 33% of the patients. The proportion of children who had an improved outcome in the present study is similar to the Malaysian cohort of Japanese encephalitis (37%), although a worse outcome at follow-up was only reported in 17% of the children from the latter study.[429] In contrast, a previous study in Nepal showed that almost 50% of children with acute encephalitis syndrome were reported to have a better functional outcome at follow-up, and only 20% of the children had a worse outcome at follow-up.[421]

Children with tuberculous CNS infection had significantly higher mortality at discharge compared to those with non-tuberculous CNS infection (75% versus 13.8%, $p<0.001$, table 6.6). One of the two (50%) children with tuberculous CNS infection had a severe neurological impairment at discharge. This child received anti-tuberculosis treatment at the hospital. Unfortunately, her family could not be contacted at the time of follow-up and therefore did not have a follow-up outcome available. Another child with tuberculous

CNS infection, subject P-003 - who had fulfilled the clinical criteria for definite TBM, demonstrated full recovery at discharge. She received anti-tuberculosis drugs, and an assessment 6 months post-discharge showed no neurological sequelae.

The median Glasgow Coma Scale (GCS) at the time of discharge among children with suspected CNS infection who were followed-up was 15 (range 6-15) (table 6.2), suggesting they are in a fully conscious/alert condition. However, the LOS assessment showed that most of them had functional impairment at discharge, and some remained impaired at community follow-up. This finding indicates GCS is less useful in identifying children with functional impairment.

6.4.2 Functional outcome following CNS infection in adults

Similar to the paediatric group, the burden of functional impairment in adults admitted to the hospital with suspected CNS infection was high. Almost 62% of adults with syndromic CNS infection had neurological impairment at hospital discharge, of whom over 42% remained having the impairment at follow-up (tables 6.8 and 6.9). These proportions are lower than those reported in children described above.

There was no difference in the proportion of functional impairment experienced by adults with syndromic CNS infection compared to those with no CNS infection (61.8% versus 69.2%, p=0.760, table 6.8). Similar to the paediatric patients, this finding suggests that adults can have a decrease in their confidence or abilities to perform daily activities following hospitalisation. Previous studies have reported similar results where patients with critical illness experience functional and cognitive impairments as well as depression.[447-450] Furthermore, this finding suggests that the adult patients might have pre-existing conditions that affected their function before hospital admission. Unfortunately, the baseline GOSE score was not assessed in this study. This finding

suggests that adults with no CNS infection can have neurological sequelae. In this study, the diagnoses of the patients with no CNS infection who had impairment at discharge included septic encephalopathy, spinal metastasis, and brain tumour; all of which have been reported to cause neurological sequelae.[449-452]

In the present study, functional improvement following syndromic CNS infection was better in adults than in children, where 52% of adults had improved functional outcomes at follow-up. The proportion of adults with worse functional outcomes at follow-up was only half of that reported in children. It should be noted, however, that the proportion of patients with syndromic CNS infection who were lost to follow-up was higher in adults than in children (22/86 (26%) versus 34/208 (16%), sections 6.3.1.1 and 6.3.1.2), and the outcome in these patients was unknown. It is possible that adults who were lost to follow-up had worse outcomes, thus less likely to participate in follow-up interviews. The overall mortality at 6 months was much higher in adults than in children (52% versus 21%, section 6.3.2.2 and 6.3.3.2). This finding, along with the former result, indicates that adults who presented at the hospital with the severe conditions were more likely to die at the hospital, whilst the paediatric group were more likely to survive at the hospital but suffer from long-term neurological sequelae. The overall adult mortality at 6 months in the present study is similar to that reported by a previous study of outcomes for CNS infection in Jakarta, Indonesia, where 57% of adults died within six months.[6]

Most adults with syndromic CNS infection and those with no CNS infection reported improved outcomes at follow-up compared to discharge (figures 6.8 and 6.10). This finding suggests that although the GOSE assessment may be subjective by nature, the adult patients can reasonably report their function accurately.

Within the adult syndromic CNS infection group, patients with tuberculous CNS infection had significantly higher mortality at discharge than those with non-tuberculous CNS infection (83% versus 39%, $p<0.001$, table 6.8). Of the four adults with tuberculous CNS infection who were alive at hospital discharge, one had severe disability (subject N-022), one had moderate disability (subject N-131), one had good recovery (subject N-149) and one did not have discharge GOSE available (subject N-029) (table 6.8). Subjects N-022, N-131, and N-149 received anti-tuberculosis treatment. Follow-up assessment showed subject N-022 had improved outcome from lower severe disability (GOSE 3) to upper moderate disability (GOSE 6), whilst subject N-131 had improved outcome from lower moderate disability (GOSE 5) to upper moderate disability (GOSE 6). Interestingly, subject N-141 had a deteriorating outcome from upper good recovery (GOSE 8) at discharge to upper moderate disability (GOSE 6). No further information was available to explain why the latter subject had a worse functional outcome at follow-up.

Most adults with suspected CNS infection who were followed-up were fully conscious when discharged from the hospital (median GCS 15 [range 5-15], table 6.4). The GOSE assessment, however, showed that most of them had neurological impairment at discharge, which could have persisted at the follow-up time. Similar to the paediatric cases, this finding suggests the GOSE is more valuable than the GCS in identifying neurological impairment.

6.4.3 Limitations of the outcome assessment

There are some limitations on the outcome assessment using the LOS and the GOSE questionnaires as follows:

1) The questions in the LOS questionnaire are largely based on the carer's opinion. As such, responses may be influenced by the carer's perception of their child's ability.

Some of the assessments in the LOS, including the child's ability to sit, stand up, walk, put both hands on the head and pick up a small-sized item, could be performed via observation by the interviewer. As described previously, such observations could not always be undertaken in the ward on the day of hospital discharge. In such cases, the assessment was based on the parent's observation.

2) All of the follow-up assessments were made by telephone interview. Again, scores reported by the carers might be influenced by parental/carer anxiety and/or increased awareness around changes in the child's function (i.e. ascertainment bias). Although other studies had successfully used the LOS questionnaire by telephone interview,[420, 421] the results would be more accurate if the assessment was made by direct observation/examination from the interviewer.
3) The GOSE questionnaire was again completed by telephone interview and depended on patient/carer reports. Again, the assessment could have been improved by asking the patients to do the required tasks while being observed by the interviewer.
4) The follow-up assessment was not done at the same follow-up interval. Ideally, all patients should be followed-up at the same time interval (i.e. six months). Due to time constraints, it was not possible to do so. Nevertheless, only four children and eight adults had a follow-up interval under six months and thus should have a minimal impact on the results.

6.4.4 Potential implications for patient management

I have shown 21% of children and 52% of adults with syndromic CNS infection died within six months. Additionally, almost 76% of children and over 42% of adults suffered long-term neurological sequelae. Among adults, the frequency and severity of neurological sequelae were higher among patients with tuberculous (TB) CNS infection.

These findings suggest opportunities to improve the care pathway for the long-term benefit of patients with CNS infection should be sought.

Although rehabilitation is available at Dr Sardjito Hospital, there are no specific guidelines for patients to be referred to the rehabilitation programme following CNS infection. The current practice at Dr Sardjito includes offering clinical evaluation at 5-7 days post-hospital discharge by the acute care team. The neurological sequelae are identified by the acute care team. The frequency, interval, and length of further follow-up are at the discretion of the team. For instance, patients diagnosed with seizures post-discharge are typically prescribed anti-epileptic drug(s) and can be monitored by the team until 3 years free-from-seizures. Patients with motor abnormalities can be referred to the physical rehabilitation programme. This programme typically involves local hospital attendance 2-3 times weekly. The programme includes passive and active exercises depending on the abnormality. Children with CNS infection are routinely screened for hearing impairment using brainstem evoked response audiometry (BERA) one month after hospital discharge.

The main type of neurological sequelae identified in children was behavioural disturbance. Children with this problem are offered follow-up with the occupational therapy department at Dr Sardjito. Additionally, there is a child psychology clinic as a part of paediatric outpatient care. Follow-up and rehabilitation programmes are all covered by national insurance. Data from my earlier chapters showed children underwent LP within a median interval of 1 day from admission to Dr Sardjito Hospital. Similarly, most children (>80%) received appropriate first-line treatment at our hospital. Nevertheless, the data from this chapter demonstrates child's long-term outcome remained unfavourable.

As stated previously, the time to presentation to our hospital was delayed (median 5 days and 14 days from symptom onset among children and adults, respectively), with the

majority of patients having been reviewed at other medical centres before attending our hospital.

Delayed presentation to our hospital may have influenced poor patient outcome in children and adults.

Among adults, the outcome was worse among patients with TB. The majority of TB CNS infection patients were not diagnosed by routine hospital testing and were often prescribed inappropriate treatment at our hospital. Consequently, as well as delayed presentation, lack of aetiological diagnosis, and inappropriate acute treatment may also have influenced poor outcome among adults.

In addition, although rehabilitation is available at the hospital, there are no specific guidelines about which patients should be referred to the rehabilitation programme following CNS infection, despite the availability of such guidelines for resource poor settings.[453] Some rehabilitation programmes (such as physical rehabilitation) request patients visit the hospital frequently. Regular attendance may be challenging for patients/families living far away and/or from low socio-economic backgrounds.

Studies on the long-term outcome following CNS infection, particularly in Indonesia, are limited. I hope dissemination/publication of the results from my thesis will help improve awareness around the long-term impact of CNS infection on children and adults, promotion of post-hospital follow-up, and consideration of referral to rehabilitation programmes for those with ongoing neurological sequelae.

6.5 Conclusion

Patients with CNS infection who were admitted to Dr Sardjito Hospital during the study period had high mortality and morbidity, not only at the time of hospital discharge but

also within six months post-discharge. The proportion of patients who died at the hospital was significantly higher in those with tuberculous CNS infection than in those with non-tuberculous CNS infection among both children (75% versus 13.8%, $p<0.001$) and adults (83% versus 39%, $p<0.001$). These findings offer information to the treating team that the management for these patients remains sub-optimal and needs improvement. A strict implementation of the recent guidelines for the diagnosis and management of CNS infection should be attempted. Systematic CSF testing, particularly the *M. tuberculosis* PCR assay, should be introduced as routine testing for patients with CNS infection in the study setting. Regular follow-up should be strongly considered to identify patients with neurological sequelae following CNS infection.

Chapter 7 – Discussion

7.1 Central nervous system infections in Yogyakarta

This study is the first cohort in Yogyakarta and the largest one in Indonesia, undertaking a broad assessment of both paediatric (aged ≥1 month to ≤18 years) and adult (aged >18 years) patients with central nervous system (CNS) infections. Patients with clinically suspected CNS infection admitted to Dr Sardjito Hospital, a large tertiary referral hospital in Yogyakarta, from February 2015 – January 2018 were recruited into the study. I reviewed the clinical presentation, laboratory features, neuroimaging results, and clinical management of the patients. I then introduced systematic cerebrospinal fluid (CSF) testing, including pathogen-specific polymerase chain reaction (PCR), antigen and antibody tests, into the local laboratory to supplement the standard hospital diagnostic tests. Target pathogens for the PCR assays included enterovirus, herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2), cytomegalovirus (CMV), varicella zoster virus (VZV), *Mycobacterium tuberculosis, Streptococcus pneumoniae, Streptococcus suis, Haemophilus influenzae, Neisseria meningitidis, Klebsiella pneumoniae, Salmonella* species*, Escherichia coli,* and *Streptococcus agalactiae*. Antigen/antibody testing was conducted for detection of dengue virus (DENV), Japanese encephalitis virus (JEV), *Orientia tsutsugamushi,* and *Cryptococcus* infections. Additionally, I assessed the patient's functional outcome at hospital discharge and at follow-up (ranging from 1-6 months). In chapter 3, patient recruitment, syndromic classification, clinical features and management are discussed. Pathogen-specific PCR assays were applied to patient samples and analysed (chapter 4). Following this, antibody testing was performed (chapter 5). Patient's short- and long-term outcomes were analysed and discussed in chapter 6.

In chapter 3, 355 children and 195 adults with clinically suspected CNS infection were recruited, and syndromic case definitions were retrospectively applied. This identified 247 (70%) children and 168 (86%) adults with syndromic CNS infection, whereas the remaining were classified as not CNS infection. The two most common types of syndromic CNS infection in both age groups were encephalitis and meningoencephalitis, accounting for 70.8% and 17.4% of paediatric and 49.4% and 22% of adult cases, respectively. Other syndromic CNS infection types in children included definite shunt infection (4%), meningitis (2.8%), brain abscess (2.4%), unclassified CNS infection (1.6%), and encephalomyelitis (0.8%). In adults, they were presumptive cerebral toxoplasmosis (7.1%), brain abscess (5.3%), myelitis (4.8%), encephalomyelitis and unclassified CNS infection (4.2% each), as well as meningitis (3%).

Patients may have been more commonly classified as encephalitis because the case definition for encephalitis applied in this study did not incorporate cerebrospinal fluid (CSF) pleocytosis (chapter 3, table 3.1). Encephalitis is often confused with encephalopathy, a state of altered mental status, including confusion, changing behaviour, disorientation, or other cognitive disturbances, with or without brain inflammation. There are multiple causes of encephalopathy, including infection, metabolic, hypoxia/ischemia, hypertension, toxins, and head injury. One frequent cause of infection-associated encephalopathy is sepsis. Septic encephalopathy cases often have electroencephalography (EEG) abnormalities and/or neuroimaging changes CSF pleocytosis or a pathogen detected in the CSF.[454] Therefore, distinguishing between septic encephalopathy and encephalitis is a challenge. It is possible that some of the cases defined as encephalitis in this study were, in fact, encephalopathy cases.

Pathogens were routinely identified using hospital standard testing which incorporated Gram stain, bacterial culture, fungal culture (only if fungi were identified from Gram stain

or if requested by the clinicians), Ziehl-Neelsen stain and Indian ink stain. Antibody tests for *Toxoplasma gondii*, rubella virus, CMV, HSV-1, HSV-2, DENV, *Salmonella,* and *Leptospira* were performed in serum but not in CSF samples and usually done based on the clinician's request. GeneXpert MTB/RIF was occasionally performed on CSF following the clinician's request. Among children with syndromic CNS infection, a causative pathogen was detected in 26/247 (11%) of the cases. These included 10/10 (100%) children with definite shunt infection, 8/43 (19%) children with meningoencephalitis, 1/7 (14.3%) children with meningitis, 1/6 (16.7%) children with brain abscess and 6/175 (3.4%) children with encephalitis. The most common causative pathogen identified by the standard hospital testing in children was *Escherichia coli* (n=4), followed by *Salmonella* species, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, and unspecified Gram-negative bacilli (n=2 each) [chapter 3, table 3.3]. Pathogen detection was much lower in adults, where only 10/168 (6%) adults with syndromic CNS infection had a causative pathogen detected by the standard hospital testing. These included 1/7 (14.3) adults with unclassified CNS infection, 1/9 (11.1%) adults with brain abscess, 3/37 (8.1%) adults with meningoencephalitis, and 5/83 (6.0%) adults with encephalitis. *Acinetobacter baumanii* and *Klebsiella pneumoniae* were the two most common aetiological agents detected in adults (n=2 each) [chapter 3, table 3.4]. Other pathogens that were identified in adults included *S. pneumoniae, Cryptococcus neoformans*, *Pseudomonas* species (unspecified), *Shigella* group (unspecified), *Staphylococcus hominis*, and *Plasmodium falciparum* (n=1 each).

In chapter 4, the systematic use of 'in-house' pathogen-specific PCR assays resulted in increased pathogen detection among both adults and children with suspected CNS infection, particularly among those with non-neurosurgical CNS infection. A marked increase in the detection of *M. tuberculosis* was achieved in this study, from one case to

eight cases in children and from zero case to 23 cases in adults. It was the most common pathogen identified by PCR in both children and adults. The other most common pathogens identified by PCR in children were *E. coli* (n=6), CMV (n=4), *S. pneumoniae* (n=3), and *S. agalactiae* (n=2); whereas in adults included *S. pneumoniae* (n=3) and HSV-2 (n=2). There was no increase in pathogen detection among neurosurgical CNS infection cases. This is not surprising, given the pathogen-specific PCR assays I applied were not designed for this group.

In chapter 5, I have shown that systematic antibody and antigen tests increased pathogen detection among children and adults. I identified new dengue, scrub typhus, and Cryptococcus cases among CNS infection patients. The two most common aetiology of CNS infection identified by antibody testing in children were dengue virus (DENV) and *Orientia tsutsugamushi* (n=4 each) [chapter 5, section 5.3.6.1]. In adults, both *Cryptococcus* and *O. tsutsugamushi* were the two most common CNS infection causes identified by the antibody and antigen tests in adults (n=3 each), followed by DENV (n=2) [chapter 5, section 5.3.6.2]. The number of *O. tsutsugamushi* cases accounted for here are those with no other pathogen identified in CSF, thus fitting into the case definition of presumptive scrub typhus CNS infection cases (chapter 3, table 3.1).

This was the first time dengue IgM was successfully detected in the CSF, and thereby the first dengue CNS infection was identified in both children and adults in Dr Sardjito Hospital. In this study, the dengue IgM enzyme-linked immunosorbent assay (ELISA) test had a slightly better agreement (when dengue PRNT was used as the gold standard) than the dengue rapid immunochromatographic test, which was used as the standard DENV testing at the local hospital (44% versus 40%) [chapter 5, section 5.3.3.1]. However, both exhibited a very high false positive rate compared to PRNT (>50%). Both tests gave results

that were inconsistent with the dengue plaque reduction neutralisation test (PRNT) results.

Therefore, a positive dengue rapid or dengue IgM ELISA result should be interpreted with caution and ideally confirmed by PRNT assay. However, the latter assay is complex to perform and less likely to be implemented in a hospital setting.

This is the first time scrub typhus has been shown to be a potential cause of CNS infection in Yogyakarta and the surrounding areas, and to a broader extent, in Indonesia. Scrub typhus is rarely studied in the country. As the kit requires a regional cut-off reference which is unavailable in Indonesia, I used an alternative approach in determining the cut-off value. Interpretation of the kit's sensitivity and specificity and the accuracy of the cut-off was not possible due to the lack of a reference test for scrub typhus in Indonesia.

In terms of *Cryptococcus* detection, I have shown that the cryptococcal antigen (CrAg) lateral flow assay was more sensitive than CSF fungal culture as it detected two additional cases which tested negative for *Cryptococcus* by culture. Moreover, CrAg testing accurately detected a case that tested positive for *Cryptococcus* by CSF culture and Indian ink testing.

Finally, in chapter 6, I observed that patients with suspected CNS infection experience high morbidity and mortality, either at hospital discharge or in the community (i.e. within 6 months of follow-up). The overall 6-months mortality in children and adults with suspected CNS infection was 18% and 50%, respectively (sections 6.3.2.2 and 6.3.3.2). Interestingly, a high proportion of suspected CNS infection patients, including those later categorised as non-infectious cases, reported a reduction in their ability to carry out routine daily activities (expected for their age) at hospital discharge (based on Liverpool Outcome Score [LOS] or Glasgow Outcome Scale Extended [GOSE]). This was reported in

88.0% of children and 61.8% of adults with syndromic CNS infection, as well as 81.7% of children and 69.2% of adults with no CNS infection (chapter 6, tables 6.6 and 6.8). However, at community follow-up, a higher proportion of patients classified with syndromic CNS infection reported problems compared to those categorised as non-infectious (75.8% versus 58.7% in children [chapter 6, table 6.7 and 42.4% versus 22.2% in adults [chapter 6, table 6.9]). The former finding suggests all patients can experience a reduction in their confidence or ability to carry out daily tasks following hospital admission. The latter finding suggests recovery of these abilities is less likely among those who have suffered CNS infection. This may reflect brain damage during the infective process.

Comparing outcomes among patients infected by different pathogens, the mortality in those with tuberculous CNS infection was significantly higher than in those with non-tuberculous CNS infection among both children (75.0% versus 13.8%, p=<0.001) [chapter 6, table 6.6] and adults (83% versus 39%, p<0.001) [chapter 6, table 6.8]. As *M. tuberculosis* was the most common pathogen identified in this study, improved testing for this pathogen would help guide clinicians to give appropriate treatment promptly and, therefore, might improve patient outcomes.

Patients with shunt infection reported low LOS scores (poor ability to undertake daily tasks). Families in this syndromic group often reported problems with lower limb function. This may indicate a continuation of problems of cerebral palsy or possibly worsening of these problems following shunt infection.

Global comparison to another Southeast Asia country

Only a few studies investigate a broad spectrum of CNS infections.[3, 6, 455] Among these, the Vietnam study by Trung *et al.*[3] is the most similar to this study as both cover CNS infections

among children and adults. We found a similar proportion of patients were classified as having no CNS infection among those with suspected CNS infection (30% versus 27% among children and 14% versus 19% among adults, respectively). The median age of patients with syndromic CNS infection in this study was younger than in the Vietnam study (1.7 versus 4 years in children and 35 versus 38 years in adults). Among the patients whose human immunodeficiency virus (HIV) status was known, the proportion of HIV-positive cases in this study was much higher than in the Vietnam study (4/8 (50%) versus 0/50 (0%) in children and 66/148 (45%) versus 10/498 (2%) in adults, respectively).

In this study, *M. tuberculosis* was the most common causative pathogen in both children and adults (described in chapter 4, section 4.3.4); whereas in the Vietnam study, JEV and *S. suis* were the most common causative pathogens in children and adults, respectively. Perhaps unsurprisingly, no cases of JEV or *S. suis* were detected in this study which is most likely because the majority of the population in this study are Muslims who do not consume pork or farm pigs. Pigs are an important host for both JEV and *S. suis*.

In terms of the outcome at discharge, mortality was remarkably higher in this study than in the Vietnam study (16% versus 7% in children and 45% versus 12% in adults, respectively). This study has the added value that patients were followed up within 6 months post-discharge, and their functional outcome was assessed, unlike the Vietnam study (chapter 6).

7.2 Clinical policy and research implications

7.2.1 Clinical implications

The introduction of pathogen-specific PCR and antibody/antigen assays increased pathogen detection among clinical samples among suspected CNS infection patients

attending Dr Sardjito Hospital by 9% (from 11% to 20%) in children and by 21% (from 6% to 27%) in adults.

However, whether increased detection could translate into improved care is less clear. My study has observed some key challenges to improving clinical care. These are:

1) Late patient presentation to Dr Sardjito hospital, particularly among adults

   The median duration of symptoms prior to admission at Dr Sardjito Hospital for children or adults with suspected CNS infection was 5 and 14 days, respectively. Therefore, even if diagnostic testing occurred on the day of arrival at our hospital, patients, particularly adults, were already relatively far into their illness course. Studies have repeatedly shown the later appropriate CNS infection treatment is started, the less effective it is.[6, 456, 457]

   Therefore, the impact of testing on clinical care would, in part, be dependent on the time of diagnostic testing relative to the patient's illness course.

2) Treatment prior to diagnostic testing (before attending Dr Sardjito hospital)

   Over 84% of children and 64% of adults recruited into my study attended another medical centre before admission to Dr Sardjito Hospital. Although not formally measured in my study, many patients had received antibiotics at the local medical centres.

   It is well established that prior antibiotic provision can reduce the detection of bacteria via culture and to a lesser extent by pathogen-specific PCR.[158, 294, 418, 419] Therefore, the relatively low number of additional bacterial pathogens detected by pathogen-specific PCR may reflect prior partial sterilisation of CSF and/or blood due to earlier initiation of antibiotics.

Again, the impact of testing on clinical care would, in part, be dependent on the time of diagnostic testing relative to the patient starting antibiotics.

3) Delayed LP in Dr Sardjito Hospital

The LP was performed one or three days (median) after admission in children or adults, respectively. The majority of patients had received antibiotics (92% children and 95% adults) and/or steroids (58% children and 46% adults) prior to LP.

The time to LP is late compared to the 17 hours (median) reported among adults in the UK.[2] Treatment may have altered CSF parameters (i.e. CSF leucocyte count, neutrophil and lymphocyte percentage, protein, and glucose) and reduced the bacterial yield, thus affecting the results of CSF culture and CSF pathogen-specific PCR assays. To my knowledge, the time to LP and the impact of delayed LP on the yield of the standard hospital diagnostic tests have not been reported in Indonesia before, although these have been frequently reported elsewhere.[2, 158, 418, 458] Therefore, the findings of my study provide essential information to local clinicians. McGill *et al.* reported that every hour of delay in LP significantly reduced the pathogen detection rate by 1% in viral meningitis (odds ratio [OR] 0.988 [95% CI 0.982-0.995], p=0.001).[2] In bacterial meningitis, the pathogen detection rate was also reduced by 1% for every hour of delay in LP, although this was not statistically significant (OR 0.995 [95% CI 0.989-1.002], p=0.16).

As previously, the impact of testing on clinical care would, in part, be dependent on the time of LP and other diagnostic testing relative to the patient starting antibiotics.

4) Clinicians not always giving appropriate treatment despite available diagnostic results

Three out of 8 (38%) children and 23 out of 27 (85%) adults who had pathogens detected by either standard hospital testing or study testing did not receive appropriate treatment and had a fatal outcome. These findings highlight the need for good communication (plus/minus education) between laboratory and clinical teams to highlight the availability of diagnostic testing and ensure that provision of diagnostic test results promotes consideration of more appropriate treatment.

Eleven children and seven adults who received appropriate treatment for the pathogens detected also had a fatal outcome. This emphasises poor clinical outcomes still occur despite appropriate treatment. As stated earlier, many patients presented late to Dr Sardjito Hospital. Therefore, these patients may have already developed complications (e.g. cerebral infarctions or raised intracranial pressure) before appropriate treatment was started.

The clinical impact of testing will always be dependent on clinicians' responding promptly and appropriately to test results. That said, clinicians will not be able to learn the benefits of appropriate treatment until they have access to routine extended diagnostic testing.

Therefore, to improve the yield of the above-mentioned diagnostic tests and in keeping with the current guidelines for the management of CNS infection, [79, 138, 273, 459] I strongly recommend local clinicians perform LP as soon as reasonably possible.

Implementing a new diagnostic technique into routine practice in hospital laboratories is not always straightforward, particularly if the technology is advanced, requires specialised training, and is not as cheap as rapid point-of-care tests, as was the case here. This study

did not assess the cost-effectiveness of the new advance diagnostic assays. Therefore, a further study is necessary to implement these assays as part of routine hospital investigations and determine their cost-effectiveness and true impact.

Suggested uptake of extended testing

Taking the number of cases, cost of assays, and clinical importance into account, I would recommend the following assays to be incorporated into routine CSF testing in the study setting:

1) The GeneXpert MTB/RIF assay, as *M. tuberculosis* was the most common pathogen identified in both children and adults, the infection requires specific treatment, the untreated cases cause a fatal outcome, and the assay kit and equipment are provided free by the government;
2) The *M. tuberculosis* PCR assay, as it has shown to be more sensitive and requires a smaller CSF volume;
3) The CrAg lateral flow assay (particularly in HIV-positive patients), as the cryptococcal infection requires specific treatment, the untreated cases cause a fatal outcome, and the test is rapid, simple, and affordable;
4) The *E. coli* (particularly in children) and the *S. pneumoniae* PCR assays, as both pathogens were among the most common pyogenic bacteria identified in the present study, the specific treatment guideline is available, the PCR assays have shown to be more sensitive than the culture, and the untreated cases lead to a fatal outcome;
5) The scrub typhus serology testing, as scrub typhus is a potential cause of CNS infection in the study setting which requires specific antibiotics and may lead to a fatal outcome;

6) The HSV-2 PCR assay (particularly in adults), as it is the most significant viral aetiology of adult CNS infection in the study setting, and a negative result could help clinicians to stop antibiotic treatment.

I have shown that CNS infection causes a substantial burden of functional impairment in both children and adults, either at hospital discharge or at follow-up. Routine follow-up is therefore necessary to identify patients who have long-term neurological sequelae and to make sure these patients receive appropriate medical rehabilitation.

7.2.2 Implications on Policy

1) Highlighting the need for early LP

   Data from this study showed there was a significant delay in LP, particularly in adults, which caused a delay in diagnosis, late initiation of appropriate treatment, and might in part contribute to the poor patient outcome. Therefore, it is essential to emphasise the need for early LP to clinicians, particularly those who work in hospitals where laboratories with CSF diagnostic testing are available.

2) Highlighting the need to refer early to hospitals that can undertake appropriate diagnostic testing

   This study showed that most patients were referred late to Dr Sardjito Hospital and might have disease complications before the initiation of appropriate treatment. Consequently, they might have a fatal outcome. This finding emphasises the need to improve the referral system where such cases should be referred as early as possible to hospitals that can undertake appropriate diagnostic testing.

3) Vaccine coverage

   The pathogens identified by systematic testing included *H. influenzae, S. pneumoniae, N. meningitidis,* and DENV. All these pathogens have vaccines

commercially available. As described in chapter 4, the vaccine against *H. influenzae type b* has been included in the Indonesian national immunisation programme since 2017, but the coverage rate in 2018 was only 61%.[24] The vaccines for the remaining pathogens have not been incorporated into the national immunisation programme, but are available through private health centres in Indonesia. Although from a small population, the findings in this thesis support consideration of these vaccinations to be included in the national immunisation programme.

7.2.3 Implications on Research

I have shown that the application of pathogen-specific PCR and antigen/antibody assays, in addition to the standard hospital diagnostic tests, have improved the detection of CNS infection aetiology in the study setting. However, a further study is required to assess the cost-effectiveness of the advanced diagnostic tests. Furthermore, once a such study is undertaken, it would also be useful to evaluate the impact of the advanced testing implementation on the length of hospitalisation and patient outcome.

To a further extend, this study has improved knowledge on the aetiology of CNS infection in Indonesia. Taking the various geographical and demographic conditions in Indonesia, further studies in other regions in Indonesia are recommended.

7.3 Study limitations

This study has some limitations as follows:

1) Case definitions were applied retrospectively; thus, I could not identify what clinicians thought when deciding whether to perform LPs on patients and what diagnostic criteria they used to establish a patient's diagnosis. Moreover, there are variations in published syndromic case definitions and CSF pleocytosis definitions, which, if used, would have changed my findings. For example, the Vietnam study[3] I

described above used a modified WHO encephalitis case definition and added their own study inclusion criteria for CSF pleocytosis.

2) The inclusion criteria did not require an LP to be performed. As this study is the first cohort investigating various aspects of CNS infection in the local hospital, I included all suspected CNS infection cases, whether the LP was performed or not. The advantage of this approach is that I can describe overall patient management, such as how many patients underwent LP, why LP was not performed in certain patients, etc. The obvious disadvantage is the unavailability of CSF samples and/or CSF parameters data from patients who did not undergo LP or those whose CSF samples could not be collected for research due to technical problems during LP. As the case definition for certain types of syndromic CNS infection did not require CSF parameters, a proportion of cases classified into the CNS infection group did not have CSF parameters and/or samples available. Consequently, systematic CSF testing could not be performed in such cases, and definite CNS infection aetiology could not be determined.

3) LP was performed much later than most other studies, and clinicians were not encouraged to perform the LP earlier. In the present study, there was no intervention in patient management, including promoting an earlier LP, as an analysis of current practice was desired. Whilst advantageous from an evaluation point of view, delayed LPs resulted in patients receiving excessive antibiotics and steroids prior to LP. This resulted in low yields of pathogen detection either by the standard hospital diagnostic or by the systematic CSF testing and the possibility of altered CSF parameters (e.g. CSF leucocyte count, neutrophil and lymphocyte percentage, etc.).

Consequently, although the PCR assays revealed an increased pathogen detection rate, this was not as high as expected, and the vast majority of the syndromic CNS infection cases remained with no pathogen identified.

4) There was a delay in storing samples in a -80 °C freezer. At the beginning of the study, I did not have access to any of the -80 °C freezers in the hospital laboratory. Consequently, samples collected in the early stage of this study were stored in a -20 °C freezer for a few months until I was granted access to a -80 °C freezer in the hospital laboratory. Nevertheless, access was limited. Thus, the samples were usually stored at -20 °C for a few weeks before being transferred to a -80 °C freezer. This delay could cause RNA degradation, which could be a reason why there were no enterovirus cases found in the study, despite enterovirus being the second most common viral aetiology of CNS infection in Vietnam and Cambodia.[3, 227]

5) The dengue IgM ELISA test had low accuracy in detecting dengue-specific IgM. Furthermore, no true-positive and true-negative dengue samples were collected from the local population in the study setting to determine the optimum cut-off for the dengue ELISA kit when the study was conducted.

6) There was no gold standard test for scrub typhus available at the study site; consequently, I cannot confirm whether the scrub typhus cases found in this study are true-positives.

7) The outcome assessment was done by telephone interview. Therefore, the response was subjective and could result in bias. To obtain more accurate results, the outcome assessment should be ideally done by direct observation. For example, to assess whether a patient could do daily activities without help, it would be more objective to ask the patient to do such activities while being observed by the interviewer rather

than ask what the patient thinks of his/her ability to do so. The downside of a direct observation/assessment is that the interviewer would need to meet the patient in person, either at the hospital or at the patient's house, thus requiring an extra cost and resource than the telephone interview. Alternatively, the direct observation method could be done in a proportion of patients as an attempt to reduce the probability of obtaining biased results.

8) The follow-up rate in adults was relatively low compared to that in children (26% versus 16%) [chapter 6, section 6.3.1.1 and 6.3.1.2]. One of the possible reasons for the loss-to-follow-up is that the patients or their families had changed their phone numbers. An attempt had been made to anticipate this by asking the patients/families to provide two contact numbers to the research team. However, some of them only provided one contact number for various reasons, such as the patients were from a low-income family and only had one mobile phone, or the patients/families did not want to share more than one contact number for personal reasons. Another approach to contacting the patients/families before the outcome assessment period to remind them about the assessment could have been tried to confirm if the phone number remained active and to ask them to notify the research team if they planned to change their phone number if they were willing to do so. Alternatively, the follow-up rate could be increased by shortening the follow-up period from 6 months to 3 months, as the longer the time between hospital discharge and follow-up, the higher the possibility of loss-to-follow-up.

7.4 Future research directions

Having obtained some notable findings in this thesis, my supervisor and I were inspired to continue this study to a further extent. We applied for the UK-Indonesia Joint Partnership

on Infectious Diseases through the Newton Fund, and our proposed project was selected to receive funding. The new project, the Diagnostics Improvements for Meningo-Encephalitis in Indonesia (DIME – Indonesia) study, commenced in March 2019 and will run for 3 years. In the new project, the work I have done in my thesis is expanded to a new study site, Dr Cipto Mangunkusumo Hospital, the national referral hospital in the Indonesian capital city, Jakarta, which is linked with Universitas Indonesia. We will also collaborate with The Eijkman Institute for Molecular Biology, one of the reference laboratories in Indonesia, by helping to optimise the pathogen detection in the new project (The Eijkman Institute enabled me to perform PRNT assays in the present study). We also want to examine the impact of introducing systematic CSF testing on outcomes for patients with CNS infection. We intend to collaborate with the University of Warwick to perform this outcome assessment.

Among the areas we want to improve in the DIME – Indonesia study are as follows:

1) Promoting earlier lumbar puncture (LP)

   As an evaluation of my finding in the present study, where the median time to LP was 1 day in children and 3 days in adults, we will encourage the clinicians to perform LP much earlier than their usual practice. Our recommendation is in line with the current guidelines for the management of patients with encephalitis and meningitis, where LP should be performed as soon as possible unless there is a contraindication.[79, 138, 273, 459] By promoting earlier LP, it is expected that the patients have not received extensive antibiotics prior to LP and the PCR yield will increase accordingly.

2) Speeding up sample transfer to -80 °C freezer

With the availability of new -80 °C freezers in the local laboratory, which we have been granted access to, we aim to store the samples in the -80 °C freezer within 4 hours after collection if the LP is done within the working hours (i.e. Monday to Friday between 8 am and 4 pm) to minimise RNA/DNA degradation. If the LP is done outside working hours when access to the -80 °C freezer is not possible, the samples are stored at -20 °C and transferred to -80 °C on the next working day.

3) Incorporating the GeneXpert MTB/RIF assay into routine hospital CSF testing

I have shown that *M. tuberculosis* is the most common pathogen identified in the study setting and the GeneXpert MTB/RIF assay increased pathogen detection compared to the traditional Ziehl-Neelsen staining. Given the availability of the GeneXpert MTB/RIF assay in the study setting is free of charge, we will introduce this assay as a routine hospital CSF testing. We would analyse whether applying the GeneXpert MTB/RIF assay as a routine test improves patient outcomes and shortens the length of hospital stay.

4) Introducing the CrAg lateral flow assay into routine hospital CSF testing

The CrAg lateral assay has been shown as rapid, simple, more sensitive and specific compared to traditional Indian ink staining. Additionally, the cost of the assay is relatively affordable. Therefore, in the next project, we aim to introduce the assay as a routine CSF testing at the hospital.

5) Introducing the pathogen-specific PCR assays to the local laboratory

We will introduce pathogen-specific PCR assays to local laboratories through capacity building and laboratory technician training in the DIME- Indonesia study. This is an initial step to assess the feasibility of integrating PCR assays into routine hospital

diagnostic testing. The target pathogens will be tested in the following order based on priorities: (1) *M. tuberculosis*, (2) *E. coli* [in children only], (3) *S. pneumoniae*, (4) HSV-2, (5) other target bacteria, and (6) other target viruses.

6) Optimising the diagnosis of dengue infection

The dengue IgM ELISA test performed in this study was shown to detect additional systemic dengue cases compared to the standard hospital diagnostic tests and, for the first time, has successfully detected dengue IgM in CSF samples at the study site. However, it remains in disagreement with the dengue PRNT assay in a few cases. Moreover, the dengue IgM test may miss the case if serum samples are collected within the first 5 days of the illness onset where dengue IgM antibodies are not yet produced in sufficient quantities (i.e. too early in the disease). Recent guidelines[85, 154] recommend that a combination of both dengue molecular tests, such as PCR, and dengue antibody tests, such as IgM ELISA, will optimise the identification of dengue infection. Therefore, in the DIME – Indonesia study, we will employ dengue PCR and dengue IgM ELISA tests to detect dengue CNS infection.

We also want to re-assess the dengue IgM ELISA kit's cut-off accuracy. Initially, we will test the samples previously collected in the present study using a dengue PCR assay. A proportion of samples that test positive and negative for DENV by PCR will be further tested by PRNT to identify the true-positives and the true-negatives. These true-positive and true-negatives samples will then be used to re-assess the accuracy of the ELISA kit's cut-off.

7) Extending the target pathogen panel

Despite the improvement achieved in identifying a pathogen by the systematic testing I introduced in this study, the majority of cases had no pathogen identified.

Whilst as many target pathogens as possible were included for the systematic CSF testing, a few pathogens known to cause CNS infection, such as *Toxoplasma gondii* and *Leptospira* species (*Leptospira* spp.), were not covered in this study. Cerebral toxoplasmosis is one of the main opportunistic infections in HIV-infected individuals.[460] In the present study, there were 12 presumed cases identified by the presence of one or more cerebral lesions on computed tomography (CT) or magnetic resonance imaging (MRI) in HIV-positive individuals with a documented clinical response to anti-toxoplasmosis treatment. Previous studies from other regions in Indonesia have successfully detected *T. gondii* by PCR assay.[6, 10] Therefore, in the DIME – Indonesia study, we will include *T. gondii* in our pathogen-specific PCR panel. Leptospirosis has been linked with sepsis and acute undifferentiated fever in Indonesia, with a case fatality rate of 16.88% in 2017.[20, 342, 461] Among six provinces which reported leptospirosis cases to the Indonesia Ministry of Health, Central Java and Yogyakarta Provinces had the highest incidence and case fatality rate in 2017, both of which are where the majority of patients admitted to Dr Sardjito Hospital come from.[20] Studies from South and Southeast Asian countries have shown that *Leptospira* spp. can cause acute encephalitis syndrome and aseptic meningitis.[462-464] However, cases of CNS disease caused by *Leptospira* spp. have never been reported in Indonesia. Therefore, we want to investigate whether *Leptospira* spp. cause CNS infections in this study setting.

8) Improving pathogen detection in the unknown aetiology cases

Many cases with unknown aetiology had marked CSF pleocytosis and other abnormal CSF parameters. It is possible that these included cases caused by the pathogens tested for in this study, but they were not detected. Alternatively, it is also possible that these were cases caused by other pathogens not tested for here. These may

include pathogens that have been known to cause CNS infection or possibly new pathogens which rarely or have never been linked with CNS infection. In an attempt to identify the causative pathogens in the unknown aetiology cases, we will perform next-generation sequencing (NGS) for selected cases, particularly those with marked CSF pleocytosis and other CSF abnormalities. The NGS will be conducted by the Eijkman Institute for Molecular Biology.

9) Distinguishing bacterial from non-bacterial CNS infection cases using a host-response assay

My thesis supervisor currently leads the TRanscripts to Identify bacterial Meningitis (TRIM) study, which aims to distinguish between bacterial and non-bacterial meningitis using host-response analysis of blood samples. The TRIM test has been tested in the UK and is currently undergoing further validation studies in a larger cohort of UK, Danish and Dutch patients. We aim to validate the TRIM test among Indonesian populations with suspected CNS infections in the DIME- Indonesia study. Furthermore, because the number of tuberculous CNS infections (including tuberculous meningitis) in Indonesia is remarkably high (as shown in my thesis and previous Indonesian studies),[6, 7] the TRIM marker set will be extended to distinguish tuberculous meningitis from other bacterial causes of meningoencephalitis. The sample type required for the TRIM test validation is whole blood stored in RNA stabilisation fluid, which should be collected within three days of hospital admission. This strict criterion is consistent with our aim to speed up the LP in the study setting.

7.5 Final conclusions

I have performed the first prospective study of suspected CNS infection among children and adults in Indonesia. Furthermore, this is the first CNS infection study in Indonesia to undertake community follow-up in both age groups.

I successfully designed my own pathogen-specific primer/probes. I initially confirmed test accuracy in the laboratory in Liverpool. I then successfully translated the assays for use in a local Indonesian setting. I also applied new antibody-based assays to the local setting.

Introduction of my 'in-house' PCR assays and antibody/antigen tests, in addition to the standard hospital diagnostic testing, have successfully increased the detection of pathogens causing CNS infection from 11% to 20% in children and from 6% to 27% in adults. I have identified new cases of *M. tuberculosis*, *S. pneumoniae*, *E. coli*, *S. agalactiae, N. meningitidis*, *H. influenzae*, HSV-2, CMV and VZV using the PCR assays, and new cases of dengue, scrub typhus and *Cryptococcus* using the antibody tests among the CNS infection patients. *M. tuberculosis* was the most common pathogen identified in CSF in both children and adults.

I have shown that patients with suspected CNS infection experience high morbidity and mortality at hospital discharge and within six months of follow-up. Mortality at hospital discharge was significantly higher in patients with tuberculous CNS infection than in those with non-tuberculous CNS infection among both children (75.0% versus 13.8%, p=<0.001) and adults (83% versus 39%, p<0.001). The majority of patients with tuberculous CNS infection did not receive anti-tuberculosis treatment as the diagnosis was not established at the hospital, which is in part due to the poor performance of the standard hospital diagnostic tests.

My study indicates that provision of molecular and antibody tests has the potential to help guide local clinicians to give more appropriate treatment (particularly for tuberculous CNS infection). In turn, this may lead to reduced mortality and morbidity among local patients with CNS infection.

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8. Appendices

8.1 Patient Information Sheet for Children

8.1.1 Patient Information Sheet for Young Persons (Age 11-18)

The UNITY-NeuroID Project
Young Persons (Age 11-18) Information Sheet
Version 1.1

Why are we doing this study?

Brain illnesses are quite common especially in children. Doctors here are trying to find the bugs that cause your brain illness and set up new tools that can detect the bugs better. Doctors also want to know the impact of the brain illness on your life.

We would like to invite you to take part in our research study. Before you decide if you want to join in, we would like you to understand why the research is being done and what it will mean for you. So please read the leaflet carefully. Talk about it with your family, friends, doctors or nurses if you want to.

Why have I been chosen?

Doctors are keeping you in hospital to look at your brain closely.

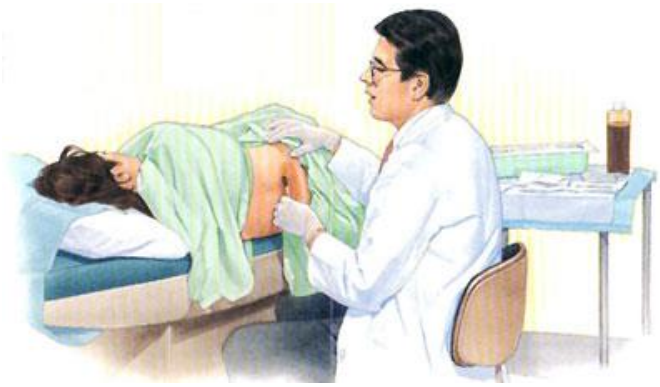
Do I have to take part?

It is up to you. We only want you to take part if you want to. If you decide not to take part it won't change how you are looked after. If you decide to take part and then change your mind that's OK too. You can stop taking part at any time, without giving a reason.

What do I have to do if I take part?

Your doctor will need to take fluid from your back with a gadget to find the bugs that cause your brain illness, whether you take part or not. You can see the pictures of how the doctor will take the fluid below. The nurse will also need to take a little of your blood to help finding out the bugs in your body.

A small needle will go into your back. Don't worry it won't stay there. There will be a little pressure on your backbone (spine) while it's in place.



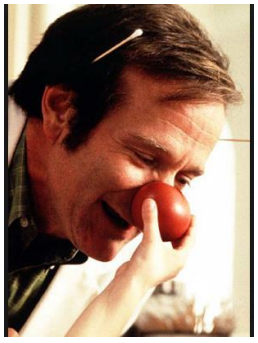
The doctor will collect the fluid from your back.

The nurse will also take a little of your blood.

At any time you or your family can ask us any questions that you have.

We might also collect some other information

Additionally, if you and your parents agree, we will collect some more information from other tests.



What are the possible benefits of taking part?

There are no benefits for you being part of the study. However, this research could help other children and adults with brain illnesses in the future.

What are the risks of taking part?

There will be no more risk to you if you decide to take part in the study, compared to not taking part.

What happens now?

If you feel like you want to take part you can write your name on the form at the end of this leaflet. This is just to say that you understand the study and what will happen. You will be given a copy of the form to keep, as well as this information leaflet. Your mum or dad or guardian will need to sign a form to agree to you taking part.

Thank you for reading this information.

The UNITY-NeuroID Project

Young Persons (Age 11-18) Assent Form

Version 1.1

Patient's name: Date of birth: [__|__] / [__|__] / [__|__|__|__]

Child (or if unable, parent on their behalf) to circle all they agree with:

Has somebody explained this study to you?
Yes/No

Do you understand what this study is about?
Yes/No

Have you asked all the questions you want?
Yes/No

Have your questions been answered in a way you understand?
Yes/No

Do you understand it's OK to stop taking part at any time?
Yes/No

Are you happy to join in? **Yes / No**

If any answers are 'no' or you **don't** want to join in, **don't** sign your name!

If you do want to take part, please write your name and today's date

Your name _____
Date_____

Your parent or guardian must write their name here too if they are happy for you to do the study

_____	_____	_____
Parent's signature	Name (in block letter)	Date

The research doctor who explained this study to you needs to sign too:

_____	_____	_____
Research doctor's signature	Name (in block letter)	Date

Thank you for your help

8.1.2 Patient Information Sheet for Parents

EPIDEMIOLOGY OF NEUROLOGICAL INFECTIOUS DISEASES IN YOGYAKARTA
Participant Information Sheet
Version 2.0

We are research team led by dr. Bardatin Lutfi Aifa, MRes from Neurology Department, Faculty of Medicine, Universitas Gadjah Mada. We would like to invite you and your child to take part in our study entitled "Epidemiology of Neurological Infectious Diseases in Yogyakarta". Epidemiology is the spread pattern of disease in communities. This study is funded by Indonesia Endowment Fund for Education (*Lembaga Pengelola Dana Pendidikan* / LPDP), Indonesia Ministry of Finance.

This study aims to identify the most common bugs that cause nervous system infection disease among population in Yogyakarta. This study also aims to know the impact of this disease to your daily activities and the money you/your family spends to treat this disease. The results of this study may help guide health care professionals on better diagnosing and treating patients with neurological infection and preventing the disease. The study will involve around 310 patients and each patient will be in the study for the duration of 6 months.

A. Participation in the study

It is entirely up to you whether you take part in this research study. If you decide to take part you are still free to withdraw from the study at any time without getting fined / sanctioned. A decision to withdraw at any time, or a decision not to take part, will not affect any future medical care in Dr Sardjito General Central Hospital.

B. Study procedure

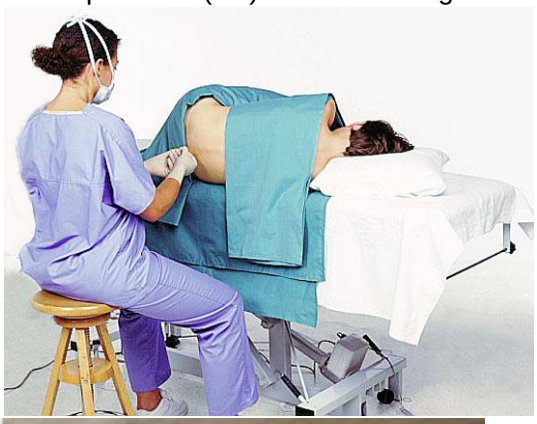
If you do decide to take part you will be given this information sheet to keep and be asked to sign the consent form in three copies, one will be kept by the investigator, one to be kept in your child's medical record, and one will be given to you for your archive.

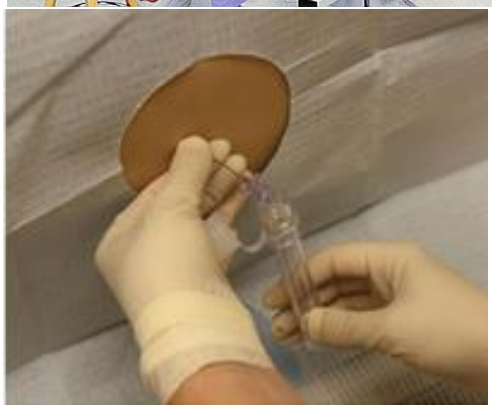
The procedure of this study is as follows:

1. You/your child will be interviewed by the research team regarding your child's identity and medical history.
2. Your child will undergo routine examinations which comprise of physical examination, laboratory test and imaging studies to establish a diagnosis.
3. The research team will record data from your child's medical record.
4. Up to 7.5 ml (equal to half spoon of liquid) extra volume of blood will be taken for research purposes. This amount of extra blood taken should not affect your child's health. These blood will be withdrawn at the same time as routine venepuncture where possible.

5. Your child will also have cerebrospinal fluid (CSF - the fluid that your brain baths in) taken as part of the standard routine care. This will be done by inserting a needle through your child's backbone (lumbar puncture -see pictures). It is routine practice for the doctors to ask for your consent on each occasion that CSF is taken. Some of your child's CSF will undergo routine tests, whilst the remaining CSF will be used for research purposes. First, the research team will use a certain method to recognise the bugs' genetic material in CSF. If this method fails to identify the bugs, we will perform genetic testing to your child's CSF and/or blood to screen for new bugs which may cause the disease.

   Pictures of the spinal needle being inserted through patient's backbone during lumbar puncture (left) and CSF being collected into a tube (right).





6. At discharge, your child will undergo an assessment to determine the impact of the disease to your child's daily activities. You will also be required to complete an economic questionnaire to assess the cost you/your family pay for the treatment of the disease.
7. At 6 months after your discharge, the research team will contact you by telephone and you/your family will be required to undertake these 2 assessments again.

C. Subject's requirement

As study participants, you and your child are required to follow the instruction for the study as written above. If there are unclear things, please contact the investigator for further queries before you decide to participate in the study.

D. Risks, adverse reactions, and their treatment

1) **Risk of giving a blood or spinal fluid sampling** - Having a blood test or CSF taken can be uncomfortable and may cause a bruise. These procedures are part of routine care and will be necessary whether or not you and your child participate in the study.
2) **Risks of genetic testing** - Some people may consider tests on their genetic code to be an invasion of privacy. The study will focus on the **genetic code of the pathogens (bugs)** infecting your child. Results will be made available to other researchers but your child will not be identified and no one will know whose genetic information it is.

   Any adverse reactions will be treated based on standard procedure applied in Dr Sardjito GCH.

E. Benefits

This study may not have a direct benefit to your child as it takes over 3 years to complete the whole study. The results of this study will help health care professional to improve the diagnosis and treatment of neurological infection in Yogyakarta, particularly in Dr. Sardjito GCH in the future.

F. Confidentiality

All information collected about your child during this study will be confidential. Only people working on the study or working to ensure the study is run correctly will have access to your child's personal information. All samples and information will be identified with codes arranged by the investigator.

G. Appreciation

Compensation in the form of money will not be given to you/your child. However, you/your child will receive a souvenir as our appreciation for your child's participation in this study.

H. Funding

All additional tests performed for research purposes will be covered by the investigator. You will not need to pay for additional costs related to the research.

I. Additional information

If you need any further information at any stage, please contact:
dr. Bardatin Lutfi Aifa, MRes.
Department of Neurology, Faculty of Medicine Universitas Gadjah Mada
Dr Sardjito General Central Hospital, Jalan Kesehatan no. 1 Sekip Utara, Yogyakarta
Phone: 085729585735

You could also get information about the study from:
Medical and Health Research Ethics Committee (MHREC)
Faculty of Medicine Universitas Gadjah Mada
Phone: *0274-588688 ext. 17225*

EPIDEMIOLOGY OF NEUROLOGICAL INFECTIOUS DISEASES IN YOGYAKARTA
Parents Consent Form

Patient's name: Date of birth: [__|__] / [__|__] / [__|__|__|__]

		Please initial box
1.	I have read all pages of the participant information sheet for the above study. I have had the opportunity to ask questions and had these answered satisfactorily. I have been given time to consider the information carefully.	
2.	I understand that my participation in this study is entirely voluntary and that I may withdraw at any time, without any consequences to my child's ongoing and future medical care at this institution.	
3.	I authorize the access of my child's medical record be provided to research team, as well as the regulatory authorities and the ethics committee of this institution for the purposes of this study only. This authorization will be valid for a period of up to 5 years	
4.	I understand that I will be given a copy of the participant information sheet and this informed consent to keep for my own information once it is signed.	
5.	My signature below indicates that I voluntarily agree to take part in this study.	
6.	I agree that my child's blood and cerebrospinal fluid will undergo genetic testing for this study.	Yes ☐ No ☐
7.	I agree that my child's data and remaining samples can be used for future research, including genetic research, approved by the ethics committee without re-asking my permission.	Yes ☐ No ☐

_____	_____	_____
Signature of Mother	Name (in block letters)	Date
_____	_____	_____
Signature of Father/legal guardian	Name (in block letters)	Date
_____	_____	_____
Signature of witness*	Name (in block letters)	Date

*if required, e.g. if parents/legal guardian are illiterate.

I confirm having met with the subject/legal guardian at the time of enrolment and I have explained the study to parents/legal guardian, answered their questions about this study, and I am sure they understand about the purpose, reason, and possible risks if they/their child participated in this study.

_____	_____	_____
Signature of Research Doctor	Name (in block letters)	Date

When completed, 1 to be kept in medical notes, 1 for participant, 1 (original) for researcher site file.

ADDITIONAL PAGE FOR TRANSLATOR

I have translated all the information contained in this consent to the above named individual and translated all questions that this individual had, as well as the answers provided by the research doctor.

_____	_____	_____
Translator's Signature	Name (in block letters)	Date

8.2 Patient Information Sheet for Adults

EPIDEMIOLOGY OF NEUROLOGICAL INFECTIOUS DISEASES IN YOGYAKARTA
Participant Information Sheet
Version 2.0

We are research team led by dr. Bardatin Lutfi Aifa, MRes from Neurology Department, Faculty of Medicine, Universitas Gadjah Mada. We would like to invite you to take part in our study entitled "Epidemiology of Neurological Infectious Diseases in Yogyakarta". Epidemiology is the spread pattern of disease in communities. This study is funded by Indonesia Endowment Fund for Education (*Lembaga Pengelola Dana Pendidikan* / LPDP), Indonesia Ministry of Finance.

This study aims to identify the most common bugs that cause nervous system infection disease among population in Yogyakarta. This study also aims to know the impact of this disease to your daily activities and the money you/your family spends to treat this disease. The results of this study may help guide health care professionals on better diagnosing and treating patients with neurological infection and preventing the disease. The study will involve around 310 patients and each patient will be in the study for the duration of 6 months.

A. Participation in the study

It is entirely up to you whether you take part in this research study. If you decide to take part you are still free to withdraw from the study at any time and without getting fined / sanctioned. A decision to withdraw at any time, or a decision not to take part, will not affect any future medical care in Dr Sardjito General Central Hospital.

B. Study procedure

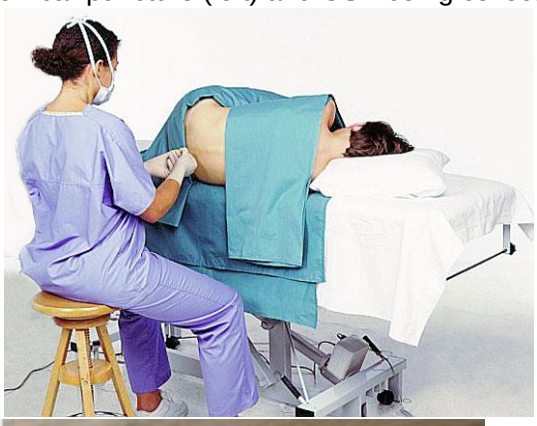
If you do decide to take part you will be given this information sheet to keep and be asked to sign the consent form in three copies, one will be kept by the investigator, one to be kept in medical record, and one will be given to you for your archive.

The procedure of this study is as follows :

1. You will be interviewed by the research team regarding your identity and medical history.
2. You will undergo routine examinations which comprise of physical examination, laboratory test and imaging studies to establish a diagnosis.
3. The research team will record data from your medical record.
4. Up to 7.5 ml (equal to half spoon of liquid) extra volume of blood will be taken for research purposes. This amount of extra blood taken should not affect your health. These blood will be withdrawn at the same time as routine venepuncture where possible.

5. You will also have cerebrospinal fluid (CSF - the fluid that your brain baths in) taken as part of the standard routine care. This will be done by inserting a needle through your backbone (lumbar puncture -see pictures). It is routine practice for the doctors to ask for your consent on each occasion that CSF is taken. Some of your CSF will undergo routine tests, whilst the remaining CSF will be used for research purposes. First, the research team will use a certain method to recognise the bugs' genetic material in CSF. If this method fails to identify the bugs, we will perform genetic testing to your CSF and/or blood to screen for new bugs which may cause the disease.

   Pictures of the spinal needle being inserted through patient's backbone during lumbar puncture (left) and CSF being collected into a tube (right).



6. At discharge, you will be required to complete an economic questionnaire to assess the cost you/your family pay for the treatment of the disease. *You will also be contacted by the research team within two weeks after discharge to complete an assessment determining the impact of the disease to your daily activities*.
7. At 6 months after your discharge, the research team will contact you by telephone and you/your family will be required to undertake these 2 assessments again.

C. Subject's requirement

As a study participant, you are required to follow the instruction for the study as written above. If there are unclear things, please contact the investigator for further queries before you decide to participate in the study.

D. Risks, adverse reactions, and their treatment

1) **Risk of giving a blood or spinal fluid sampling** - Having a blood test or CSF taken can be uncomfortable and may cause a bruise. These procedures are part of routine care and will be necessary whether or not you participate in the study.
2) **Risks of genetic testing** - Some people may consider tests on their genetic code to be an invasion of privacy. The study will focus on the **genetic code of the pathogens (bugs)** infecting you. Results will be made available to other researchers but you will not be identified and no one will know whose genetic information it is.

   Any adverse reactions will be treated based on standard procedure applied in Dr Sardjito GCH.

E. Benefits

This study may not have a direct benefit to you as it takes over 3 years to complete the whole study. The results of this study will help health care professional to improve the diagnosis and treatment of neurological infection in Yogyakarta, particularly in Dr. Sardjito GCH in the future.

F. Confidentiality

All information collected about you during this study will be confidential. Only people working on the study or working to ensure the study is run correctly will have access to your personal information. All samples and information will be identified with codes arranged by the investigator.

G. Appreciation

Compensation in the form of money will not be given to you. However, you will receive a souvenir as our appreciation for your participation in this study.

H. Funding

All additional tests performed for research purposes will be covered by the investigator. You will not need to pay for additional costs related to the research.

I. Additional information

If you need any further information at any stage, please contact:
dr. Bardatin Lutfi Aifa, MRes.
Department of Neurology, Faculty of Medicine Universitas Gadjah Mada
Dr Sardjito General Central Hospital, Jalan Kesehatan no. 1 Sekip Utara, Yogyakarta
Phone: 085729585735

You could also get information about the study from:
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Faculty of Medicine Universitas Gadjah Mada
Phone: *0274-588688 ext. 17225*

EPIDEMIOLOGY OF NEUROLOGICAL INFECTIOUS DISEASES IN YOGYAKARTA
Participant Consent Form

Patient's name: Date of birth: [__|__] / [__|__] / [__|__|__|__]

	Please initial box
1. I have read all pages of the participant information sheet for the above study. I have had the opportunity to ask questions and had these answered satisfactorily. I have been given time to consider the information carefully.	
2. I understand that my participation in this study is entirely voluntary and that I may withdraw at any time, without any consequences to my ongoing and future medical care at this institution.	
3. I authorize the access of my medical record be provided to research team, as well as the regulatory authorities and the ethics committee of this institution for the purposes of this study only. This authorization will be valid for a period of up to 5 years	
4. I understand that I will be given a copy of the participant information sheet and this informed consent to keep for my own information once it is signed.	
5. My signature below indicates that I voluntarily agree to take part in this study.	
6. I agree that my blood and cerebrospinal fluid will undergo genetic testing for this study.	Yes ☐ No ☐
7. I agree that my data and remaining samples can be used for future research, including genetic research, approved by the ethics committee without re-asking my permission.	Yes ☐ No ☐

_____ _____ _____
Signature of Participant Name (in block letters) Date

_____ _____ _____
Signature of witness/consultee* Name (in block letters) Date

*if required, e.g. if participant's condition is not able to give consent or if participant is illiterate.

I confirm having met with the participant at the time of enrolment. I have explained the study to participant, answered his/her questions about this study, and I am sure he/she understands about the purpose, reason, and possible risks if they participated in this study.

_____	_____	_____
Signature of Research Doctor	Name (in block letters)	Date

When completed, 1 to be kept in medical notes, 1 for participant, 1 (original) for researcher site file.

ADDITIONAL PAGE FOR TRANSLATOR

I have translated all the information contained in this consent to the above named individual and translated all questions that this individual had, as well as the answers provided by the research doctor.

_____	_____	_____
Translator's Signature	Name (in block letters)	Date

8.3 Liverpool Outcome Score for Assessing Children at Discharge

Subject ID:._____ Date of birth (dd/mm/yy): __ __ / __ __ /__ __

Age: _____ Sex: Male ☐ Female ☐

Today's date (dd/mm/yy): ___ ___ /___ ___ /___ ___

Date of admission to hospital (dd/mm/yy): ___ /___ /____

Date of discharge from hospital (dd/mm/yy): ___ /___ /____

Relationship of person with child (e.g., mother/aunt): _____

Name of person completing form – please print: _____

Job Title: _____

Answer each question. <u>Circle or underline the correct answer, and write the score in the column</u>

<u>Ask the parent or caregiver the following questions:</u>

For some of these questions, you ask the parent or caregiver how the child compares with how
they were immediately before the illness (irrespective of length of time in hospital).

<u>1. Speech or communication</u>

Compared with before the illness, is the child's speech or communication:

- The same as before (5)
- Changed or reduced (3)
- Not speaking or communicating (2) _____

<u>2. Feeding</u>

The child's feeding is:

- The same as before illness (5)
- Occasionally needs help (3)
- Always needs more help (2) _____

3. Leaving Alone

Before the illness, could this child be left alone without coming to harm?

- If **No** score 5 (5)

If **Yes**, can this child now be left alone?

- Yes (5)
- Yes briefly in familiar environment (3)
- No (2) _____

4. Behaviour

Compared with before the illness do the caregivers think the child's behaviour is altered?

- No completely normal (5)
- Gets angry easily (4)
- Other behavioural problems (4)
- Severely abnormal (2) _____

If abnormal give details: _____

_____

5. Recognition

Could the child recognise their family members, other than their main carer, before the illness?

- If **No**, score 5 (5)

If **Yes**, can this child now recognise their family members, other than their main carer?

- Yes (5)
- Some (3)
- None (2) _____

6. School and working

Before the illness, was the child at school or working?

If **Yes**, do the carers think the child will go back to school or work?

- Yes (5)
- No (3)

If **No,** do the carers think the child will still able to do the same tasks at home, follow the
same routine, or play normally?

- Yes (5)
- Not able to (3) _____

7. Epilepsy/ Seizures

Did the child have any seizures during this illness?

- If **No**, score 5 (5)

If **Yes**, is the child still having seizures?

- No seizures and not on anti-epileptic drugs (5)
- No seizures and on anti-epileptic drugs (4)
- Yes still having seizures (3)
- Yes, seizures most days (2) _____

8. Dressing

Can other children of this age dress themselves?

- If **No**, score 5 (5)

If **Yes**, can the child dress themselves since their illness?

- Yes, the same as before illness (5)
- Occasionally needs extra help (3)
- Needs more help than before (2) _____

9. Bladder and Bowel control

Is urinary and faecal continence:

- The same as before the illness (5)
- Occasionally needs more help or occasionally is incontinent (4)
- Needs more help or is incontinent of bowel or bladder (2) _____

10. Hearing

Does the parent think this child's hearing is:

- Normal (5)
- Reduced in one or both ears (4)
- Cannot hear at all (3) _____

Observation of the child's abilities

For these questions you observe what the child can do. If you cannot get the child to cooperate,
answer these questions based on what the caregiver says.

11. Sitting

Could the child sit before the illness?

- If **No**, score 5 (5)

If **Yes**, observe, can this child sit?

- Yes independently (5)
- Needs help (3)
- Not at all (2) _____

12. Standing up

Could the child get from sitting to standing before the illness?

- If **No**, score 5 (5)

If **Yes**, observe, can the child get from sitting to standing?

- Yes, independently (5)
- Needs help (3)
- Not at all (2) _____

13. Walking

Could the child walk before the illness?

- If **No**, score 5 (5)

If **Yes**, observe this child walking 5 metres across room. The child walks:

- Normally (5)
- Abnormally, but independently +/- crutches/stick (3)
- Not able to walk (2) _____

14. Hands on head

Put both your hands on your head, and ask the child to copy you. Child is:

- Too young (5)
- Normal both hands (5)
- Abnormal one or both hands (4)
- Unable one or both hands (3) _____

15. Picking Up

Ask child to pick up pea-sized ball of paper or small coin. Child is:

- Too young (5)
- Normal pincer grasp both hands (5)
- Unable one hand (3)
- Abnormal one hand or both hands (3)
- Unable both hands (2) _____

Outcome Score =Lowest score for any single question (range 2-5):_____

Total Score = all the individual scores added up (range 33 –75): _____

(If the child died, the score = 1)

Any other Comments: _____

_____

_____

The Final Liverpool Outcome Score is the lowest number scored for any question single question

Score interpretation:

5 = Full recovery

4 = Minor sequelae with no effect, or only minor effects, on physical function; or personality change; or on medication.

3 = Moderate sequelae mildly affecting function, probably compatible with independent living

2 = Severe sequelae, impairing function sufficient to make patient dependent

1 = Death

Please feel free to use the score. We would recommend contacting Dr Penny Lewthwaite (pennylewthwaite@doctors.org.uk or penny.lewthwaite@liv.ac.uk) for the latest information regarding the score. We request that data from use of the score be submitted, so that we can continue to improve and develop the score. www.liv.ac.uk/braininfections

8.4 Liverpool Outcome Score (LOS) for assessing children at Follow-Up

Subject ID . _____

Date of birth (dd/mm/yy): __ __ / __ __ /__ __

Age: _____

Today's date (dd/mm/yy): __ __ /__ __ /__ __ Sex: Male ☐ Female ☐

Date of original admission to hospital (dd/mm/yy): __ __ /__ __ /__ __

Date discharged from hospital (dd/mm/yy): __ __ /__ __ /__ __

Relationship of person with child (e.g., mother/aunt): _____

Name of person completing form – please print: _____

Job Title: _____

Answer each question. Circle or underline the correct answer, and write the score in the column

Ask the parent or caregiver the following questions:

For some of these questions, you ask the parent or caregiver how this child compares with other children of a similar age in their locality, e.g., how does this child compare in speaking or walking or talking to other children of the same age n the community.

1. Speech or communication:

Compared with other children the same age in the community, is the child's speech or
communication:

- The same as other children of this age (5)
- Changed or reduced (3)
- Not speaking or communicating (2) _____

2. Feeding

The child's feeding is:

- The same as other children (5)
- Occasionally needs help (3)
- Always needs more help (2) _____

3. Leaving Alone

Before the illness, could a child of this age be left alone without coming to harm?

- If **No** score 5 (5)

If **Yes**, can this child be left alone now?

- Yes (5)
- Yes briefly in familiar environment (3)
- No (2) _____

4. Behaviour

Compared with other children of this age, do the caregivers think the child's behaviour is altered?

- No, completely normal (5)
- Gets angry easily (4)
- Other behavioural problems (4)
- Severely abnormal (2) _____

If abnormal give details_____

5. Recognition

Can other children of this age recognise their relatives, other than their main carer?

- If **No**, score 5 (5)

If **Yes,** can this child recognise their relatives, other than their main carer?

- Yes (5)
- Some (3)
- None (2) _____

6. School and working

Are other children of the same age at school or working?

If **Yes**, is the child

- Now back to normal at school or work (5)
- Not doing as well (4)
- Dropped a school grade or no longer attending school or work (3)

If **No**, is the child:

- Still able to do the same tasks at home, follow the same routine, or play normally? (5)
- Not able to do as well as before (4)
- Not able to do at all (3) _____

7. Epilepsy/ Seizures

Has the child had any seizures in the last 2 months?

- No seizures and not on anti-epileptic drugs (5)
- No seizures and on anti-epileptic drugs (4)
- Yes, has had seizures (3)
- Yes, seizures most days (2) _____

8. Dressing

Can other children of this age dress themselves?

- If **No**, score 5 (5)

If **Yes**, can this child dress themselves?

- Yes (5)
- Occasionally needs more help (3)
- Always needs more help than other children of the same age (2) _____

9. Bladder and Bowel control

Is urinary and faecal continence:

- The same as other children the same age (5)
- Occasionally needs more help or occasionally is incontinent (4)
- Needs more help or is incontinent of bowel or bladder (2) _____

10. Hearing

Does the parent think this child's hearing is:

- Normal (5)
- Reduced in one or both ears (4)
- Cannot hear at all (3) _____

Observation of the child's abilities

For these questions you observe what the child can do. If you cannot get the child to cooperate, answer these questions based on what the caregiver says.

11. Sitting

Can other children of the same age sit?

- If **No,** score 5 (5) _____

If **Yes**, observe, can this child sit?

- Yes, independently (5)
- Needs help (3)
- Not at all (2) _____

12. Standing up

Can other children of this age get from sitting to standing?

- If **No**, score 5 (5) _____

If **Yes**, observe, can the child get from sitting to standing?

- Yes, independently (5)
- Needs help (3)
- Not at all (2) _____

13. Walking

Can other children of this age walk?

- If **No**, score 5 (5)

If **Yes**, observe this child walking 5 metres across room. The child walks:

- Normally (5)
- Abnormally, but independently +/- crutches/stick (3)
- Not able to walk (2) _____

14. Hands on head

Put both your hands on your head, and ask the child to copy you. Child is:

- Too young (5)
- Normal both hands (5)
- Abnormal one or both hands (4)
- Unable one or both hands (3) _____

15. Picking Up

Ask child to pick up pea-sized ball of paper or small coin:

- Normal pincer grasp both hands (5)
- Unable one hand (3)
- Abnormal one hand or both hands (3)
- Unable both hands (2) _____

Outcome Score =Lowest score for any single question (range 2-5)_____

Total Score = all the individual scores added up (range 33 –75) _____

(If the child died, the score = 1)

Any other Comments: _____

_____

_____

The Final Liverpool Outcome Score is the lowest number scored for any question single question

5 = Full recovery

4 = Minor sequelae with no effect, or only minor effects on physicial function; or personality change; or on medication.

3 = Moderate sequelae mildly affecting function, probably compatible with independent living

2 = Severe sequelae, impairing function sufficient to make patient dependent

1 = Death

Please feel free to use the score. We would recommend contacting Dr Penny Lewthwaite

(pennylewthwaite@doctors.org.uk or penny.lewthwaite@liv.ac.uk) for the latest information regarding the score. We request that data from use of the score be submitted, so that we can continue to improve and develop the score.
www.liv.ac.uk/braininfections

8.5 Glasgow Outcome Scale Extended (GOSE)

Subject ID: _____ Date of interview (dd/mm/yy): __ __ / __ __ /__ __

Date of birth : __ __ / __ __ /__ __ Date of injury: __ __ / __ __ /__ __

Sex: Male ☐ Female ☐

Age at injury: _____ Interval post-injury: _____

Respondent:

Patient alone ☐

Relative/friend/carer alone ☐

Patient + relative/friend/carer ☐

Interviewer: _____

Conciousness:

1. Is the head-injured person able to obey simple commands or say any words?

☐Yes ☐No (VS)

Note: anyone who shows the ability to obey even simple commands or utter any word or communicate specifically in any other way is no longer considered to be in vegetative state. Eye movements are not reliable evidence of meaningful responsiveness. Corroborate with nursing staff and/or other caretakers. Confirmation of VS requires full assessment.

Independence at home:

2a. Is the assistance of another person at home essential every day for some activities of daily living?

☐Yes ☐No If no: go to 3

Note: for a NO answer they should be able to look after themselves at home for 24 hours if necessary, though they need not actually look after themselves. Independence includes the ability to plan for and carry out the following activities: getting washed, putting on clean clothes without prompting, preparing food for themselves, dealing with callers and handling minor domestic crises. The person should

be able to carry out activities without needing prompting or reminding and should be capable of being left alone overnight.

2b. Do they need frequent help of someone to be around at home most of the time?

☐Yes (lower SD) ☐No (upper SD)

Note: for a NO answer they should be able to look after themselves at home up to eight hours during the day if necessary, though they need not actually look after themselves.

2c. Was the patient independent at home before the injury?

☐Yes ☐No

Independence outside home:

3a. Are they able to shop without assistance?

☐Yes ☐No (upper SD)

Note: this includes being able to plan what to buy, take care of money themselves and behave appropriately in public. They need not normally shop, but must be able to do so.

3b. Were they able to shop without assistance before?

☐Yes ☐No

4a. Are they able to travel locally without assistance?

☐Yes ☐No (upper SD)

Note: they may drive or use public transport to get around. Ability to use a taxi is sufficient, provided the person can phone for it themselves and instruct the driver.

4b. Were they able to travel locally without assistance before the injury?

☐Yes ☐No

Work:

5a. Are they currently able to work (or look after others at home) to their previous capacity?

☐Yes If yes, go to 6 ☐No

5b. How restricted are they?

a. Reduced work capacity? ☐ a. (Upper MD)

b. Able to work only in a sheltered workshop or non-competitive ☐b. (Lower MD)

job or currently unable to work?

5c. Does the level of restriction represent a change in respect to the pre-trauma situation?

☐Yes ☐No

Social and Leisure activities:

6a. Are they able to resume regular social and leisure activities outside home?

☐Yes If yes, go to 7 ☐No

Note: they need not have resumed all their previous leisure activities, but should not be prevented by physical or mental impairment. If they have stopped the majority of activities because of loss of interest or motivation, then this is also considered a disability.

6b. What is the extent of restriction on their social and leisure activities?

a. Participate a bit less: at least half as often as before injury ☐ a. (Lower GR)

b. Participate much less: less than half as often ☐ b. (Upper MD)

c. Unable to participate: rarely, if ever, take part. ☐ c. (Lower MD)

6c. Does the extent of restriction in regular social and leisure activities outside home represent a change in respect or pre-trauma

☐Yes ☐No

Family and friendships:

7a. Has there been family or friendship disruption due to psychological problems?

☐Yes ☐No If no, go to 8

Note: typical post-traumatic personality changes are: quick temper, irritability, anxiety, insensitivity to others, mood swings, depression and unreasonable or childish behaviour.

7b. What has been the extent of disruption or strain?

a. Occasional - less than weekly ☐ a. (Lower GR)

b. Frequent - once a week or more, but not tolerable ☐ b. (Upper MD)

c. Constant - daily and intolerable ☐ c. (Lower MD)

7c. Does the level of disruption or strain represent a change in respect to pre-trauma situation?

☐Yes ☐No

Note: if there were some problems before injury, but these have become markedly worse since the injury then answer yes to question 7c

Return to normal life:

8a. Are there any other current problems relating to the injury which affect daily life?

☐Yes (Lower GR) ☐No (Upper GR)

Note: other typical problems reported after head injury: headaches, dizziness, sensitivity to noise or light, slowness, memory failures and concentration problems.

8b. If similar problems were present before the injury, have these become markedly worse?

☐Yes ☐No

What is the most important factor in outcome?

☐ a. Effects of head injury

☐ b. Effects of illness or injury to another part of the body

☐ c. A mixture of these

Scoring: The patient's overall rating is based on the lowest outcome category indicated on the scale.

Outcome Score =Lowest score for any single question: _____

Score interpretation:

1 = Death

2 = Vegetative State (VS)

3 = Lower Severe Disability (Lower SD)

4 = Upper Severe Disability (Upper SD)

5 = Lower Moderate Disability (Lower MD)

6 = Upper Moderate Disability (Upper MD)

7 = Lower Good Recovery (Lower GR)

8 = Upper Good Recovery (Upper GR)